Dietary Effects on Lipoproteins and
Thrombogenic Activity

Manual of Operations
Protocol 2 Version 1.0
September 30, 1994

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CHAPTER ONE: Hypotheses and Endpoints

1.0 PROTOCOL 2 HYPOTHESES AND ENDPOINTS

The purpose of this study is to determine the effects of 2 diets, both low in saturated fatty acids but differing in carbohydrate and monounsaturated fatty acid content, on plasma lipids and lipoproteins, and on hemostatic factors. In particular, we are interested in determining whether carbohydrate or monounsaturated fatty acids are the best replacement for saturated fat in people with high plasma triglycerides and/or low HDL cholesterol and/or high insulin levels.

1.0.1 Specific Aims

1. To compare the effects of three diets, differing in total fat, saturated and monounsaturated fatty acids and total carbohydrates on fasting plasma lipids and lipoproteins men and women at increased risk to develop atherosclerotic cardiovascular disease and diabetes.

2. To compare the effects of these diets on plasma hemostatic factors in these men and women.

3. To determine the effects of these diets on postprandial lipids and lipoproteins, and on plasma glucose and insulin levels.

1.0.2 Study Outcomes

1. The primary lipid and lipoprotein endpoints will be plasma concentrations of total cholesterol and triglycerides, low density lipoprotein cholesterol, low density lipoprotein size, high density lipoprotein cholesterol, and apoproteins **B** and **A-I**.

2. A second group of lipid and lipoprotein endpoints will include apo E genotypes, HDL subfractions lipoprotein (a) and very low density lipoprotein cholesterol.

3. The primary hemostatic endpoints will be factor VII and plasminogen activator inhibitor I, and beta thromboglobulin.

4. A second group of hemostatic factors may include fibrinogen and platelet aggregation by flow cytometry.

5. Fasting plasma insulin and glucose levels will also be primary endpoints.

6. Other secondary endpoints will include postprandial lipids, lipoproteins, insulin, and glucose levels.

7. Several ancillary studies may be carried out to test sub-hypotheses.

1.0.3 Rationale for endpoints

The rationale for the study design derives from controversy concerning the optimum diet for individuals with insulin resistance/hypertriglyceridemia/low HDL cholesterol (Syndrome X by Reaven). In particular, although diets low in saturated fat will reduce LDL cholesterol levels, replacement of saturated fat by carbohydrate has been shown, in some populations (particularly diabetics), to increase plasma triglycerides and lower HDL cholesterol levels. In addition, plasma insulin and glucose levels may be higher on high carbohydrate diets. There is little or no information about the effects of these diets on hemostatic factors or lipoprotein (a). Postprandial studies may provide information concerning the effects of these diets on chylomicron remnant metabolism as well as on the daily integrated lipid, glucose and insulin levels on high carbohydrate vs. high monounsaturated fat diets.

1.1 ORGANIZATION AND ADMINISTRATION

The organizational structure of this study will include the following main components: the Field Centers, the Coordinating Center, the Steering Committee, and an external Protocol Review Committee to provide oversight and advice to the NHLBI at various stages of the study. The structure and function of these study components are described below.

1.1.1 Field Centers

There will be four Field Centers responsible for the recruitment, feeding and investigation of study participants. Each Field Center will consist of a team of investigators who will provide the necessary skills and effort to develop and carry out this protocol successfully. The principal investigator and designated key co-investigators from each Field Center will participate in protocol development and in decisions concerning the conduct of the study and the analysis and publication of its results via the Steering Committee and its subcommittees.

1.1.2 Coordinating Center

The Coordinating Center will have primary responsibility for coordinating the efforts of the study investigators and for editing, storing, and analyzing the data generated by the Clinical Centers and by central laboratories established for key study measurements. Its investigators and staff will have a central role in designing the data collection system and in monitoring data quality.

1.1.2.1 Food Analysis Laboratory Control Center (FALCC)

FALCC is the central laboratory for food analysis and associated research; it will receive, composite, assay and archive diet samples from the study.

The FALCC will undertake the following tasks:

1. Develop the necessary protocols for collecting, storing, shipping and compositing the diet samples.

2. Develop, modify and validate the various analytical methods needed for the assay of the food components of concern in the diet composites.

3. Participate in the diet validations as appropriate, including the assay of selected diet-menu-calorie level combinations for the nutrients of concern in this protocol.

4. Participate in the diet monitoring to verify that the menus have the nutrients as planned for the particular experimental period, that the diets being fed at the different centers are virtually identical, and documenting any drift in the composition of the diets over the period of the study.

The director will be a member of the Diet Subcommittee and will participate as a non-voting member of the Steering Committee.

1.1.2.2 Nutrient Composition Laboratory (NCL) U.S. Department of Agriculture (USDA)

The NCL will provide standard reference materials for quality control of the food assays. It will provide external quality control of FALCC. It will also provide expert consultation on all aspects of the control of diet composition. The Director will be a non-voting member of the Steering Committee.

1.1.3 Steering Committee

The Steering Committee is primarily responsible for all decisions pertaining to the design and conduct of the studies undertaken in the DELTA project. It will determine the scientific objectives of the studies, design various studies to attain these objectives -- e.g., this protocol -- and oversee the scrupulous implementation of the study protocols. The voting members of the Steering Committee are the principal investigators of each Field Center and the Coordinating Center and the NHLBI Project Officer. Each has a single vote; a simple majority decides. However, approval of the external Protocol Review Committee will be required for any significant changes in protocol, including the initiation of ancillary studies that are recommended by the Steering Committee during the course of the study. The chairman of the Steering Committee will be appointed by the Director, Division of Heart and Vascular Diseases, NHLBI.

The Steering Committee will meet at least 6 times during the first year and quarterly in subsequent years. It will designate the following subcommittees of investigators and/or staff as needed to oversee aspects of the study that require more frequent attention and/or special expertise. Each subcommittee will have appropriate representatives from each field center and the Coordinating Center:

1. Protocol Subcommittee: oversees the development of study protocols for implementing study objectives identified by the Steering Committee. The Protocol Subcommittee develops study design, sample size calculations, eligibility criteria, data variables and sequencing of measurements. It reviews relevant data collections forms, consent forms, and manuals of operation for each study protocol.

2. Diet Subcommittee: oversees the development and testing of the study diets, identifies food composition assays, evaluates nutrient databases, reviews relevant data collection forms and manuals of operation, coordinates procurement and donations of study foods.

3. Manual(s) of Operation and Forms Subcommittees: provides editorial assistance and final approval for manual(s) of operation, provides content input for data collection forms, approves design and format of forms, reviews suggested or required changes to forms and procedures after the start of the study.

4. Laboratory Subcommittee: the Laboratory Subcommittee advises the Steering Committee on appropriate laboratory measurements to achieve study objectives, and monitors performance of the hemostasis, lipid and lipoprotein laboratories and oversees training and certification of phlebotomists, and laboratory standardization via reports from the Coordinating Center. This committee reviews relevant manual(s) of operation and data forms.

5. Publications Subcommittee: advises the Steering Committee on publication policy. It oversees the preparation and review of abstracts and manuscripts emanating from the study to assure proper distribution of analysis topics and authorship credit among study investigators. It ensures that collaborative manuscripts represent the study accurately.

6. Conflict of Interest Subcommittee: drafts guidelines regarding outside activities of study investigators that represent potential conflicts of interest and collects annual disclosure statements from investigators regarding relevant activities.

   DELTA investigators in cooperation with the NHLBI project office seeks donations of products, equipment and services to support specific activities of the study.

   We follow the guidelines established by the NHLBI for obtaining third party support.

   A major effort has been made to get food donations from national companies in order to defray the cost of feeding the diets across clinics. Other potential third party support includes cholesterol screening machines and various participant incentives.

7. Ancillary Studies Subcommittee: reviews and makes recommendations regarding the merit and feasibility of ancillary studies that are proposed by study investigators or other interested parties.

The Steering Committee will retain the prerogative to add, combine, delete, or redefine subcommittees as the study evolves and its needs change.

1.1.4 External Oversight

1. Protocol Review Committee: This committee of at least five experts not otherwise affiliated with the study has been appointed by the Director, NHLBI to review this protocol, to recommend revisions as needed, and to advise the Institute as to its acceptability. This study was not implemented until the Director, NHLBI, acting with the advice of this committee, approved this protocol.

   This committee will meet prior to initiation of each new study and additionally as necessary to carry out its oversight responsibility.

   The Chairman of the Steering Committee, Project Officer, and designated NHLBI staff will participate in these meetings in an ex-officio capacity, to facilitate communication between the Protocol Review Committee and Steering Committee.

   The principal investigator of the Coordinating Center and designated Coordinating Center staff will attend these meetings (but will not have a vote) and will be responsible for preparing and presenting up-to-date statistical reports on the progress of the study. These reports will include data on recruitment and randomization, as well as statistical tests and special analyses requested by the committee.

1.1.5 Human subjects protection

A. Informed consent

Informed consent will be obtained from each participant before they are enrolled in the study. Field Center Informed Consent forms are found in the Appendix to this Manual. The consent form will describe the risks and benefits of participating in the study, as well as the responsibilities of the participants and the investigators.

B. Privacy

Privacy in the context of this study includes confidentiality of data and personal information at the field center (see individual site descriptions below) and in handling and reporting of data by the Coordinating Center (see Chapter 9). It also includes discretion on the part of field center staff and arrangements for physical privacy during interviews and examinations.

C. "Right-to-know"

Information obtained at screening, before enrollment, would be available at that time. Abnormal values found at screening would be reported to the participant and also, upon request, to his or her personal physician. During the course of the study, participants occasionally are curious about their progress and about

changes in a variable such as serum cholesterol level. Such data will not be available until the very end of the study, because the protocol calls for batch analysis of all samples collected throughout. Information will be provided at the end of the study to participants who are interested.

D. Safety

The DELTA study will evaluate the effects of various diets on blood lipids, and lipoproteins, clotting parameters and related variables. The diets will have varying composition but will not differ greatly from those consumed by many members of the public. All diets will be nutritionally adequate and meet subjects' calorie needs (they will by designed specifically to prevent weight change). The experimental diets will be planned using the RDA for men and women 25-50.

The experimental treatments for the DELTA study are not expected to pose any particular risk. Since participants of this study are at increased risk of developing coronary heart disease we will provide group instruction on the principles of a Step 1 diet at the end of each feeding period. Subjects will be encouraged to follow this diet during the breaks between the feeding periods. At the end of the study, all subjects will receive either individual or group instruction on the implementation of healthy dietary practices. Individuals for whom the diets are contraindicated because of pre-existing conditions (for example, liver disease, kidney disease, clotting disorder) would not be eligible for participation according to defined exclusion criteria.

Information about food allergies will be collected during screening.

The collection of biological specimens and other information carries a small risk. For example, phlebotomy carries a risk of bruising and discomfort. This will be explained to the participant in the consent form.

Food safety procedures will be rigorously applied in order to protect participants against illness due to microbiological contamination of food. The DELTA field centers will guard against this through rigorous application of food handling standards appropriate for their particular institution (hospital-based kitchen; other setting). Individuals will be carefully instructed in the handling and storage of any take-home food or meals. Coolers and "blue-ice" will be provided and time-temp strips will be enclosed with instructions not to consume certain foods if the strip indicates that holding temperatures have not been appropriate. The consent form will include an explanation of the participant's obligation to handle take-out food according to instructions they will be given to maintain adequate sanitary conditions.

Safety oversight

Safety concerns will be addressed during protocol review by the NHLBI-appointed committee and by the individual field center IRBs. After enrollment,

the principal investigators will monitor safety issues continuously and report any problems to the Coordinating Center, which will inform the NHLBI Medical Officer. Each field center will have an assigned Medical Officer to ensure the satisfactory disposition of Study-related adverse events (referral to physician; treatment; or decision to drop from Study for medical reasons, which must be made with P.I.).

MEDICAL OFFICERS:

Columbia:	Dr. Henry Ginsberg Dr. Neil Shachter
Minnesota:	Dr. Aaron Folsom
Penn State:	Dr. W. C. Nicholas
Pennington:	Dr. Donna Ryan

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CHAPTER TWO: Recruitment

2.0 RECRUITMENT GOALS

The Dietary Effects on Lipoproteins and Thrombogenic Activity 2nd protocol (DELTA) will recruit men and women between the ages of 21 and 68 years of age. The study population will include approximately 50% and 50% men, and approximately 15% African American.

Table 1 gives an example of possible recruitment outcome. In this chapter we will use "Black" to refer to African American subjects, and "White" to refer to all other subjects in the study. We anticipate that this latter category may include a few Asian and Hispanic subjects.

Each DELTA center has experience in recruitment and will approach the recruitment phase using sources, methods and strategies that have been most successful for them in the past. Recruitment will take place over approximately two months time and will include the use of flyers, brochures, advertisements and announcements to attract study participants. In addition, lists prepared from recruitment for previous studies, university employee directories and student and community organizations listings will provide targeted sources for mailing purposes. Recruitment will include a variety of sources to produce a wide range in participant age and cholesterol levels.

Table 1

Assumed Sample Design

Center	Gender	Black	White	Total
Columbia U.	F+	1	4	5
	F-	2	4	6
	M	6	8	14
Pennington	F+	3	4	6
	F-	2	3	5
	M	7	7	14
U. Minn.	F+	2	4	6
	F-	1	4	5
	M	3	11	14
Penn. State	F+	2	4	6
	F-	1	4	5
	M	3	11	14
Total	F+	8	15	23
	F-	6	15	21
	M	19	37	56
		33	67	100

M: Males
F+: Premenopausal Females
F-: Postmenopausal Females
Black: African American subjects
White: Non - African American subjects (mostly Caucasian)

2.1 RECRUITMENT METHODS AND SOURCES BY FIELD CENTER

2.1.1 University of Minnesota DELTA Recruitment

2.1.1.1 Sources

Recruitment sources will include lists from previous studies, university employees, and age eligible students. Recruitment efforts will be coordinated with a University of Minnesota health clinic which serves employees in an attempt to identify individuals who are most likely to meet eligibility criteria. Care will be taken to target recruitment toward individuals to whom such a study is feasible in an effort to reduce the overall recruitment cost and produce a yield of participants who are likely to be retained throughout the diet periods.

2.1.1.2 Methods and Strategies

The University of Minnesota has successfully recruited subjects for feeding studies through informational advertising on the campus. Flyers, posters, employee and student letters and advertisements in the campus newspapers and newsletters will serv as a base of methods to be utilized to attract interest among the campus population. A University of Minnesota health clinic population will be surveyed to obtain lists of likely eligible individuals to whom study recruitment information will be mailed. Recruitment of the surrounding community will involve mailings to specific zip code areas, posters in community locations and advertisements and feature articles in targeted community newspapers.

A thorough description of the feeding study and the expectations and commitment necessary will be provided in the beginning phase of recruitment in the effort to minimize otherwise eligible individuals who are not interested in the rigors of a feeding trial. This effort will reduce the professional staff time and laboratory costs and will likely minimize study dropouts.

2.1.2 Columbia University DELTA Recruitment

2.1.2.1 Population and Sources

Subjects will be recruited for this protocol from the large number of employees at Columbia Presbyterian Medical Center. The overwhelming response we had with the first protocol has indicated to us that our employees will attend the DELTA Health Fair and for lipid screening. Potential subjects can also be selected from the first and second year classes at the Medical and Dental schools and the School of Nursing as has been done in the past several years for our studies.

Potential candidates will also be identified through the division of General Medicine in the Department of Medicine which provides primary care for 8000 - 10,000 patients. Sixty-five percent of these patients live within 5 miles of the medical center. We also have the SCOR lipid clinic. Dr. Henry Ginsberg is the director of this clinic. Three hundred new patients are evaluated yearly. Some of these individuals may be eligible for this study.

2.1.3 Pennington Biomedical Research Center DELTA Recruitment

2.1.3.1 Sources

The PBRC Field Center will recruit subjects from the following sources: PBRC Volunteer Data Base. Over the course of the last 3 years, the PBRC Clinic has received over 4,000 responses from individuals wishing to participate in ongoing clinical studies. In excess of 750 individuals have expressed specific interests in participating in dietary studies. These potential subjects will be pre-screened for eligibility and targeted for recruitment.

Louisiana State University. The LSU campus in Baton Rouge is located within 4 miles (15 minute drive) of PBRC. The campus population is comprised of 22,000 undergraduate students, 5,000 graduate students and 5,000 employees. Many of the students and employees of LSU live in the community immediately surrounding the PBRC Field Center.

Southern University. The Southern University Campus is located approximately 20 minutes away from the PBRC Field Center. Southern University is an historically African American university and currently has an enrollment of over 9,000 students. The PBRC has an established record of interaction with Southern University.

Surrounding Baton Rouge Community. To assure adequate subject volunteers, the PBRC Field Center has cultivated a constituency among the Baton Rouge community including ties to its civic, social and religious organizations.

2.1.3.2 Methods and Strategies

All recruiting will be coordinated through a full-time Clinical Subject Recruiter. Advertisements describing the study will be placed in the university and community newspapers.

Publications Director, Ben Phillips, a veteran journalist, will contact local newspapers and the radio and television stations to publicize the study and volunteer recruitment. Public Education Director, Ruth Patrick, Ph.D., performs a weekly five minute spot on a local television station on nutrition-related issues. Her presentation of research activities at the PBRC will be used to aid in subject recruitment.

2.1.4 Penn State University DELTA Recruitment

2.1.4.1 Sources

Recruitment sources will include University employees and students, lists from previous studies, patients of physicians in the local and surrounding communities and the general population within the surrounding community. The University's Office of Health Promotions (OHP) willing shares lists of employees who will be targeted. In addition, the Office of Minority Affairs is a source of contact to assist with the recruitment of minority (e.g.; African Americans) subjects into the study. Local physicians have expressed an interest i the study. They will be contacted (via a letter followed by a phone call) to provide assistance with recruitment efforts.

2.1.4.2 Methods and Strategies

Various methods have proved to be very effective in recruiting subjects for previous feeding studies at Penn State. Most notably, personalized letters sent to the University community (employees and age-eligible students) that describe the study and encourage participation in it have been highly effective. Lists and mailing addresses of employees and graduate students will be obtained from Office of Administrative Systems and the Penn State registrar. In addition, other successful recruitment approaches that have been used in previous feeding studies will be used. These include announcements to various organizations and off campus, flyers, posters, radio announcements (public service and advertisements), newspaper advertisements and articles (campus and local newspapers), television announcements (C-Net), E-mail, electronic bulletins, and newspapers. Recruitment meetings will be scheduled for potentially interested subjects. These are held at different times to inform potential subjects about the requirements of the study. This has been an effective strategy for recruiting friends and significant others of interested individuals.

Physicians in the local community, especially those who have participated in other Penn State studies, will be informed about the study. Their participation in recruiting eligible students via word-of-mouth will be sought. In addition, flyers and posters will be placed in these physicians' offices.

Finally, lists of individuals who have participated in other nutrition studies or in programs sponsored by the OHP over the years will be collected. These persons will e contacted via mailings and informed about the DELTA Study Protocol 2.

2.2 RECRUITMENT FORMS

A recruitment source log is used by the DELTA centers to record all methods utilized to recruit participants. Each method used receives a code. Prospective participants will be asked to indicate where their knowledge of the study originated, and the appropriate code will be included on their eligibility forms.

2.2.1 Recruitment Source Log

The recruitment source log would be used to record any and all methods employed to recruit participants. Each method used would receive a code and additional information would be recorded (including where a poster, flyer, article or advertisement was placed, the date, and the cost).

The code is derived using a three digit system where the first digit would indicate the major method category (i.e., written materials, network publicity, mailings or word of mouth), the second digit could indicate the method within a category (i.e., flyer, poster, newspaper advertisement, etc.) and the third digit could be center-specific for tracking of the response rate on a particular poster, advertisement, etc.

The shaded areas to the right of the form allow information regarding number of responses generated and actual number of scheduled visits resulting from a particular method to be recorded.

The log, itself, would not require data entry. It would serve as a record of recruitment methods and sources used. The assigned code would be major importance as a description of the recruitment method and source utilized at your field center. Each center will be required to report recruitment methods and sources utilized at your center. Also, copies of each recruitment material will be sent to the Coordinating Center for archival purposes. This information will not be entered into the data entry system (DES); however, the Coordinating Center will take the raw data sent to the Coordinating Center and report results.

Prospective participants would be asked to indicate where their knowledge of the study originated on the Telephone Screening Form.

Because of the different recruitment methods and different populations at each site, it is felt that a multicenter approach cannot be taken with this. Each site will be able to generate cost comparisons on method and yield for each center-specific site. Information gained would be extremely valuable in the ongoing recruitment process for Protocol 1 and will be used to target recruitment efforts to improve yield and cost effectiveness.

2.2.2 Weekly Recruitment Tracking Form (WRT)

During recruitment, the WRT is filled in every Friday by each study coordinator and faxed to the Coordinating Center. Cumulative summaries are prepared by the Coordinating Center and faxed to the Steering Committee.

2.2.3 Eligibility Monitoring Form (EMF)

The EMF is used to monitor recruitment by eligibility category. It is completed weekly at each field center for potential participants who qualify after completing EV2. It is faxed to the Coordinating Center where cumulative summaries are prepared and faxed to the Steering Committee.

2.2.4 DELTA retention form (DRF)

The DRF is used to track retention rates study. It is filled in weekly at each center and forwarded to the Coordinating Center (Form to be added).

Figure 2.2.6 Recruitment Source Log

RECRUITMENT SOURCE LOG

Recruitment Period: Diet Protocol 1

Written Materials Method	Code	Date	Additional Information	Cost	#Resp	#Sched
Flyers						
1.						
2.						
3.						
4.						
Posters						
1.						
2.						
3.						
4.						
Newspaper Advertisements						
1.						
2.						
3.						
4.						
Newsletter Articles						
1.						
2.						
3.						
4.						
Other						
1.						
2.						
3.						
4.						

RECRUITMENT SOURCE LOG

Recruitment Period: Diet Protocol 1

Network Publicity Method	Code	Date	Additional Information	Cost	#Resp	#Sched
Radio PSA						
1.						
2.						
3.						
4.						
Radio Talk Show						
1.						
2.						
3.						
4.						
Television PSA						
1.						
2.						
3.						
4.						
Television Talk Show						
1.						
2.						
3.						
4.						
Other						
1.						
2.						
3.						
4.						

RECRUITMENT SOURCE LOG

Recruitment Period: Diet Protocol 1

Mailings Method	Code	Date	Additional Information	Cost	#Resp	#Sched
Existing Lists						
1.						
2.						
3.						
4.						
Check Stuffers						
1.						
2.						
3.						
4.						
Department Mailboxes						
1.						
2.						
3.						
4.						
Other						
1.						
2.						
3.						
4.						
Word of Mouth						

Figure 2.2.7 Weekly Recruitment Tracking Form

DELTA WEEKLY RECRUITMENT TRACKING FORM

Field Center: _____

Date: _____

Counts This Week | **Cumulative Counts**

Inquiries (overall count) [ ] [ ]

Counts This Week | **Cumulative Counts**

	WM <40	BM <40	WM >40	BM >40	WF+	BF+	WF-	BF-	N	WM <40	BM <40	WM >40	BM >40	WF+	BF+	WF-	BF-	N
Completed TSV																		
Completed EV1																		
Completed EV2																		
Selected for Run-In																		
Completed Run-In																		
Randomized																		
Projected																		

Legend:

- B = African Americans
- M<40 = Males 22-40 years
- F+ = Premenopausal Females
- N = Overall count across age, race, gender, and menopausal status
- W = All Other Races
- M>40 = Males over 40 years
- F- = Postmenopausal Females

Notes:

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Figure 2.2.8 Eligibility Monitoring Form

ELIGIBILITY MONITORING FORM

For subjects entered into the study this week, indicate the number of subjects that qualified under the appropriate inclusion category.

		INCLUSION CATEGORY							
		↓HDL	↓HDL + ↑TG	↓HDL + ↑INS	↑TG	↑TG + ↑INS	↑INS	↓HDL + ↑TG + ↑INS	TOTAL
This Week	COL								
	MN								
	PBRC								
	PSU								
Total	COL								
	MN								
	PBRC								
	PSU								
Total	ALL CENTERS								

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CHAPTER THREE: Visit Schedule and Description of Eligibility Visits

3.0 VISIT SCHEDULE AND DESCRIPTION OF ELIGIBILITY VISITS

There are three encounters with the applicant (Telephone Screen, Eligibility Visit 1 and Eligibility Visit 2) where eligibility is determined. The schedule of these visits and an outline of important features and requirements of these visits are in this chapter. Instructions for completing all data collection forms are also found in this chapter.

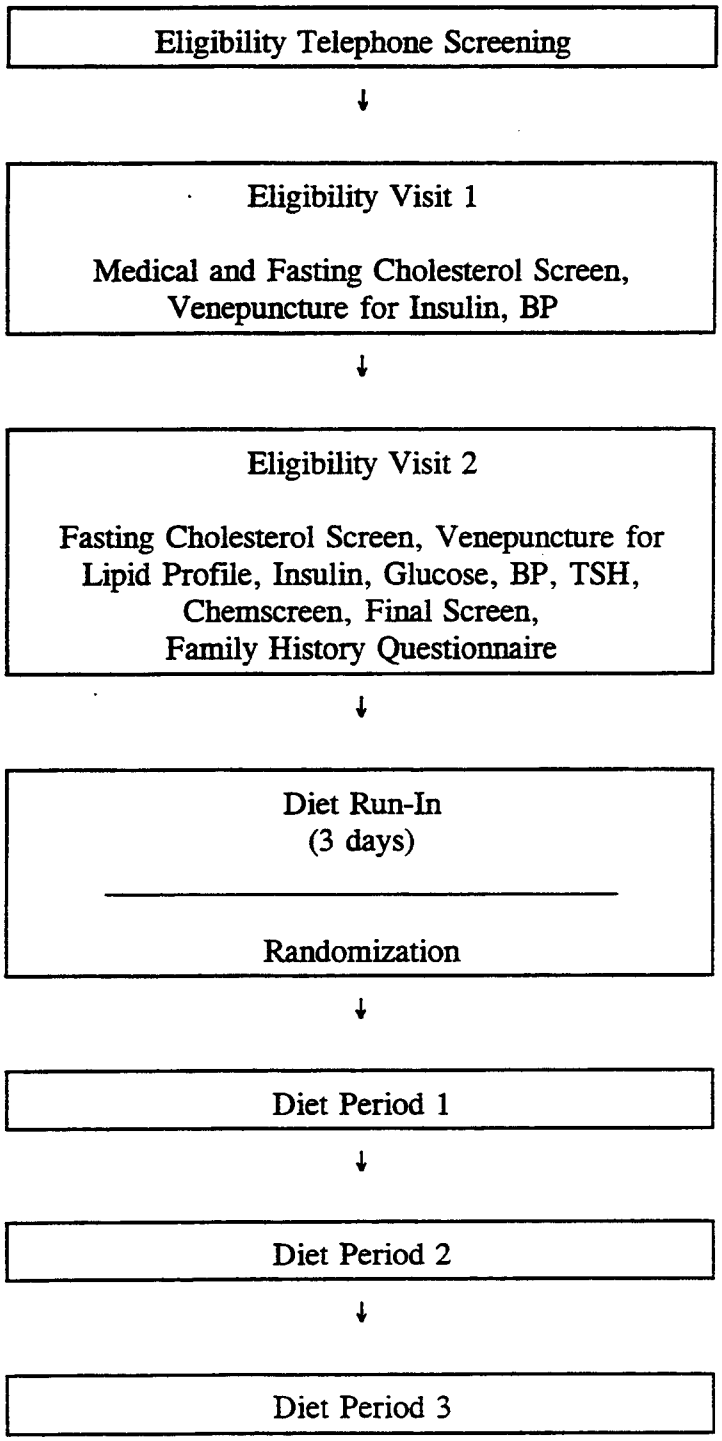
All items on the study forms that require medical review must be reviewed by medical personnel before proceeding. Forms should NOT be completed retrospectively from clinic notes or memory. If corrections are made to the form after the data are eventually keyed into the data entry system (DES), give the form to the person who is responsible for keying data at your site to make the necessary corrections.

SCHEDULE

Screening Episode	Timeframe
Telephone Screening Form	Anytime
Eligibility Visit 1 (EV1)	At least one week after initial contact from potential participant
Eligibility Visit 2 (EV2)	One to four weeks after EV1

3.1 VISIT FLOW CHART

DELTA Protocol 2 Design Overview - Visit Sequence



3.2 GENERAL INSTRUCTIONS

Please use the following guidelines when completing any of the data collection forms:

1. Use a black ball-point pen and **print** neatly.

2. List all dates numerically as month/day/year, e.g. March 21, 1961 is 03/21/61.

3. Data collected on April 3, 1993 would be entered as Today's Date: 04/03/93

   NOTE: DELTA usually records time using a 12-hour clock, with AM or PM indicated separately. Colons (":") are used as the separator character for hours and minutes. The format to be used is indicated under the space. Use leading zeros within each time unit (hour or minute) so that each space is filled. Note that midnight is recorded as 12:00 AM, and noon is recorded as 12:00 PM.

   Example: A time of fasting determination of 8:05 in the morning is entered as:

   a. Time: 08:05 b. AM (A) or PM (P): A

4. Make corrections by drawing a single line through the incorrect entry and then indicate the correct entry as near to the incorrect entry as possible. Initial and date the correction.

   Example: 10/14/92 6:45

   Do not use correction tape or fluid.

5. If data requested are not applicable to the patient, please indicate by NA (Not Applicable). If data requested are not known or if particular procedure was not performed, please enter ND (No Data).

   The following forms should be completed at the field centers at the time indicated:

Form	Time for completion
Telephone Screening Form (TSF)	Phone interview
Eligibility Visit 1 Form (EV1)	Visit 1
Eligibility Visit 2 Form (EV2)	Visit 2
Family History (EV2)	
Additional forms to be completed include:	
Participant Agreement Form	
Consent Form	

6. Read all forms instructions and the actual forms prior to beginning an interview. This will greatly enhance flow of the interview and the accuracy of the information collected.

7. **Always** complete the subject ID number, your code number, and the date the form is being collected first. This information is requested at the beginning of each form.

8. Ask the questions exactly as they are written on the forms.

9. Please retain all the subject data for at least 5 years following the completion of the trial. Before destroying any records, please contact the DELTA Coordinating Center.

DO NOT HESITATE TO CALL SUSAN BLACKWELL AT (919)-962-3092 IF YOU HAVE ANY QUESTIONS REGARDING THESE FORMS!

3.3 TELEPHONE SCREENING

3.3.1 Purpose

1. To introduce the study and answer questions the participant may have.

2. To have the interviewer complete the Telephone Screening Form.

3. To determine initial eligibility and complete general medical condition questionnaire.

4. To determine alcohol consumption.

5. To obtain a list of current medications.

6. To obtain weight and height and compare against upper weight limit table.

7. To determine if women in child-bearing age are pregnant, planning to become pregnant, breastfeeding or have borne a child within the last 6 months.

8. To give a general description of the study and determine further interest.

9. To schedule EV1 if patient remains eligible.

3.3.2 Items Needed for Telephone Screen

1. TSF questionnaire to be completed by the interviewer.

3.3.3 Telephone Screening Form Instructions

Instructions for Completing the Telephone Screening Form (TSV), Version B

IMPORTANT: Read both the General Instructions and the Instructions related to the Data Management System (located in the DELTA Forms Guide) before beginning completion of all forms.
GENERAL REMINDERS: Please use black ball-point pen. Print all responses legibly. Initial and date all corrections. Be sure to respond to all applicable questions. Enter NA or ND where applicable.
Before beginning, thoroughly read the instructions about assigning an appropriate DELTA ID. DO NOT assign the DELTA ID until the end of the Telephone Screening Interview.

Enter the date of the interview.

Question 1: Personnel code number of person completing the form

1. Enter your personnel code number.

Questions 2-5: Applicant name, address, home telephone number and work telephone number

2-5. Enter the applicant's complete name, address, home telephone number and work telephone number. Please ask for correct spelling if necessary. Read this information back to the applicant to ensure accuracy. Ask about the best time to try to reach the applicant and if it is allowable to call at his/her work number.

Questions 6-9: Demographics

6. Enter the applicant's birth date numerically as month, day, and year. For example, March 14, 1965 should be entered as 03/14/65.

7. Enter the applicant's age in years in the space provided.

8. If uncertain, ask applicant what is his/her gender and circle M for male or F for female.

9. a. It is possible that some applicants may be offended by this question. If this occurs assure the applicant that the information is needed solely for demographic reasons. Allow the applicant not to respond if he/she feels strongly. Circle the letter representing the response. For example, for Caucasian circle A. **Please note** "Did not respond" M is an option for this answer.

   b. Responses coded L for Other, should be described with information the applicant provides.

Question 10: Advertisement of Study

10. a. Circle letter representing response. For example, for Radio PSA (Public Service Announcement) , circle E.

b. Responses coded 0 should be described in the space provided.

Question 11: Availability for the next year

Circle YES or NO.

If YES, go to question 22.

If NO, the applicant has become ineligible for the study. Thank the applicant for his/her time and interest, terminate the interview, and complete questions 12-13.

(At this point in the interview, the interviewer proceeds to question #22 to continue.)

Questions 12-13: Administrative Information

This section is to be completed at the end of the telephone interview. At this point in the interview, proceed to question 22. Return to this section at the end of the interview and follow the instructions below.

12. a. Indicate whether applicant is eligible for the study by circling Y, N, or R.

b. - d. If the response is R, list question numbers requiring medical review.

(If any medications are listed under items 24-29, the applicant must be reviewed by medical personnel before a decision can be made regarding eligibility. Please check the accuracy of the applicant's telephone number for future contacts.)

e. - g. If applicant is eligible for the study at this time, schedule a date and time for the first screening visit. Remind applicant to bring all medications (prescription and non-prescription including oral contraceptives) to the first visit.

h. After Medical Review has been completed, circle YES or NO and complete additional information required.

13. To be completed at the point in time when the reason for an applicant's exclusion is known.

a. Circle the letter for the appropriate response.

b. - d. List exclusion code number(s) from the Exclusion Code List provided in the DELTA Forms Guide.

Questions 14-21: Applicant's Lab and Measurement Results

This section must be completed after the eligibility visits if Lab and Measurement data were obtained at these visits.

Question 22: Medical Conditions

a. - o. For question 22, read the entire list of medical exclusions to the applicant and circle Y for yes, N for no **or** never tested, **or** U for unsure.

Listed below are a number of medical terms associated with certain diseases which the applicant may note when answering this question. This list is meant to help you complete this form accurately, it is **not** a comprehensive list.

Heart Disease: myocardial infarction, coronary occlusion, coronary thrombosis, or congestive heart failure.

Diabetes: sugar in the blood or urine

High Blood Pressure: hypertension, high blood

Renal Disease or Kidney Disease: nephritis, pyelonephritis, glomerulonephritis, chronic kidney infections

Gastrointestinal Condition: Crohn's disease, irritable bowel syndrome, ulcer problems, ulcerative colitis, acute ulcer, gastric resection, bowel surgery

Blood Clotting Disorders: Prolonged bleeding, any condition requiring use of coumadin.

Liver Disease: cirrhosis, alcoholic liver disease, chronic hepatitis

Conditions that require steroid medication: asthma, allergies

Gout requiring treatment: If applicant has been told in the past that they have gout but they are not taking any medication for this problem, they are still eligible for the study.

Depression or Mental Illness: Bipolar disorder, SAD (Seasonal Affective Disorder), manic-depressive illness, panic disorder, nerves, chronic depression severe enough to be treated with antidepressant medication.

Sickle Cell Anemia: This does **not** include sickle cell trait

If any of the items in question 22 (except item J) are answered YES, the applicant has become ineligible for the study. Please thank the applicant for his/her interest, terminate the interview, and complete questions 12-13. If only item J is answered YES, medical review by qualified personnel will be required to determine eligibility status. If any item in question 22 is marked UNSURE, medical review by qualified personnel will be required to determine eligibility status.

Question 23: Other Medical Conditions

a. Circle YES or NO. If NO, go to question 24.

b.-d. If YES, list the medical condition noted in the blanks provided. Please attempt to obtain the technical term for the medical condition rather than a common term, e.g. hypertension or high blood pressure instead of "high blood". If any medical condition is listed, review by medical personnel is required.

Questions 24-29: Medications

Circle YES or NO. If NO, go to question 30.

a.-c. If YES, under items 25-29 enter all medications taken, either prescribed by a doctor or self-prescribed. Ask applicant for correct spelling. If possible, have applicant read spelling directly off of the product label. For each entry, indicate whether the medication is prescribed by a doctor by circling Y or N. Be sure to list the reason for taking the medication.

Reminder: If any entries are made to this section, the information must be reviewed by medical personnel before a determination of eligibility can be made.

Question 30: Food Allergies

a. Circle YES or NO. If NO, go to question 31.

b.-d. If YES, please attempt to obtain as complete information as possible, e.g. if milk is noted, please ask if allergic to all dairy products; if nuts are noted, please ask if allergic to all nuts or just certain types. If any food allergy is listed, review by medical personnel is required.

Question 31: Refusal to Eat Specific Foods

a. Circle YES or NO. If NO, go to question 32.

b.-d. If YES, list the specific foods mentioned.

Question 32: Special Diets

a. Circle YES or NO. If NO, go to question 33.

b.-f. If YES, read through the list provided and circle YES or NO, or UNSURE. If YES or UNSURE is circled for any special diet, review by medical personnel is required.

g. Responses coded F should be described in more detail.

Question 33: Alcohol Consumption

a. Circle YES or NO. If NO, go to question 34.

b. If YES, enter the number of drinks the applicant consumes in a seven day week (weekdays and weekends together). Note: 1 drink = 1, 5 oz. glass of wine; 1, 12 oz. serving of beer; or a 1.5 oz. shot of liquor. Enter 0 for less than 1 drink per week. If the answer is greater than 12, the applicant has become ineligible. Thank the applicant for his/her interest, terminate the interview, and complete questions 12 - 13.

Questions 34-36: Body Mass Index (BMI)

34. Enter the applicant's height in feet and inches. Height should be without shoes rounded to the nearest inch.

35. a. Enter applicant's weight in pounds. Weight should be without shoes, rounded to the nearest pound.

    b. Find the applicant's height in the BMI table provided in the DELTA Forms Guide. Circle YES or NO.

    If the applicant's weight is greater than the upper limit listed for his/her height the applicant is ineligible for the study. Thank the applicant for his/her time and interest, terminate the interview, and complete questions 12-13.

36. Circle YES or NO. If the applicant indicates NO, emphasize again that this study requires participants **not** to lose or gain weight. If the applicant affirms NO to this question he/she has become ineligible for the study. Thank the applicant for his/her time and interest, terminate the interview, and complete questions 12-13.

Questions 37-39: Women born after 1943 only

Circle YES or NO. If YES to any of these questions, the applicant is no longer eligible for the study. Thank the applicant for her time and interest, terminate the interview, and complete questions 12-13.

At this point in the interview, if the applicant remains eligible for the study, read him/her the general description of the DELTA study located in the DELTA Forms Guide.

Question 40: Further Interest

a. Circle YES or NO.

If YES go to questions 41 & 42. Schedule the applicant for Eligibility Visit 1 and **enter this information under question 12 of this form.**

b.-k. If NO, circle YES or NO for all reasons listed and **complete questions 12-13 of this form.**

Question 41: Scheduling Eligibility Visit 1

Schedule the date and time for EV1 in the spaces. Be sure to circle AM or PM.

Instruct the applicant to bring all medications he/she is taking to the visit. This includes both prescription and over the counter medications, diet supplements, and for females, oral contraceptives.

Read the instructions about Fasting to the applicant.

Question 42: Need for Medical Review

Circle YES or NO.

If YES, tell the applicant he/she will be called back.

(Page 12 of the form is provided for additional notes if needed.)

Telephone Screening Form

Form Code: TSV
Version B 5/10/94

INTERVIEWER: Do not assign the DELTA ID until the Telephone Screening Visit is completed and you determine if the applicant is or is not eligible for Eligibility Visit 1. The assignment of the DELTA ID is as follows:

- **If the applicant is eligible for Eligibility Visit 1, then assign an ID from the set of IDs with sequence numbers 0100-0499. This ID will be used for this applicant throughout the screening visits until randomization. Blood tube labels are provided for these IDs.**
- **If the applicant is not eligible following the Telephone Screening Visit, then assign an ID from the set of IDs with sequence numbers 0500-1599. No other forms labels or blood tube labels are provided for these IDs.**

DELTA ID_____ Today's Date ____/____/____

1. Code Number of personnel completing form: _____

2. _____
   a. First Name b. MI c. Last Name

3. _____
   a. Street

   _____
   b. City c. State d. Zip Code

4. Home Telephone: _____ 5. Work Telephone: _____
   area-###-#### area-###-####

   When is the best time to call? _____

   Can you receive a call at work? YES NO

6. Date of Birth: _____ 7. Age: _____ 8. Gender: M (male) F (female)
   mm/dd/yy

3.3.4 Telephone Screening Form

Telephone Screening Form

Form Code: TSV
Version B 5/10/94

9. a. What do you consider your race to be? **[Circle letter preceding selection]**

A Caucasian (white)	F American Indian	K Pacific Islander
B African American (black)	G Asian Indian	L Other
C Hispanic	H Korean	M Did not respond
D Mixed Race	I Vietnamese	
E Chinese	J Japanese	

b. If Other, describe:_____

10. a. How did you hear about this study? **[Circle letter preceding selection]**

A Flyer	F Radio Talk Show	K Letter in Dept. Mailbox
B Poster	G TV PSA	L E-Mail
C Newspaper Ad	H TV Talk Show	M Physician or Nurse
D Newsletter Clip	I Letter by Mail	N Word of Mouth
E Radio PSA	J Flyer in Paycheck	O Other

b. If Other, describe:_____

11. Do you plan to remain in the area for the next year? YES NO

[If the answer TO QUESTION 11 is NO, then the applicant has become ineligible. Terminate the interview and complete questions 12-13.]

Telephone Screening Form

INTERVIEWER: CONTINUE WITH QUESTION 22. COMPLETE THIS PAGE AFTER THE TELEPHONE SCREENING VISIT IS COMPLETE!

12a. Is applicant eligible following telephone screening?

[Circle the letter preceding selection.]

Y YES
N NO
R NEEDS MEDICAL REVIEW **[Enter question number(s) to be reviewed.]**

b.____, c.____, d.____

e. If YES to 12a, date of EV1 : _____ (mm/dd/yy) f. Time: _____ (hh:mm) g. AM PM

h. If NEEDS MEDICAL REVIEW to 12a, does the applicant remain eligible?
YES NO

__/__/__ (date of medical review) _____(initials of MD)

13a. If this applicant has been excluded from the study, at which time point was the applicant excluded? **[circle letter preceding selection]:**

A Telephone Screening
B Eligibility Visit 1
C Eligibility Visit 2

[SEE EXCLUSION CODE LIST in the Forms Guide. Enter reason code numbers below.]

b._____, c._____, d._____

Telephone Screening Form

INTERVIEWER: SKIP THIS PAGE DURING THE TELEPHONE SCREENING INTERVIEW.

After Eligibility Visit 1 and Eligibility Visit 2 have been completed, return to this form and record the applicant's lab values, blood pressure, and height and weight in the spaces provided below.

APPLICANT'S LAB AND MEASUREMENT RESULTS

	EV1	EV2
14. TC	a. mg/dl	b. mg/dl
15.LDL	a. mg/dl	b. mg/dl
16.HDL	a. mg/dl	b. mg/dl
17. TG	a. mg/dl	b. mg/dl
18. INS	a. μU/ml	b. μU/ml
19. BP	a. systolic b. diastolic	c. systolic d. diastolic
20. HT	a. ft b. in	c. ft d. in
21. WT	a. lbs	b. lbs

Telephone Screening Form

MEDICAL CONDITIONS

22. Because certain medical conditions will interfere with our study, we need to ask the following questions. Do you have any of the following medical conditions?

[Interviewer: Read list of medical conditions and circle response YES (Y), NO (N) if NO or NEVER TESTED, or UNSURE (U)]

a. heart disease	Y	N	U
b. diabetes	Y	N	U
c. high blood pressure or hypertension treated with medication	Y	N	U
d. renal or kidney failure	Y	N	U
e. gastrointestinal condition (Crohn's disease, irritable bowel syndrome, ulcer problems, bowel surgery)	Y	N	U
f. history of blood clotting disorders	Y	N	U
g. liver disease (cirrhosis)	Y	N	U
h. condition that requires use of steroid medication	Y	N	U
i. gout requiring treatment	Y	N	U
j. recent history of depression or mental illness requiring medication or treatment within last 6 months	Y	N	U
k. anemia	Y	N	U
l. sickle cell anemia	Y	N	U
m. lung disease, chronic bronchitis, emphysema	Y	N	U
n. positive HIV test or Acquired Immune Deficiency Syndrome (AIDS)	Y	N	U
o. cancer (active within last 5 years)	Y	N	U

[If any medical condition was circled UNSURE, or item J was circled YES, then review by medical personnel is required to determine eligibility status. If any item, other than J, was circled YES, then the applicant has become ineligible. If so, terminate the interview and complete questions 12-13.]

OTHER MEDICAL CONDITIONS

23. a. Do you have any other medical conditions not listed above?
YES NO **[If NO go to question 24]**

[If YES] Please list other medical conditions **[enter one per line]:**

b. _____

c. _____

d. _____

[If any medical condition is listed, review by medical personnel is required.]

MEDICATIONS

24. Do you take any type of doctor or self-prescribed medications? YES NO
[If NO go to question 30]

[If YES] What is the name of the medication that you take?

[Record both doctor and self-prescribed medications. Ask for spelling of medication if necessary.]

a. Medication	b. Prescribed by Doctor YES (Y) or NO (N)	c. Reason for taking medication
25.	Y N	
26.	Y N	
27.	Y N	
28.	Y N	
29.	Y N	

[If YES was circled for any medication, review by medical personnel is required.]

FOOD ALLERGIES

30. a. Do you have any food allergies? YES NO **[If NO go to question 31]**

[If YES] What foods are you allergic to?

b. _____

c. _____

d. _____

[If any food allergy is listed, review by medical personnel is required.]

31. a. Are there any foods you refuse to eat? YES NO **[If NO go to question 32]**
[If YES] What foods will you absolutely not eat? **[List below]**

b. _____ c. _____ d. _____

SPECIAL DIETS

32. a. Are you on a special diet prescribed by a doctor for a medical condition?
YES NO **[If NO go to question 33]**

[If YES] Is it for:

[Read list of special diets and circle response YES, NO, UNSURE]

b. diabetes	YES	NO	UNSURE
c. heart disease	YES	NO	UNSURE
d. hypertension or high blood pressure	YES	NO	UNSURE
e. renal or kidney disease	YES	NO	UNSURE
f. any other disease	YES	NO	UNSURE

g. if other, specify_____

[If any special diet is circled YES or UNSURE, then review by medical personnel is required.]

Telephone Screening Form

ALCOHOL CONSUMPTION

DEFINITION:1 drink = a 5 oz. glass of wine, a 12 oz. can of beer, or 1.5 oz. of liquor, **(enter 0 for less than 1 drink per week)**

33. a. Do you drink alcoholic beverages? YES NO **[If NO go to question 34]**

b. **[If YES]** How many drinks do you usually have in a 7-day week? _____

[If the applicant usually drinks over 12 drinks in a 7-day week, then the applicant has become ineligible. If so, terminate the interview and complete questions 12-13.]

HEIGHT AND WEIGHT

[See Height and Weight cutpoint tables in the Forms Guide.]

34. What is your height without shoes? a. ft: ____ b. in: ____

35. a. What is your weight without shoes? lbs: ____

b. Is the applicant's weight recorded in question #35a greater than the upper weight limit for the applicant's height in the height/weight tables? YES NO

36. It is important that our participants not lose or gain weight in this study. Are you willing to participate in a study where your weight is maintained at the same level it is now?

YES NO

[If the answer to question 35b is yes, or the applicant is not willing to maintain the same weight during the study, then the applicant has become ineligible. If so, terminate the interview and complete questions 12-13].

WOMEN BORN AFTER 1943 ONLY

37. Are you pregnant or planning to become pregnant within the next year? YES NO

38. Are you breastfeeding? YES NO

39. Have you had a baby within the last 6 months? YES NO

If the answer to either question 37, or 38, or 39 is YES, then the applicant has become ineligible. If so, terminate the interview and complete questions 12 and 13.

Telephone Screening Form

[Interviewer: If applicant is still eligible at this point, read the general description of the DELTA Study in the Forms Guide and continue the interview.]

FURTHER INTEREST

40a. Based on your understanding of the study at this point, would you be interested in coming to the center to learn more about this study and to have some blood work done and your blood pressure checked to determine if you remain eligible?

YES **(go to question 41)** NO

[If NO] What is the reason? **[Circle YES or NO for reasons for not scheduling Eligibility Visit 1]:**

b.	Uninterested in general study protocol	YES NO
c.	Unwilling to commit due to length of study	YES NO
d.	Unwilling to come to feeding center for 2 meals each day for 5 days each week	YES NO
e.	Unwilling to eat study food	YES NO
f.	Unwilling to limit intake to study foods only	YES NO
g.	Unwilling to allow maintenance of current body weight	YES NO
h.	Lives too far from feeding center	YES NO
i.	Travels out of town as part of job position or has travel plans for study period	YES NO
j.	Unwilling to submit to frequency of blood draws	YES NO
k.	Other (1. specify: _____)	YES NO

[Interviewer: If applicant is not interested, terminate interview and complete questions 12-13.]

Telephone Screening Form

SCHEDULING ELIGIBILITY VISIT 1

41. If applicant is eligible at this point, schedule Eligibility Visit 1:

a. date:_____ b. time:_____ c. AM PM
(mm/dd/yy) (hh:mm)

Interviewer: Ask applicant to bring all medications, including diet supplements, over the counter medications, and any contraceptives, to Eligibility Visit 1. Inform applicant that he/she will need to fast before the visit. Read the following to the applicant:

"Fasting for DELTA means that you should not eat or drink anything except water for 10 hours before coming in for Eligibility Visit 1. Additionally, you should not use alcohol of any type for 48 hours before the visit."

42. Do the applicant's responses need medical review? YES NO

If applicant is interested, but responses need medical review, then tell applicant that he/she will be called back.

RETURN TO PAGE 3 AND COMPLETE QUESTIONS 12-13.

Telephone Screening Form

NOTE PAGE

DELTA ID:_____ DATE:___/___/___

NAME: _____
a) first b) middle c) last

PERSONNEL CODE NUMBER _____

3.4 ELIGIBILITY VISIT DESCRIPTION AND DATA FLOW

1. Prepare the EV1 and EV2 items before the applicant arrives. Place labels on all the forms.

2. Greet the applicant and give a brief description of what will be done at this visit. Answer any questions he/she may have. Collect all the medications brought in at EV1 and ask the participant if he/she has left any medications at home.

3. Give the participant the consent form before he/she begins Part 1 of EV1. Have him/her read it and sign on the bottom. The signing must be witnessed by one other person. Have the witness sign the consent form after the applicant has signed.

4. All forms should be reviewed for completeness by the study coordinator and given to the data entry person for further editing when the DES is made available.

5. Pages 1 - 4 of the Telephone Screening Form will be entered for all applicants.

6. All pages of EV1 and EV2 forms will be entered for all patients who are randomized into the study.

3.4.1 Eligibility Visit 1 (EV1)

3.4.1.1 Purpose

1. To answer additional questions the applicant may have about the study.

2. To have the participant complete Part I (Questions #1-21) of the EVI Form.

3. To have the interviewer complete Part II (Questions #22-64).

4. To determine continued eligibility and complete general medical condition questionnaire.

5. To determine alcohol consumption and rule out people who drink more than 5 drinks in a week.

6. To obtain a list of current physician-prescribed and self-prescribed medications.

7. To measure weight and height and compare against upper weight limit table.

8. To determine if there are any medical, personal, or professional reasons that would keep an applicant from participating in DELTA.

9. To determine women's menstrual status.

10. To measure blood pressure.

11. To measure fasting cholesterol by fingerstick method (using Cholestech machine) and Insulin by venepuncture.

12. To schedule EV2 if patient remains eligible.

3.4.1.2 Items Needed for EV1

1. EV1 questionnaire to be completed by the interviewer.

2. Blood pressure cuff.

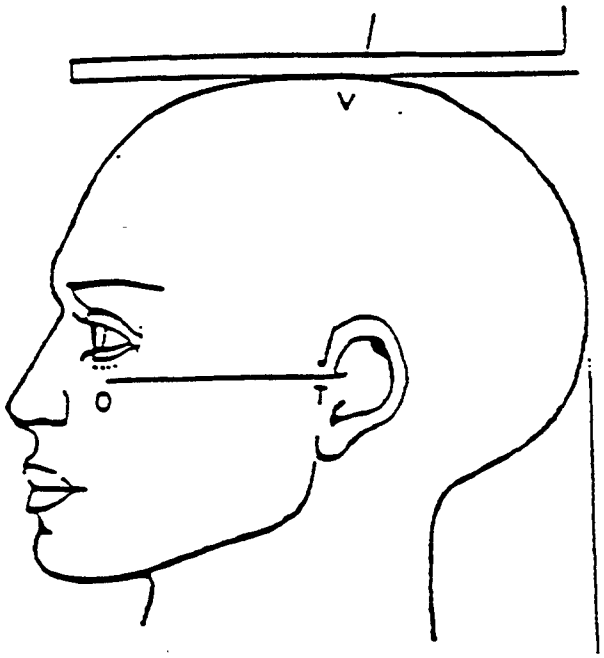
3. Cholestech Machine and cassettes.

4. Collect medications from applicant to verify medicines.

3.5 PHYSICAL MEASUREMENTS

3.5.1 Standing Body Height

The participant stands erect on the floor or the horizontal platform with his/her back against the vertical mounted metal centimeter ruler, heels together and against the vertical ruler, looking straight ahead with his/her head in the Frankfort horizontal plane (the horizontal plane which includes the lower margin of the bony orbit -- the bony socket containing the eye -- the most forward point in the supratragal notch -- the notch just above the anterior cartilaginous projections of the external ear) (**Figure 1**). The right angle is brought down snugly but not tightly on the top of the head. A foot stool is used if the examiner is shorter than the participant such that the examiner's view is level with the point of measurement on the head of the participant. The participant's height is recorded to the centimeter or inch, rounding down.



ORBITAL:	Lower margin of eye socket
TRAGION:	Notch above tragus of ear or at upper margin of zygomatic bone at that point
FRANKFORT PLANE:	Orbital-tragion line horizontal

Figure 3.5.1.1: Frankfort Plan or Measuring Body Height

Figure 3.5.1.2: Body Weight Scales Calibration Form

BODY WEIGHT SCALES CALIBRATION FORM

A) **DAILY:** At the beginning of each day, scales should be checked to see that they read zero when there is no weight on them.

B) **WEEKLY:** Calibrate the scales using the 50 pound known weight. This calibration is performed again whenever the scales are moved. If the scales are outside the 49.5 to 50.5 range an independent service technician is called in to recalibrate the scales. Calibration with the 50 pound weight is performed for both balance arms (light and heavy) on the scale.

C) **ANNUALLY:** The scales are certified annually by an independent scale technician.

The Anthropometry Equipment Calibration Form should be filled out and sent to the Coordinating Center bi-weekly.

1. Calibration Check of Scales with 50 lb weight		Initials: _____
Reading of scales with 50 lb weight	_____	
If reading is outside of 49.5 to 50.5 range, scale should be serviced.		
If service is requested, give	Date ___-___-___	
Recalibration by independent service technician	Technician: _____	
2. Repeat Calibration because of moving of scales		
Scales moved:	1. Date ___-___-___ Initials _____	2. Date ___-___-___ Initials _____
Calibration:	1. Date ___-___-___ Initials _____	2. Date ___-___-___ Initials _____

Today's Date ___-___-___

Completed by _____

Center _____

3.5.2 Body Weight

Before a participant is weighed, the scale is balanced so that the indicator is at zero when no weight is on the scale. The scale must be level and on a firm surface (not a carpet). The participant is instructed to stand in the middle of the platform of the balance scale with head erect and eyes looking straight ahead. Adjust the weight on the indicator until it is balanced. Record the results down to the pound or kg, rounding down. To maintain accuracy, the scale is zeroed daily and must be calibrated with a known weight (50 lbs.) every week or whenever the scale is moved.

3.5.3 Blood Pressure

3.5.3.1 Cuffs and Bulbs

Proper size of the cuff is essential for accurate blood pressure measurement. Field Centers have four standardized cuffs available - small adult, regular adult, large adult, and thigh cuff.

The range markings on commercial cuffs overlap from size to size and do not offer a precise guideline. In the DELTA study arm size is measured, and the cuff size is selected as follows:

Table 1. Determination of cuff size based on arm circumference

Cuff	Size	Arm Circumference
Small	Adult/Child	<24.5 cm
Regular	Adult	24.5-33.0 cm
Large	Adult	33-40 cm
Thigh		>40 cm

3.5.3.2 Blood Pressure Measurement Instructions

The Coordinating Center is presenting the ARIC procedure at this time in lieu of any final decisions on blood pressure measurements as endpoint determinations (pending Steering Committee decision). Some of the many extraneous factors influencing blood pressure are controlled by standardizing the measurement technique and the environment in which the measurement is made. Blood pressures should be taken before any attempts to draw blood.

3.5.3.3 Staff Preparation for Participant Visit

In relating to the DELTA participants, remember that participation in the study is voluntary. Participants are given full explanation and instructions about the preparation for the blood pressure examination and an opportunity for questions. The setting in which blood pressure measurements are made is standardized and takes place in a separate, quiet room where no other activity is taking place, and where temperature fluctuations are minimal. Clinic scheduling procedures establish consistent appointment times to minimize as much as possible the impact of daily blood pressure variation.

3.6 INSTRUCTIONS FOR COMPLETING ELIGIBILITY VISIT 1 (EV1) FORM VERSION B

IMPORTANT: Read both the General Instructions and the Instructions related to the Data Management System (located in the DELTA Forms Guide) before beginning completion of all forms.
GENERAL REMINDERS: Please use black ball-point pen. Print all responses legibly. Initial and date all corrections. Be sure to respond to all applicable questions. Enter NA or ND where applicable.
Enter the applicant's ID number and today's date in the spaces. Other information in the box at the top of the first page will be completed at the end of the visit.

Part I. Self-administered Questionnaire

Be sure the applicant reads and understands the screening consent form. He/she must sign the consent form before completing this questionnaire!

Give the applicant brief instructions on how to complete the form, (e.g.: use a black ball-point pen, print legibly, and to note all questions he/she is not certain how to complete). After the applicant has completed the form to the best of his/her ability, review the answers quickly for any omissions or questions. Use the following instructions to assist you with each question.

Questions 1-3: General Information

The applicant is to complete his/her name, birth date and emergency information. If the TSV is available, check to see that the birth date is in agreement. Also check that the applicant has completed all information requested. Ask for spelling or clarification if necessary.

Questions 4-5: Level of Education and Employment Status

The applicant is to circle the letter denoting the appropriate response. If the appropriate response to Question 5 is I Other, the applicant must give description in 5.b.

Questions 6: Availability

The applicant is to circle YES or NO. Remind the applicant that the study requires participants to consume two meals, 5 days a week at the center.

Question 7: Food Allergies

a.-k. The applicant is to read and respond to each of the foods listed by circling Y for yes, N for no or U for unsure.

l.-n. If k. is answered YES or UNSURE, the applicant is to list specific foods.

Question 8: Refusal to eat certain foods

The applicant is to list any foods they would refuse to eat.

Questions 9-13: Alcohol consumption

This study requires that participants consume no more than 5 alcoholic beverages per week. This series of questions is designed to address this issue. Be sure the applicant understands the definition of one drink as it relates to these questions. (The DELTA definition is on the EV1 form.)

Questions 9 & 11 ask for a total number of alcoholic beverages across particular days. Question 10 & 12 ask for a maximum number of alcoholic beverages in any one given day.

For example, if the applicant consumes 4, 12 oz. cans of beer and one, 1 oz. shot of liquor, 5 is the correct response.

However, if the applicant consumes 4, 16 oz. cans of beer and one, 1 oz. shot of liquor, 6 is the correct response (4, 16 oz cans is approximately equal to 5, 12 oz. cans).

For question 13, the applicant must circle YES or NO. (Unsure is not an option for this question.)

Questions 14-15: Nutritional Supplements

The applicant is to circle YES or NO.

Question 16: Special Diets

The applicant is to circle YES or NO for each of the diets listed.

If item j is circled YES, applicant needs to include a brief description in the space for item k.

Question 17: Self-prescribed Diets

a. The applicant is to circle YES or NO.

b. If the applicant circles YES in part a., be sure he/she includes an adequate description of the self-prescribed diet. Ask for clarification if necessary.

Question 18: Weight History

The applicant is to circle YES or NO.

Questions 19-21: Smoking and Exercise Habits

19.a. All applicants must circle YES OR NO.

If the applicant circles YES for 19.a. he/she must complete 19.b. by filling the average # of cigarettes smoked/day. Also, if the applicant circles YES for 19.a. he/she does not need to answer 19.c. and 19.d.

If the applicant circles NO for 19.a. he/she skips part b. but must complete 19.c. by circling YES or NO. If the applicant circles YES to 19.c. he/she must answer 19.d. **NOTE:** Question 19.b. asks for the total number of cigarettes smoked per day not the number of packs per day.

20. a. Should be completed by circling YES or NO. If NO go to question 21. If the applicant circles YES be sure they specify what type of exercise or sport they perform, and for how long (in hours and minutes/week) in items 20. b-i.

21. a. Should be completed by circling YES or NO. If YES, the applicant should describe the type of physical labor in part b.

The self-administered section of this form ends here and the applicant is instructed to stop working and return the form to the DELTA interviewer.

Part II. Clinic Data Form

This section of the EV1 Form is to be completed by clinic personnel.
[Note to Interviewer: return to question 8. Be sure this item has been read by the applicant and completed. If the applicant has left this item blank because there are no foods that he/she refuses to eat, enter NA is the left most space.]

Question 22: Medical Exclusions

Read each of the medical conditions listed to the applicant and circle Y for YES, N for NO **or** NEVER TESTED, or U for UNSURE. Since this information is used for exclusion purposes, please attempt to obtain as accurate information as possible.

Question 23: Thyroid Disease

Circle Y, N, or U for all three parts of this question. (**Note:** Parts b. & c. must be answered even when Part a. is answered N.

Thyroid disease is difficult to diagnose. Please attempt to obtain as accurate information as possible. **If any part of questions 22 or 23 is marked Y or U, medical review is required and should be verified in the spaces provided after Questions 23.**

Questions 24-26: Other Exclusions

a. Circle YES or NO. If NO, go on to the next question.

b. If YES, briefly describe the reason given by the applicant.

Questions 27-31: Menstrual Status (to be completed by women applicants only)

27. Circle YES or NO for part a. and then complete either part b. or c.

28-30. Circle R for Regular or Normal, I for Irregular, or N for Not Menstruating. If response is R, go to question 30. If response is I or N complete the related part(s) of Question 29.

31. Circle YES or NO for part a. and then complete either b. or c.

Questions 32-42: Medication Use

32-33. Circle the letter consistent with the applicant's response. (Note: Occasionally means less often than weekly.)

34. Circle YES or NO. If NO go to question 41.

35-40. (a., b., c., & d.). If YES to question 34, list all doctor-prescribed medications taken in the past six months. The applicant was instructed to bring all current medications to the interview. At this point in the interview ask to see the medications and confirm correct spelling and complete other required information.

41. a. Circle YES or NO. If NO go to questions 43.

b-g. If YES TO 41.a., list names of all self-prescribed medications and/or nutritional supplements.

42. Circle YES or NO (UNSURE is not a response option for this question.)

Question 43-47: Body Mass Index (BMI)

Complete either questions 43 & 44 or 45 & 46 based on whether your Field Center will be using customary or metric units of measure.

43. a. & b. Enter the applicants height in feet and inches. Height should be without shoes rounded to the nearest inch.

44. a. Enter applicant's weight in pounds. Weight should be without shoes and rounded to the nearest pound.

b. Find the applicant's height in the BMI table provided, check the information, and circle YES or NO.

OR

45. Enter the applicant's height in centimeters. Height should be without shoes and rounded to the nearest centimeter.

46. a. Enter the applicant's weight in kilograms. Weight should be without shoes and rounded to the nearest kg.

    b. Find the applicant's height in the BMI table provided, check the information, and circle YES or NO.

47. Circle YES or NO. (UNSURE is not a response option for this question.)

NOTE: If the applicant's weight is greater than the upper weight limit, or the applicant is not willing to maintain the same weight, then the applicant has become ineligible. If so, thank the applicant for his/her time and interest, and terminate the interview.

Questions 48-54: Sitting Blood Pressure

48. Measure the applicant's arm circumference in centimeters and record this value in the space provided.

49. Select the appropriate cuff size and circle the corresponding letter (e.g.: if a applicant's arm is 31 cm circle R for regular adult size.)

50. Obtain pulse obliteration value by inflating cuff until pulse can no longer be heard. Add 30 to this value to obtain peak inflation level and enter the sum in Part b.

51. Obtain the applicant's 30 second pulse, record this value in the left most space provided, multiply by 2, and enter the product in the right most space provided.

After applying the blood pressure cuff, the applicant must sit quietly and remain seated with legs uncrossed for 5 minutes before the blood pressure measurements are obtained.

52. a.-b. Record both the systolic and diastolic values for the first blood pressure measurement in the appropriate spaces.

Wait 30 seconds after the first blood pressure reading before taking the second reading.

53. a.-b. Record both the systolic and diastolic values for the second blood pressure measurement in the appropriate spaces.

54. a.-b. Compute the average systolic and diastolic blood pressure as indicated in the example below and record these values in the appropriate space. Round to the nearest whole number.

Sample computations:

First blood pressure measurement	a. Systolic:	112	b. Diastolic	64
Second blood pressure measurement	a. Systolic:	109	b. Diastolic	62
	Total =	221		126
	Divide by 2 =	110		63

55. Circle YES if the applicant's average systolic blood pressure exceeds 140 **or** the average diastolic blood pressure exceeds 90. Using the sample computations above, you would circle NO since the average blood pressures do not exceed these values.

Questions 56-60: Blood Drawing

Prior to sending the applicant for blood drawing, complete questions 56-59. Be sure to circle AM or PM.

If the applicant has not fasted for at least 10 hours do not draw blood, and reschedule the applicant for a fasting blood draw in question 60.

If the applicant has fasted for at least 10 hours, send him/her for blood drawing.

Questions 61 & 62: Rapid Cholesterol Screen and Insulin Level

61. a.-d. Obtain a rapid cholesterol check on the applicant and record the values (mg/dl) in the spaces provided.

    e. Circle YES or NO. To remain eligible for the study, the applicant's cholesterol level must fall within the normal range for his/her age, race, and gender as indicated in the Lipid Cutpoint Tables or by the Lipid/Insulin Eligibility Program result.

62. a. Obtain insulin value and record the value in the space provided when lab assay is completed.

    b. Circle YES or NO. To remain eligible for the study, the applicant's insulin level must fall within the normal range for his/her age, race, and gender as indicated in the Insulin Cutpoint Tables or by the Lipid/Insulin Eligibility Program result.

NOTE: If the applicant's lipid or insulin level is not within range for his/her gender, race, and age; the applicant has become ineligible. Terminate the interview. Call the applicant to inform him/her that he/she is no longer eligible.

Scheduling Eligibility Visit 2

Read the DELTA definition of Fasting to the applicant.

Respond to each question by circling Y for YES or N for NO. If all questions in this section are answered Y (YES) the applicant can be scheduled for Eligibility Visit 2.

Question 63-64: Administrative Information

63. Enter your personnel code.

64. a. Enter the date for the applicant's Eligibility Visit 2.
    b. Enter the time for the applicant's Eligibility Visit 2.
    c. Circle AM or PM.

(INTERVIEWER: Return to the first page of this form to complete the remaining information in the top section. Also, return to the TSV Form to record the lab values from questions 61 and 62 on this form. If the applicant has become ineligible at this point, complete questions 13 on the TSV Form.)

(Page 15 of the form is provided for additional notes if needed.)

Eligibility Visit 1

Form Code: EV1
Version B 5/10/94

DELTA ID:_____ **TODAY'S DATE:**_____

Medical review needed?	NO	YES		
Medical review done?	NO	YES	DATE_____	MD INITIALS_____
Eligible after EV1?	NO	YES		
COMMENTS:				

Part I (Questions 1 - 21) - To be completed by applicant

Have you read and signed the screening consent form? If you haven't, contact DELTA staff member.

1. _____
   a. First Name b. Middle Name c. Last Name
2. Date of Birth (mm/dd/yy):_____
3. Contact in case of an emergency:
   a. Name:_____
   b. Address:_____
   c. Town/City:_____ d. State:_____ e. Zip Code _____
   f. Home Telephone:_____ g. Work Telephone:_____
   area-###-#### area-###-####

3.6.1 Eligibility Visit 1 Form

4. What is your highest level of education completed? **[Please circle the letter preceding your selection.]**

   A Eighth grade or less
   B Trade school or business school instead of high school
   C Some high school
   D High school graduate
   E Trade school or business school after graduating from high school
   F Some college including 2-year degree
   G Received bachelor's degree
   H Graduate or professional education beyond the bachelor's degree
   I Graduate or professional degree

5. a. What is your current employment status? **[Please circle the letter preceding your selection.]**

   A Working a full-time job
   B Working a part-time job
   C Full-time or part-time student, not working
   D Student working full-time or part-time
   E Homemaker/Volunteer
   F Retired
   G Unemployed
   H Disabled
   I Other

   b. If Other, describe: _____

6. Do you plan to remain in the area for the next year? **[Circle answer]** YES NO

7. Do you have any allergies or sensitivities to any of the following foods? **[Read each of the following foods and circle your response YES (Y), NO (N), or UNSURE (U)]**

a. meat, fish or poultry	Y	N	U
b. shellfish	Y	N	U
c. milk or dairy products	Y	N	U
d. If, YES to milk or dairy products, is this a milk allergy?	Y	N	U
e. If YES to milk or dairy products, is this a lactose intolerance?	Y	N	U
f. eggs	Y	N	U
g. fruit	Y	N	U
h. vegetables	Y	N	U
i. nuts	Y	N	U
j. chocolate	Y	N	U
k. other foods	Y	N	U

If YES to Other foods, list below:

l._____, m._____, n._____

8. Are there any foods that you absolutely won't eat? **[List each separately.]**

a._____ b._____ c._____

We would like to ask you a few questions about your alcohol consumption...
DEFINITION: 1 drink = a 5 oz. glass of wine, a 12 oz. can of beer, or 1.5 oz. of liquor.

9. What is the total number of alcoholic drinks that you drink Monday through Thursday?_____

10. What is the maximum number of alcoholic drinks that you usually drink in any one day Monday through Thursday? _____

11. What is the total number of alcoholic drinks that you drink Friday, Saturday, and Sunday?_____

12. What is the maximum number of alcoholic drinks that you usually drink in any one day Friday, Saturday, or Sunday? _____

13. Would you be willing to limit your intake of alcohol to no more than 5 drinks per week for the duration of the study? **[Circle your response.]** YES NO

14. Are you taking any vitamins, minerals or other nutritional supplements? **[An interviewer will ask you to list any nutritional supplements in Part II.]** YES NO

15. Because some nutritional supplements may interfere with study results, would you be willing to stop taking this supplement if you qualify for this study? YES NO

16. Are you currently on any of the following special diets prescribed by a doctor for a medical condition? **[For each special diet listed below, circle YES or NO]**

a. Weight loss	YES	NO
b. Low salt or low sodium	YES	NO
c. Diabetic	YES	NO
d. Heart disease	YES	NO
e. Lower blood pressure	YES	NO
f. Weight gain	YES	NO
g. Vegetarian	YES	NO
h. Renal disease	YES	NO
i. Allergy	YES	NO
j. Other	YES	NO

k. If Other, describe: _____

17. a. Are you on a self-prescribed diet? YES NO

b. If YES, describe the self-prescribed diet: _____

18. Have you lost or gained more than 10 pounds within the past two months? YES NO

19. a. Do you currently smoke cigarettes? YES **[If YES go to 19b]** NO **[If NO go to 19c]**

b. If YES to 19a, on average, how many cigarettes do you smoke per day? _____ **[go to 20]**

c. Have you ever smoked cigarettes? YES **[If YES go to 19d]** NO **[If NO go to 20]**

d. If YES to 19c, how long has it been since your last cigarette? **[Circle letter preceding answer.]**

A Less than 1 year B 1 year or more

20. a. Do you exercise more than once a week or play sports regularly? YES NO

[If NO, go to question 21.]

If YES, describe the activity and enter the amount of time spent **per week** at this activity:

ACTIVITY (ENTER TIME IN HOURS AND MINUTES)

b. _____ c. _____:_____

d. _____ e. _____:_____

f. _____ g. _____:_____

h. _____ i. _____:_____

21a. Does your job require heavy physical labor? YES NO

b. If YES, describe_____

STOP!

PLEASE HAND THIS FORM TO THE DELTA INTERVIEWER TO INITIATE THE REMAINDER OF THE CLINIC VISIT.

Part - II Clinic Data Form [Interviewer: Review Part I for any automatic exclusions. Questions? See Coordinator.]

22. Because certain medical conditions will interfere with our study, we need to ask the following questions. Do you have any of the following medical conditions? [Read list of medical conditions and circle response YES (Y), NO (N) if NO or NEVER TESTED, or UNSURE (U)]

a. heart disease	Y	N	U
b. diabetes	Y	N	U
c. high blood pressure or hypertension	Y	N	U
d. renal or kidney disease	Y	N	U
e. gastrointestinal condition (Crohn's disease, irritable bowel syndrome, ulcer problems, bowel surgery)	Y	N	U
f. history of blood clotting disorders	Y	N	U
g. liver disease (cirrhosis)	Y	N	U
h. condition that requires steroid medication	Y	N	U
i. gout requiring treatment	Y	N	U
j. recent history of depression or mental illness requiring treatment or medication within last 6 months	Y	N	U
k. anemia	Y	N	U
l. sickle cell anemia	Y	N	U
m. lung disease, chronic bronchitis, emphysema	Y	N	U
n. acquired immune deficiency syndrome (AIDS) or positive HIV test	Y	N	U
o. cancer (active within 5 years)	Y	N	U

Please be sure to answer 23 a, and b, and c!

23 a. Do you have thyroid disease or a thyroid problem? Y N U

b. Have you ever had treatment, such as radioactive iodine or surgery for a thyroid problem? Y N U

c. Are you taking any medication for your thyroid? [If unsure, check medications.] Y N U

[If any medical condition was circled YES or UNSURE, then review by medical personnel is required to exclude applicant from participation.]

Medical reviewer Initials:_____ Eligible: YES NO Date ____/____/____

24. a. Are there any medical reasons that might interfere with your ability to participate?
(Examples: hospitalized on a regular basis, scheduled surgery, family medical problems)
YES NO **[If NO go to question 25.]**

b. If YES, describe:_____

25. a. Are there any personal reasons that would keep you from participating?
(Examples: family problems, vacation scheduled during study period, child care difficulties, religious reasons) YES NO **[If NO go to question 26.]**

b. If YES, describe:_____

26. a. Are there any professional reasons that would keep you from participating?
(Examples: job related travel out of town, work irregular shifts or night shift)
YES NO **[If NO go to question 27.]**

b. If YES, describe:_____

If the answer to questions 24, or 25, or 26 is YES, inform Study Coordinator.

WOMEN ONLY

27. a. Are you currently taking an oral contraceptive? YES NO

    b. If YES to 27a, are you planning to stop? YES NO

    c. If NO to 27a, are you planning to start? YES NO

[Circle the letter preceding the response.]

28. What is your current menstrual status?
    - R Regular (normal) **[go to question 30]**
    - I Irregular **[go to question 29a]**
    - N Not menstruating **[go to question 29c]**

29. a. If you are menstruating irregularly, what is the reason?
    - A Undergoing menopause
    - B Other

    b. If Other, describe_____

    c. If you are not menstruating, what is the reason?
    - A Natural menopause
    - B Hysterectomy
    - C Medication stopped period
    - D Other (d. describe_____)

30. When did you have your last period?
    - A Less than 2 months ago
    - B 2 months to 6 months ago
    - C 6 months to 1 year ago
    - D 1 year but less than 3 years ago
    - E At least 3 years ago

31. a. Are you taking or have you ever taken estrogen? [Estrogen or female hormones for hot flashes or symptoms of menopause] YES NO

    b. If YES to 31a, are you currently taking estrogen? YES NO

    c. If NO to 31a, do you plan to start taking estrogen? YES NO

RESUME ASKING QUESTIONS OF ALL APPLICANTS.

32. How often do you take antacids? **[Circle the letter preceding the response]**
D Daily W Weekly O Occasionally N Never

33. How often do you take laxatives? **[Circle the letter preceding the response]**
D Daily W Weekly O Occasionally N Never

34. Within the past six months, have you taken any medications on a regular basis prescribed by a doctor? YES NO **[Go to question 41.]**

If YES, Specify doctor-prescribed medications, including oral contraceptives, one per line: **[Enter names of medications with correct spellings.]**

	a. Medication	b. Reason for Taking Medication	c. Date When Stopped (mm/dd/yy)	d. Plan To Resume
35.	_____	_____	_____	YES NO
36.	_____	_____	_____	YES NO
37.	_____	_____	_____	YES NO
38.	_____	_____	_____	YES NO
39.	_____	_____	_____	YES NO
40.	_____	_____	_____	YES NO

[Applicant's doctor-prescribed medications must be confirmed at this time.]

41. a. Within the past six months, have you taken any self-prescribed medication or nutritional supplements on a regular basis? YES NO

If YES, please list self-prescribed medications or supplements, one per line: **[Enter names of medications with correct spellings.]**

b._____ c._____ d._____

e._____ f._____ g._____

42. If you are taking self-prescribed medications or supplements, would you be willing to discontinue use of the self-prescribed medication or supplement for the duration of this study? YES NO

If the answer to question 42 is YES, inform the Study Coordinator.

HEIGHT AND WEIGHT [SEE HEIGHT / WEIGHT CUTPOINT TABLES in the Forms Guide]

[Choose whether you will enter height/weight in customary units (ft-in/lb) or metric units (cm/kg). Only enter responses for questions 43-44 or 45-46.]

Customary Units

43. Height (without shoes) a. ft: _____ b. in: _____

44. a. Weight (without shoes) lbs: _____

b. Is the applicant's weight recorded in question 44a greater than the upper weight limit for applicant's height in the ht/wt table? YES NO

Metric Units

45. Height (without shoes) cm: _____

46. a. Weight (without shoes) kg: _____

b. Is the applicant's weight recorded in question 46a greater than the upper weight limit for applicant's height in the ht/wt table? YES NO

47. It is important that our participants not lose or gain weight in this study. Are you willing to participate in a study where your weight is maintained at the same level it is now? YES NO

If the applicant's weight is greater than the upper weight limit, or the applicant is not willing to maintain the same weight, then the applicant has become ineligible. If so, terminate the interview.

SITTING BLOOD PRESSURE

[Measure the applicant's arm circumference and choose the appropriate cuff.
After applying the cuff, the applicant must be quiet and remain continuously seated without legs crossed for 5 minutes before the two measurements. Wait 30 seconds after the 1st reading before taking the 2nd reading.

48. Arm circumference (cm): _____

49. Cuff Size: **[Circle the letter by your selection.]**
    - P Pediatric (<24.5 cm)
    - R Regular adult (24.5-33 cm)
    - L Large adult (33-40 cm)
    - X X-large (>40 cm)

50. Pulse obliteration (a) _____ + 30 = peak inflation level (b) _____

51. Pulse: beats in 30 seconds _____ x 2 = _____ beats/minute

52. First blood pressure measurement: a. Systolic: _____ b. Diastolic: _____

53. Second blood pressure measurement: a. Systolic: _____ b. Diastolic: _____

54. Calculated average of first and second blood pressure measurements:

    Add the two values: _____ _____

    Divide sum by 2: a. Systolic: _____ b. Diastolic: _____

55. Is average systolic blood pressure > 140 or average diastolic blood pressure > 90? YES NO

BLOOD DRAWING

56. When was the last time you ate or drank anything except water?

a. Time (hh:mm): _____ b. AM PM

57. How many hours since you last drank any alcohol? _____

58. Enter the current time: a. Time (hh:mm): ____:____ b. AM PM

59. Number of hours fasted: _____

[If applicant has not fasted for at least 10 hours, or has consumed alcohol within 48 hours, do not draw blood. Reschedule applicant in question 60.]

60. a. Has applicant been rescheduled for blood drawing? YES NO

If YES, enter scheduled date:

b. Date: _____ (mm/dd/yy) c. Time: ____:____ (hh:mm) d. AM PM

If the applicant remains eligible, has had no alcohol in the last 48 hours, and has fasted at least 10 hours, send him/her for blood drawing.

LIPID SELECTION CRITERIA [See Lipid and Insulin Cutpoint Tables in the Forms Guide.]

61. a. TC _____ mg/dl
b. HDL _____ mg/dl
c. TG _____ mg/dl
d. LDL _____ mg/dl (calculated)
e. Are all lipid levels within eligible range for applicant's gender, race, age? YES NO

62. a. INS _____ μU-ml
b. Is insulin level within eligible range for applicant's gender, race, age? YES NO

If the applicant's lipid and insulin levels are not within the eligible range, then the applicant will be ineligible. If so, terminate the interview. If the levels ARE within the eligible range, explain that continued eligibility will be dependent on the laboratory values meeting DELTA criteria for Protocol 2.

Eligibility Visit 1

Form Code: EV1
Version B 5/10/94

SCHEDULING ELIGIBILITY VISIT 2

[Read the following to the applicant before scheduling for Eligibility Visit 2.]

"Fasting for DELTA means that you should not eat or drink anything except water for 10 hours before coming in for the Eligibility Visit 2. Additionally, you should not use alcohol of any type for 48 hours before your visit."

The following questions should all be answered "YES" before the applicant is scheduled for Eligibility Visit 2.

Did the applicant read and sign the consent screening form?	Y	N
Was Part I of Eligibility Visit 1 completed?	Y	N
Was the DELTA Study explained and questions addressed?	Y	N
Were applicant's doctor-prescribed medications confirmed?	Y	N
Does applicant remain eligible for Eligibility Visit 2?	Y	N

ADMINISTRATIVE INFORMATION

63. Code Number of personnel completing this form _____

64. a. Date scheduled for Eligibility Visit 2 (mm/dd/yy): _____

b. Time scheduled for Eligibility Visit 2 (hh:mm): _____ **c. AM PM**

REMINDER: Return to the Telephone Screening Visit form and record the lab values (questions 61 and 62 on this form) for all applicants. If the applicant has been excluded at this point, also complete question 13 on the TSV form.

Eligibility Visit 1

NOTES

DELTA ID:_____ DATE:____/____/____

NAME:_____
first middle last

CODE NUMBER of personnel completing this form _____

3.6.2 Pennsylvania State University Participant Agreement

PENNSYLVANIA STATE UNIVERSITY
DELTA STUDY

Participant Agreement

I understand that if I agree to be a participant in Dietary Effects on Lipoproteins and Thrombogenic Activity (**DELTA**), I will be expected to do the following:

1. Come to the Mateer Room for my lunch and dinner, Monday through Friday. The study will last 8 months, and I will eat 3 different diets lasting 7 weeks each during that time. Between Diet periods 1 and 2, I will have a 7 week break where I can eat any food and during which I do not need to come to the study facility. Between Diet periods 2 & 3, I will have a 4 week break where I can eat any food and during which I do not need to come to the study facility.

2. Pick up prepackaged containers of one meal and a snack for daily weekday use, as well as prepackaged meals for use during weekends.

3. Eat <u>all</u> foods provided. Eat <u>only</u> foods provided.

4. Weigh in at the Feeding Center to allow adjustment of calories to maintain my current body weight.

5. Avoid or limit use of all alcoholic beverages during each 7 week diet period.

6. Allow blood samples to be drawn 3 times during each diet period.

My signature indicates that I have read and understand the above description of my responsibilities in the **DELTA** study.

_____

Prospective Participant

3.6.3 Columbia University Participant Agreement

COLUMBIA UNIVERSITY
DELTA STUDY

Participant Agreement

I understand that if I agree to be a participant in Dietary Effects on Lipoproteins and Thrombogenic Activity (**DELTA**), I will be expected to do the following:

1. Come to Bard Hall (B1) - 60 Haven Avenue for my lunch and dinner, Monday through Friday. The study will last 8 months, and I will eat 3 different diets lasting 7 weeks each during that time. Between Diet periods 1 and 2, I will have a 7 week break where I can eat any food and during which I do not need to come to the study facility. Between Diet periods 2 & 3, I will have a 4 week break where I can eat any food and during which I do not need to come to the study facility.

2. Pick up prepackaged containers of one meal and a snack for daily weekday consumption, as well as prepackaged meals for my consumption during weekends.

3. Eat <u>all</u> foods provided. Eat <u>only</u> foods provided.

4. Weigh in at the Feeding Center twice weekly (Monday and Thursday - before dinner) to allow adjustment of calories to maintain my current body weight.

5. Avoid use of alcoholic beverages during each 7 week diet period.

6. Allow blood samples to be drawn 3 times during each diet period.

My signature indicates that I have read and that I understand the above description of my responsibilities in the **DELTA** study.

_____

Prospective Participant

THE UNIVERSITY OF MINNESOTA
DELTA STUDY

Participant Agreement

I understand that if I agree to participate in Dietary Effect of Lipoprotein and Thrombogenic Activity (DELTA), I will be expected to do the following:

1) Come to the Moos Tower Feeding Center for breakfast and dinner, Monday through Friday. The study will include 3 diet periods of eight weeks each. I will eat 3 different diets during that time. After each 7 week diet period, I will have a break of several weeks where I can eat any food and during which I do not need to come to the study facility.

2) Pick up prepackaged containers of lunch and snacks for weekday use, as well as prepackaged meals for use during weekends.

3) Avoid all foods other than those provided or allowed by the Feeding Center and eat all foods provided by the Center.

4) Weigh in at the Feeding Center to allow adjustment of calories to maintain my current body weight.

5) Avoid or limit use of all alcoholic beverages to no more than 5 per week during each 7 week diet period.

6) Allow blood samples to be drawn 3 times during each diet period.

I understand that I may be dismissed from the study and will not receive the stated monetary compensation if I become unwilling or unable to comply with the conditions stated above.

My signature that indicates that I have read and understand the above description of my responsibilities in the DELTA Study.

Signed_____Date_____

PENNINGTON BIOMEDICAL RESEARCH CENTER DELTA STUDY

Participant Agreement

I understand that if I agree to participate in Dietary Effect of Lipoprotein and Thrombogenic Activity (DELTA), I will be expected to do the following:

1) Come to the Pennington Center for two meals each day, Monday through Friday. The study will last 8 months, and I will eat 3 different diets lasting 8 weeks each during that time. After each 7 week diet period, I will have a break where I can eat any food and during which I do not need to come to the study facility.

2) Pick up prepackaged containers of lunch and snacks for weekday use, as well as prepackaged meals for use during weekends.

3) Avoid all foods other than those provided or allowed by the Feeding Center and eat all foods provided by the Center.

4) Weigh in at the Feeding Center to allow adjustment of calories to maintain my current body weight.

5) Limit use of all alcoholic beverages to no more than 5 per week during each 7 week diet period.

6) Allow blood samples to be drawn 3 times during each diet period.

7) I understand that I may be dismissed from the study and will not receive the stated monetary compensation if I become unwilling or unable to comply with the conditions stated above.

My signature that indicates that I have read and understand the above description of my responsibilities in the DELTA Study.

Signed_____Date_____

3.7 ELIGIBILITY VISIT 2 (EV2)

3.7.1 Purpose

1. To answer additional questions the applicant may have about the study.

2. To determine continued eligibility.

3. To measure blood pressure.

4. To measure weight and height circumferences.

5. To determine appliance availability.

6. To perform the fasting blood draw for Lipid profile, Insulin, Glucose, TSH, Chemscreen.

7. To schedule callback to applicant to communicate eligibility for the Diet Run-In Visits, Randomization and Feeding Period following determination of eligibility.

3.7.2 Items Needed for EV2

1. EV2 questionnaire to be completed by the interviewer.

2. Blood pressure cuff.

3. Beam balance to measure weight.

4. Instrument for measuring height.

5. Retractable inelastic metric measuring tape

IMPORTANT: Read both the General Instructions and the Instructions related to the Data Management System (located in the DELTA Forms Guide) before beginning completion of all forms.
GENERAL REMINDERS: Please use black ball-point pen. Print all responses legibly. Initial and date all corrections. Be sure to respond to all applicable questions.
Enter the applicant's ID number, and today's date at top of page. Check the applicant's chart to ensure that they have signed a Screening Consent Form. This form must be signed prior to continuing this visit!

Questions 1-8 Sitting Blood Pressure

1. Record the applicant's arm circumference in centimeters.

2. According to the guidelines select the appropriate cuff size and circle the letter corresponding to this value, e.g., if a applicant's arm is 31 cm circle R for regular adult size.

3. Obtain the applicant's 30 second pulse, record this value in the left most space. Multiply the applicant's 30 second pulse by 2 and enter the product in the right most space.

4a.-b. Apply blood pressure cuff. Obtain pulse obliteration value by inflating cuff until pulse can no longer be heard. Add 30 to this value to obtain peak inflation level and enter the sum in Part b.

5. The applicant must sit quietly and remain seated with legs uncrossed for 5 minutes before the blood pressure measurements are obtained.

a.-b. Record both the systolic (a) and diastolic (b) values for the first blood pressure measurement in the appropriate spaces.

Wait 30 seconds after the first blood pressure reading before taking the second reading.

6a.-b. Record both systolic (a) and diastolic (b) values for the second blood pressure measurement in the appropriate spaces.

7a.-b. Compute the average systolic (a) and diastolic (b) blood pressure as indicated in the example below and record these values in the appropriate spaces.

Sample computations:

1st blood pressure measurement	a. Systolic:	112	b. Diastolic	64
2nd blood pressure measurement	a. Systolic:	109	b. Diastolic	62
	Total =	221		126
	Divide by 2 =	110		63

According to this sample computation, the average values to record in question 6 are 110 for systolic and 63 for diastolic.

8. If the applicant's average systolic blood pressure exceeds 140 **or** their average diastolic blood pressure exceeds 90, circle YES. Using the sample computations above, you would Circle NO since the average blood pressures do not exceed these values.

Questions 9-10: Waist & Hip Circumference

9.a-b. Follow the Procedure for Measuring Waist and Hip Circumference (which follows these instructions) in the DELTA Forms Guide. The measurements should be obtained using a metric measuring tape. Take two measurements of the applicant's waist circumference (in centimeters) and record each measurement in the correct space.

9.c. Compute the average of 9a and 9b and record the result.

10.a-b. Take two measurements of the applicant's hip circumference (in centimeters) and record each measurement in the correct space.

10.c. Compute the average reading of 10a and 10b and record the result.

Questions 11 - 12: Appliances Availability

Circle either YES or NO for each appliance listed.

(Note to interviewer: If any one part of questions 11 or 12 is answered NO, inform the Study Coordinator.)

Questions 13 - 17: Blood Drawing

Prior to sending the applicant for blood drawing, complete questions 13 - 16. Be sure to circle AM or PM.

If the Applicant has not fasted for at least 10 hours do not draw blood, and reschedule the applicant for a fasting blood draw in question 17.

If the applicant has fasted for at least 10 hours, send him/her for blood drawing.

Questions 18 - 19: Lipid and Insulin Selection Criteria

18. a.-e. Record Lipid and Insulin results in the correct spaces.

19. a. Circle YES or NO based on the results of the Lipid/Insulin Eligibility Program for the average of EV1 and EV2 lipid measurements.

b. Circle YES or NO based on the results of the Lipid/Insulin Eligibility Program for the average of EV1 and EV2 insulin measurements.

Circle NA if the insulin measurement from EV1 was not processed.

Question 20: Administrative Information

Enter your personnel code number.

(Remember to return to the TSV Form, Page 4 to record EV2 lab results. If applicant is excluded after EV2, remember to complete question 13 on page 3 of the TSV form.)

(Page 5 of the form is provided for additional notes if needed.)

3.7.3.1 Procedure for Waist and Hip Circumference

A. Waist Circumference

1. The examiner will stand in front of the subject. The subject will stand erect with the abdomen relaxed, arms at sides and feet together. The subject will expose the waist with underpants pulled below the waist. A retractable inelastic tape should be placed around the subject in a horizontal plane at the level of the natural waist (the narrowest part of the torso). Specifically, this area is mid-way between the inferior border of the rib cage and the superior border of the iliac crests. In the obese it may be difficult to locate the waist narrowing. In this instance the smallest horizontal circumference is to be measured mid-way between the ribcage and iliac crest.

2. Examiner one and two must make certain the measuring tape is horizontal and that its diameter touches the entire circumference of the abdomen without compressing tissue.

3. The examiner will locate the point on the tape where the zero aligns with the other end of the loop. The measurement should be taken at the end of normal expiration. This measurement will be recorded to the nearest 0.1 cm.

Reliability:

The technical error of measurement for adolescents has been found to be 1.31 cm. (intramеasurer) and 1.56 cm. (intermeasurer). The technical error in the elderly has been found to be 0.48 cm. in men and 1.15 cm. in females.

3.7.3.2 Hip (Buttocks) Circumference

1. This is a measure of the circumference of the hips at the level of the trochanters (maximal extension of the buttocks).

2. The subject will stand erect with arms at sides and feet together. The examiner will squat at the side of the subject so the level of maximum extension of the buttocks can be visualized.

3. The inelastic tape is placed by the examiner around the maximal extension of the buttocks in a horizontal plane without causing tissue compression or indention. Examiner two will help in positioning the tape on the opposite side of the subject's body.

4. The measurement will be recorded to the nearest 0.1 cm.

5. In the obese, the abdominal wall may sag and could accidently be included in this measurement. This is a potential problem with this measurement.

Reliability:

Little is known about the reliability of this measurement. A correlation of 0.99 between measurements one day apart was found in young men.

Eligibility Visit 2

Form Code: EV2
Version B 5/10/94

DELTA ID: _____ Today's Date: ___/___/___

NAME: _____
First Middle Last

Has the applicant read and signed the screening consent form? YES NO

SITTING BLOOD PRESSURE

Measure the applicant's arm circumference and choose the appropriate cuff. After applying the cuff, the applicant must be quiet and remain continuously seated without legs crossed for 5 minutes before the two measurements. Wait 30 seconds after the 1st reading before taking the 2nd reading.

1. Arm circumference (cm): _____

2. Cuff Size: **[Circle the letter by your selection]**

   P Pediatric (<24.5 cm)
   R Regular adult (24.5-33 cm)
   L Large adult (33-40 cm)
   X X-large (>40 cm)

3. Pulse: beats in 30 seconds _____ x 2 = _____ beats/minute

4. Pulse obliteration (a) _____ + 30 = peak inflation level (b) _____

5. First blood pressure measurement: a. Systolic: _____ b. Diastolic: _____

6. Second blood pressure measurement: a. Systolic: _____ b. Diastolic: _____

7. Calculation of average blood pressure:
   (add two values) _____ _____
   (divide sum by 2) a. Systolic: _____ b. Diastolic: _____

8. Is average systolic blood pressure >140 or average diastolic blood pressure >90?

   YES NO

[If average systolic blood pressure is >140 or average diastolic blood pressure is >90 at both Eligibility Visit 1 and Eligibility Visit 2, then the applicant has become ineligible. If so, terminate the interview.]

WAIST AND HIP CIRCUMFERENCE

[See the DELTA Forms Guide for the procedure for measuring waist and hip circumference. Round the readings and average to the nearest whole numbers.]

	a. Reading 1	b. Reading 2	c. Average
9. Waist circumference (cm)	a. _____	b. _____	c. _____
10. Hip circumference (cm)	a. _____	b. _____	c. _____

APPLIANCES AVAILABILITY

11. Does the applicant have access to the following appliances at home?
[For each appliance listed below, circle YES or NO]

a. refrigerator	YES	NO
b. freezer	YES	NO
c. microwave or oven or toaster oven	YES	NO

12. Does the applicant have access to the following appliances at work or school?
[For each appliance listed below, circle YES or NO]

a. refrigerator	YES	NO
b. freezer	YES	NO
c. microwave or oven or toaster oven	YES	NO

[If any of the answers to questions 11-12 are NO, inform Study Coordinator.]

BLOOD DRAWING

13. When was the last time you ate or drank anything except water?

    a. Time (hh:mm): ____:____ b. AM PM

14. How many hours since you last consumed any alcohol? _____

15. Enter the current time:

    a. Time (hh:mm): ____:____ b. AM PM

16. Number of hours fasted: _____

[If applicant has not fasted for at least 10 hours or has consumed alcohol in the last 48 hours, do not draw blood. Reschedule applicant in question 17.]

17. a. Has applicant been rescheduled for blood drawing? YES NO

If YES, enter scheduled date:

b. Date: _____ (mm/dd/yy) c. Time: ____:____ (hh:mm) d. AM PM

If the applicant remains eligible, send him/her for blood drawing.

LIPID and INSULIN SELECTION CRITERIA

18. a. TC _____ mg/dl

    b. HDL _____ mg/dl

    c. TG _____ mg/dl

    d. LDL _____ mg/dl (calculated)

    e. INS _____ μU-ml

Use the Lipid/ Insulin Eligibility Program to answer question 19. The EV2 Lipid and Insulin cutpoint tables are provided in the Forms Guide. **These cutpoints are based on the AVERAGE of EV1 and EV2 measurements.]**

19a. Based on the Lipid / Insulin Eligibility Program results for the average of EV1 and EV2 lipid measurements, is the applicant still eligible?

[Circle answer] YES NO

b. Based on the Lipid / Insulin Eligibility Program results for the average of EV1 and EV2 insulin measurements, is the applicant still eligible?

[Circle answer] YES NO NA (not applicable)

REMINDER: Return to page 4 of the Telephone Screening Visit Form and record the EV2 lab results from question 18 on this form for all applicants. If the applicant has been excluded after EV2, also complete question 13 on the Telephone Screening Visit Form.

20. Code number of personnel completing this form_____

NOTE PAGE

DELTA ID:_____ DATE:___/___/___

NAME:_____
first middle last

PERSONNEL CODE NUMBER _____

Family Medical History

Form Code: FMH
Version B 5/16/94

DELTA ID _____ **NAME** _____ **Today's Date:** ____ / ____ / ____

INSTRUCTIONS: Please give the following information about YOUR blood-related family members. For each health problem, circle the appropriate answer: *Y for YES, N for NO, U for Unsure.*

	PARENTS		GRANDPARENTS				BROTHERS or SISTERS			
Relatives	Father	Mother	Father's Father	Father's Mother	Mother's Father	Mother's Mother	#1	#2	#3	#4
Alive	Y N U	Y N U	Y N U	Y N U	Y N U	Y N U	Y N U	Y N U	Y N U	Y N U
If Alive, Age										
Age at death, if deceased										
Diabetes	Y N U	Y N U	Y N U	Y N U	Y N U	Y N U	Y N U	Y N U	Y N U	Y N U
Heart Attack	Y N U	Y N U	Y N U	Y N U	Y N U	Y N U	Y N U	Y N U	Y N U	Y N U
Death From Heart Disease before Age 50	Y N U	Y N U	Y N U	Y N U	Y N U	Y N U	Y N U	Y N U	Y N U	Y N U
Stroke	Y N U	Y N U	Y N U	Y N U	Y N U	Y N U	Y N U	Y N U	Y N U	Y N U
Elevated cholesterol and/or triglycerides	Y N U	Y N U	Y N U	Y N U	Y N U	Y N U	Y N U	Y N U	Y N U	Y N U
High blood pressure	Y N U	Y N U	Y N U	Y N U	Y N U	Y N U	Y N U	Y N U	Y N U	Y N U

3.8 MENSTRUAL CALENDAR

This information will be kept in the participant's file to ensure that accurate dates of the menstrual cycle are recorded on the Participant Weekly Monitoring Form (PWM).

The calendars should be kept current during the 7 weeks of the diet trial. If you distribute them to the subjects, you should plan to collect them at the final (week 7) blood draw for each subject. Alternatively, you may choose to keep the calendar in the subject file and update it at each visit.

Menstrual Calendar - Feeding Period 1

DELTA ID Number _____ **Usual cycle length** _____ **days**
(typical length = 28 days)

On the calendar below put an X in the block corresponding to the first day of your period (first day of bleeding). Check ✓ each day that bleeding continues enough to require a pad or tampon.

SEPTEMBER / OCTOBER						
SUN	MON	TUE	WED	THU	FRI	SAT
					30	1
2	3	4	5	6	7	8
9	10	11	12	13	14	15
16	17	18	19	20	21	22
23	24	25	26	27	28	29
30	31					
NOVEMBER						
SUN	MON	TUE	WED	THU	FRI	SAT
		1	2	3	4	5
6	7	8	9	10	11	12
13	14	15	16	17	18	

Please submit this completed calendar to study personnel at the last blood drawing

Menstrual Calendar - Feeding Period 2

DELTA ID Number _____

Usual cycle length _____ **days**
(typical length = 28 days)

On the calendar below put an X in the block corresponding to the first day of your period (first day of bleeding). Check ✓ each day that bleeding continues enough to require a pad or tampon.

JANUARY						
SUN	MON	TUE	WED	THU	FRI	SAT
					6	7
8	9	10	11	12	13	14
15	16	17	18	19	20	21
22	23	24	25	26	27	28
29	30	31				
FEBRUARY						
SUN	MON	TUE	WED	THU	FRI	SAT
			1	2	3	4
5	6	7	8	9	10	11
12	13	14	15	16	17	18
19	20	21	22	23	24	

Please submit this completed calendar to study personnel at the last blood drawing

Menstrual Calendar - Feeding Period 3

DELTA ID Number _____ **Usual cycle length** _____ **days**
(typical length = 28 days)

On the calendar below put an X in the block corresponding to the first day of your period (first day of bleeding). Check ✓ each day that bleeding continues enough to require a pad or tampon.

MARCH / APRIL						
SUN	MON	TUE	WED	THU	FRI	SAT
					31	1
2	3	4	5	6	7	8
9	10	11	12	13	14	15
16	17	18	19	20	21	22
23	24	25	26	27	28	29
30						
MAY						
SUN	MON	TUE	WED	THU	FRI	SAT
	1	2	3	4	5	6
7	8	9	10	11	12	13
14	15	16	17	18	19	

Please submit this completed calendar to study personnel at the last blood drawing

3.9 RUN-IN ENERGY ESTIMATE FORM

This form will be completed during the 3-day run-in phase of the study prior to participant randomization. Data from this form will not be entered into the data management system.

Instructions
DELTA Run-In Energy Estimate (RIE)

Use black ball-point pen, date and initial all changes, do not use liquid collection fluid, and follow all other general instructions for completing DELTA forms. This form is placed in the participant's file at the field center and is not entered into the DELTA DMS. **Day 1** items (2. - 6.) are completed for the first day of the Run-In period, **Day 2** items (7. - 11.) are completed for the second day of the Run-In period, and **Day 3** items (12. - 16.) are completed for the third day of the Run-In period.

Record the participant's DELTA ID.

List the field center location.

1a,b,c. Record the participant's first, middle, and last name.

DAY 1

2. Record the date of the first day of run-in.

3. Record that day's kcal level.

4a. or 4b. Record the participant's weight in pounds (a) or kilograms (b).

5. Circle the letter of the correct choice based on the participant's response.

6. Record comments if appropriate.

Information for **Days 2 and 3** of the Run-In period is recorded similarly in items 7. - 11. for **Day 2** and in items 12. - 16. for **Day 3**.

When completed file the form in the participant's record file.

Run-In Energy Estimate

Form Code: RIE
Version: B 8/30/94

DELTA ID: _____ **Center:** _____

1. a. First name: _____

   b. Middle name: _____

   c. Last name: _____

Day 1 2. Date: _____ (mm/dd/yy) 3. Calorie level: _____

4. Weight, either in lbs. or kg: a. lbs: _____ b. kg: _____

5. Circle correct response:
   - A Food was enough
   - B Food was too much
   - C Food was not enough

6. Comments: _____

Day 2 7. Date: _____ (mm/dd/yy) 8. Calorie level: _____

9. Weight, either in lbs. or kg: a. lbs: _____ b. kg: _____

10. Circle correct response:
    - A Food was enough
    - B Food was too much
    - C Food was not enough

11. Comments: _____

Day 3 12. Date: _____ (mm/dd/yy) 13. Calorie level: _____

14. Weight, either in lbs. or kg: a. lbs: _____ b. kg: _____

15. Circle correct response:
    - A Food was enough
    - B Food was too much
    - C Food was not enough

16. Comments: _____

3.9.1 Run-In Energy Estimate Form

TABLE OF CONTENTS

CHAPTER FOUR: Adherence

4.0 ADHERENCE

Compliance with the study protocol and the study diet is a challenge equal to or greater than participant recruitment. In DELTA, assessment of adherence is important in interpreting results of the study. There is good reason to believe that our study population will be particularly responsive to a supportive and welcoming atmosphere at the clinic sites. Thus, personal interactions between the staff and participants will be very important in maintaining adherence.

In spite of our best efforts, some participants may drop out; and it will be important to record their reason for doing so on the Drop-out Form.

Instructions for Completing the Drop-Out Form

The Drop-Out Form will be completed at the time that a randomized participant drops out of the study for any reason. Data from this form will be entered into the data management system.

DELTA ID AND TODAY'S DATE

Apply the pre-printed DELTA ID label or write in the 5-character ID number.

Record the date the form is being completed as month/day/year using leading zeros to fill in all spaces.

Question 1: Date of Last Visit

Record the date of the participant's last visit to the study site. Enter the date as month/day/year using leading zeros to fill in all spaces.

Question 2: Participant's Name

Record the participant's first name, middle name, and last name in 2a-c, respectively.

Question 3: Reason for Drop-Out

Circle only one letter corresponding to the reason for drop out from the study.

Question 4: Detailed Reason for Drop-Out

Describe the reason for drop-out in the space provided, write legibly and avoid abbreviations.

Question 5: Personnel Code Number

Record the personnel code number assigned to the DELTA study staff for the person completing this form.

Drop Out Form

Form Code: DPO
Version B 6/30/94

DELTA ID: _____ **Today's Date**___/___/___

1. Date of last visit: _____
   (mm/dd/yy)

2. a. First name: _____

   b. Middle name: _____

   c. Last name: _____

3. Reason for drop-out (circle only one):

   A Failure to comply with protocol (missing 2 meals, eating 3 self-selected meals greater than 40% fat, exceeding alcohol limits more than 1 time in a period)

   B Serious illness or death

   C Voluntary withdrawal

4. Any comments? Y [Yes] N [No] [Circle answer.] If Y, enter detailed reason or comments below:

_____

_____

_____

_____

_____

_____

_____

5. Personnel code number: _____

Figure 4.0.1.1: Drop Out Form

4.1 MOTIVATORS TO COMPLIANCE

4.1.1 Perceptions of Personal Benefit

For the participant to perceive personal benefit from participation in the study, these aspects should be emphasized:

- close medical, dietary and weight surveillance,
- dietary counseling,
- free food,
- free hematology panels,
- being part of a group of peers who are helping to advance medical knowledge,
- extended breaks between each of the feeding periods, and inclusion of an optional self-selected meal once a week.

4.1.2 Endorsement from Peers and Leaders

Consensual validation of the study itself and of any individual's participation in it may be a key component both in recruitment and adherence for the participants. The study investigators can obtain appropriate endorsement by developing and maintaining close associations with leaders in the community, with organizations and also with grass roots activists.

All the professional staff working in the study should be willing to provide endorsements of DELTA to the media or to interest groups. Conversely, all staff should be aware of the influence and authority they bring to such interviews or presentations and should guard against promoting negative perceptions or unfounded expectations concerning the benefits of DELTA.

4.1.3 Regular Feedback

Feedback is an effective reinforcer. Participants should be given regular feedback on their own adherence, and on the progress of the study in general and the importance of their role in its success.

4.2 INHIBITORS TO COMPLIANCE

To be written by Dr. Elmer

4.3 MASKING OF THE DIETS

The diet assignments will be masked to the principal investigators, coordinators, and laboratory technicians of each site; however, kitchen staff will know assignments so the correct food and beverages can be plated for service.

4.4 MANAGEMENT OF POOR ADHERENCE

Apart from the specifics discussed above, a number of general precepts apply:

Much of the potential for poor adherence can be avoided early on by adequate explanations of what the study entails and what the participant's role will be.

Prevention of poor adherence, as well as its management after the fact, will depend in large part on the relationships built between individual participants and staff. Your interpersonal skills will have a crucial impact on the participants' desire to return for each visit. Similarly, relationships among the participants will be a crucial factor; and strategies that foster group bonding should be explored and initiated.

Family and friends can be important allies both in preventing and turning around poor adherence. In fact, if they are not for you, they may well be against you, so pay attention to the participants' support systems.

Although you are expected to combat poor adherence energetically, be sensitive to the potential for inadvertently crossing the line into undue pressure or coercion.

4.5 PARTICIPANT WEEKLY MONITORING

Figure 4.5.1

Instructions for Completing the Participant Weekly Monitoring Form

---

Note that questions 7-13 on this form are asked at the first weekly interview concerning the participant's activity during the previous week.

Data from this form will be entered into the data management system.

DELTA ID

Apply the pre-printed DELTA ID label or write in the 5-character ID number.

Monday's Date

Enter the current Monday's date which is usually the first day each week that a participant is interviewed. Enter the date as month/day/year using leading zeros to fill in all spaces.

Question 1: Personnel Code Number

Record the personnel code number assigned to the DELTA study staff for the person completing this form.

Questions 2-4: Blood Draw

These items are completed only during weeks 5, 6, and 7.

2. Circle the number of the Feeding Period.

3. Circle the number of the week into the Feeding Period.

4. Fill in the specific date of the blood draw. Enter the date as month/day/year using leading zeros to fill in all spaces.

Questions 5-6: Weight

The participant will be interviewed twice each week, usually on Monday and Thursday, to record weight and other vital information.

5a-d. Enter the date, weight, and current calorie level from the first weekly interview, usually on Monday. In 5a, enter the date as month/day/year using leading zeros to

fill in all spaces. In 5b or 5c, record the participant's current weight either in pounds (lbs) or kilograms (kg), respectively. Weigh the participant before the dinner meal, without shoes or coats. In 5d, circle the calorie level based on the body weight in 5b or 5c. The calorie level is adjusted such that the participant's weight does not vary 2.2 pounds (1 kg) or more than the target weight. *(Note to the Interviewer: For target weight, use the weight recorded on the first day of feeding period 1 or the first weekly weight recorded during the first week of feeding period 1.)*

6a-d. Enter the date, weight, and circle the current calorie level from the second weekly interview, usually on Thursday. In 6a, enter the date as month/day/year using leading zeros to fill in all spaces. In 6b or 6c, record the participant's current weight either in pounds (lbs) or kilograms (kg), respectively. Weigh the participant before the dinner meal, without shoes or coats. In 6d, circle the calorie level based on the body weight in 6b or 6c. The calorie level is adjusted such that the participant's weight does not vary 2.2 pounds (1 kg) or more than the target weight. *(See previous note under question 5 denoting target weight.)*

Question 7: Exercise (Answered based on information from the week preceding the first weekly interview.)

In 7a, circle either YES or NO. If the response is NO, go to question 8. If the response is YES, continue with 7b and circle one letter only corresponding to the correct response. *(Note to interviewer: If 7a is answered YES, inform the Study Coordinator.)*

Questions 8-9: Medications

In 8a circle YES or NO. If 8a is answered NO go to question 9. If 8A is answered YES complete b-g listing the name(s) of the medications(s) and the total weekly amount(s) taken. Be sure to list the reason the participant took the medication.

In 9a circle YES or NO. If 9A is answered NO go to question 10. If 9a is answered YES complete b-e listing the name of the supplement(s) and the total weekly amounts(s) taken. *(Note to interviewer: If 8a or 9a is answered YES, inform the Study Coordinator.)*

Questions 10: Illness *(Answered based on information from the week preceding the first weekly interview.)*

10. Circle either YES or NO. If the response is NO, skip to question 12 to continue.

If the response is YES, describe the illness in the space provided, writing legibly and avoiding abbreviations. Also, proceed to answer question 11.

Question 11: Change in Eating as a Result of Illness

In 11a circle YES or NO. If 11 a is answered NO go to question 12. If 11 a is answered YES, describe the reasons in the spaces provided and complete b and c by circling YES or NO. *(Note to the interviewer: For 11b and 11c, any diet history or action would be taken by the Study Coordinator or other DELTA personnel trained to do so.)*

Question 12: Smokers Only *(Answered based on information from the week preceding the first weekly interview.)*

In 12a, circle either YES or NO. A change in smoking habits is defined as started smoking, stopped smoking, or increased or decreased smoking by at least 50 percent. If the response to 12a is NO, go to question 13. If the response to 12a is YES, then proceed with 12b and circle one letter only corresponding to the correct response. *(Note to interviewer: If 12a is answered YES, inform the Study Coordinator.)*

Question 13: Women Only *(Answered based on information from the week preceding the first weekly interview.)*

In 13a, circle either YES or NO. If the response to 13a is YES, then proceed with 13b and 13c, recording the date and circling the day of the week the participant began menstruating. (*Note to interviewer: This question should be skipped completely for men participants as well as for clearly postmenopausal women participants. NA is not needed as a response.*)

Reminder to All Study Personnel: Be sure to complete this form for each participant at the time of the last blood draw of the feeding period.

Participant Weekly Monitoring

Form Code: PWM
Version B 5/10/94

DELTA ID: _____ **Monday's DATE:** ____/____/____
[THIS WEEK] (mm/dd/yy)

1. Code Number of personnel completing this form: _____

BLOOD DRAW
[Complete numbers 2-4 during weeks 5, 6, 7.]

2. Period **1 2 3 [Circle correct number]**

3. Week **5 6 7 [Circle correct number]**

4. Date of blood draw ____/____/____
mm/dd/yy

WEIGHT [THIS week]
[Participants are weighed before dinner, without shoes or coats.]

5. a. Date of first weekly weight: _____
(mm/dd/yy)

First weekly weight, either in lbs or kg:

b. lbs: _____ or c. kg: _____

d. Current calorie level: **1500 2000 2500 3000 3500 [Circle correct number]**

6. a. Date of second weekly weight: _____
(mm/dd/yy)

Second weekly weight, either in lbs or kg:

b. lbs: _____ or c. kg: _____

d. Current calorie level: **1500 2000 2500 3000 3500 [Circle correct number]**

Be sure to administer this form at the last blood draw of the period!

Figure 4.5.2: Participant Weekly Monitoring

Participant Weekly Monitoring

[Interviewer: Ask the participant the rest of the questions based on their activities during the past week.]

EXERCISE [Exercise is recorded at the first weekly visit following the weekend.]

7. a. In the past week, has your exercise level changed? YES NO **[If NO go to question 8]**

   b. If YES, how has your exercise level changed: **[Circle letter preceding your selection]**

   A...More active B...Less active C...No exercise

If the answer to question 7a is YES, inform the Study Coordinator.

MEDICATION

8. a. Have you taken any medications in the last week? YES NO **[If NO, go to question 9]**

   If YES, specify the name of the medication and amount of medication:

   b. Medication: _____ c. Total weekly amount:_____

   Reason _____

   d. Medication: _____ e. Total weekly amount: _____

   Reason _____

   f. Medication: _____ g. Total weekly amount: _____

   Reason _____

9. a. Have you taken any vitamin, mineral or other nutritional supplements in the past week?
   YES NO **[If NO go to question 10]**

   If YES, specify name and amount:

   b. Name: _____ c. Total weekly amount: _____

   d. Name: _____ e. Total weekly amount: _____

If the answer to either question 8 or 9 is YES, inform the Study Coordinator.

ILLNESS

10. Have you been ill in the last week? YES NO **[If NO go to question 12]**

If YES, describe illness: _____

_____

11. a. In the past week did your eating change as a result of any illness?
YES **[If Yes, enter reasons in space provided.]** NO **[If NO go to question 12]**

_____

_____

b. If YES to 11a, was a diet history taken? YES NO

c. If YES to 11a, was any action taken? YES NO

If the answer to question 11 is YES, inform the Study Coordinator.

SMOKING HABITS

12. a. In the last week, have your smoking habits changed? YES NO **[If NO go to question 13]**

[A change in smoking habits is defined as started smoking, stopped smoking, or increased or decreased smoking by at least 50 percent.]

b. If YES, how have your smoking habits changed? **[Circle letter preceding the selection.]**

A...Smoking more
B...Smoking less
C...Quit smoking
D...Started smoking

If participant reports any changes in smoking habits, inform Study Coordinator.

WOMEN ONLY

13. a. Did you begin menstruating during the last week? YES NO

b. If YES to 13a, what date did you begin menstruating: _____
(mm/dd/yy)

c. If YES to 13a, what day of the week did you begin menstruating?
(Circle the number preceding the answer)

1. Monday 2. Tuesday 3. Wednesday 4. Thursday

5. Friday 6. Saturday 7. Sunday

Be sure to administer this form at the last blood draw of the period!

4.6 MEDICATIONS THAT CAN BE USED OCCASIONALLY

Participants will be instructed to report the use of any medications to their Study Coordinators.

Headache medications: Aspirin, Tylenol, Nsaids (Motrin, Naprosyn):

Pain medications: same as listed above

Infections: antibiotics for short term

Sleep medications and sedatives: antihistamines, hydroxyzine, over the counter drugs

Cold and allergy medications: antihistamines with/without ephedrine or other beta adrenergies -- many of these are over the counter combinations that should be ok as long as they do not contain steroids.

Laxatives: Milk of Magnesia, senna and cascara -- only occasional use

Antidiarrheal: Lomotil, Kaopectate -- only occasional use

Cough medicines: Robitussin, Ny-Quil

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CHAPTER FIVE: Diet

5.0 DESCRIPTION OF DIET

Three experimental diets will be used to test the hypotheses presented in Protocol 2. The nutrient specifications are as follows:

	Average American Diet	High Mono Diet	New Step One Diet
	Diet "D"	Diet "E"	Diet F"
Total fat, % Kcal	37	37	30
SFA, % Kcal	16	8	8
MFA, % Kcal	14	22	15
PFA, % Kcal	7	7	7
Fiber g/1000 Kcal	7.5	7.5	15
Simple CHO, % of CHO	40	40	*
Cholesterol	300	300	300
*Kept at same absolute level as "D" and "E" but with increased starch			

Diet D, the Average American Diet, was representative of the U.S. diet during the collection of NHANES II data from 1976 to 1980. (The newly released NHANES III data indicate that total fat and saturated fat intake have decreased to 34% and 13% of calories, respectively). The average American Diet, Diet A, was one of the experimental diets used in Protocol 1. Thus, it will be possible to examine the lipid/lipoprotein and hemostatic responses to an Average American Diet in an insulin resistant population and a healthy population studied previously.

Diet E is high in MFA and low in SFA. (It was designed to meet the lower range of the SFA recommendations of the Step-One Diet of the National Cholesterol Education Program Adult Treatment Panel-II Report). Because of the enrichment of this diet in MFA, the total fat content of Diet E is the same as that for Diet D.

Diet F meets the nutrient specifications of the Step-One Diet. Diets E and F differ in the amount of MFA and, therefore, total fat; the contribution of SFA and PFA to the diets is the same. Diet F is higher in dietary fiber and complex carbohydrates.

All experimental diets provide 300 mg of cholesterol per day to enable comparisons to be made between diets on the basis of differences in only the amount of MFA, complex carbohydrate and dietary fiber. The design of the experimental diets will answer the question of whether diet E or F is preferable for individuals who are insulin resistant. Since total fat and MFA are lower, and dietary fiber and the proportion of complex to simple carbohydrate are both higher in Diet F, versus Diet E, it will not be possible to identify the dietary factor that accounts for this. Rather, the diet design will permit comparisons between two different dietary patterns that both meet the ATP-II recommendations for SFA and cholesterol (e.g., a high complex carbohydrate, high fiber and lower fat diet versus a high fat and high MFA diet).

5.0.1 Terms used in this chapter

Diet: This term is used to describe the experimental diets that will be fed to participants. It is defined by the macronutrient composition, levels of fatty acid classes, cholesterol, dietary

fiber, and proportion of complex to simple carbohydrates. Each experimental diet will meet the nutrient criteria specified but will vary in energy (to meet the different calorie needs of the participants).

Menu: A description of foods served at a meal. Menus refer to descriptions of a single meal or meals for one or more days.

Meal: Food served/eaten at breakfast, lunch, dinner, or snack.

Menu Cycle: The period of time within which a complete set of menus is served. An 8-day menu cycle is used. Since the feeding periods will be 7 weeks long, there will be 6 menu cycles during each feeding period. This 8-day cycle is comprised of one 6-day weekday cycle and one 2-day weekend cycle.

Unit Food: A food such as a muffin or a roll that is formulated to have the same ratio of control nutrients to calories. It can be eaten as desired by the participant without altering the composition of the diet. It is used as a calorie adjuster or given to participants who wish to have a snack.

Fat Blend: A mixture of natural fats that have a similar fatty acid profile as the experimental diets. The fat blends are used to prepare unit foods.

5.1 GENERAL INSTRUCTIONS

Rigorous control of the diet is critical to the success of the DELTA study. Procedures are directed at controlling sources of variability to the maximum extent compatible with practicality and compliance and sampling the diets for chemical verification of the composition and its variability. Sources of variability include procurement, preparation, (weighing, cooking) and consumption. Each of these procedures is described in detail below.

5.1.1 Procurement of food

Fat sources (cheese, meat, eggs, cooking oils, fat spreads, nuts, olives, and canned gravies and soups) must be procured centrally from a single lot. Foods with a stable shelf life may also be procured centrally. Skim milk is procured locally from a dairy that can guarantee skim milk with a fat content $< 0.5\%$. Whole milk is also procured locally and will provide 3.25 (e.g.: 3.3) % of fat. Perishable fruits and vegetables are procured locally according to standard specifications.

5.1.2 Specifications for food

All food whether purchased or received through donation will be procured according to the specifications listed in figure 5.1.2.

Figure 5.1.2.1

DELTA-2 Food Specifications

Fats & Oils	Specifications	Procurement
Coconut oil	AARHUS	Central*
Olive oil	extra light; Bertolli	Central*
Safflower oil	linoleic; over 70%	Central*
Corn oil	Mazola	Central*
Palm oil	Palm Oil Research Inst. of Malaysia	Central*
Margarine	regular, hard; Parkay	Central*
Margarine	corn oil, hard; Mazola	Central*
Margarine	soft; Promise	Central*
Butter	w/salt; Land O'Lakes	Central*
Lard	Park Corporation	Central*
Fat blends	DELTA; Kraft	Central*
Mayonnaise	Soybean oil; w/salt; Hellman's	Central*
Other Fat Sources	**Specifications**	**Procurement**
Cream of Chicken Soup	Campbell's	Central*
Beef gravy	Pepperidge Farm Hearty	Central*
Turkey gravy	Pepperidge Farm	Central*
Beef Bouillon	Wylers / any brand	Local
Chicken Bouillon	Wylers / any brand	Local
Nuts & Peanut Butter	**Specifications**	**Procurement**
Peanuts	dry roasted; w/salt; Planters	Central*
Almonds	unblanched, dry roasted, w/salt	Central*
Pecans	dried	Central*
Hazelnuts	unblanched, dry roasted, w/salt	Central*
Peanut butter	smooth, Skippy	Central*

*Indicates item that must be centrally procured (i.e., from same production lot # and/or date code), due to fat, oil, protein, or cholesterol content. Other items may be centrally or locally procured.

Dairy (other than cheese)	Specifications	Procurement
Whole milk	3.3%; any brand	Local
2% milk	2% fat w/added Vitamin A; any brand	Local
Skim milk	w/added Vitamin A; any brand	Local
Yogurt	Fruit on the bottom; low fat, 9gr protein/8 oz.; Dannon	Local
Jell-O Free Pudding	Kraft	Central
Fat free sour cream	Real Dairy/any brand	Local
Cheeses	**Specifications**	**Procurement**
Mozzarella	whole milk, natural; Kraft	Central*
Cheddar	American domestic, natural; Kraft	Central*
Parmesan	grated, natural; Kraft	Central*
Cottage Cheese	fat free; Healthy Choice/any brand	Local
Eggs & Egg Products	**Specifications**	**Procurement**
Whole frozen eggs	Sunnyfresh	Central*
Egg yolk powder	dried; Henningsen	Central*
Egg white powder	dried; Henningsen	Central*
Pastas, Rice, Cereals	**Specifications**	**Procurement**
rice	white; long grain; converted; Uncle Ben's	Central
rice	brown; long grain; converted; Uncle Ben's	Central
noodles	egg; enriched; Best Foods varieties	Central*
spaghetti	enriched; w/salt; Ronzoni; Hershey Pasta Group	Central
macaroni	enriched; Best Foods varieties	Central
Rolled Oats	instant; Quaker	Central
Raisin Bran	General Mills; Total	Central
Corn Flakes	General Mills; Country	Central
Cheerios	General Mills	Central

*Indicates item that must be centrally procured (i.e., from same production lot # and/or date code), due to fat, oil, protein, or cholesterol content. Other items may be centrally or locally procured.

Fiber One	General Mills	Central
Rice Cereal	General Mills; Triples	Central
All Bran	Kelloggs	Central
Complete Bran	Kelloggs	Central
Breads, Crackers, Cookies	**Specifications**	**Procurement**
Country Classic Rolls	Pepperidge Farm	Local
Whole Wheat Bread	Pepperidge Farm; sandwich-sliced or thin-sliced	Local
French Rolls	Pepperidge Farm	Local
White Bread	Pepperidge Farm; sandwich-sliced or thin-sliced	Local
Onion Sandwich Buns	Pepperidge Farm	Local
Classic Hoagie Rolls	Pepperidge Farm; w/sesame seeds	Local
English Muffins	Pepperidge Farm	Local
Whole Wheat English Muffin	Thomas'; Cooper's	Local
Breadsticks	Pillsbury; refrigerated; plain	Central
French Toast	Quaker; Aunt Jemima; frozen	Central
Pancakes	Pillsbury; Hungry Jack; frozen; plain (inc. buttermilk)	Central
Graham Crackers	Nabisco; Honey Maid	Central
Saltine Crackers	Nabisco	Central
Nilla Wafers	Nabisco	Central
Pretzels	Nabisco, salted	Central
Frito Corn Chips	Frito-Lay	Central
Potato Chips	Frito-Lay	Central
Bread crumbs	plain; Progresso/any brand	Local
Canned/Frozen Fruits/Vegetables	**Specifications**	**Procurement**
Fruit cocktail	unsw. canned in juice; sol. & liq.; Del Monte/any brand	Local
Peaches	unsw. canned in juice; sol. & liq.; Del Monte/any brand	Local
Pears	unsw. canned in juice; sol. & liq.; Del Monte/any brand	Local

*Indicates item that must be centrally procured (i.e., from same production lot # and/or date code), due to fat, oil, protein, or cholesterol content. Other items may be centrally or locally procured.

Pineapple	unsw. canned in juice; sol. & liq.; Del Monte/any brand	Local
Mandarin Oranges	unsw. canned in juice; sol. & liq.; Del Monte/any brand	Local
Applesauce	canned; sweetened; fruit combo; Motts/any brand	Local
Applesauce	canned; unsweetened; Motts/any brand	Local
Blueberries	frozen; unsweetened; Birdseye/any brand	Local
Raisins	seedless; Nabisco / Del Monte/any brand	Local
Pumpkin	canned; unsweetened; Libby/any brand	Local
Mushrooms	canned; sliced; Del Monte/any brand	Local
Corn	frozen; yellow; sweet; off cob; unprepared; Birdseye	Central
Green Peas	frozen; unprepared; Birdseye	Central
Green Beans	frozen; snap; unprepared; Birdseye	Central
Broccoli	frozen; spears; unprepared; Birdseye	Central
Carrots	frozen; sliced; unprepared; Birdseye	Central
Potatoes	frozen; mashed; butter flavor; Ore-Ida	Local
Potatoes	canned; new; Finast/any brand	Local
Potatoes	canned; sweet; in heavy syrup; Taylors/any brand	Local
Pimento	canned; any brand	Local
Olives	canned; black; sliced; Vlassic-Campbell's	Central*
Beans	canned; kidney; Progresso/any brand	Local
Tomatoes	canned; whole; peeled; Del Monte	Central
Tomato Sauce	canned; Del Monte	Central
Pickle Relish	sweet; Del Monte	Central
Pasta Sauce	Healthy Choice Traditional	Central
Fruit/Vegetable Juices	**Specifications**	**Procurement**
Grape Juice	unsweetened; any brand	Local
Apple Juice	unsweetened; any brand	Local
Orange Juice	unsweetened; Ca-fortified; Minute Maid	Local
V-8 Juice	Campbell's	Local
Lemon Juice	any brand	Local

*Indicates item that must be centrally procured (i.e., from same production lot # and/or date code), due to fat, oil, protein, or cholesterol content. Other items may be centrally or locally procured.

Spices & Other Baking Ingredients	Specifications	Procurement
Ground cinnamon	McCormick	Central
Ground ginger	McCormick	Central
Ground cloves	McCormick	Central
Ground sage	McCormick	Central
Ground thyme	McCormick	Central
Ground oregano	McCormick	Central
Ground black pepper	McCormick	Central
Dried rosemary	McCormick	Central
White pepper	McCormick	Central
Salt	McCormick	Central
Dried basil	Any brand	Local
Paprika	McCormick	Central
Parsley Flakes	Any brand	Local
Chili Powder	McCormick	Central
Onion Powder	McCormick	Central
Garlic Powder	McCormick	Central
Baking Soda	Any brand	Local
Baking Powder	Any brand	Local
White sugar	granulated, Domino/any brand	Local
Light Brown sugar	Domino/any brand	Local
Lemon Peel	McCormick	Central
Vanilla Extract	McCormick	Central
Molasses	Brer Rabbit/any brand	Local
Gelatin	powdered, unsweetened, unflavored	Local
Corn meal	Quaker	Local
Flour	white; all purpose; Gold Medal	Central

*Indicates item that must be centrally procured (i.e., from same production lot # and/or date code), due to fat, oil, protein, or cholesterol content. Other items may be centrally or locally procured.

Flour	whole wheat; Gold Medal	Central
High Gluten Flour		Local
Wheat bran	any brand	Local
Cream of Tartar	powdered	Local
Banana Puree'		Local
Cocoa	powdered; unsweetened; Hershey's	Central
Promod	Protein supplement; Ross Labs	Central*
Cooking spray	Mazola/any brand	Local
Condiments	**Specifications**	**Procurement**
Yellow Mustard	Kraft	Central
Fat Free Mayonnaise	Kraft	Central
Fat Free Salad Dressing	Kraft	Central
Pancake Syrup	table blend; Kraft	Central
Pancake Syrup	reduced-calorie; Log Cabin, Smucker's/any brand	Local
Jelly	all flavors; Kraft	Central
Dietetic Jelly	all flavors; Kraft; artificially sweetened	Central
Miscellaneous	**Specifications**	**Procurement**
Cider vinegar	Any brand	Local
Jell-O Gelatin	prepared; Kraft	Central
Worcestershire sauce	Any brand	Local
Tabasco Sauce	Any brand	Local
Soy sauce	Any brand	Local
Fresh Produce	**Specifications**	**Procurement**
Iceberg lettuce	raw	Local
Romaine lettuce	raw	Local

*Indicates item that must be centrally procured (i.e., from same production lot # and/or date code), due to fat, oil, protein, or cholesterol content. Other items may be centrally or locally procured.

Spinach leaves	raw	Local
Tomatoes	red, ripe, raw	Local
Carrots	raw	Local
Spring onions	tops & bulbs, raw	Local
Onions	raw	Local
Green peppers	sweet, raw	Local
Celery	raw	Local
Parsley	raw	Local
Garlic	raw; minced (if purchased already minced; stored in water, not oil)	Local
Bananas	raw, medium 170-210g each as purchased	Local
Meat, Poultry, Fish	**Specifications**	**Procurement**
Beef, sirloin tips	beef, short loin, top sirloin, Select, separable lean, raw, 1" cubes to be used w/out further trimming; Penn State Meat Lab	Central*
Beef, cooked, sliced, sirloin	beef, short loin, top sirloin, Select, cooked, "0" fat, to be used w/out further trimming; Penn State Meat Lab	Central*
Beef, cooked, sliced, top round	beef round, top round, Select separable lean & Fat, "0" fat, to be used w/out further trimming; Penn State Meat Lab	Central*
Beef, top round (for chili)	beef, round, top round, Select, separable lean, raw; USDA Select only, do not substitute USDA Choice; trim all visible fat	Local
Chicken breast, raw	chicken, broiler or fryer, breast meat only raw; preferred brands in order are Tyson, Perdue, Holly Farms (if these brands are not available, Ed Mills will be contacted). Remove all skin and loose fat (a small amount of visible fat should remain in the seams between muscles of the breast.)	Local
Chicken breast, cooked	chicken, broiler or fryer, breast meat only, cooked/roasted, diced, skinless w/ or w/out broth, fat content 1%; preferred brands in order are Healthy Choice and Blue Coach - another national brand is acceptable if fat content is 1% (cooked & diced product must be frozen for at least 24 hrs. before using to insure a consistent fluid purge). Pat dry with a paper towel before weighing.	Local

*Indicates item that must be centrally procured (i.e., from same production lot # and/or date code), due to fat, oil, protein, or cholesterol content. Other items may be centrally or locally procured.

Turkey Breast, cooked	turkey, all classes, light meat, cooked/roasted; Butterball Skinless Turkey breast w/broth, 99% fat free, subjected to one freeze-thaw cycle before using to insure consistent fluid purge. Pat dry with a paper towel before weighing. (If Butterball is not available, Ed Mills should be contacted to discuss acceptable alternatives.)	Local
Pork, center loin (for chops & stir-fry)	pork, fresh loin, center loin, separable lean, raw, boneless, one typical boneless center loin should not weigh more than 6 lbs; IMPS #412 B or 414. Preferred brands in order are IBP, EXCEL, IPC, Hatfield (other national brands are acceptable as long as the loins are from "typical" market weight animals; now sows. (Trimming instructions are included at the end of this list.)	Local
Pork, cured ham	pork, cured ham, boneless, extra lean, approx. 5% fat, w/water added, label claim 95% fat free; one suggested brand name is Wilson Corn King Lean or other national brands are acceptable.	Local
Meat Balls	pre-cooked, ½ oz portion size, Mrs. DiFillippos (Mrs. D's) Mild Italian Meat Balls. Procurement and shipping will be handled by the Penn State Meat Lab.	Central*
Turkey salami	15% fat, 85% fat free (a nutritional labeling panel showing 10 to 15% fat); cooked or cotto varieties are acceptable, **hard or genoa are not acceptable even if the products meet other requirements**. One suggested brand name is Butterball Cooked Turkey Salami.	Local
Breakfast sausage	brown & serve, sausage, pork links or bulk, pre-cooked, Hormel Little Sizzlers 10 links/7 oz. box (7g fat/.7 oz link; UPC Code 3760043734) or bulk quantities are available through food service divisions (these may be called "broil & serve").	Local
Shrimp	fish/shellfish, shrimp, mixed species, cooked - moist heat, large salad, deveined, no tails 100 to 200/pound. PSU kitchen (Abir Farhat) will be centrally procuring for all centers.	Central

Pork trimming instructions: All surface fat and "tail" should be removed, leaving only the main muscle (longissimus). Leave most surface connective tissue intact on the muscle. The large end of the trimmed muscle can be cut into chops, the smaller end into stir-fry strips. Larger accessory muscles may be used for stir-fry after trimming away visible fat. The expected yield of usable trimmed meat should be about 70% of starting weight.

*Indicates item that must be centrally procured (i.e., from same production lot # and/or date code), due to fat, oil, protein, or cholesterol content. Other items may be centrally or locally procured.

5.1.3 Food Procurement Directory

COMPANY	PRODUCTS	DELTA CONTACT / TELEPHONE #	
Campbell	cr. of chicken soup canned beef gravy canned turkey gravy canned black olives	Penny Kris-Etherton Abir Farhat	(814) 863-2923 (814) 863-9746
Best Foods	corn oil margarine corn oil mayonnaise macaroni egg noodles peanut butter	Susan Blackwell	(919) 962-3092
General Mills	Triples Fiber One Total Raisin Bran Cheerios Country Corn Flakes all purpose flour whole wheat flour	Joanne Slavin	(612) 624-7234
PSU Lab/Other possible meat companies	beef products meatballs	Ed Mills Susan Blackwell	(814) 863-0069 (919) 962-3092
Hormel	sausage links	Nancy Van Heel	(612) 624-1497
Singleton Seafood Co.	shrimp	Penny Kris-Etherton Abir Farhat	(814) 863-2923 (814) 863-9746
Nabisco	Saltines Nilla wafers graham crackers pretzels peanuts	Wahida Karmally	(212) 305-6639
PMK Associates	pecans hazelnuts almonds	Wahida Karmally	(212) 305-6639
Del Monte	tomato sauce	Wahida Karmally Abir Farhat	(212) 305-6639 (814) 863-9746

M & M Mars/ Uncle Ben's	white rice brown rice	Marlene Windhauser	(504) 765-2663
Quaker	dry oats French toast sticks	Joanne Slavin	(612) 624-7234
Frito Lay corn chips	potato chips corn chips	Penny Kris-Etherton Abir Farhat	(814) 863-2923 (814) 863-9746
Hershey	Spaghetti dry cocoa	Penny Kris-Etherton Abir Farhat	(Same as above)
Henningsen Foods	egg whites egg yolk powder	Vikkie Mustad	(814) 863-0772
Kelloggs	Complete Bran All Bran	Joanne Slavin	(612) 624-7234
Pillsbury	bread sticks frozen pancakes	Joanne Slavin	(Same as above)
Ross Labs	Promod	Penny Kris-Etherton Abir Farhat	(814) 863-2923 (814) 863-9746
Healthy Choice	pasta sauce	Penny Kris-Etherton Abir Farhat	(Same as above)
Kraft General Foods	fat blends Parkay margarine mozzarella cheese parmesan cheese cheddar cheese Jell-O Free Pudding Jell-O (prepared) yellow mustard fat-free salad dr. fat free mayonnaise jelly dietetic jelly pancake syrup	Penny Kris-Etherton Abir Farhat	(Same as above)
Birdseye	frozen vegetables	Penny Kris-Etherton Abir Farhat	(Same as above)
McCormick	all spices vanilla extract	Penny Kris-Etherton Abir Farhat	(Same as above)

AARHUS	coconut oil	Lynn Martin	(919) 962-3096
Palm Oil Res. Ins.	palm oil	Lynn Martin	(Same as above)
Bertolli	ex. lt. olive oil	Lynn Martin	(Same as above)
Park Corporation	lard	Lynn Martin	(Same as above)
VanDenBergh Foods	soft promise margarine	Lynn Martin	(Same as above)
Land O'Lakes	butter	Lynn Martin	(Same as above)

5.1.4 Calibration of Food Balances

In order to minimize variability contributed by weighing of food portions in individual centers, Nutrient Composition Laboratory (NCL) has provided identical sets of weights to each center, including the FALCC. These weights have been calibrated against an NIST weight set (Class P Certified). Please provide the simple instructions to your staff members for their daily use. If you have any questions please call Carol Davis, NCL 301-504-8356.

First Weighing (beginning of each day):

- Wear fat-free, powder-free gloves
- Clean balance pan (with damp cloth or damp paper towel)
- Level balance if necessary
- Zero balance
- Use gloves when handling weights:

Weigh 1, 10, and 100 gram weights and record their weights on the calibration form (see page 15). For the 100g weight be sure to use both hands and forceps (one pair for each side).

Acceptable Ranges:

Weights you obtain should fall within the following ranges for the nominal weights assigned to each. **If weight falls outside of the acceptable range repeat weighing procedure to verify; then call for service.**

The supervisor or co-worker should verify the calibration and initial the calibration form at the beginning of each day.

NOMINAL WEIGHT	TOLERANCE	2 X TOLERANCE	
1 gram		.005g	.01g
10 gram		.020g	.04g
100 gram		.030g	.06g

1a. If balance is accurate to tenths and w = weight, then:

NOMINAL WEIGHT	ACCEPTABLE RANGE*	ROUNDED TO TENTHS	ACC/RANGE
1 gram		0.99 - 1.01	1.0 - 1.0
10 gram		9.96 - 10.04	10.0 - 10.0
100 gram		99.94 - 100.06	99.9 - 100.1

1b. If balance is accurate to hundredths and w = weight, then:

NOMINAL WEIGHT	ACCEPTABLE RANGE*	ROUNDED TO HUNDREDTHS	ACC/RANGE
1 gram		0.99 - 1.01	.99 - 1.01
10 gram		9.96 - 10.04	9.96 - 10.04
100 gram		99.94 - 100.06	99.94 - 100.06

*(The acceptable weight range is calculated by allowing two times the tolerance range.)

5.1.4.1 DELTA Calibration Form for Food Balances

Center Name: _____ Scale #: _____

Please complete the following form every morning. Use a separate sheet for each scale. These data may be needed by the Coordinating Center. If first weight is out of range, supervisor or co-worker should repeat the calibration. If second weight is out of range, service is required. Do not use scale until calibration is within range.

Date	1 gram Range 0.99-1.01 First Wt.	Second Wt.	10 gram Range 9.96-10.04 First Wt.	Second Wt.	100 gram Range 99.94-100.06 First Wt.	Second Wt.	Calibration Checked by (Please Initial)	Service Required (Yes/No)

5.2 WEIGHING FOODS

5.2.1 Weighing fat and cholesterol containing items

Fat sources should be weighed to the nearest 0.10 gram (except for milk which is provided as a unit pack). The following items are considered to be fat/cholesterol sources:

Meats, poultry, shellfish
Cheese
Oils and solid fats, regular mayonnaise and salad dressings
Olives, nuts
Unit food and cookie dough/batter
Frozen eggs
Egg yolk powder

Procedure for weighing egg yolk powder:

1. Following the weighing of the batch serving, mayonnaise, or Healthy Choice Marinara sauce into the individual serving dish, the scale should be zeroed.

2. Slowly and evenly sprinkle the egg yolk powder over the ingredients while closely monitoring the increasing weight of the powder.

3. When the amount of powder is reaching the desired weight, allow the powder to form a small pile in a dry portion of the serving container.

4. If too much powder is added, the excess can be removed by carefully scooping from the top of the pile.

5. After the exact weight has been reached, gently shake the individual serving container to promote even distribution of the powder, or carefully mix into the mayonnaise or Healthy Choice Marinara sauce using a rubber spatula.

6. Double check the weight before zeroing the scale and going on to the next item on the recipe.

5.2.2 Weighing casserole items

1. Place the individual serving dish on the scale platform. Zero the scale.

2. Weigh the specified portion of the batch mixture or pasta item into the serving dish.

3. If applicable, measure the spice mix and sprinkle evenly over the batch mixture. Zero the scale.

4. Carefully weigh to the ± 0.1 gram the egg yolk powder on to the batch mixture, distributing evenly over the individual portion (see procedure above for weighing egg yolk powder). Zero the scale.

5. Portion the diced/cubed meat into the individual serving dishes, distributing evenly and weighing to the $\pm$0.1 gram. Zero the scale.

6. Add the oils, using a squirt bottle or eye dropper to carefully add drop by drop when approaching the desired weight. Weigh to the $\pm$0.1 gram.

7. If necessary, mix using a rubber spatula. If mixing is required, carefully wipe the spatula on the inside of the individual container, or use an additional spatula to return all ingredients to the mixture and to prevent loss of oil or egg yolk powder.

5.2.3 Foods that can be weighed or measured by volume

Milk, juice, and lettuce can be weighed as either a gram weight or household measure.

5.2.4 Foods that can be weighed to +/- 1 gram

The following foods can be weighed to +/- 1 gram:

Menu 1

Breakfast
- Orange Juice
- Triples
- Bran Flakes
- Jelly

Lunch
- Lettuce
- Ginger Cookie Ingredients:
  - Sugar
  - Molasses
  - Vinegar
  - Flour
  - Baking Soda
  - Ginger, Cinnamon, Cloves
- Peaches

Dinner
- Egg Noodles
- Gravy
- Corn
- Carrot
- Tomato
- Lettuce
- Applesauce

Snack
- Raisins
- Pretzels

Menu 2

Breakfast
- Tangerines
- Raisin Bran
- Fiber One

Lunch

Shrimp Pasta Salad Ingredients:
- Spiral-shaped Pasta
- Green Onion
- Celery
- Broccoli
- Tomato
- Lemon Juice
- Vinegar
- Spice Mix
- Egg White

Oatmeal Cookie Ingredients:
- Light Brown Sugar
- White Sugar
- Flour
- Baking Powder
- Rolled Oats

Dinner

Chicken Jambalaya Ingredients:
- Tomato
- Onion
- Green Peppers
- Celery
- Garlic
- Sugar
- Parsley
- Black Pepper
- Rice

Lettuce
Spinach
Green Onion
Fruit Cocktail

Menu 3

Breakfast
- Orange Juice
- Cheerios
- Jelly

Lunch

Chicken Salad Ingredients:
- Celery
- Sweet Pickle Relish
- Onion
- Lemon Juice

Salt & Pepper
Lettuce
Tomato
Pineapples

Dinner
Spaghetti
Lettuce
Tomato
Green Pepper
Rolled Oat Macaroon Ingredients:
Sugar
Rolled Oats
Baking Powder
Vanilla
Salt

Menu 4

Breakfast
Orange Juice
Bran Flakes
Jelly

Lunch
Macaroni Salad Ingredients:
Macaroni
Celery
Sweet Pickle Relish
Green Onions
Tomato
Lemon Juice
Vinegar
Parsley
Salt
Peaches

Dinner
Turkey Almond Casserole Ingredients:
Egg Noddles
Spice Mix
Bread Crumbs
Green Beans
Lettuce
Tomato
Ginger Cookie Ingredients (See Menu 1)

Snack
Raisins
Pretzels

Menu 5

Breakfast
- Apple Juice
- Cheerios
- Fiber One
- Jelly

Lunch
- Pork Stir Fry Ingredients:
  - Carrot
  - Peas
  - Green Pepper
  - Garlic
  - Rice
- Rolled Oat Macaroon Ingredients (See Menu 3)

Dinner
- All Seasoned Bread Crumbs Ingredients
- Pasta Sauce
- Spaghetti
- Lettuce
- Tomato
- Pears

Menu 6

Breakfast
- Orange Juice
- Corn Flakes
- Fiber One
- Jelly
- Blueberry Muffin Ingredients:
  - Flour
  - Sugar
  - Blueberries
  - Baking Powder
  - Cinnamon

Lunch
- Chili Ingredients:
  - Tomato
  - Kidney Beans
  - Onions
  - Celery
  - Chili Powder
  - Garlic
  - Salt & Pepper
- Carrots
- Jell-O

Dinner — Lemon Sage Chicken Spice Mix
Rice Pilaf Ingredients:
- Rice
- Mushrooms
- Pimento
- Onions
- White Pepper

Broccoli
Pineapples

Snack — Brownie Ingredients:
- Sugar
- Flour
- Cocoa Powder
- Salt
- Baking Powder

Menu W1

Breakfast — Grape Juice
Syrup

Lunch — Lettuce
V-8 Juice
Peaches

Dinner — Sweet Potato
Broccoli
Pineapples

Snack — Pretzels

Menu W2

Breakfast — Applesauce
Syrup

Lunch — Lettuce
Carrot
Fruit Cocktail

Dinner — Pasta Sauce
Spaghetti
Corn

Snack — Jelly

Menu W3

Breakfast	Orange Juice
	Syrup
	Fiber One
Lunch	Pasta Sauce
	Carrot
	Pears
Dinner	Gravy
	Green Beans
	Fruit Cocktail

Menu W4

Breakfast	Syrup
Lunch	Lettuce
	V-8 Juice
	Pineapple
Dinner	Gravy
	Potato
	Broccoli
	Peaches
Snack	Jelly

5.2.5 Portion Control Unit Packs

DELTA-2 menus have been calculated to incorporate single serving unit packs wherever possible for milk and non-fat items. Centers may use unit packs or weigh out servings from bulk quantities as long as the bulk item conforms to the specifications for the unit packs.

Note: Calcium-fortified orange juice used on weekday menus is not available in unit packs and must be measured or weighed. Use reconstituted frozen concentrate.

5.2.6 Bread Products

All Pepperidge Farm and other brand bread products are served as units. The following constitute 1 unit:

White Bread, 1 slice (sandwich sliced)
Whole Wheat Bread, 1 slice (thin sliced)
French Rolls, 1 roll
Country Classic Rolls, 1 roll
Onion Sandwich Rolls, 1 roll
Sesame Seeded Buns, 1 bun
English Muffins, 1 muffin
Wh. Wheat English Muffins, 1 muffin

Note: DO NOT USE END PIECES ON LOAF BREAD.

5.3 PREPARATION

5.3.1 Containers

In order to avoid losses in transferring food, it is preferable to weigh directly into the serving container. Cooked foods may be cooked and served in the same container. This is particularly important where there may be fat loss during cooking. If the food is not served in its cooking container, a rubber spatula should be used to transfer any food adhering to the cooking container onto the serving dish. Food must be cooked in non-stick cookware or cookware sprayed with "Mazola No-Stick" spray. Follow specifications in recipe.

5.3.2 Meat, Fish, Poultry

Meat, fish and poultry are stored frozen. **Do not weigh any items not previously frozen.** These items should always be thawed in the refrigerator. **Remove any visible fat, drain and pat dry** with paper towels before weighing. Chicken should be washed and dried before weighing. Meat products packed in vacuum pack plastic or precooked products do not need washing. Shrimp should be thawed in a colander in the refrigerator; pat dry to remove remaining water before weighing.

5.3.3 Fat Blends for Unit Foods

Store fat blends, including the unopened cans, in the refrigerator. Before use, melt and aliquot each of the fat blends according to the following procedure:

1. Open the fat blend can and remove the lid. Cover the can with aluminum foil.

2. Melt the blend either in a hot water bath or over low heat for until it is completely liquified. The melting time depends on the temperature of the hot plate/water bath, room, and the composition of the blend.

3. Stir occasionally with a spoon.

4. When the fat is completely melted, cool for two-five minutes, mix with a spoon until homogeneous.

5. Aliquot the warm fat blend into two-quart opaque plastic containers. Fill only 2/3 of each plastic container.

6. Cover the containers, label the sides and lids (write your labels with dry ink on masking tape), and store in the refrigerator.

When you are ready to bake the unit foods, melt the aliquoted fat blend in the microwave. Melt at medium for two minutes at a time until the blend is completely melted. Stir with a spoon until mixture is homogenous. Weigh out the required amount of melted warm fat blend. Weighed out fat blend should be in the liquid state.

5.3.4 Canned Fruits and Frozen Vegetables

Fruits are generally incorporated in the menus in portion pack amounts. Centers may elect whether to use portion packs. In some cases it may be more aesthetically pleasing, and/or less expensive to weigh out servings from bulk quantities. If portions are weighed, do not drain the fruit before weighing. Stir with a non-slotted spoon to distribute liquid in fruit. Spoon out portions using a non-slotted spoon.

Frozen vegetables should be weighed in the frozen state and then microwaved according to directions in the recipe.

5.3.5 Instructions for Cooking Pasta

American Beauty - Vermicelli

1. Bring 5 quarts of water to a boil.
2. Empty contents of package into boiling water and stir.
3. Return to a boil and boil, uncovered, for 6 to 8 minutes. For best results, AVOID OVERCOOKING.
4. Drain, cool to room temperature.

Rainbow - Egg Noodles

1. Add ½ contents of package (approximately 4 C.) to 2 quarts rapidly boiling water.
2. Add 1 tsp. salt, stir.
3. Cook 6-8 minutes, or to taste. Stir often to prevent sticking.
4. Drain, cool to room temperature.

Mueller's - Elbows

1. Bring 4 to 6 quarts water to a rapid boil. If desired, add up to 2 teaspoons salt. (Use 3 quarts water and up to 1 teaspoon salt when cooking 8 ounces or less.)
2. Add elbows and stir to separate; return to boil.
3. Boil uncovered, stirring occasionally, 9 to 12 minutes or until desired tenderness.
4. Drain, cool to room temperature.

5.3.6 Instructions for Cooking Rice

Stove Top:

1. Use the chart below to measure. Bring water to a boil. Add rice and salt (optional). Stir and cover.
2. Reduce heat to low, and simmer 20 minutes. Do not lift lid while cooking. Steam cooks rice.
3. Remove from heat. Let stand covered until all water is absorbed, about 5 minutes.
4. Cool to room temperature.

Approximate number of servings	1-2	3-4	5-6	7-8
Rice (cups)	1/2	1	1 1/2	2
Water (cups)	1 1/3	2/1/2	3/1/3	4 1/4
Salt (teaspoon) *OPTIONAL	1/4	1/2	3/4	1

Microwave Directions:

1. Using the chart above, combine hot water, rice and salt* in microwavable dish.
2. Cover and microwave on high (full power) for 5 minutes. Reduce to medium power (50%) and microwave for 20 minutes.
3. Remove from microwave. Let stand covered until all water is absorbed, about 5 minutes.
4. Cool to room temperature.

MICROWAVE OVENS VARY.
TIMES GIVEN ARE APPROXIMATE.

5.3.7 Fresh Fruit

Bananas are specified as medium, unpeeled, approximately 170-210 grams each. Use larger bananas in the bunch for the higher calorie level.

5.3.8 Batch Preparation

Wherever possible, non-fat food mixtures are produced in batches. The individual servings are then weighted out. Fat sources are then weighed out individually into each portion to complete recipe. Follow the recipe directions exactly as written.

5.3.9 Sandwich spread w/egg yolk (W1 lunch)

The following is a list of the quantities of the sandwich spread ingredients for each diet and calorie level. (The quantities of fat-free mayo and dijon mustard, may be adjusted to enhance the acceptability at each field center):

Diet, Kcal	Mayo (g)	Yolk (g)	Fat-free mayo	Dijon mustard (g)	Olive oil
1500 D*	7.5	5.0	1 tsp	1 tsp	0.0
1500 E*	7.0	7.5	2 tsp	1 tsp	0.0
1500 F*	0.0	7.5	3 tsp	1 tsp	0.0
2000 D	10.0	3.0	0.0	1 tsp	0.0
2000 E	7.0	6.0	1 tsp	1 tsp	0.0
2000 f	0.0	6.0	2 tsp	1 tsp	0.0
2500 D**	14.0	0.5	0.0	1 tsp	0.0
2500 E	10.0	4.0	0.0	1 tsp	0.0
2500 F	0.0	4.0	2 tsp	1 tsp	5.0
3000 D**	12.0	0.0	0.0	1 tsp	0.0
3000 E	10.0	2.0	0.0	1 tsp	0.0
3000 F	0.0	2.0	2 tsp	1 tsp	5.0
3500 D**	15.0	0.0	0.0	1 tsp	0.0
3500 E**	12.5	0.0	0.0	1 tsp	0.0
3500 F	0.0	0.0	2 tsp	1 tsp	5.0

*May need to give the spread in a separate container. It was very hard to put all the spread on the roll. It was also not possible to cut down on the amount of fat-free ingredients without affecting the acceptability.

**Fat-free mayo may be used.

5.3.10 Reconstitution of egg yolk from egg yolk powder

For 1.0 gram of egg yolk powder add 1.25 grams of water. Example: combine 100.0 grams of egg yolk powder with 125.0 grams of water to make 225.0 grams of reconstituted egg yolk.

5.3.11 Reconstitution of egg white from egg white powder

For 1.0 gram of egg white powder add 7.5 grams water. Example: combine 50.0 grams of egg white powder with 375.0 grams of water to make 425.0 grams of reconstituted egg white.

5.4 DIET, MENU, AND CALORIE ASSIGNMENT

5.4.1 Run-in

During the run-in period, each participant will be fed one day of each experimental diet over a three-day period. In order to maintain blinding, a different menu will be fed for each of the diets. Choice of the menus is optional at each center. The calorie level selected for the run-in should be on the generous side to avoid any negative reaction to the study because of hunger. It is not necessary to use DELTA procured foods for the run-in.

5.4.2 Randomization

Each participant who is randomized after run-in will be assigned a diet sequence and initial calorie level. All participants will begin the study on Friday with menu 2 at the dinner meal. This will allow the kitchen personnel time to make up production schedules. Each subsequent feeding period will begin at Friday dinner with menu 2.

5.4.3 Menu cycles

Weekday menus are numbered 1 through 6. Weekend menus are numbered W1 through W4. Menus will be rotated sequentially as shown in the schedule on pages 30-37 of this manual.

Feeding begins at dinner on September 30, with menu 2. On any given day each center is feeding the same menu. All participants will receive the same menu except during the post prandial studies in week 6 or in unusual circumstances.

5.4.4 Unit foods

Four unit foods have been formulated in 150 kcal units that contain the same carbohydrate and fiber composition and the same fatty acid profile of the diets. Unit foods are used to fine tune energy adjustment (see section 5.6) and to provide extra snacks if a subject gets hungry outside of scheduled meals. The unit foods are: banana muffin, pumpkin muffin, corn muffin and yeast roll.

5.4.5 Estimating Energy Requirements

The estimated calorie level is calculated from the height and weight obtained at EV1 according to the Harris Benedict formulae:

Harris-Benedict Equations (HBE) for Basal Energy Expenditure (BEE):

BEE (Females) = 655 + 9.46 Wt (kg) + 1.86 Ht (cm) - 4.68 Age (yr)
BEE (Males) = 66.47 + 13.75 Wt (kg) + 5 Ht (cm) - 6.76 Age (yr)

TC = BEE x 1.6	Moderate activity*
TC = BEE x 1.5	Light activity
TC = BEE x 1.3	Sedentary
TC = Total Calories	

*Note: Current experience suggests that most participants will require a factor of at least 1.6 X BEE.

5.4.6 Energy Adjustment

Adjustments in the calorie level may be made after the run-in period based on the participant's perception of whether the amount of food is too much or too little. Thereafter, adjustments will be made based on body weight monitoring. See weekly monitoring (PWM) form. Participants should remain on the initial calorie level for about one week before adjusting calories unless they are unusually hungry or full.

Menus are calculated at 5 standard calorie levels 1500, 2000, 2500, 3000 and 3500. If the participant's weight varies $\geq$ 1 Kg. (2.2 lbs) from the baseline weight, the calorie level is lowered or raised accordingly.

Weight gain in premenopausal women is evaluated in relation to the menstrual period. If weight gain occurs during the menstrual period, reduction in energy intake should be made only if the weight gain persists one week after the menstrual period.

5.4.7 Adjusting calories in feeding periods 2 and 3

Participants will be given guidelines for eating between feeding periods (see section 5.12) and cautioned to maintain their weight. Participants should be placed on the same calorie level they stabilized at during feeding period 1 and allowed to fluctuate during the first two weeks, before making adjustments.

Figure 5.4.7.1: DELTA Menu Schedule - Protocol 2

Feeding Period 1
Menu Schedule for DELTA - 2
SEPTEMBER, 1994

SUN	*MON*	*TUE*	*WED*	*THU*	*FRI*	*SAT*
				[1]	[2]	[3]
[4]	[5]	[6]	[7]	[8]	[9]	[10]
[11]	[12]	[13]	[14]	[15]	[16]	[17]
[18]	[19]	[20]	[21]	[22]	[23]	[24]
[25]	[26]	[27]	[28]	[29]	[30] Begin *(2)* *cycle 1*	

Menu Schedule for DELTA - 2
OCTOBER, 1994

SUN	*MON*	*TUE*	*WED*	*THU*	*FRI*	*SAT*
						[1] W1 *cycle 1*
[2] W2 *cycle 1*	[3] 3 *cycle 1*	[4] 4 *cycle 1*	[5] 5 *cycle 1*	[6] 6 *cycle 1*	[7] 1 *cycle 2*	[8] W3 *cycle 1*
[9] W4 *cycle 1*	[10] 2 *cycle 2*	[11] 3 *cycle 2*	[12] 4 *cycle 2*	[13] 5 *cycle 2*	[14] 6 *cycle 2*	[15] W1 *cycle 2*
[16] W2 *cycle 2*	[17] 1 *cycle 3*	[18] 2 *cycle 3*	[19] 3 *cycle 3*	[20] 4 *cycle 3*	[21] 5 *cycle 3*	[22] W3 *cycle 3*
[23] W4 *cycle 3*	[24] 6 *cycle 3*	[25] 1 *cycle 4*	[26] 2 *cycle 4*	[27] 3 *cycle 4*	[28] 4 *cycle 4*	[29] W1 *cycle 4*
[30] W2 *cycle 4*	[31] 5 *cycle 4*					

Menu Schedule for DELTA - 2
NOVEMBER, 1994

SUN	*MON*	*TUE*	*WED*	*THU*	*FRI*	*SAT*
		[1] 6 *cycle 4*	[2] 1 *cycle 5*	[3] 2 *cycle 5*	[4] 3 *cycle 5*	[5] W3 *cycle 5*
[6] W4 *cycle 5*	[7] 4 *cycle 5*	[8] 5 *cycle 5*	[9] 6 *cycle 5*	[10] 1 *cycle 6*	[11] 2 *cycle 6*	[12] W1 *cycle 6*
[13] W2 *cycle 6*	[14] 3 *cycle 6*	[15] 4 *cycle 6*	[16] 5 *cycle 6*	[17] 6 *cycle 6*	[18] 1	[19]
[20]	[21]	[22]	[23]	[24]	[25]	[26]
[27]	[28]	[29]	[30]			

Feeding Period 2
Menu Schedule for DELTA - 2
JANUARY, 1995

SUN	*MON*	*TUE*	*WED*	*THU*	*FRI*	*SAT*
[1]	[2]	[3]	[4]	[5]	[6] Begin Per. 2 2 *cycle 1*	[7] W1 *cycle 1*
[8] W2 *cycle 1*	[9] 3 *cycle 1*	[10] 4 *cycle 1*	[11] 5 *cycle 1*	[12] 6 *cycle 1*	[13] 1 *cycle 2*	[14] W3 *cycle 1*
[15] W4 *cycle 1*	[16] 2 *cycle 2*	[17] 3 *cycle 2*	[18] 4 *cycle 2*	[19] 5 *cycle 2*	[20] 6 *cycle 2*	[21] W1 *cycle 2*
[22] W2 *cycle 2*	[23] 1 *cycle 3*	[24] 2 *cycle 3*	[25] 3 *cycle 3*	[26] 4 *cycle 3*	[27] 5 *cycle 3*	[28] W3 *cycle 3*
[29] W4 *cycle 3*	[30] 6 *cycle 3*	[31] 1 *cycle 4*				

Menu Scheduling for DELTA - 2
FEBRUARY, 1995

SUN	MON	TUE	WED	THU	FRI	SAT
—			[1] 2 *cycle 4*	[2] 3 *cycle 4*	[3] 4 *cycle 4*	[4] W1 *cycle 4*
[5] W2 *cycle 4*	[6] 5 *cycle 4*	[7] 6 *cycle 4*	[8] 1 *cycle 5*	[9] 2 *cycle 5*	[10] 3 *cycle 5*	[11] W3 *cycle 5*
[12] W4 *cycle 5*	[13] 4 *cycle 5*	[14] 5 *cycle 5*	[15] 6 *cycle 5*	[16] 1 *cycle 6*	[17] 2 *cycle 6*	[18] W1 *cycle 6*
[19] W2 *cycle 6*	[20] 3 *cycle 6*	[21] 4 *cycle 6*	[22] 5 *cycle 6*	[23] 6 *cycle 6*	[24] 1	[25]
[26]	[27]	[28]				

Feeding Period 3
Menu Schedule for DELTA - 2
MARCH, 1995

SUN	*MON*	*TUE*	*WED*	*THU*	*FRI*	*SAT*
			[1]	[2]	[3]	[4]
[5]	[6]	[7]	[8]	[9]	[10]	[11]
[12]	[13]	[14]	[15]	[16]	[17]	[18]
[19]	[20]	[21]	[22]	[23]	[24]	[25]
[26]	[27]	[28]	[29]	[30]	[31] **Begin Per. 3** **2** *cycle 1*	

Menu Scheduling for DELTA - 2
APRIL, 1995

SUN	*MON*	*TUE*	*WED*	*THU*	*FRI*	*SAT*
						[1] W1 *cycle 1*
[2] W2 *cycle 1*	[3] 3 *cycle 1*	[4] 4 *cycle 1 .*	[5] 5 *cycle 1*	[6] 6 *cycle 1*	[7] 1 *cycle 2*	[8] W3 *cycle 1*
[9] W4 *cycle 1*	[10] 2 *cycle 2*	[11] 3 *cycle 2*	[12] 4 *cycle 2*	[13] 5 *cycle 2*	[14] 6 *cycle 2*	[15] W1 *cycle 2*
[16] W2 *cycle 2*	[17] 1 *cycle 3*	[18] 2 *cycle 3*	[19] 3 *cycle 3*	[20] 4 *cycle 3*	[21] 5 *cycle 3*	[22] W3 *cycle 3*
[23] W4 *cycle 3*	[24] 6 *cycle 3*	[25] 1 *cycle 4*	[26] 2 *cycle 4*	[27] 3 *cycle 4*	[28] 4 *cycle 4*	[29] W1 *cycle 4*
[30] W2 *cycle 4*						

Menu Schedule for DELTA - 2
MAY, 1995

SUN	*MON*	*TUE*	*WED*	*THU*	*FRI*	*SAT*
	[1] 5 *cycle 4*	[2] 6 *cycle 4*	[3] 1 *cycle 5*	[4] 2 *cycle 5*	[5] 3 *cycle 5*	[6] W3 *cycle 5*
[7] W4 *cycle 5*	[8] 4 *cycle 5*	[9] 5 *cycle 5*	[10] 6 *cycle 5*	[11] 1 *cycle 6*	[12] 2 *cycle 6*	[13] W1 *cycle 6*
[14] W2 *cycle 6*	[15] 3 *cycle 6*	[16] 4 *cycle 6*	[17] 5 *cycle 6*	[18] 6 *cycle 6*	[19] 1 END	[20]
[21]	[22]	[23]	[24]	[25]	[26]	[27]
[28]	[29]	[30]	[31]			

5.5 GUIDELINES FOR PARTICIPATION IN DELTA

Each Center should provide participants with written instructions for participation in DELTA. Below is an example of Minnesota guidelines.

5.5.1 Minnesota DELTA Participant Guidelines

Welcome to the DELTA study! We appreciate your participation in this very important and interesting research study.

As a participant of DELTA, **you have agreed to eat all food provided by DELTA and to avoid eating any food not provided by the study.** Breakfast and your evening dinner meal will be eaten at the feeding center Monday through Friday between the following times:

Breakfast	6:30-9:30 AM
Dinner	4:30-6:30 PM

It is very important that you eat breakfast and dinner at the center. We can ensure that the food is stored and served at the correct temperature and we can respond to any needs you may have. Additionally, it is important that we make certain that all food served is eaten!

We know everyone has an emergency once in a while, and we will do our best to meet your needs in the event of a crisis. However, please realize that a situation such as being late for work or a last minute fishing trip is not an emergency. Should a meal away from the center become unavoidable, please call us as soon as possible to allow time for a packed meal to be arranged.

The following guidelines will help you to participate within defined limits set by the study protocol.

1. When you arrive for your breakfast and dinner meal, please **identify yourself to the kitchen staff.** This will assure that the correct tray is assembled and will help to facilitate the serving of your meal as soon as possible.

2. **Please help yourself to** use of the microwave, toaster, coffee, diet beverages, ice water, sugar-free sweetener, additional napkins and any other **items provided within the dining area.**

3. Notice that your tray utensils include a small spatula to allow you to **finish eating everything.** To help in eating "every last bit" of the food, it may also help to save some soft bread for the end of the meal to make a final wipe over salad bowls, plates, etc. For this study, it is much more important to be an excellent member of the Clean Plate Club than to have impeccable table manners!

4. The following seasonings are allowed for your use as desired: salt, pepper, Mrs. Dash, seasoned salt, lemon pepper, tabasco, soy sauce, vinegar, and lemon juice. Sugar-free chewing gum is also allowed.

5. **Beverages that are unlimited in their use include:** water, calorie-free mineral water, diet caffeine-free soda pop, decaffeinated coffee and caffeine-free teas. (Any sweetener added to the coffee and teas must be sugar-free.)

6. **Caffeinated beverages are limited to 5 cups of coffee per day or the equivalent, as listed:**

Coffee:	up to 5 cups per day (1 cup = 6 oz.)
Instant coffee:	up to 9 tsp per day
Brewed Tea:	up to 10 cups per day (1 cup = 6 oz.)
Instant Tea:	up to 16 tsp per day
Diet Soda Pop:	up to 5 caffeinated cans per day (1 can = 12 oz.)

(Examples: Diet Coke, Diet Cherry Coke, TAB, Diet Dr. Pepper, Diet Rite Cola, Diet 7-Up Gold, Diet Pepsi, Diet Mountain Dew, Diet Mello Yello)

Non-caffeinated diet or unsweetened beverages may be consumed in any amount desired.

Alcoholic Drinks: limited to no more than 5/WEEK
(1 drink = 5 oz. wine, 12 oz. beer, or 1 1/2 oz. shot glass of liquor. Liquor must be mixed with only those beverages that are allowed.) **NO ALCOHOLIC DRINKS ARE PERMITTED 48 HOURS BEFORE BLOOD DRAWING.**

7. In case of emergency situation, please call the following numbers in order:

Nancy Van Heel, Project Coordinator	624-1497(Home: 559-1190)
Kay Fritz, Research Assistant	624-2192(Home: 641-3366)
Christine Wold, Research Dietitian	624-1999(Home: 866-8281)
Dining Room	625-0944
Kitchen	626-3605

ANY QUESTIONS?? Always feel free to ask DELTA staff.

5.6 FEEDING

All centers will serve two meals a day Monday through Friday at the feeding site. **One of the meals must be dinner.** Meals not eaten at the center, snacks and free beverages will be packaged in suitable leak-proof containers for consumption off site (see Food Safety; pages 59-62). Participants must eat and drink all study foods provided except for discretionary items (see next section). Participants should be given instruction in how to use either bread or a rubber spatula to be sure they consume all food residues. Rubber spatulas should be provided for home use.

5.6.1 Discretionary foods, beverages and seasonings:

Discretionary foods

1 - 2 TBSP fat free salad dressing/day

Up to 2 c. iceberg lettuce/day
1 tsp. mustard/day
Fat-free Salad Dressings are interchangeable

Discretionary beverages

A limited number of alcoholic and caffeinated beverages are allowed. Alcoholic beverages may be consumed up to 5 drinks per week. One drink is defined as:

1 shot (1.5 oz.) hard liquor
1 - 12 oz. can of beer
1 - 5 oz. glass of dry table wine
Cordials (liqueurs) and mixed drinks containing sugar are not permitted.
No alcoholic beverages are permitted 48 hours prior to blood drawing.

Caffeinated beverages not containing sugar may be consumed in amounts up to the following limits:

5 (6 oz.) cups coffee or tea
5 12 oz. glasses iced tea
5 12 oz. cans diet soda: Diet Coke, Diet Cherry Coke, Diet 7-UP Gold, TAB, Diet Rite Cola, Diet Dr. Pepper, Diet Cherry Cola, Diet Pepsi, Jamaica Cola, Canada Dry, Mountain Dew, Mellow Yellow

Non-caffeinated diet or unsweetened beverages may be consumed in any amount.

Sugar-free, calorie-free chewing gum is allowed.

Discretionary seasonings:

The following seasonings are allowed for discretionary use: salt, pepper, Mrs. Dash's, Seasoned salt, Lemon pepper, Tony's (Louisiana), Tabasco or other red pepper sauce, vinegar, lemon juice, and soy sauce limited to 1 portion pack per day.

The following seasonings are not allowed for discretionary use: Worcestershire or similar steak sauces containing fermented products and/or anchovy.

5.7 QUALITY CONTROL OF DIET

DELTA will implement a standard procedure for assessing compliance and for monitoring the composition of the experimental diets during the study. It is essential that DELTA is able to document that all participants were provided food with the composition stated in the protocol and that participant compliance was assessed. **Each center must be able, if requested, to produce evidence that on any given day a particular participant received the food, ate it, and did not eat non-study food, or alternatively, that departures from the protocol occurred and were documented.**

Key elements of quality assurance in the field centers include (1) training and supervision (2) organization and security of records (3) Food Production Forms (4) Tray Assembly Check Sheets (5) Food and Beverage Intake Report (6) Compliance Check Sheet (7) Diet Deviation Form.

5.7.1 Training

Each center is responsible for implementing a training program for food production personnel. This training will encompass all procedures outlined in Chapter 5 of the DELTA Manual of Procedures (MOP).

5.7.2 Organization and security of records

See Chapter 10.

5.7.3 Diet Documentation Forms

DELTA must be able to document that the correct food has (1) been prepared, (2) served to the participant and (3) consumed by the participant. Standard DELTA forms are used by all the centers. A sample of each form and instructions appear at the end of this chapter.

The Food Production Form (FPF) and the associated **recipes** are used in the kitchen for preparing the foods. There is a separate form for each menu and diet. The forms are color coded by diet (Green/diet D, Buff/diet E, Salmon/diet F). These forms are retained in the center.

The Tray Assembly Check List (TAC) is used to verify that each item has been served to the participant. There is a separate form for each diet and calorie level (15 forms) for each

day. Each diet is printed on a different color paper (Green/diet D, Buff/diet E, Salmon/diet F). These forms are retained in the center, but may be requested by the Coordinating Center.

The Compliance Check Sheet (CCS) is used to verify that all study food has been consumed and records discretionary unit foods, alcoholic beverages and whether a **DDD** was filled out. **This form is keyed.**

The Participant Food and Beverage Intake Report (FBI) is used by the participants to report their daily compliance, number of unit foods, and alcoholic beverages. The FBI is distributed at the dinner meal to record activity for the previous day. FBI forms are also included in the weekend packed meals and returned to the Center Monday morning. Information from the FBI is transferred to the CCS before the CCS is keyed.

The DELTA Diet Deviation (DDD) form is used by only diet staff to record deviations that occur during tray inspection at on-site meals or problems that arise off site where diet staff have advised substitutions. Information is transferred to the CCS before the CCS is keyed.

The Packed Meal Form (PMF) and Snack Form will be attached to each carry out meal and snack. These forms are used by the participant to verify that he/she has received all food items and drinks for that meal/snack.

Each Recipe form lists all ingredients that can be prepared in a batch mode, (non-fat containing ingredients), and then lists all item fat containing ingredients that are added to each serving for each calorie level. Ingredients in the recipes do not appear on any other form, but are listed on the **FPF** and **TAC** forms as 1 sv.

5.7.4 Labels and Masking Diet Assignment

Each food service facility has in place standard procedures for producing and delivering the diet and these will be maintained in the DELTA study. The critical factors the procedures should ensure are verification that the subject has received the correct diet and that masking of the subject is maintained. Masking of nutritionists is not feasible because they will have supervisory and counseling/compliance responsibilities. Nutrition personnel will be instructed 1) to maintain the same level of activity and interaction with each participant regardless of diet assignment and 2) to avoid revealing a participant's diet assignment. Diets are designated by a letter code and a color. Participants may know the diet code but staff should be careful not to reveal the composition corresponding to the diet code.

Described below are the procedures each center will use to verify the diet and maintain blinding of the participants.

5.7.5 Columbia

DELTA Food Production Forms are used to weigh the individual foods for each calorie and fat level. DELTA recipes are used to prepare combination foods. Each food is labeled with the participant's name, I.D., and calorie level.

Each menu item (e.g., peaches) for each diet and calorie level is weighed and labeled with the participant's name and I.D. and placed directly in the refrigerator.

Each participants' set of labeled foods is assembled as the participant arrives for his/her meal. This system maintains masking of the experimental diets.

For packaged meals, the bags are labeled with the name of the participant and stapled with the DELTA Packed Meal Form. The corner of the form indicating the diet assignment is cut off before the participant receives the packed meal. This form lists the food items in the bag to help the subject verify receipt of all items.

5.7.6 Pennington Biomedical Research Center (PBRC)

Labels for meal trays and carry-out boxes are printed with the participant's name, ID number and study.

All products received are labelled with the study name and dated. As recipes are prepared, the foods are placed into appropriate storage containers, labelled with the study name, food item, and treatment. Color dots are used to identify treatments.

As meal trays are assembled, the DELTA Tray Assembly Check Sheet (TAC) are followed. The TACs are color-coded to match color dots used for each treatment. Any identifying mark (i.e.: color dots) are removed when a food item is placed on individual trays or in carry-out boxes.

Packed meals (individual and weekend) are assembled similarly. All labels on each food are removed before the food is packed to mask the diet. A packed meal slip is enclosed with the packed meal(s). Participants will return the packed meal slips to the feeding center.

5.7.7 Minnesota DELTA Coding or "Masking" System

The coding or "masking" of the DELTA diets has been based on a color code system. Each diet has a specific color to be used for Food Production Forms, Tray Assembly Check Sheets, and food container labels (Diet D = Green, Diet E = Buff, Diet F = Salmon). Staff do not reveal to the participants the composition of the diet as it relates to the color code. The food items will be weighed using the Food Production Form and are marked as they are prepared with a label containing the color code and the caloric level. Meal trays will be assembled using the Tray Assembly Check Sheet. Prepared baked items will be individually labeled by color code and caloric level before storage. Weekend packed meals and snacks will be labeled with color code, caloric level, day, and meal. Each meal will be separately packed using the Tray Assembly Check Sheet.

5.7.8 Penn State

For each meal we will use the DELTA Food Production Form for each experimental diet for each calorie level.

Each menu item will be portioned out by weight for all experimental diets and calorie levels. We will wrap each dish with saran wrap, label it with the diet and calorie level, assemble them on a tray and put the tray in the refrigerator.

Thirty minutes before meal time, we will start to assemble the participants' trays. The individual trays for a specified diet and calorie level will be assembled at one time, followed by participants' trays for the next experimental diet and calorie level, etc., until all the trays have been assembled. We will assemble the trays using the DELTA Tray Assembly Check Sheet. The trays for each experimental diet and calorie level will be placed on a rack in the holding refrigerator that is labeled with the diet and calorie level. When a participant comes for his/her meal, we will remove the tray corresponding to his/her diet and calorie level from the refrigerator, re-heat the hot items and remove all the labels. A food service staff will take the tray to the participant (we will have at least 2 people working so that each one of them will be in charge of the re-heating of the food, the removal of the labels and the delivery of the tray to one particular participant). This system will maintain blinding of the experimental diets.

5.8 COMPOSITION OF EXPERIMENTAL DIETS

The composition of the experimental diets will be monitored through continuous sampling. Each center will sample two 8-menu cycles during each feeding period.

Rationale

The underlying principle for assaying the samples is to verify the composition of the diets actually fed to the participants. Samples sent to FALCC should be an accurate representation of what the participants receive. Food items for the sample should be assembled and plated in a manner identical to that for an actual participant. This means that they are assembled at the

same time as the participants' food, and taken from the same source. If food is assembled in a way that identifies it clearly as a monitoring sample, it may not reflect the composition of the participants' diet.

Sampling: Each center prepares 1 menu per day according to the following schedule.

Table 5.8.1: Resulting Composites to be Assayed

Composite ID Number	Feeding Period	8-Day Menu Cycle	Field Center	Diet	Calorie Level
1	1	1	L	*D*	1
2	1	1	M	*D*	4
3	1	2	P	*D*	2
4	1	3	C	*E*	2
5	1	4	L	*E*	5
6	1	4	M	*E*	3
7	1	5	P	*F*	3
8	1	6	C	*F*	1
9	2	1	C	*E*	4
10	2	2	L	*F*	4
11	2	2	M	*D*	1
12	2	3	P	*E*	1
13	2	4	C	*D*	5
14	2	5	L	*D*	3
15	2	5	M	*F*	2
16	2	6	P	*F*	5
17	3	1	P	*E*	3
18	3	2	C	*F*	3
19	3	3	L	*F*	1
20	3	3	M	*F*	4
21	3	4	P	*D*	4
22	3	5	C	*D*	2
23	3	6	L	*E*	2
24	3	6	M	*E*	5

In this example, Pennington would prepare Diet D 1500 Kcal 8 menus in cycle 1. In cycle 4 Pennington would prepare Diet E, 3500 Kcal 8 menus. Pennington would not sample again until feeding period 2.

Center specific sampling plans are shown on pages 47-78. The procedure is being developed for sampling the unit foods.

5.8.2 Procedure for Menu Sampling

1. Identify the correct diet to be sampled (D-1, D-2, etc.) from the sampling schedule for your center. See Sampling Plan Calendars pages 79 - 110.

2. Include this diet as an extra "participant" in your production sheets and production line. All portions for a given food item for this diet/calorie level should be weighed/measured out before being assembled.

3. The QC sample is assembled at the same time as the participant's meal or snack.

4. Assemble the QC sample by selecting at random each food item for the meal. Be sure you have the correct container. Verify that the correct diet has been sampled.

5. Fill out the Tray Assembly Check Sheet as for a participant, checking the boxes in the left column.

Sampling Plan Calendar
SEPTEMBER, 1994

MINNESOTA

SUN	*MON*	*TUE*	*WED*	*THU*	*FRI*	*SAT*
				[1]	[2]	[3]
[4]	[5]	[6]	[7]	[8]	[9]	[10]
[11]	[12]	[13]	[14]	[15]	[16]	[17]
[18]	[19]	[20]	[21]	[22]	[23]	[24]
[25]	[26]	[27]	[28]	[29]	[30] **Begin** ***(2)*** **Period 1**	

LEGEND

Period	Cycle	Sample Diet
1	1	D-4
1	4	E-3
2	2	D-1
2	5	F-2
3	3	F-4
3	6	E-5

Sampling Plan Calendar
OCTOBER, 1994

MINNESOTA

SUN	*MON*	*TUE*	*WED*	*THU*	*FRI*	*SAT*
						[1] W1 D-4
[2] W2 D-4	[3] 3 D-4	[4] 4 D-4	[5] 5 D-4	[6] 6 D-4	[7] 1	[8] W3 D-4
[9] W4 D-4	[10] 2	[11] 3	[12] 4	[13] 5	[14] 6	[15] W1
[16] W2	[17] 1	[18] 2	[19] 3	[20] 4	[21] 5	[22] W3
[23] W4	[24] 6	[25] 1 E-3	[26] 2 E-3	[27] 3 E-3	[28] 4 E-3	[29] W1 E-3
[30] W2 E-3	[31] 5 E-3					

Key: D-4, Diet D, 3000 kcal
E-3, Diet E, 2500 kcal
Sample Menus in shaded areas only!

Sampling Plan Calendar
NOVEMBER, 1994

MINNESOTA

SUN	*MON*	*TUE*	*WED*	*THU*	*FRI*	*SAT*
		[1] 6 E-3	[2] 1	[3] 2	[4] 3	[5] W3
[6] W4	[7] 4	[8] 5	[9] 6	[10] 1	[11] 2	[12] W1
[13] W2	[14] 3	[15] 4	[16] 5	[17] 6	[18] 1	[19]
[20]	[21]	[22]	[23]	[24]	[25]	[26]
[27]	[28]	[29]	[30]			

Key: E-3, Diet E, 2500 kcal

Sample Menus in shaded areas only!

Sampling Plan Calendar
JANUARY, 1995

MINNESOTA

SUN	*MON*	*TUE*	*WED*	*THU*	*FRI*	*SAT*
[1]	[2]	[3]	[4]	[5]	[6] Begin Per. 2 2	[7] W1
[8] W2	[9] 3	[10] 4	[11] 5	[12] 6	[13] 1 D-1	[14] W3
[15] W4	[16] 2 D-1	[17] 3 D-1	[18] 4 D-1	[19] 5 D-1	[20] 6 D-1	[21] W1 D-1
[22] W2 D-1	[23] 1	[24] 2	[25] 3	[26] 4	[27] 5	[28] W3
[29] W4	[30] 6	[31] 1				

Key: D-1, Diet D, 1500 kcal

Sample Menus in shaded areas only!

Sampling Plan Calendar
FEBRUARY, 1995

MINNESOTA

SUN	*MON*	*TUE*	*WED*	*THU*	*FRI*	*SAT*
			[1] 2	[2] 3	[3] 4	[4] W1
[5] W2	[6] 5	[7] 6	[8] 1 F-2	[9] 2 F-2	[10] 3 F-2	[11] W3 F-2
[12] W4 F-2	[13] 4 F-2	[14] 5 F-2	[15] 6 F-2	[16] 1	[17] 2	[18] W1
[19] W2	[20] 3	[21] 4	[22] 5	[23] 6	[24] 1	[25]
[26]	[27]	[28]				

Key: F-2, Diet F, 2000 kcal

Sample Menus in shaded areas only!

Sampling Plan Calendar
MARCH, 1995

MINNESOTA

SUN	*MON*	*TUE*	*WED*	*THU*	*FRI*	*SAT*
			[1]	[2]	[3]	[4]
[5]	[6]	[7]	[8]	[9]	[10]	[11]
[12]	[13]	[14]	[15]	[16]	[17]	[18]
[19]	[20]	[21]	[22]	[23]	[24]	[25]
[26]	[27]	[28]	[29]	[30]	[31] **Begin Per. 3** **2**	

Key:

Sampling Plan Calendar
APRIL, 1995

MINNESOTA

SUN	*MON*	*TUE*	*WED*	*THU*	*FRI*	*SAT*
						[1] W1
[2] W2	[3] 3	[4] 4	[5] 5	[6] 6	[7] 1	[8] W3
[9] W4	[10] 2	[11] 3	[12] 4	[13] 5	[14] 6	[15] W1
[16] W2	[17] 1 F-4	[18] 2 F-4	[19] 3 F-4	[20] 4 F-4	[21] 5 F-4	[22] W3 F-4
[23] W4 F-4	[24] 6 F-4	[25] 1	[26] 2	[27] 3	[28] 4	[29] W1
[30] W2						

Key: F-4, Diet F, 3000 kcal

Sample Menus in shaded areas only!

Sampling Plan Calendar
MAY, 1995

MINNESOTA

SUN	*MON*	*TUE*	*WED*	*THU*	*FRI*	*SAT*
	[1] 5	[2] 6	[3] 1	[4] 2	[5] 3	[6] W3
[7] W4	[8] 4	[9] 5	[10] 6	[11] 1 E-5	[12] 2 E-5	[13] W1 E-5
[14] W2 E-5	[15] 3 E-5	[16] 4 E-5	[17] 5 E-5	[18] 6 E-5	[19] 1 END	[20]
[21]	[22]	[23]	[24]	[25]	[26]	[27]
[28]	[29]	[30]	[31]			

Key: E-5, Diet E, 3500 kcal

Sample Menus in shaded areas only!

Sampling Plan Calendar
SEPTEMBER, 1994

PSU

SUN	*MON*	*TUE*	*WED*	*THU*	*FRI*	*SAT*
				[1]	[2]	[3]
[4]	[5]	[6]	[7]	[8]	[9]	[10]
[11]	[12]	[13]	[14]	[15]	[16]	[17]
[18]	[19]	[20]	[21]	[22]	[23]	[24]
[25]	[26]	[27]	[28]	[29]	[30] **Begin** ***(2)*** **Period 1**	

LEGEND

Period	Cycle	Sample Diet
1	2	D-2
1	5	F-3
2	3	E-1
2	6	F-5
3	1	E-3
3	4	D-4

Sampling Plan Calendar
OCTOBER, 1994

PSU

SUN	*MON*	*TUE*	*WED*	*THU*	*FRI*	*SAT*
						[1] W1
[2] W2	[3] 3	[4] 4	[5] 5	[6] 6	[7] 1 D-2	[8] W3
[9] W4	[10] 2 D-2	[11] 3 D-2	[12] 4 D-2	[13] 5 D-2	[14] 6 D-2	[15] W1 D-2
[16] W2 D-2	[17] 1	[18] 2	[19] 3	[20] 4	[21] 5	[22] W3
[23] W4	[24] 6	[25] 1	[26] 2	[27] 3	[28] 4	[29] W1
[30] W2	[31] 5					

Key: D-2, Diet D, 2000 kcal

Sample Menus in shaded areas only!

DELTA

Sampling Plan Calendar
NOVEMBER, 1994

PSU

SUN	*MON*	*TUE*	*WED*	*THU*	*FRI*	*SAT*
		[1] 6	[2] 1 F-3	[3] 2 F-3	[4] 3 F-3	[5] W3 F-3
[6] W4 F-3	[7] 4 F-3	[8] 5 F-3	[9] 6 F-3	[10] 1	[11] 2	[12] W1
[13] W2	[14] 3	[15] 4	[16] 5	[17] 6	[18] 1	[19]
[20]	[21]	[22]	[23]	[24]	[25]	[26]
[27]	[28]	[29]	[30]			

Key: F-3, Diet F, 2500 kcal

Sample Menus in shaded areas only!

Sampling Plan Calendar
JANUARY, 1995

PSU

SUN	*MON*	*TUE*	*WED*	*THU*	*FRI*	*SAT*
[1]	[2]	[3]	[4]	[5]	[6] Begin Per. 2 2	[7] W1
[8] W2	[9] 3	[10] 4	[11] 5	[12] 6	[13] 1	[14] W3
[15] W4	[16] 2	[17] 3	[18] 4	[19] 5	[20] 6	[21] W1
[22] W2	[23] 1 E-1	[24] 2 E-1	[25] 3 E-1	[26] 4 E-1	[27] 5 E-1	[28] W3 E-1
[29] W4 E-1	[30] 6 E-1	[31] 1				

Key: E-1, Diet E, 1500 kcal

Sample Menus in shaded areas only!

Sampling Plan Calendar
FEBRUARY, 1995

PSU

SUN	*MON*	*TUE*	*WED*	*THU*	*FRI*	*SAT*
			[1] 2	[2] 3	[3] 4	[4] W1
[5] W2	[6] 5	[7] 6	[8] 1	[9] 2	[10] 3	[11] W3
[12] W4	[13] 4	[14] 5	[15] 6	[16] 1 F-5	[17] 2 F-5	[18] W1 F-5
[19] W2 F-5	[20] 3 F-5	[21] 4 F-5	[22] 5 F-5	[23] 6 F-5	[24] 1	[25]
[26]	[27]	[28]				

Key: F-5, Diet F, 3500 kcal

Sample Menus in shaded areas only!

Sampling Plan Calendar
MARCH, 1995

PSU

SUN	*MON*	*TUE*	*WED*	*THU*	*FRI*	*SAT*
			[1]	[2]	[3]	[4]
[5]	[6]	[7]	[8]	[9]	[10]	[11]
[12]	[13]	[14]	[15]	[16]	[17]	[18]
[19]	[20]	[21]	[22]	[23]	[24]	[25]
[26]	[27]	[28]	[29]	[30]	[31] Begin Per. 3 2	

Key:

Sampling Plan Calendar
APRIL, 1995

PSU

SUN	*MON*	*TUE*	*WED*	*THU*	*FRI*	*SAT*
						[1] W1 E-3
[2] W2 E-3	[3] 3 E-3	[4] 4 E-3	[5] 5 E-3	[6] 6 E-3	[7] 1	[8] W3 E-3
[9] W4 E-3	[10] 2	[11] 3	[12] 4	[13] 5	[14] 6	[15] W1
[16] W2	[17] 1	[18] 2	[19] 3	[20] 4	[21] 5	[22] W3
[23] W4	[24] 6	[25] 1 D-4	[26] 2 D-4	[27] 3 D-4	[28] 4 D-4	[29] W1 D-4
[30] W2 D-4						

Key: E-3, Diet E, 2500 kcal
D-4, Diet D, 3000 kcal
Sample Menus in shaded areas only!

Sampling Plan Calendar
MAY, 1995

PSU

SUN	*MON*	*TUE*	*WED*	*THU*	*FRI*	*SAT*
	[1] 5 D-4	[2] 6 D-4	[3] 1	[4] 2	[5] 3	[6] W3
[7] W4	[8] 4	[9] 5	[10] 6	[11] 1	[12] 2	[13] W1
[14] W2	[15] 3	[16] 4	[17] 5	[18] 6	[19] 1 END	[20]
[21]	[22]	[23]	[24]	[25]	[26]	[27]
[28]	[29]	[30]	[31]			

Key: D-4, Diet D, 3000 kcal

Sample Menus in shaded areas only!

Sampling Plan Calendar
SEPTEMBER, 1994

PBRC

SUN	*MON*	*TUE*	*WED*	*THU*	*FRI*	*SAT*
				[1]	[2]	[3]
[4]	[5]	[6]	[7]	[8]	[9]	[10]
[11]	[12]	[13]	[14]	[15]	[16]	[17]
[18]	[19]	[20]	[21]	[22]	[23]	[24]
[25]	[26]	[27]	[28]	[29]	[30] Begin *(2)* Period 1	

LEGEND

Period	Cycle	Sample Diet
1	1	D-1
1	4	E-5
2	2	F-4
2	5	D-3
3	3	F-1
3	6	E-2

Sampling Plan Calendar
OCTOBER, 1994

PBRC

SUN	*MON*	*TUE*	*WED*	*THU*	*FRI*	*SAT*
						[1] W1 D-1
[2] W2 D-1	[3] 3 D-1	[4] 4 D-1	[5] 5 D-1	[6] 6 D-1	[7] 1	[8] W3 D-1
[9] W4 D-1	[10] 2	[11] 3	[12] 4	[13] 5	[14] 6	[15] W1
[16] W2	[17] 1	[18] 2	[19] 3	[20] 4	[21] 5	[22] W3
[23] W4	[24] 6	[25] 1 E-5	[26] 2 E-5	[27] 3 E-5	[28] 4 E-5	[29] W1 E-5
[30] W2 E-5	[31] 5 E-5					

Key: D-1, Diet D, 1500 kcal
E-5, Diet E, 3500 kcal
Sample Menus in shaded areas only!

Sampling Plan Calendar
NOVEMBER, 1994

PBRC

SUN	*MON*	*TUE*	*WED*	*THU*	*FRI*	*SAT*
		[1] 6 E-5	[2] 1	[3] 2	[4] 3	[5] W3
[6] W4	[7] 4	[8] 5	[9] 6	[10] 1	[11] 2	[12] W1
[13] W2	[14] 3	[15] 4	[16] 5	[17] 6	[18] 1	[19]
[20]	[21]	[22]	[23]	[24]	[25]	[26]
[27]	[28]	[29]	[30]			

Key: E-5, Diet E, 3500 kcal

Sample Menus in shaded areas only!

Sampling Plan Calendar
JANUARY, 1995

PBRC

SUN	*MON*	*TUE*	*WED*	*THU*	*FRI*	*SAT*
[1]	[2]	[3]	[4]	[5]	[6] Begin Per. 2 2	[7] W1
[8] W2	[9] 3	[10] 4	[11] 5	[12] 6	[13] 1 F-4	[14] W3
[15] W4	[16] 2 F-4	[17] 3 F-4	[18] 4 F-4	[19] 5 F-4	[20] 6 F-4	[21] W1 F-4
[22] W2 F-4	[23] 1	[24] 2	[25] 3	[26] 4	[27] 5	[28] W3
[29] W4	[30] 6	[31] 1				

Key: F-4, Diet F, 3000 kcal

Sample Menus in shaded areas only!

Sampling Plan Calendar
FEBRUARY, 1995

PBRC

SUN	*MON*	*TUE*	*WED*	*THU*	*FRI*	*SAT*
			[1] 2	[2] 3	[3] 4	[4] W1
[5] W2	[6] 5	[7] 6	[8] 1 D-3	[9] 2 D-3	[10] 3 D-3	[11] W3 D-3
[12] W4 D-3	[13] 4 D-3	[14] 5 D-3	[15] 6 D-3	[16] 1	[17] 2	[18] W1
[19] W2	[20] 3	[21] 4	[22] 5	[23] 6	[24] 1	[25]
[26]	[27]	[28]				

Key: D-3, Diet D, 2500 kcal

Sample Menus in shaded areas only!

Sampling Plan Calendar
MARCH, 1995

PBRC

SUN	*MON*	*TUE*	*WED*	*THU*	*FRI*	*SAT*
			[1]	[2]	[3]	[4]
[5]	[6]	[7]	[8]	[9]	[10]	[11]
[12]	[13]	[14]	[15]	[16]	[17]	[18]
[19]	[20]	[21]	[22]	[23]	[24]	[25]
[26]	[27]	[28]	[29]	[30]	[31] Begin Per. 3 2	

Key:

Sampling Plan Calendar
APRIL, 1995

PBRC

SUN	*MON*	*TUE*	*WED*	*THU*	*FRI*	*SAT*
						[1] W1
[2] W2	[3] 3	[4] 4	[5] 5	[6] 6	[7] 1	[8] W3
[9] W4	[10] 2	[11] 3	[12] 4	[13] 5	[14] 6	[15] W1
[16] W2	[17] 1 F-1	[18] 2 F-1	[19] 3 F-1	[20] 4 F-1	[21] 5 F-1	[22] W3 F-1
[23] W4 F-1	[24] 6 F-1	[25] 1	[26] 2	[27] 3	[28] 4	[29] W1
[30] W2						

Key: F-1, Diet F, 1500 kcal

Sample Menus in shaded areas only!

Sampling Plan Calendar
MAY, 1995

PBRC

SUN	*MON*	*TUE*	*WED*	*THU*	*FRI*	*SAT*
	[1] 5	[2] 6	[3] 1	[4] 2	[5] 3	[6] W3
[7] W4	[8] 4	[9] 5	[10] 6	[11] 1 E-2	[12] 2 E-2	[13] W1 E-2
[14] W2 E-2	[15] 3 E-2	[16] 4 E-2	[17] 5 E-2	[18] 6 E-2	[19] 1 END	[20]
[21]	[22]	[23]	[24]	[25]	[26]	[27]
[28]	[29]	[30]	[31]			

Key: E-2 Diet E, 2000 kcal

Sample Menus in shaded areas only!

Sampling Plan Calendar
SEPTEMBER, 1994

COLUMBIA

SUN	*MON*	*TUE*	*WED*	*THU*	*FRI*	*SAT*
				[1]	[2]	[3]
[4]	[5]	[6]	[7]	[8]	[9]	[10]
[11]	[12]	[13]	[14]	[15]	[16]	[17]
[18]	[19]	[20]	[21]	[22]	[23]	[24]
[25]	[26]	[27]	[28]	[29]	[30] **Begin** ***(2)*** **Period 1**	

LEGEND

Period	Cycle	Sample Diet
1	3	E-2
1	6	F-1
2	1	E-4
2	4	D-5
3	2	F-3
3	5	D-2

Sampling Plan Calendar
OCTOBER, 1994

COLUMBIA

SUN	*MON*	*TUE*	*WED*	*THU*	*FRI*	*SAT*
						[1] W1
[2] W2	[3] 3	[4] 4	[5] 5	[6] 6	[7] 1	[8] W3
[9] W4	[10] 2	[11] 3	[12] 4	[13] 5	[14] 6	[15] W1
[16] W2	[17] 1 E-2	[18] 2 E-2	[19] 3 E-2	[20] 4 E-2	[21] 5 E-2	[22] W3 E-2
[23] W4 E-2	[24] 6 E-2	[25] 1	[26] 2	[27] 3	[28] 4	[29] W1
[30] W2	[31] 5					

Key: E-2, Diet E, 2000 kcal

Sample Menus in shaded areas only!

Sampling Plan Calendar
NOVEMBER, 1994

COLUMBIA

SUN	*MON*	*TUE*	*WED*	*THU*	*FRI*	*SAT*
		[1] 6	[2] 1	[3] 2	[4] 3	[5] W3
[6] W4	[7] 4	[8] 5	[9] 6	[10] 1 F-1	[11] 2 F-1	[12] W1 F-1
[13] W2 F-1	[14] 3 F-1	[15] 4 F-1	[16] 5 F-1	[17] 6 F-1	[18] 1	[19]
[20]	[21]	[22]	[23]	[24]	[25]	[26]
[27]	[28]	[29]	[30]			

Key: F-1, Diet F, 1500 kcal

Sample Menus in shaded areas only!

Sampling Plan Calendar
JANUARY, 1995

COLUMBIA

SUN	*MON*	*TUE*	*WED*	*THU*	*FRI*	*SAT*
[1]	[2]	[3]	[4]	[5]	[6] Begin Per. 2 2	[7] W1 E-4
[8] W2 E-4	[9] 3 E-4	[10] 4 E-4	[11] 5 E-4	[12] 6 E-4	[13] 1	[14] W3 E-4
[15] W4 E-4	[16] 2	[17] 3	[18] 4	[19] 5	[20] 6	[21] W1
[22] W2	[23] 1	[24] 2	[25] 3	[26] 4	[27] 5	[28] W3
[29] W4	[30] 6	[31] 1 D-5				

Key: E-4, Diet E, 3000 kcal
D-5, Diet D, 3500 kcal
Sample Menus in shaded areas only!

Sampling Plan Calendar
FEBRUARY, 1995

COLUMBIA

SUN	*MON*	*TUE*	*WED*	*THU*	*FRI*	*SAT*
			[1] 2 D-5	[2] 3 D-5	[3] 4 D-5	[4] W1 D-5
[5] W2 D-5	[6] 5 D-5	[7] 6 D-5	[8] 1	[9] 2	[10] 3	[11] W3
[12] W4	[13] 4	[14] 5	[15] 6	[16] 1	[17] 2	[18] W1
[19] W2	[20] 3	[21] 4	[22] 5	[23] 6	[24] 1	[25]
[26]	[27]	[28]				

Key: D-5, Diet D, 3500 kcal

Sample Menus in shaded areas only!

Sampling Plan Calendar
MARCH, 1995

COLUMBIA

SUN	*MON*	*TUE*	*WED*	*THU*	*FRI*	*SAT*
			[1]	[2]	[3]	[4]
[5]	[6]	[7]	[8]	[9]	[10]	[11]
[12]	[13]	[14]	[15]	[16]	[17]	[18]
[19]	[20]	[21]	[22]	[23]	[24]	[25]
[26]	[27]	[28]	[29]	[30]	[31] Begin Per. 3 2	

Key:

Sampling Plan Calendar
APRIL, 1995

COLUMBIA

SUN	*MON*	*TUE*	*WED*	*THU*	*FRI*	*SAT*
						[1] W1
[2] W2	[3] 3	[4] 4	[5] 5	[6] 6	[7] 1 F-3	[8] W3
[9] W4	[10] 2 F-3	[11] 3 F-3	[12] 4 F-3	[13] 5 F-3	[14] 6 F-3	[15] W1 F-3
[16] W2	[17] 1	[18] 2	[19] 3	[20] 4	[21] 5	[22] W3
[23] W4	[24] 6	[25] 1	[26] 2	[27] 3	[28] 4	[29] W1
[30] W2						

Key: F-3, Diet F, 2500 kcal

Sample Menus in shaded areas only!

Sampling Plan Calendar
MAY, 1995

COLUMBIA

SUN	*MON*	*TUE*	*WED*	*THU*	*FRI*	*SAT*
	[1] 5	[2] 6	[3] 1 D-2	[4] 2 D-2	[5] 3 D-2	[6] W3 D-2
[7] W4 D-2	[8] 4 D-2	[9] 5 D-2	[10] 6 D-2	[11] 1	[12] 2	[13] W1
[14] W2	[15] 3	[16] 4	[17] 5	[18] 6	[19] 1 END	[20]
[21]	[22]	[23]	[24]	[25]	[26]	[27]
[28]	[29]	[30]	[31]			

Key: D-2 **Diet D,** **2000 kcal**

Sample Menus in shaded areas only!

5.8.4 Procedure for Collection and Shipping of Menus

PROCEDURE FOR COLLECTION AND SHIPPING OF MENUS

DELTA Protocol 2

Food Analysis Laboratory Control Center (FALCC)
Standard Operating Procedure

SOP #1027-0
Revision: New

15-SEP-94

Dept. of Biochemistry and Anaerobic Microbiology
Virginia Tech
Blacksburg, VA 24061-0308

PLEASE READ THIS PROCEDURE COMPLETELY, PRIOR TO FOOD COLLECTION

Scope

This procedure applies to menu collection for DELTA Protocol 2 (1994-95).

Purpose

To describe the procedure for collecting prepared menus and shipping the foods to FALCC in order to monitor the composition of experimental diets during feeding trials.

Overview

Menus will be collected according to the **sampling plan in the Manual of Operations and distributed by the Coordinating Center** and sent to the FALCC to be assayed. Foods to be sent to the FALCC for assay are to be prepared and handled in the same manner as foods actually fed to subjects, so that the assayed menus will most accurately reflect the composition of the diets consumed.

Materials

At Field Center:

- prepared foods from menus
- refrigerator (0-6°C)
- freezer (-20°C or lower)
- heavy paper (e.g. brown paper or newspaper)
- TAC for each menu to be collected (see sampling plan)[1]
- **dry ice (ca. 5 lbs per cooler)**

Food Collection and Shipping Materials (supplied by FALCC):

- Rubbermaid containers - **prelabeled**
- stainless steel spatula(s)
- cryogenic marker
- fat-free powder-free gloves (disposable)
- Form #F001 (sample transfer), with example form filled out
- Forms #F002 (deviation from SOP)
- shipping cooler
- packing tape[2]
- Federal Express dry ice identification stickers
- pre-addressed Federal Express shipping labels (1 per cooler)

PROCEDURES

NOTE: **Follow these procedures exactly. If a deviation occurs in preparation, packaging, ingredients, shipping, etc., fill out form #F002 and include it with the food shipment. TAKE THE SAME CARE SERVING AND PACKING MENUS FOR FOOD**

---

[1] May be provided by the FALCC

NOTE: The FALCC will supply each Field Center with a reasonable amount of packing tape for shipping foods to the FALCC - please reserve the tape for this use only.

ANALYSIS AS YOU DO FOR DELTA PARTICIPANTS.

Receipt of Shipping Materials:

Make sure you received all items listed above. If there is a discrepancy or if you should need replenishment of supplies, immediately notify the FALCC at (703)231-4361, FAX (703)231-9070, or E-mail: FALCC@vtvm1.cc.vt.edu

A. Collection of each Total Menu

NOTE: Each container has been prelabeled at the FALCC with the Diet, Menu#, Cycle #, and Center name.

- READ THROUGH THIS ENTIRE PROCEDURE PRIOR TO FOOD COLLECTION
- PERFORM THE FOLLOWING STEPS FOR the daily menu for the specified diet you are sampling, each day
- Treat foods shipped to FALCC just like another "participant"

NOTE: At the start of Menu Collection, obtain a TAC for the menu to be collected.

Breakfast

1. Assemble all foods from the breakfast menu. **Include** milk and juices, but **NOT** *ad lib* beverages (e.g. coffee, tea, water, diet soft drinks).

2. Retrieve the Rubbermaid container prelabeled with the diet, cycle, and menu identification for the menu you are collecting; **enter the date and your initials** on the label using the **cryogenic marker** (supplied).

3. While wearing fat-free powder-free gloves and using a clean stainless steel spatula (included in shipping kit), scrape **all** of the food into the container. If bread or a muffin is a part of the meal being collected, set it aside and use it to scrape plate, then add to collection container.

   Check off each item on the TAC as it is added to the container.

   NOTE: **It is as CRITICAL that all food residues are collected as it is that each subject eats all of the food**; if not, analytical values will not reflect the composition of the diet.

4. Completely seal the container, and place the container in the refrigerator (0-2°C) until collection of total menu is complete.

NOTE: Collection of total menu must be completed within the same timeframe as it would be consumed by participants.

- READ THROUGH THIS ENTIRE PROCEDURE PRIOR TO FOOD COLLECTION

- PERFORM THE FOLLOWING STEPS FOR EACH MENU OF FOODS TO BE COLLECTED:

Lunch

1. Assemble all foods from the lunch menu. **Include** milk and juices, but **not** *ad lib* beverages (e.g coffee, tea, water, diet soft drinks).

2. Retrieve the container containing breakfast foods from the same menu from the refrigerator. **CHECK THE LABEL AND MAKE SURE YOU HAVE THE CORRECT CONTAINER FOR THE MENU YOU ARE COLLECTING.**

3. While wearing fat-free powder-free gloves, scrape **all** of the lunch food into the container (use a clean stainless steel spatula to obtain **all** food residues; if bread or a muffin is a part of the meal being collected, set it aside and use it to scrape plate, then add to collection container).

   Check off each item on the TAC as it is added to the container.

   NOTE: **It is CRITICAL that all food residues are collected**, or else analytical values will not reflect the composition of the menu.

4. Completely seal the container, and place it in the refrigerator (0-2°C) until collection of total menu is complete.

- READ THROUGH THIS ENTIRE PROCEDURE PRIOR TO FOOD COLLECTION

- PERFORM THE FOLLOWING STEPS FOR EACH MENU OF FOODS TO BE COLLECTED:

Dinner

1. Assemble all foods from the dinner menu. **Include** milk and juices, but **not** *ad lib* beverages (e.g coffee, tea, water, diet soft drinks).

2. Retrieve the container containing breakfast and lunch foods from the same menu from the refrigerator. **CHECK THE LABEL AND MAKE SURE YOU HAVE THE CORRECT CONTAINER FOR THE MENU YOU ARE COLLECTING.**

3. While wearing fat-free powder-free gloves, scrape **all** of the dinner food into the container (use a clean stainless steel spatula to obtain **all** food residues; if bread or a muffin is a part

of the meal being collected, set it aside and use it to scrape plate, then add to collection container).

Check off each item on the TAC as it is added to the container.

NOTE: <u>It is CRITICAL that all food residues are collected</u>, or else analytical values will not reflect the composition of the menu.

4. Completely seal the container, and place it in the refrigerator (0-2°C) until collection of total menu is complete ($\leq$ 24 hrs).

- READ THROUGH THIS ENTIRE PROCEDURE PRIOR TO FOOD COLLECTION

- PERFORM THE FOLLOWING STEPS FOR <u>EACH</u> MENU OF FOODS TO BE COLLECTED:

Snacks

1. Assemble all snacks from the menu. **<u>Include</u>** milk and juices, but **<u>not</u>** *ad lib* beverages (e.g coffee, tea, water, diet soft drinks).

2. Retrieve the container for the corresponding breakfast, lunch, and dinner menu items. **CHECK THE LABEL AND MAKE SURE YOU HAVE THE CORRECT CONTAINER FOR THE MENU YOU ARE COLLECTING.**

3. While wearing fat-free powder-free gloves, scrape **all** of the snack food into the container (use a clean stainless steel spatula to obtain **<u>all</u>** food residues; if bread or a muffin is a part of the meal being collected, set it aside and use it to scrape plate, then add to collection container).

Check off each item on the TAC as it is added to the container.

NOTE: <u>It is CRITICAL that all food residues are collected</u>, or else analytical values will not reflect the composition of the menu.

4. Insure that the lid is completely sealed to the container (it is not necessary to tape around the edges of container) and place it in the **FREEZER (-20°C or less)**. All foods from the menu should now be in the container.

5. The food must be **frozen at -20°C or less at least overnight prior to shipment**.

SHIPPING * * * * * * **DO NOT SHIP ON FRIDAY!** * * * * * *

Contact FALCC before preparing coolers for shipping. During times of potential severe storms which may interfere with usual Fed Ex schedules, it may be prudent to delay shipment to avoid potential loss of samples.

DO NOT LET PACKED COOLERS SIT AT AMBIENT TEMPERATURE FOR AN EXTENDED TIME PERIOD PRIOR TO FED EX PICK-UP

1. Assemble containers of food to be shipped: **FROZEN solid (at least OVERNIGHT at -20°C) prior to shipment**.

2. Ensure that each container is completely sealed.

3. Fill out a sample transfer form (#F001) for each cooler. Include all required information (see sample form included). Make a copy for your records.

4. **Wrap EACH container of food in several layers of brown paper, newspaper, or other cushioning wrap. This is necessary in order to prevent container breakage during transit.**

5. Place wrapped containers in the cooler, then pack wads of brown paper, newspaper or other cushioning material around each container.

6. Place a layer of brown paper, newspaper, or other cushioning material on top of containers, then add a minimum of 5 pounds of dry ice. **USE CAUTION WHEN HANDLING DRY ICE; WEAR APPROPRIATE PROTECTIVE APPAREL AND INSULATED GLOVES.**

7. Pack wads of newspaper or brown paper to fill out cooler and prevent movement.

8. Place completed **sample transfer form (#F001)**, Deviation from SOP (Form(s) #F002, if any), and completed TACs for each menu shipped in a sealed zip-lock bag (to protect from moisture), and place in cooler, on top.

9. Tightly seal around the seam of the cooler and lid with packing tape.

10. Fill out all information on the dry ice stickers (included in shipping kit) required for Federal Express shipping: Make sure to include your complete address and make sure that the dry ice weight agrees on all stickers for the same cooler.

11. Affix a pre-addressed pre-paid FedEx shipping label to the box, and send via Federal Express overnight delivery to FALCC:
    Dr. K. Stewart, Dept. of Biochemistry, 304 Engel Hall, Virginia Tech, Blacksburg, VA 24061-0308

12. **Notify FALCC of shipment:** Phone: (703) 231-4361 **or** FAX:(703) 231-9070 **or** E-mail: FALCC@vtvm1.cc.vt.edu

5.8.5 Unit Food Sampling and Assay Plan

Documentation of unit food composition

Unit food composition will be documented by diet-center-feeding period. Fat blend composition will be documented by the diet-center twice during the study: once at the end of feeding period 2 and once during feeding period 3.

The FALCC will check that a sample was received from each unit food batch entered on the log sheets. Any deviations will be documented. The unit foods will be composited (FALCC SOP #5038) by diet-center-feeding period (no separation by unit food type will be made). Thus 12 unit food composites per feeding period will result (3 diets x 4 centers). The total number of constituent units and total weight of each composite will be documented.

Each unit food composite will be assayed for total fat, moisture, protein (Kjeldahl Nx6.25), and ash. Moisture will be determined by vacuum drying at 60°C (FALCC SOP #5002). Total fat, protein and ash will be assayed by the same methods used for diet composites (MOP Section 7). Mean total kcal (calculated from proximates) per unit and total fat %kcal will be available as calculated values.

Each fat blend sample will be assayed in triplicate for fatty acid composition, using the gas chromatographic method employed for assay of fatty acids in diets (FALCC SOP #5025). Each fatty acid (C12:0, C14:0, C16:0, C18:0, C18:1, C18:2, C18:3n-3, C20:5n-3, C20:6n-3) will be reported in weight percent, corresponding to the units in which fat blends were formulated.

PROCEDURE FOR COLLECTION AND SHIPPING OF UNIT FOODS- DELTA Protocol 2 DIET MONITORING

Food Analysis Laboratory Control Center (FALCC)
Standard Operating Procedure

SOP #1030
Revision: (New)

01-MAR-95

Dept. of Biochemistry and Anaerobic Microbiology
Virginia Tech
Blacksburg, VA 24061-0308

PLEASE READ THIS PROCEDURE COMPLETELY, PRIOR TO FOOD COLLECTION

Scope

This procedure applies to collection of unit foods samples for DELTA Protocol 2 diet monitoring, as part of documentation of diet composition (DELTA Protocol 2 (1994-95).

Purpose

To describe the procedure for collecting samples from unit food batches prepared for DELTA Protocol 2 and shipping the samples to FALCC for compositing and assay.

Overview

Each center will maintain a log of all unit food batch preparations for each feeding period. This log will include, for each batch, the date of preparation, unit food name, diet, and size of batch. Each center will randomly select one unit from every batch of unit food prepared for each of the three diets D, E, and F. Each of these units will be individually packaged in an airtight container (e.g. zip-lock bag), labelled, and frozen at -20°C. At the end of each feeding period, unit food samples will be shipped frozen, on dry ice, to the FALCC where they will be composited and assayed.

Materials

At Field Center:

- prepared unit foods
- freezer (-20°C or lower)
- heavy paper (e.g. brown paper or newspaper)
- dry ice
- DELTA Unit Food Batch Preparation Log

Food Collection and Shipping Materials (supplied by FALCC):

- zip-lock bags
- labels
- cryogenic marker
- fat-free powder-free gloves (disposable)
- Form #F001 (sample transfer), with example form filled out
- Forms #F002 (deviation from SOP)
- shipping cooler
- packing tape[3]
- Federal Express dry ice identification stickers
- pre-addressed Federal Express shipping labels (1 per cooler)

NOTE: **Follow these procedures exactly. If any deviation occurs in preparation, packaging, ingredients, sampling, shipping, etc., fill out form #F002 and include it with the food shipment.**

[3]NOTE: The FALCC will supply each Field Center with a reasonable amount of packing tape for shipping foods to the FALCC - please reserve the tape for this use only.

TAKE THE SAME CARE SERVING AND PACKING FOODS FOR ASSAY AS YOU DO FOR FOODS FOR PATIENTS.

Receipt of Shipping Materials:

Make sure you received all items listed above. If there is a discrepancy or if you should need additional labels or supplies, immediately notify the FALCC at (703)231-4361, or FAX (703)231-9070 or E-mail: FALCC@VTVM1.CC.VT.EDU.

Documentation of Unit Food Batch Preparation

The purpose of batch documentation is to facilitate ensuring that all batches prepared are sampled and inlcuded in the assay composite. The "Unit Food Batch Preparation Log" serves this purpose.

1. At the time a batch of unit food is prepared, do the following:

   Record the date of preparation, batch ID number (if any), the unit food name, diet, and batch size (e.g. total number of units or total weight), and your initials.

2. Maintain the preparation log at your center, and **send a copy to the FALCC with the shipment of unit foods.**

Sample Collection

For each batch of each type of unit food prepared for each diet throughout the feeding period, do the following:

1. **Randomly select** one (1) unit and

2. Place unit into a zip-lock bag **(There should be only one unit in each zip-lock bag)**

3. Enter in the appropriate spaces on a cryogenic label (provided): the name of your Center, the unit food name, Diet (D, E, or F), batch preparation date, sampling date (=current date), and your initials.

4. Affix label to the sample bag, **INSIDE.**

NOTE: It is very important to place the label inside the bag, as the label may detach at -20°C.

5. Seal each bag completely and check seal integrity.

6. Freeze and hold all samples at -20°C or lower prior to shipment.

Please return any unused labels and ziplock bags to the FALCC at the end of the feeding period al with sample shipment (thank you).

SHIPPING * * * * * * **DO NOT SHIP ON FRIDAY!** * * * * * *

DO NOT LET PACKED COOLERS SIT AT AMBIENT TEMPERATURE FOR AN EXTENDED TIME PERIOD PRIOR TO FED EX PICK-UP

1. Assemble all samples to be shipped: **FROZEN solid (at least OVERNIGHT at -20°C) prior to shipment**.

2. Ensure that each bag is completely sealed and properly labeled.

3. Fill out a sample transfer form (#F001). Include all required information (see sample form included); **there should be one entry for each sample enclosed in the cooler**. Make a copy of the form for your records.

4. Place all bags in the cooler, then pack wads of brown paper, newspaper or other cushioning material around them.

5. Place a layer of brown paper, newspaper, or other cushioning material on top of containers, then add a minimum of 5 pounds of dry ice.

   USE CAUTION WHEN HANDLING DRY ICE; WEAR APPROPRIATE PROTECTIVE APPAREL AND INSULATED GLOVES.

6. Pack wads of newspaper or brown paper to fill out cooler and prevent movement.

7. Place completed sample transfer form (**#F001**), Deviation from SOP (Form(s) **#F002**, (if any), and a **copy** of the Unit Food Batch Preparation Log (Form **#F031**) in a sealed zip-lock bag (to protect from moisture), and place in cooler, on top.

8. Tightly seal the lid of the cooler with packing tape around seam.

9. Fill out all information on the dry ice stickers (included in shipping kit) required for Federal Express shipping: Make sure to include your complete address and make sure that the dry ice weight agrees on all stickers for the same cooler.

10. Affix a pre-addressed pre-paid FedEx shipping label to the box, and send via Federal Express overnight delivery to FALCC:

    Dr. K. Stewart
    Dept. of Biochemistry
    304 Engel Hall
    Virginia Tech
    Blacksburg, VA 24061-0308

11. **Notify FALCC of shipment:** Phone: (703) 231-4361 **or** FAX: (703) 231-9070 or E-mail: FALCC@VTVM1.CC.VT.EDU

PROCEDURE FOR COLLECTION AND SHIPPING OF FAT BLEND SAMPLES- DELTA Protocol 2 DIET MONITORING

Food Analysis Laboratory Control Center (FALCC)
Standard Operating Procedure

SOP #1031
Revision: (New)

01-MAR-95

Dept. of Biochemistry and Anaerobic Microbiology
Virginia Tech
Blacksburg, VA 24061-0308

PLEASE READ THIS PROCEDURE COMPLETELY, PRIOR TO FOOD COLLECTION

Scope

This procedure applies to collection of unit food fat blend samples for DELTA Protocol 2 diet monitoring (DELTA Protocol 2 (1994-95).

Purpose

To describe the procedure for collecting unit food fat blend samples for DELTA Protocol 2 and shipping the samples to FALCC for assay, as part of documentation of diet composition.

Overview

The centrally prepared unit food fat blend for each of the three diets (D,E,F) will be sampled twice at each center: one sample taken between feeding periods 2 and 3 and one sample during feeding period 3. The sampling schedule is shown in the table below (DELTA Protocol 2 MOP, 3/95 addition). Each fat blend sample will consist of 10-15 grams of the fat blend, aliquotted after being liquefied according to the procedure for unit food preparation (per MOP). Sample containers will be provided by the FALCC. Fat blend samples will be frozen at -20°C and shipped frozen on dry ice to the FALCC, where they will be stored at -20°C or lower until assayed.

Sampling Schedule for fat blend monitoring.

Center (all diets)	Feeding Per. 1	Feeding Per. 2	Feeding Per. 3
PSU	not sampled	week of 3/5/95	week of 4/23/95
MINN	not sampled	week of 3/12/95	week of 4/30/95
COL	not sampled	week of 3/19/95	week of 5/7/95
PBRC	not sampled	week of 3/26/95	week of 5/14/95

Materials

At Field Center:

fat blend for each diet (D,E,F)
freezer (-20°C or lower)
heavy paper (e.g. brown paper or newspaper)
dry ice

Food Collection and Shipping Materials (supplied by FALCC):

small glass jars with Teflon lined lids (6)
zip-lock bags
labels
cryogenic marker
fat-free powder-free gloves (disposable)
Form #F001 (sample transfer), with example form filled out
Forms #F002 (deviation from SOP)
shipping cooler
packing tape[4]
Federal Express dry ice identification stickers
pre-addressed Federal Express shipping labels (1 per cooler)

NOTE: **Follow these procedures exactly. If any deviation occurs in preparation, packaging, sampling, shipping, etc., fill out form #F002 and include it with the food shipment.**

TAKE THE SAME CARE SAMPLING AND PACKING SAMPLE FOR ASSAY AS YOU DO IN PREPARING UNIT FOODS for PATIENTS.

Receipt of Shipping Materials:

Make sure you received all items listed above. If there is a discrepancy or if you should need additional labels or supplies, immediately notify the FALCC at (703)231-4361, or FAX (703)231-9070 or E-mail: FALCC@VTVM1.CC.VT.EDU.

[4]NOTE: The FALCC will supply each Field Center with a reasonable amount of packing tape for shipping foods to the FALCC - please reserve the tape for this use only.

A. Sample Collection

Sample the fat blend once for each diet (D,E,F) at each of the times specified in the Table on p. 3, as follows. Wear clean powder free gloves (provided) during sampling.

1. Liquefy fat blend according to MOP, as if portion were being taken for preparation of unit foods.

2. Aliquot 10-15 grams of the liquefied blend into one of the clean glass jars provided, **using the same aliquotting technique employed for weighing fat blend for unit food recipe.**

3. Enter in the appropriate spaces on a cryogenic label provided: the name of your Center, Diet (D, E, or F), sampling date (=current date), lot number of fat blend (if any), and your initials.

4. Seal jar tightly and **tape completely around jar/lid seam with packing tape.**

5. Affix label to jar. NOTE: If oil has gotten on outside of jar, may have to clean outside of jar so label will stick. **Make sure lid is securely sealed first.**

6. Place jar into a zip-lock bag and seal bag. Jar is placed inside bag so label will stay with sample if it were to fall off the jar.

7. Freeze and hold all samples at -20°C or lower prior to shipment.

B. SHIPPING

NOTE: Ship fat blend samples along with a menu shipment (FALCC SOP #1027) or unit food shipment (FALCC SOP #1030) to save on shipping costs.

1. Make sure lids are sealed tightly and **taped** onto sample jars to be shipped.

2. Wrap jars in brown paper, newspaper, or other wrap to prevent breakage.

3. Make sure to enter each sample on the sample transfer form (#F001) enclosed with the shipment.

NOTE: Enter all items in the shipment (menus and fat blend samples) individually on a single sample transfer form.

4. Follow procedure for menu or unit food shipment (FALCC SOP #1027 or #1030). Make sure to include deviation forms, if any, for the fat blend samples.

If you have any questions, contact FALCC: Phone: (703) 231-4361; FAX: (703) 231-9070; E-mail: FALCC@VTVM1.CC.VT.EDU

UNIT FOOD BATCH PREPARATION LOG
CENTER _____

Batch Prep. Date	Internal I.D. No. (If any)	Unit Food Name	Diet (D,E,F)	Batch Size	Initials

5.9 SPECIAL PROCEDURES FOR DEPARTURES FROM PROTOCOL

5.9.1 Log Book

Every center will keep a daily diary **log book** for recording all unusual circumstances involving any of the procedures in diet management and food production. This log serves as a means of communication among staff and may also be used to interpret unusual data points in analysis.

Example of log entries are shown below:

DELTA DIET LOG BOOK		
Date	**Time**	**Message**
10/5/94	6 pm	Mary Jane has a stomach virus and vomited her lunch. G.I.
10/6/94	8a.m.	John Doe says he is hungry despite a constant weight. I gave him one cup chopped lettuce with 1tb fat free French dressing. Hope Wright
10/17/94	8a.m.	Began using centrally procured Parkay margarine today. T.M. Real

5.9.2 Missed meals

If a participant is absent for a meal, without a valid reason, he/she may be dismissed from the study. If the participant did not eat any non-study food during the absence, a replacement meal may be provided to be eaten during the remainder of the day.

5.9.3 Food that is left

If the participant is still at the feeding site, give any food that is left back to the participant to eat. If the participant cannot eat all of the meal, it may be packaged for later consumption or it may be added to the next meal. Foods that cannot be safely or aesthetically saved for another meal should be discarded and a replacement provided. Food that is not eaten by the end of the day should be recorded on the form DDD by the kitchen staff or on form FBI by the participant.

5.9.4 Illness

Illnesses that interfere with dietary compliance should be reported immediately to the study coordinator.

5.9.5 Special Requests for single meal carry out

Centers have the option of responding to special requests for packed meals and each center should develop its specific policy. It is important that participants understand the limitations of this policy at the outset. Following are policy guidelines from 3 centers.

Figure 5.9.5.1 PSU Policy for Special Request Packed Meals

PSU Policy for Special Request Packed Meals

Dear DELTA Participant,

Welcome to DELTA II and thank you very much for participating in our large multi-center feeding study.

This is a reminder that you have **agreed** to eat only foods provided by the center and not to eat any additional foods not provided by us. You have also **agreed** to come for your on site meals during the designated hours throughout the whole study. If you need a take-out meal(s) for an unforeseen event, we will evaluate the situation and try to accommodate you.

We need **3 work days** advance notice if it is impossible for you to come for one day or less.

We need **5 work days** advance notice if it is impossible for you to come for more than one day.

An advance notice **does not guarantee** that your request will be granted.

If your life circumstances change in such a way that you will request packed meals very frequently, the central diet subcommittee will re-evaluate your whole file and you may be discontinued from the study.

Many thanks in advance for your cooperations and understanding!

Sincerely,

Principal Investigator

Figure 5.9.5.2 PBRC Policy for Special Request Packed Meals

PBRC Policy for Special Request Packed Meals

1) The nurse coordinator will outline our expectations with the participants during the recruiting processing.

2) The dietetic coordinator will meet with the participants during Screening 3 to review the menus and discuss our expectations.

3) There will be an orientation meeting with the PI, the nurse coordinator, and the dietetic coordinator for all participants before feeding begins. At this meeting, the "Kitchen Connection" brochure will be reviewed, our expectations will be reaffirmed, and questions will be answered.

4) The established guidelines are:

   a) A participant may take-out 2 "on-site" meals during each diet period.

   b) Because food safety is our greatest concern, a participant is restricted to a maximum of 3 consecutive days of take-out meals.

   c) A "Take-Out Meal Request" will be completed and approved by both the nurse and the dietetic coordinators. (See attached).

   d) For compliance assessment, a participant will be expected to return all food containers and uneaten foods to the metabolic kitchen.

   e) Participants can take-out more meals than allowed only when approved by the PI, the nurse coordinator, and the dietetic coordinator. This procedure will allow for emergencies and for other special cases.

Figure 5.9.5.3 Take Out Meal Request (PBRC)

TAKE-OUT MEAL REQUEST
PENNINGTON BIOMEDICAL RESEARCH CENTER

(Participant name/ID) _____, enrolled in the _____ study requests permission to take-out the following meals on the dates specified below:

Date: _____	B	L	D	Snack
Date: _____	B	L	D	Snack
Date: _____	B	L	D	Snack

The meals should be ready by _____ (time) on _____ (date).

(Participant name/ID) _____ has been informed of his/her responsibility for handling the food in a prudent manner to assure food safety. He/She also has been told to return the empty food containers and any uneaten foods to the metabolic kitchen for compliance assessment.

_____ _____
Study Coordinator Date

_____ _____
Dietetic Coordinator
Date

Figure 5.9.5.4 Columbia Policy for Special Request Packed Meals

Columbia Policy for Special Request Packed Meals

Dear DELTA Participant,

The first weekend of the DELTA study is just around the corner. We will be packing the following meals for you:

Saturday's - Breakfast
Lunch
Dinner
Snack

Sunday's - Breakfast
Lunch
Dinner
Snack and

Monday's - Breakfast
Lunch

You are allowed to eat only one meal on your own with our guidelines.

THE "FREE" MEAL COULD BE EATEN AS DINNER IN PLACE OF SATURDAY'S LUNCH OR SUNDAY'S LUNCH.

Please let us know by Wednesday each week if your are planning to eat on your own.

WEEKDAY TAKE-OUT MEAL GUIDELINES

1. You may take-out 1 "one-site" meal during each diet period.
   You must request 3 days ahead of time for the take out-meal to Rebecca or Maliha.
2. It is your responsibility to keep the food safe.

PLEASE REFRIGERATE THE FOOD IMMEDIATELY.

Please bring empty containers on Monday or the following day.

Please complete the 'PACKED MEAL FORMS' and give it back to the DELTA Staff.

All of you will be given food records for the weekend. On these food records please write down everything you eat and drink on Saturday and Sunday, and return it to us when you come for your lunch on Monday. We will weigh you every Monday and Thursday before lunch.

Please follow the guidelines and instructions carefully as weekend eating is just as important as a weekday.

Thank you for your cooperation.

5.9.6 Missing, lost or spoiled food

If the mishap occurs during the periods of meal service, a replacement item can be provided at the next meal. If the mishap occurs in the evening or on the weekend, the participant is instructed to telephone the person on call. The person on call will either contact the dietitian or provide guidance directly to the participant on appropriate substitutions. Wherever possible, DELTA study food will be provided. Emergency meals may be used here **(see section 5.9.9)**.

5.9.7 Special Travel Meals

Occasionally it may be necessary for a participant to travel out of town. The following guidelines and special menus were developed for this purpose. Only 2000 kcal and 3000 kcal menus will be offered. Participants on 1500 kcal maintenance will be given the 2000 kcal diet. Unit foods will be used to adjust the calories for other participants if necessary.

1. Make menus simple and easy to prepare, store and package.

2. Include, as much as possible, food items that are easy to consume on the run (while driving or waiting in an airport).

3. Include a hot meal to break the monotony of cold food consumption.

4. Keep in mind that as long as the participants consume all their travel foods, the order in which they consume the meals or the individual foods does not matter.

5. Include appropriate vehicles for solid and liquid fats.

6. Use food items that are already available on site in order to minimize or eliminate special shopping trips.

5.9.8 DELTA TRAVEL MENUS

Menu 1

BREAKFAST

Applesauce
Sandwich:
- White Bread
- Butter or Margarine
- Jelly

Milk

LUNCH

Turkey Salami Sandwich
V8 Juice
Fruit Cocktail

DINNER

Spaghetti and Meatballs
Corn
Bread Sticks or Whole Wheat Bread
Butter or Margarine
Banana Chips

SNACK

Cheese and Crackers OR
Hazelnuts OR
Bread and Jelly and Hazelnuts

Menu 2

BREAKFAST

Grape Juice
Golden Grahams cereal
Bread
Butter or Margarine
Jelly
Milk

LUNCH

Sausage and Egg Sandwich
V8 Juice
Pineapple

DINNER

Ham and Turkey Sandwich
Mashed Potatoes
Peaches
Fig Newtons

SNACK

Pretzels and peanuts OR
Peanut Butter and Jelly Sand.

5.9.9 Emergency meals

In rare instances, a participant may not be able to get to the feeding site because of an unavoidable, unplanned emergency. Examples of such genuine emergencies might be a critically ill child or household problem requiring the participant to say at home over a meal period. Field centers may choose to provide an emergency frozen meal pack to each participant as a backup. However, it is preferable to arrange for delivery of emergency meals to participants in cases where the participant cannot get to the feeding site. Pending natural disasters (hurricanes and snowstorms) usually allow adequate warning for advance preparation. Each field center should have a plan for dealing with these events. (Specific plans for each center follow.)

If an emergency back up frozen entree is provided for each participant, any unused entrees must be returned to the feeding site at the end of each diet period to avoid mixing up of diets. The emergency meal is a Healthy Choice™ meal and low fat accompaniments.

5.9.9.1 Emergency Meal Plans

Columbia

Most of our participants live around the dining facility, so it is convenient for them to come or have their friends pick-up their meals. For those who live far away we pack meals. We also use "free meal" allowance during the week instead of the weekend.

Pennington (PBRC)

At PBRC, advance warning of natural disasters (heavy rain or hurricanes) is expected. In the event of pending flooding, meals will be prepared in advance and packaged for take-out. The participants will take their meals with them before the event occurs. If a participant is unable to leave their home, every effort will be made to deliver the meals to them.

Minnesota

In the event that an overnight major snowfall or severe temperature/wind chill is forecast where individuals are advised to stay in their homes due to danger encountered with travel or exposure, the University of Minnesota DELTA Center will pack meals for the following day. Breakfast and the pre-packed lunch will be sent with participants whose job position would not necessitate their coming to the University the following morning. (A number of employees are considered critical to the function of the University medical departments, and are exempt to such an advisory.) In the case of an overnight snowfall, it would be likely that most participants would be able to come to the center for their evening meal the next day as snow removal takes place in only a matter of hours in this area of the country. Participants who are living long distances from the center AND who would have no other purpose to come to the center will be given their packed breakfast and lunch meal along with Healthy Choice™ entree(s) and the remaining fruit, vegetable, bread and fat items that would have otherwise made up that evening meal. The DELTA kitchen is considered a

medical research unit and is exempt to days/shifts canceled due to weather conditions. Employees are available to serve meals for those participants who are willing and able to eat at the center.

Penn State

Two work days prior to each feeding period, the Penn State DELTA-Metabolic-Kitchen staff will prepare and pack one complete weekend menu 1 for each participant. On the first day of each feeding period, the packaged meals along with storage instructions will be distributed to the participants. The participants will be allowed to use these extra meals when they are unable to get to the feeding site due to a natural disaster. Any participant who uses the extra meals before the end of the feeding period will be provided with another day's worth of weekend menu 1 packaged meals. All unused meals must be returned to the feeding site at the end of each feeding period.

5.9.10 Participant refuses to eat an item

If the participant can no longer tolerate some of the fruits and vegetables, substitutions can be made for these items only. The dietitian will be responsible for determining substitutions using the **MENu database** and logging the substitution in the **log book**.

5.10 FOOD SAFETY

Food safety is a serious concern in every feeding study. In **DELTA**, participants will receive virtually all of their food from the field centers.

Each center is responsible for implementing appropriate procedures and training of personnel to protect participants from any food born illness. "Critical control points are those areas in the chain of food production, from raw materials to finished products, where the loss of control can result in an unacceptable food safety risk."[5]

Critical areas to be addressed include treatment of foods, personal hygiene and health of the food handlers, and misuse of food taken off site by the participants.

Procurement of fresh and wholesome foods that meet study specifications is the first step in food safety. Perishable foods should be dated and refrigerated or frozen immediately upon receipt. Refrigerators must be kept at 34° - 40° F and freezers at 0° or lower. All food should be labelled with date of receipt and stored so that "first in is first out." All foods, cooked or raw, will be stored in closed containers.

Raw meats, poultry and fish require extra safety measures. These food products should be wrapped securely and placed on a tray so they do not leak and contaminate other foods and surfaces. Keep all meats, poultry and fish refrigerated until ready to be cooked. All poultry needs to have the skin removed and be rinsed and patted dry before cooking. All frozen meats, poultry and fish should be thawed in the refrigerator.

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[5]Adapted from a publication in process by Elaine Ayers, MS, RD, LD, *Metabolic Diet Studies in Humans: A Practical Guide to Design and Management*, P.15

Cooked and raw foods must be stored separately. Precision weighed perishable food must be refrigerated immediately after weighing. Cooked foods that are served cold are weighed into individual servings and refrigerated or frozen immediately. Foods that are served hot must be served immediately after cooking. The cooked food should be checked at each meal service to insure that it has reached the proper internal temperature to kill microbial contaminants. (See Table 5.9.1). "The time elapsed between tray assembly and delivery is in compliance with food service standards. Tray assembly and delivery time should not exceed 20 minutes to maintain appropriate food temperature, appearance and palatability."[6]

Proper storage of shelf stable food is also crucial in food safety. Foods should be stored off the floor in closed containers, and in well ventilated areas. These storage areas should only be accessible to kitchen staff and **DELTA** personnel. All foods should be labelled with date of receipt and stored so that "first in is first out."

Personal hygiene is also critical for food safety. Food handlers should wash their hands with hot soapy water prior to any handling of food items. This same procedure must be followed before **AND** after handling any raw meats and poultry. Any food handler who is sick with a diarrheal illness will not prepare or serve food.

Participants who take food off site also need to follow guidelines to ensure food safety. Meals that will be eaten off site should be packed in insulated bags and coolers. Perishable foods will be kept cold or frozen before they are packed. Keep packed food in cool place and out of direct sunlight until it can be refrigerated or frozen or is ready for consumption.

Each center is responsible for providing information on keeping food safe and insuring that participants have adequate facilities for storing food safely off site.

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[6]Ayers, op. cit. p.19

Table 5.10.1 Principles of Time/Temperature Control of Potentially Hazardous Foods

Principles of Time/Temperature Control of Potentially Hazardous Foods[7]

1. Cook food to a minimum temperature:

165°	Poultry and Stuffing
150°	Pork
140°	Other entrees and casseroles

2. Reheating foods for research diets:

165°	minimum

3. Cool foods (liquid formulas) rapidly to 45° in FOUR HOURS using:

   Shallow pans (2-3" depth)
   Ice bath
   Agitation
   Loose fitting covers
   No stacking
   Placement of food in coldest part of cooling unit

4. Equipment maintenance

   Refrigeration units 35° F - 45° F
   Freezer units 0° F or below

5. Provide thermometers to be used for checking foods for proper and safe temperatures.

[7]Ayers, op. cit. p.15

5.10.2 Instructions for packing DELTA meals

Weekend Meals:

- Use one Glad Zip-Lock bags filled with ice or reusable freeze-packs to keep the coolers cold.
- Place two ice bags or six freeze-packs on the bottom of each cooler.
- Place two plastic bags in each cooler, one for Saturday's meals and the other for Sunday's meals.
- Pack the individual coolers for a specified diet and caloric level at one time, followed by coolers for the next experimental diet and calorie level, etc. Make sure to keep the **perishable items** as **close** as possible to the ice and to keep the coolers closed as much as possible.
- Pack one meal at a time starting with Saturday's breakfast.
- Place one ***Time Temperature Indicator*** sticker on one of the **cold perishable** items for **each day**.
- While packing the coolers, complete the Packed Meal Forms (PMF) according to instructions. Put each form in the corresponding plastic bag in each cooler. Tie the bags in each cooler.
- Put **one freeze-pack** between the two weekend bags. **Close** the cooler tightly.

Weekday Packed Meals and Snacks

- Use brown paper bags to pack the weekday meals and snacks.
- Pack the individual meals for a specified diet and caloric level at one time, followed by meals for the next experimental diet and calorie level, etc.
- Place **one** ***Time Temperature Indicator*** sticker on one of the **perishable items** of the meal.
- While packing the bags, complete the packed meal forms (PMF) according to instructions. Put each form in the corresponding bag.
- Bags containing **perishable items must be placed in the refrigerator** prior to pick-up.

Figure 5.10.2.1: Time-Temperature Indicator

ATTENTION

Dear Participant,

Fresh-Check® Time-Temperature Indicator (TTI) labels are being used to insure the freshness and quality of the food you will be using in the Delta Study. **Please follow the guidelines below for handling foods with *Fresh-Check* labels.**

GUIDELINES FOR HANDLING PRODUCTS WITH FRESH-CHECK LABELS

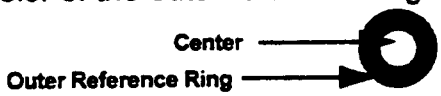
Fresh-Check *labels will change color over a period of 5 days at 40 °F, 1 day at 50 °F and 45 minutes at room temperature. To ensure maximum product shelf life:*

1) BRING FOOD HOME IMMEDIATELY*, do not let food sit in car or out on counter top.*

2) REFRIGERATE FOOD IN COLDEST SECTION OF REFRIGERATOR *(40 °F or cooler).*

HOW TO READ THE FRESH-CHECK LABEL

The ***Fresh-Check*** Indicator is read by comparing the color of the indicator center to the color of the outer reference ring.



1. If the indicator "center" is lighter than the outer reference ring, ***THE FOOD IS FRESH.*** *See example below.*

2. If the indicator matches the outer ring, ***FOOD IS STILL FRESH BUT APPROACHING EXPIRATION AND SHOULD BE USED IMMEDIATELY.*** *See example below.*

3. If the indicator center is darker than outer ring, ***FOOD IS STARTING TO SPOIL AND SHOULD BE DISCARDED.*** *See example below.*

DELTA: FOOD SERVICE SELF-INSPECTION

Instructions for filling out form: This form is to be filled out during weeks 1, 3, and 5 of each feeding period. The Supervisor of each site will inspect the personnel and the facility. Any departures from these guidelines and any items that are checked "NO" must be referred to the Principal Investigator or his/her designate.

Date: _____ Inspected by: _____

Inspection Instructions	Yes	No	Comments
I. *Personal Hygiene and Habits*			
1. Hands clean, fingernails short - washed as frequently as necessary in an approved hand-washing facility.			
2. Employees free of burns, cuts, boils, infection.			
3. Employees free of acute respiratory illness or diarrheal illness or other communicable disease.			
4. Clean outer garments worn at start of shift.			
5. No smoking or eating in food preparation or serving areas.			
6. No rings (except wedding band) or jewelry on hands or wrist.			
7. Hair nets worn. No beards or moustache.			
8. Employees using disposable tissues instead of handkerchiefs.			
9. Hands kept away from mouth, nose, hair, etc.			
II. *Food Preparation, Holding, Handling*			
10. Potentially hazardous food kept below 40° or above 150°F – not held at room temperature more than 2 hours, cumulatively.			
11. Food preparation area generally clean, free of debris.			
12. Fruits, raw foods and vegetables washed prior to preparation.			
13. Frozen food thawed under refrigeration, in the microwave, under cold running water, or cooked directly – no refreezing of thawed foods. All frozen meats should be thawed in refrigerator.			
14. Cooked leftovers thoroughly reheated.			
15. Food covered or protected from contamination (dust, sneezing, coughing).			
16. Self-service utensils dispensed so that only handles can be touched.			

DELTA: FOOD SERVICE SELF-INSPECTION

Inspection Instructions	Yes	No	Comments
17. Sugar, condiments, seasonings, dressings for self-service: in individual packets or from protected dispensers.			
18. Raw and cooked or ready-to-serve foods not being prepared on same work surface (cutting board) without washing and sanitizing between changed use.			
19. Food handlers should wear plastic disposable gloves when preparing fresh foods (salads or sandwiches) that will NOT be heated before serving.			
20. Raw meats should be cut on a sanitized cutting board that will not be used for any other ingredients. Hands washed before and after handling raw meat & poultry.			
21. Preparation equipment cleaned and sanitized between changed uses (especially pertains to grinders, slicers, choppers, mixers, knives).			
22. Kitchen equipment clean and properly stored.			
23. Food contact surfaces and utensils free of corrosion, pitting, cracks, crevices.			
24. Thermostats and thermometers accurate and operating.			
25. Stoves, griddles, broilers, fryers, etc., free of grease and properly hooded with filters and vented.			
26. Hood and filters free of accumulation of grease and condensation drippings.			
27. Unused equipment kept clean or removed (equipment not used at least occasionally should not be taking up space in food preparation, service, or storage areas).			
III. *Ware Washing and Storage*			
28. Sufficient hot water to meet washing requirements.			
29. Dishwasher machine water clean; proper amount of detergent used.			
30. Dishes, utensils pre-scraped and rinsed.			
31. Wash temperature kept between 140°F and 160°F.			
32. Jets and nozzles in dishwasher kept free of food particles and other obstructions and contaminants.			
33. No overloading or improper racking.			
34. Automatic detergent and rinse dispensers operating.			
35. Rinse temperature at least 180°F being maintained.			

DELTA: FOOD SERVICE SELF-INSPECTION

Inspection Instructions	Yes	No	Comments
36. Thermometers on dishwasher maintained and operating properly.			
37. Removal from racks and sorting done in sanitary manner.			
38. Wash hands between handling soiled table ware and sanitized ware.			
39. All equipment and utensils air dried -- no toweling. Cutting boards sanitized daily. Keep boards used for meats separate from other cutting boards.			
40. Cleaned and sanitized wares stored off floor in a clean, dry location.			
41. Ware washing equipment cleaned after each day's use to remove chemicals, food particles, soil, debris.			
IV. Storage			
A. Dry (food, equipment, supplies)			
42. Food stored above floor -- on shelves, racks, or platforms.			
43. Floor clean and free of spilled food and debris.			
44. Shelves high enough -- at least six inches -- to permit cleaning, or area beneath shelves enclosed to preclude dirt.			
45. Shelves clean, durably finished, free of dust and debris.			
46. Food supplies dated upon receipt and stored to insure "first in first out" use.			
47. Stable food (sugar, flour, etc.) stored in containers with tight lids or in original package -- properly identified.			
48. Loose and unwrapped food or food where original package is broken stored in pest-proof containers or enclosed in plastic bag and tied -- properly identified.			
49. Single service items stored properly.			
50. Canned goods with large dents segregated for return to distributor.			
51. Area dry -- free from dampness.			
52. Non-food supplies stored separately from food items.			
53. Equipment and supplies stored in neat and orderly manner.			

DELTA: FOOD SERVICE SELF-INSPECTION

Inspection Instructions	Yes	No	Comments
54. All toxic materials, including any pesticides, properly used from original containers only. When not in use stored in cabinets which are used for no other purpose, or in a place which is outside the food storage, food preparation and cleaned equipment areas.			
55. Cleaning equipment and sanitary chemicals available, properly maintained and stored.			
56. Clean and soiled linens stored separately.			
B. Refrigerator Units			
57. Temperature 40°F or below.			
58. Properly functioning thermometer in each unit.			
59. Clean, free from mold, objectionable odors, spills.			
60. All food stored off the floor in orderly manner.			
61. Storage shelves allow adequate air circulation.			
62. Panned raw or cooked foods, on shelves, covered to prevent contamination.			
63. Open "tin" cans not used for food storage.			
64. Potentially hazardous foods stored in chillable quantities (i.e.; in small, shallow containers).			
65. Leftovers which will not be used within 24-36 hours, frozen.			
66. Raw foods stored apart from and below prepared food.			
67. No units overloaded.			
68. Food dated and stored in manner to permit "first in first out" use.			
C. Freezer Units			
69. Properly functioning thermometer in each unit.			
70. Temperature 0°F or below.			
71. Food stored to insure adequate air circulation.			
72. Walls or coils defrosted.			
73. Food off the floor.			
74. Clean, free of debris.			
75. Food labelled with date of receipt and stored in manner to permit "first in, first out" use.			

DELTA: FOOD SERVICE SELF-INSPECTION

Inspection Instructions	Yes	No	Comments
V. Waste Disposal - Garbage and Trash			
76. Nonabsorbent containers clean (using plastic bags), good repair; paper cartons not being used.			
77. Tight-fitting lids on containers not in continuous (actual) use, or when filled or stored.			
78. Sufficient number of containers to hold waste.			
79. Storage area adequate, clean, and separate from food preparation and food storage areas.			
80. Disposed in approved manner, frequently. Trash containers need to be disinfected weekly.			
VI. General Sanitation			
81. Floors clean, dry (no grease or water); good repair.			
82. Walls, ceilings clean; good repair.			
83. Lighting fixtures clean; good repair, shielding protecting against broken glass; lighting adequate.			
84. Wiping cloths (including sponges) clean, rinsed out in sanitizing solution during use; those used for food contact surfaces restricted to that use.			
85. Pests (roaches, flies, rodents, etc.) effectively controlled in all areas.			
86. Equipment with insufficient space provided for easy cleaning behind or between the unit sealed to adjoining equipment or wall.			
87. Electrical and plumbing fixtures maintained and operated properly.			
88. No exposed sewer or water lines over food preparation or storage areas.			
89. Dustless methods used to clean floors and walls at time when least amount of food exposed, except in emergency.			
90. Ventilation systems adequate to control heat, steam, condensation, vapors, fumes; maintained.			
91. Planned sanitary maintenance programs used.			
92. No animals in establishment, except guide dogs.			

5.11 SELF-SELECTED MEAL

Participants are allowed one self-selected meal in place of the Saturday evening meal. Participants are given guidelines to select a meal that is 30% fat. See Figure 5.11.1. Use of the self-selected meal is optional. Participants may choose to have all weekend meals provided by the field center.

Each center will use it's own procedure for ascertaining the composition of the self-selected meal. Examples of good choices from common restaurant types can be found on pages 117-120.

Figure 5.11.1 Guidelines for the Self-Selected Saturday Evening Meal

DELTA Guidelines for the Self-Selected Saturday Evening Meal

To prevent your self-selected meal from interfering with the DELTA Study results, **please use the following guidelines in making your selections.**

1. Avoid fried and breaded foods such as fried chicken, breaded fish, french fries, etc.
2. Remove all visible fat from meat and skin from chicken before eating.
3. Avoid the use of gravy, cream sauces, cheese, sauces, and butter. Use margarine and limit the amount to one-two teaspoons.
4. Limit the use of oils, salad dressings mayonnaise, and the like to one-two tablespoons.
5. Limit meat serving size to four-six ounces or one-two pieces of chicken (skin removed).
6. Avoid high fat meats and cheeses. Hamburgers should be without cheese. Pizza should be vegetable or ground beef with usual (not extra) amount of cheese.

Examples of foods that would be considered good choices for some common restaurant types are listed below:

Chinese Restaurant	
Good Choices:	Won Ton Soup
	Chicken or Shrimp Chow Mein, Chop Suey, Moo Goo Gai Pan
	Chicken or seafood and vegetable dish
	Steamed rice
	Steamed or stir fried vegetables
	Fortune cookie, sherbet
	Tea or other allowed beverages
Avoid:	Egg Drop Soup
	Egg Rolls, Fried Wonton, Ribs
	Egg Foo Young
	Fried Rice
	Menu items made with ingredients fried in hot oil

Italian Restaurant	
Good Choices:	Cooked spaghetti or pasta
	Tomato sauce
	Meatballs (limit to 3-4, 1" diameter)
	Salad greens, tomato, onion, fresh vegetables
	Italian or French dressing
	Fresh fruit
	Plain hard rolls, French bread
	Allowed beverages
Avoid:	Alfredo, cream and cheese sauces
	Cheese based main dishes
	Pre-buttered breads and rolls
	Feta cheese (in salads, etc)
	Excessive margarine, olive oil, salad dressing, cheese

Mexican Restaurant	
Good Choices:	Chicken taco, tastado or fajita
	Tortillas made with cornmeal or soft flour tortillas
	Salad greens, tomato, onion, avocado
	Spanish rice
	Salsa
	Allowed beverages
Avoid:	Fried tortilla shells, Nachos
	Steak or beef filling
	Refried beans
	Excessive Cheese and sour cream

Seafood Restaurant	
Good Choices:	Broiled, boiled or steamed fish, crab, scallops, shrimp, lobster
	Rice Pilaf
	Baked potato (limit margarine to 1 tsp)
	Dinner rolls
	Steamed vegetables
	Tossed salad (request dressing on the side and limit to 1-2 TB)
	Allowed beverages
Avoid:	Fried and breaded items
	Seafood casseroles
	Butter, cream, and cheese sauces (use lemon or cocktail sauce instead)
	Excessive margarine and sour cream

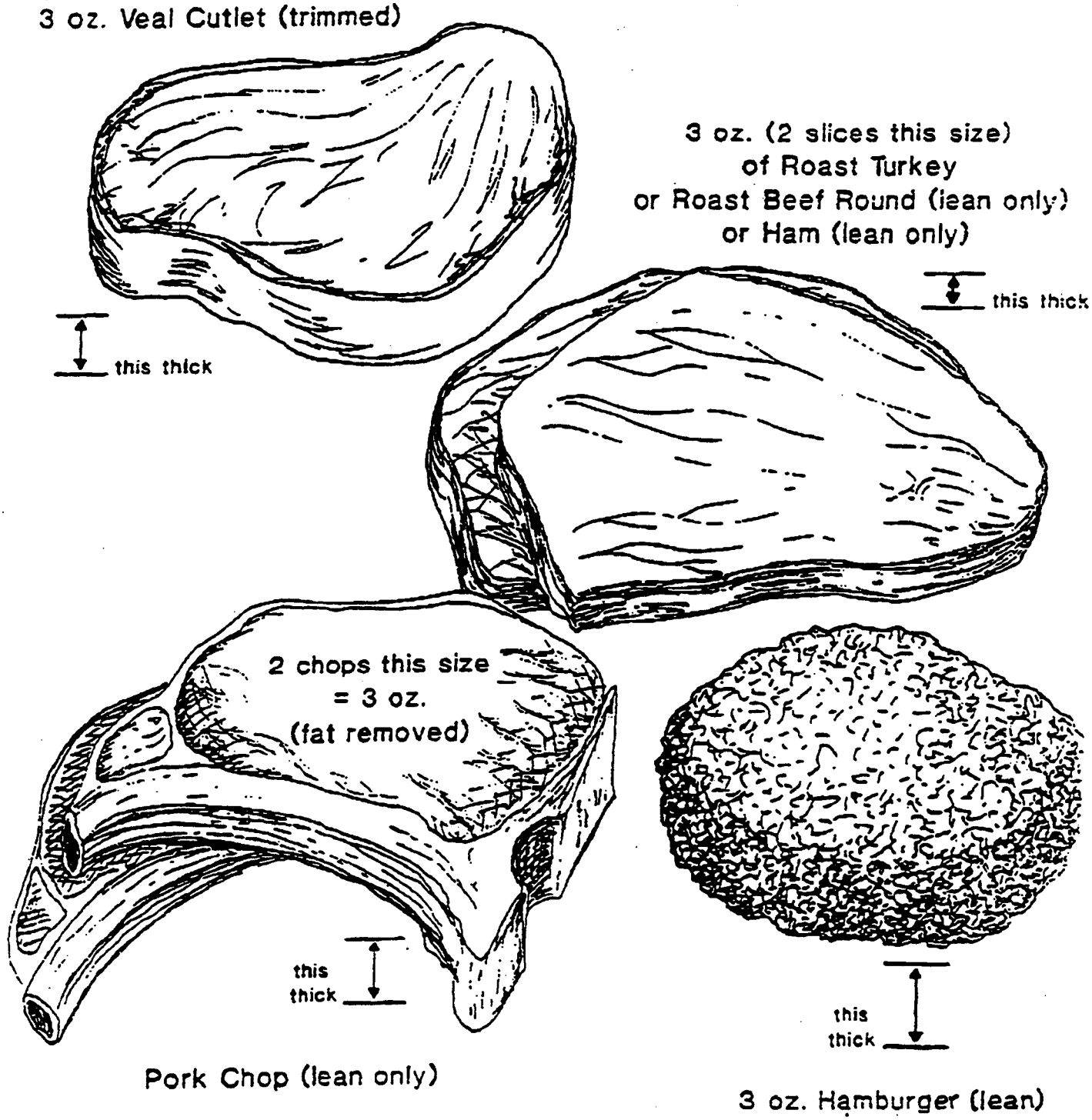
Steak Restaurant	
Good Choices:	Broiled steak with fat well trimmed
	Baked Potato (limit margarine to 1 tsp)
	Dinner rolls
	Steamed vegetables
	Tossed Salad (request dressing on the side and limit to 1-2 TB)
Avoid:	French or steak fries
	Au gratin potatoes
	Prime rib, meat fat
	Excessive margarine and sour cream

Submarine Shop	
Good Choices:	Ham, Roast Beef or Turkey Sub (Avoid cheese or reduce amount)
	Tomato, Lettuce, Pickles, Peppers, Mustard
	White or wheat bun
	Oil or mayonnaise dressing (limit to 1 TB)
	Allowed beverages
Avoid:	Meatball or Lunchmeat Sub
	Cheese
	Snack Chips

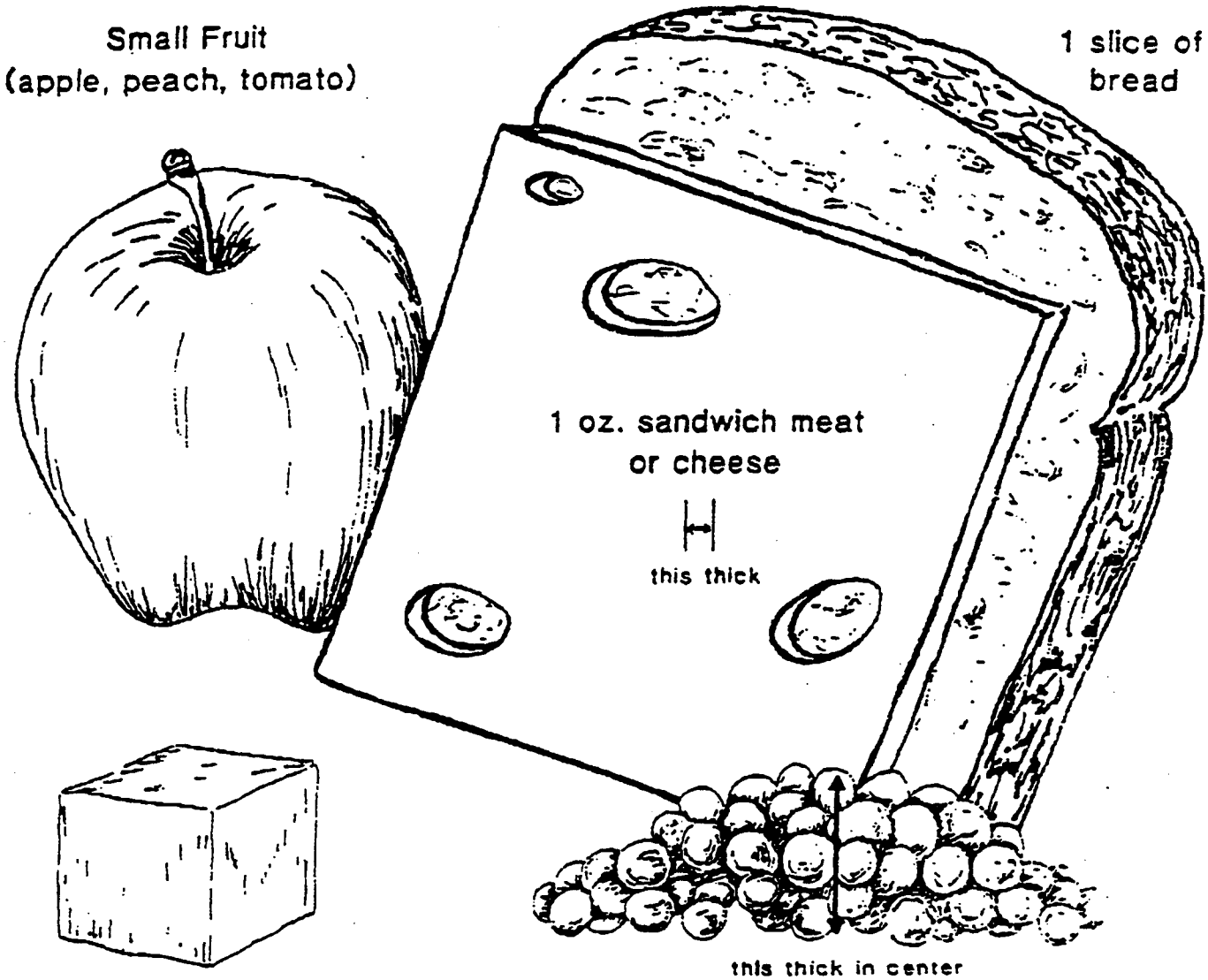
Fast Food Restaurant	
Good Choices:	Plain burger w/ketchup, mustard, luttuce, tomato, onion &/or pickles, if desired
	Broiled chicken sandwich w/ketchup, mustard, lettuce, tomato, onion &/or pickles, if desired. Avoid cheese and special dressings
	Baked potato 1/1 pat of margarine or 1 tsp sour cream
	Mixed green salad w/1 TB salad dressing
	Frozen yogurt. Avoid toppings
	Allowed beverages
Avoid:	Cheeseburger
	Fried chicken and Fried Fish sandwiches
	Specialty burgers like Big Mac, Whopper, etc. and large sandwiches

Pizza Parlors	
Good Choices:	Plain cheese pizza. Avoid deep pan pizza
	Vegetable toppings
	Mixed green salad w/1 TB salad dressing
	Italian ice
	Allowed beverages
Avoid:	Meat toppings
	Extra Cheese
	Bread sticks, garlic bread and fried mozzarella sticks
	Pizza made with pastry dough

SERVING SIZES OF MEATS



COMMON SERVING SIZES

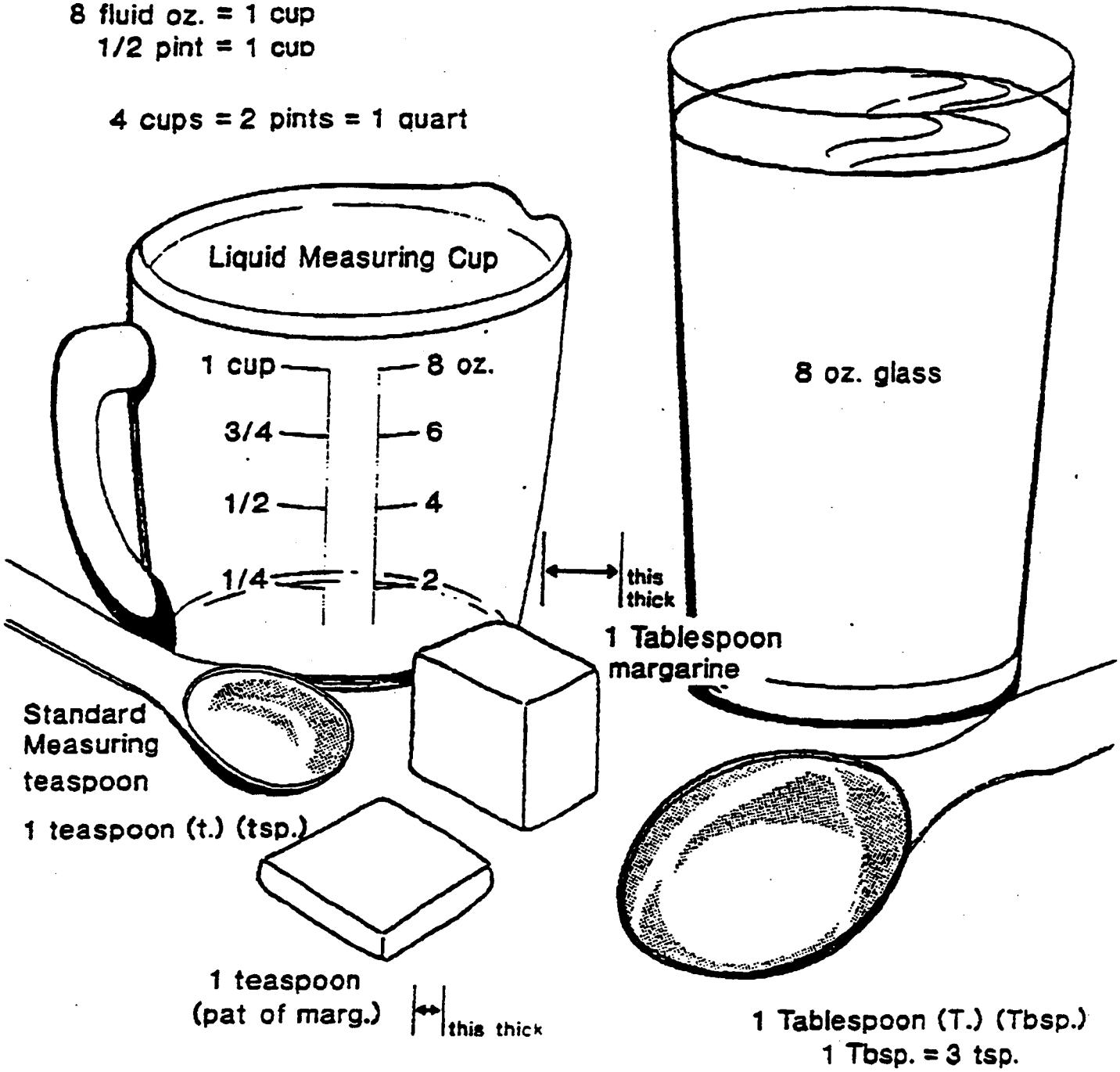


COMMON SERVING SIZES

4 Tbsp. = 1/4 cup
5 1/3 Tbsp. = 1/3 cup

16 Tbsp. = 1 cup
8 fluid oz. = 1 cup
1/2 pint = 1 cup

4 cups = 2 pints = 1 quart



5.12 EATING BETWEEN FEEDING PERIODS

Participants are counselled to maintain a similar lifestyle during the periods between study feeding so that weight remains stable. Fig. 5.12.1 is an example of a printed instruction to be given to the participant.

Figure 5.12.1 Guidelines for Between Feeding Periods

DELTA Guidelines for Between Feeding Periods

Between diet periods, it is important to maintain your weight and lifestyle habits. A change in weight of more than three-four pounds and/or a change in lifestyle habits could affect the results of the DELTA study. Since weight and lifestyle habits maintenance are two top priorities, the following guidelines have been prepared to help you achieve these goals.

Weight

1. Eat a wide variety of foods and limit the use of large amounts of fats and concentrated sweets.

2. Weigh yourself on a weekly basis. If you gain more than two pounds within a week, you will find these tips helpful in controlling your weight.

   - Eat generous portions of starchy foods like bread, rice, pasta, cereals, beans, and potatoes. Prepare and eat these foods with little or no added fat.
   - Eat plenty of fruits and vegetables. Prepare and eat these foods with little or no added fat and/or sugar.
   - Eat moderate portions of lean meat, fish, and poultry (remove poultry fat and trim visible fat from meat). A moderate portion is no more than four-six oz. of meat, fish, or poultry each day.
   - Use salad dressing or oil in moderation (1-2 TB/day). Use fat-free dressing if you need more.
   - Use fat spreads such as butter, margarine, cream cheese, and mayo-type dressing in moderation (1-2 tsp/day).
   - Avoid deep fried foods, cream sauces, cheese sauces, gravy, regular ice cream, and rich desserts or limit them to once each week.
   - Drink skim or 1% milk instead of 2% or whole milk. Use milk instead of cream in your coffee.
   - Limit alcohol use to one -- two drinks per week.

3. Weight loss is seldom a problem between diet periods. However, if you lose more than three-four pounds, increase your portion sizes at meals and include snacks liberally.

Lifestyle

1. If you are not smoking, please do not start.

2. Plan to maintain your current level of physical activity. A decrease in an activity should be replaced by an increase in a comparable activity. Avoid beginning new activity routines that result in a major change in either intensity or duration.

If you have any questions or concerns regarding your weight, food intake, smoking and physical activity, please call _____ at _____.

HAVE A WONDERFUL BREAK!!

See you for dinner (Month, Day)

APPENDIX I:

DELTA - 2 DIET FORMS

Instructions
Food Production Form (FPF)

The Food Production Form (FPF) is used in conjunction with the RECIPES for portioning all menu items. There is a separate FPF form for each diet and menu. The forms are color coded (Green: Diet D, Buff: Diet E, Salmon: Diet F).

1. **Prepare the Food Production Form:** Fill in the date corresponding to the menu that will be served. Menu sequence is shown in the Manual of Procedures, pages 28-35.

   Circle the day of the week corresponding to the date.

   Count the number of participants receiving each calorie level for the corresponding diet and fill in this number in the box above each calorie designation.

2. **Portion the items as listed in the food production sheet:** When all the portions for a given item have been weighed/portioned out, the staff responsible will initial the item.

FOOD PRODUCTION FORM

DIET D MENU 1						
DATE: ☐☐ ☐☐ ☐☐ mm dd yy DAY: M T W T F S S	Staff Initials	1500	2000	2500	3000	3500
BREAKFAST						
Orange juice		124.0	124.0	124.0	124.0	248.0
Triples		23.0	23.0	23.0	46.0	46.0
White Bread (PF)		22.7	45.4	90.4	90.4	90.4
Butter		8.0	9.0	15.0	20.0	20.0
Sweets; jellies		0.0	10.0	10.0	10.0	10.0
Jellies, dietetic		14.2	0.0	0.0	0.0	0.0
Milk, whole		245.0	245.0	245.0	490.0	490.0
LUNCH						
Sandwich package:						
*Turkey breast meat		35.0	50.0	56.7	56.7	75.0
*Mayonnaise, regular		4.3	5.0	6.0	9.0	9.0
*Iceberg lettuce		0.0	0.0	0.0	10.0	10.0
*White bread (PF)		45.4	45.4	45.4	45.4	90.8
Iceberg lettuce		24.0	24.0	24.0	40.0	40.0
Olive oil		0.0	2.0	2.0	10.0	8.0
Peaches, juice pack		127.6	127.6	127.6	127.6	127.6
Ginger Cookie		14.0	22.0	22.0	22.0	22.0

	Staff Initials	1500	2000	2500	3000	3500
DINNER						
Sirloin Tips With Gravy		1 sv	1 sv	1 sv	1 sv	1 sv
Corn, frz.		60.0	100.0	120.0	155.0	175.0
Iceberg lettuce		30.0	30.0	30.0	40.0	40.0
Tomatoes, raw		20.0	20.0	20.0	30.0	30.0
Carrots, raw		15.0	15.0	15.0	20.0	20.0
Country Classic rolls		18.9	37.8	56.7	75.6	75.6
Butter		9.0	9.0	21.0	25.0	20.0
Applesauce, unsweetened		113.0	113.0	226.0	226.0	226.0
SNACK						
Peanuts		12.0	24.0	28.0	35.0	45.0
Raisins		8.0	12.0	25.0	28.0	30.0
Pretzels, plain		15.0	18.0	24.0	24.0	35.0

Instructions
Tray Assembly Check Sheet (TAC)

The Tray assembly Check Sheet (TAC) is filled out each day for all participants in the study. There are separate forms for each calorie level, each menu and each diet (total 150). The forms are color coded by diet (Green: Diet D, Buff: Diet E, Salmon: Diet F).

1. **Prepare the form:** Select the menu corresponding to the date on which it will be served. Check the Manual of Procedures, pp 28-35 for the correct menu designation. Record the DATE on the TAC corresponding to the day it will be served and circle the corresponding DAY.

   Write in the Name or DELTA ID for each participant on the corresponding diet/calorie TAC.

2. **Assemble the tray:** Verify the diet and calorie level of the participant with the portioned items. Then place each item on the tray as listed on the TAC. When the tray is assembled, check off each item in the corresponding box for that participant to verify that all items are provided and enter your (staff) initials.

   Follow a similar procedure for packed meals, checking off each item as it is placed in the bag/ cooler, and initialing the completed meal assembly.

TRAY ASSEMBLY CHECK SHEET

Form Code: TA(
Version A 9/15/9

DIET D **MENU 1** **1500 KCAL**

DATE: ☐☐ ☐☐ ☐☐
mm dd yy
DAY: M T W T F S S

CENTER: _____

	NAME OR DELTA I.D.							
BREAKFAST								
Orange juice								
Triples (1 PC)								
White bread (1 slice)								
Butter								
Dietetic jelly (1/2 oz.)								
Whole milk (1 PC)								
Staff Initials								
LUNCH								
Turkey sandwich package								
Salad								
Salad Dressing								
Peaches (1 PC)								
Ginger Cookie (1 small)								
Staff Initials								
DINNER								
Sirloin Tips With Gravy								
Corn								
Salad								
Salad Dressing								
Rolls (1)								
Butter								
Applesauce, unsweetened (1 PC)								
Staff Initials								
SNACK								
Trail Mix								
Staff Initials								

Instructions
Participant Food and Beverage Intake Report Form (FBI)

Purposes:

1. To record the number of unit foods eaten and alcoholic beverages consumed so that total calories may be computed.

2. To compute a compliance score for deviations from the diet protocol.

   Step 1: Prepare the form. Fill in the name, DELTA ID, day, date (that the B, L, D, S is eaten) and center. The participant will be completing the form for the time beginning **AFTER** the previous nights dinner through the end of the dinner meal being eaten.

   Step 2: Place the form FBI on the participant's tray at dinner each day.

   Step 3: Instruct the participant to fill in the form for the food and beverage they consumed since dinner on the previous night through the end of the meal being eaten.

   Step 4: Retrieve the form at the end of the meal when the tray is returned. Resolve any questions at the next meal service.

   Step 5: Code the form. Convert amount of alcohol into number of drinks. 12 oz. beer, 3 oz. dry wine, 1 shot (45 ml) hard liquor = 1 drink. Enter the number of drinks.

   Calculate the kcal in the food not eaten and enter. If the total kcal $>$ 100, enter 2 on the code line for question 3. If the kcal $\leq$ 100, enter 0.

   Calculate the kcal in food reported in question 4 and enter. If the kcal $>$ 100, enter 2 on the code line for question 4. If it is $\leq$ 100 kcal, enter 0.

   Step 6: Transfer the information from form FBI to form CCS for the corresponding day.

Participant Food and Beverage Intake Report

Please complete this form each day and return it to the DELTA kitchen staff or Study Coordinator.

Name _____ DELTA ID # _____

Date: ____ / ____ / ____
mm dd yy

1. How many unit foods did you eat on this day? ☐

2. Did you drink any alcoholic beverages on this day?

☐ No ☐ Yes If yes, list.

Specify type (Beer, Wine, Hard Liquor, etc.)	Amount	# Drinks (For administrative Use)

Total _____

3. Were there any study foods you did not eat/drink on this day?
Reasons include **missing, spilled, or inedible food**, illness or other.

☐ No ☐ Yes If yes, list.

Food	Amount	Reason	Kcal (For administrative use)

Total Kcal _____

4. Did you eat anything other than study foods or drink more than 5 caffeine containing beverages on this day?

☐ No ☐ Yes If yes, list.

Food or Drink	Amount	Kcal (For administrative use)

Total Kcal _____

DELTA
Dietary Effects on Lipoproteins and Thrombogenic Activity

Instructions for completing the DELTA Diet Deviation Form (DDD)

Purposes: To provide a record of deviations observed during on-site meal service, and to record deviations called in to DELTA staff members during weekends.

For on-site deviations:

Step 1: Check the meal tray. If any study food or beverage is left, fill in a DDD. All other information on deviations is obtained from form FBI filled out by the participant). If there are discrepancies between observations by the kitchen staff and information reported by the participant on the FBI, those should be adjudicated by the dietitian before filling in the CCS.

Step 2: If food is not eaten by the end of the day, calculate the kcal value of the food and enter in question _3_ of the DDD. This information will be used for calculating the compliance code.

For deviations called in during weekends:

Step 1: Fill in a DDD if a participant calls a DELTA staff member to report a deviation during a weekend (ex. missing, lost, spoiled food). All other information on deviations is obtained from form FBI filled out by the participant). If there are discrepancies between observations by the kitchen staff and information reported by the participant on the FBI, those should be adjudicated by the dietitian before filling in the CCS.

Step 2: If food is not eaten by the end of the day, calculate the kcal value of the food and enter in question _3_ of the DDD. This information will be used for calculating the compliance code.

DELTA DIET DEVIATION FORM

To be used by kitchen personnel for reporting ON-SITE and WEEKEND packed meal deviations ONLY!

Name of participant: _____ D E L T A I D : _____

Date of deviation: ___ / ___ / ___ Day: M T W Th F Meal: B L D S
mm dd yy

1. List the food/beverage left on the tray and the amount (for on-site meals) **OR** List the items and amounts that participants called the DELTA Staff member about (for weekend meals).

_____

_____

_____

_____

2. What was done with the food?

a) Given back to the participant to eat? YES ☐ NO ☐

b) Replaced at the next meal and eaten? YES ☐ NO ☐

c) Given back/replaced, but still not eaten? YES ☐ NO ☐

d) Not replaced, therefore not eaten? YES ☐ NO ☐

e) (Weekend) replaced with non-study food? YES ☐ NO ☐

f) Other, describe _____

_____

3. What was the kcal value of the uneaten or non-study food? ☐☐☐☐

Instructions
DELTA Compliance Check Sheet (CCS)

Purpose:

The DELTA Compliance Check Sheet (CCS Version A) has four purposes:

1. To document meal by meal that all study food provided was consumed, or if not consumed to document the deviations.

2. To record the number of unit foods consumed.

3. To record the number of alcoholic beverages consumed.

4. To record a compliance code.

Step 1: Prepare the CCS. Write in the Center name and the date when the food is to be eaten. Enter each participant's name and DELTA ID number.

Step 2: For on site meals, check the breakfast tray to assess whether any food was left. If all study food and beverage were eaten, enter N in the B (Breakfast) column. If food was left, enter D in the B column and fill in a DELTA Diet Deviation form (DDD).

Repeat this step for each participant.

Enter the personnel code or initials of the person checking breakfast.

Step 3: Repeat step 2 for lunch. If breakfast or lunch was a packed meal, refer to the corresponding Participant Food and Beverage Intake Report (form FBI) question 3. If question 3 was answered No, then enter N in Column L (Lunch or B for packed breakfast). If question 3 was answered Yes, enter D in column L (or B).

Step 4: Each participant will be given an FBI form to complete for the previous 24 hours. Repeat step 2 for dinner.

Step 5: Refer to form FBI for information on snacks and enter either N or D in column S (Snack).

For weekend packed meals, refer to the FBI Form for information for filling in columns B, L, D, S.

Instructions
DELTA Compliance Check Sheet (CCS)

Step 6: Refer to form FBI, question 1 and fill in the number of unit foods eaten. Enter the personnel code for the person filling in this column.

Step 7: Refer to form FBI, question 2, and fill in the number of alcoholic beverages reported. Enter the personnel code for the person filling in this column.

Step 8: Compute the total kcal value of deviations from form FBI and the deviation form (if any). Enter the total in column "Total kcal of deviations."

The total value is the sum of negative and positive values, e.g., 1 piece of bread (70 kcal) not eaten at breakfast, and a small apple (80 kcal) eaten in addition to study foods would = 150 kcal worth of deviations.

If more than 5 caffeine containing beverages were consumed in one day, notify study coordinator.

If there is a discrepancy between question 3 from FBI and the deviation form, it must be resolved with the participant before completing form CCS.

Step 9: Check the form carefully to be sure all information has been completed correctly before the form is sent for keying.

DELTA

Dietary Effects on Lipoproteins and Thrombogenic Activity

FORM CODE: CCS
VERSION A: 10/25/94

COMPLIANCE CHECK SHEET

CENTER ID: C20000

TODAY'S DATE: ____ / ____ / ____

(1)	Participant Name *(Mask all participant names before sending this form to the Coordinating Center.)*	DELTA I.D. No. (2)	Any food or beverages left? Enter N for none, D for deviation.				# of Unit Foods (from FBI form) (3)	# of Alc. bev. (from FBI form) (4)	Total kcal value of deviations (from deviations form) (5)
			B	L	D	S			
1.									
2.									
3.									
4.									
5.									
6.									
7.									
8.									
9.									
10.									
11.									
12.									
13.									
14.									
15.									
	Personnel code # or Initials ▸								

Shaded area must be keyed into the DELTA DMS

TURN OVER FOR SIDE 2 ▸

FORM CODE: CCS
VERSION A: 10/25/94

DELTA

Dietary Effects on Lipoproteins and Thrombogenic Activity

COMPLIANCE CHECK SHEET

CENTER ID: C20000

(1)	Participant Name *(Mask all participant names before sending this form to the Coordinating Center.)*	DELTA I.D. No. (2)	Any food or beverages left? Enter N for none, D for deviation.				# of Unit Foods (from FBI form) (3)	# of Alc. bev. (from FBI form) (4)	Total kcal value of deviations (from deviation form) (5)
			B	L	D	S			
16.									
17.									
18.									
19.									
20.									
21.									
22.									
23.									
24.									
25.									
26.									
27.									
28.									
29.									
30.									
		Personnel code # or Initials ▸							

Shaded area must be keyed into the DELTA DMS

SIDE 2

Instructions
DELTA Packed Meal (PMF) and Snack Forms

The **DELTA PACKED MEAL** (PMF) form and/or the **SNACK** form will be used for all carry out foods. These forms are a check list for the participant to verify what items have been packed in their carry out containers.

Before filling in the form, first verify that the menu number matches the corresponding menu number on the **TAC**.

Enter the first and last name of the participant, ID number, day and the date on which the food will be eaten.

Kitchen personnel will check off each item as it is put into the container or bag, and enter the packers code number. Before attaching the PMF to the container, be sure to cut off the corner identifying the diet. A Fresh-Check™ Time-Temperature Indicator (TTI) label will be placed in each cooler or bag that the participant takes off site. Each Participant will be given a copy of the TTI instruction sheet and instructed on food safety.

The **PMF** and/or **SNACK** form will be attached to the container. Instruct the participant to contact a DELTA staff member if any item checked off on the form is missing from the container.

Snack Form

Menu 1

Name: _____ ID: _____ Day: _____

Date: ___ / ___ / ___ Packed by: _____ Contact Tel. #: ___-___-_____
mm / dd / yy

Packed

- ☐ Trail Mix
- ☐ _____
- ☐ _____
- ☐ Free beverage

NOTE: If any part of your snack is missing or inedible be sure to note it on your Food and Beverage Intake Record for the day.

Snack Form

Menu 1

Name: _____ ID: _____ Day: _____

Date: ___ / ___ / ___ Packed by: _____ Contact Tel. #: ___-___-_____
mm / dd / yy

Packed

- ☐ Trail Mix
- ☐ _____
- ☐ _____
- ☐ Free beverage

NOTE: If any part of your snack is missing or inedible be sure to note it on your Food and Beverage Intake Record for the day.

PACKED MEAL FORM

D

MENU 1, 1500 Kcal

Name _____ I.D. _ _ _ _ _ _ _ _ _ Telephone Contact (___) _____ - _____

Day M T W T F Other _____ Date ___ ___ ___ Packed by _____
(Circle One) (Specify) mm dd yy

BREAKFAST	LUNCH	DINNER
Packed ☐ Orange juice ☐ Triples (1 PC) ☐ White bread (1 slice) ☐ Fat spread ☐ Dietetic jelly (1/2 oz.) ☐ Whole milk (1 PC) ☐ Free beverage	Packed ☐ Turkey sandwich package ☐ Salad ☐ Salad Dressing ☐ Peaches (1 PC) ☐ Ginger Cookie (1 small) ☐ Free beverage	Packed ☐ Sirloin Tips with Gravy ☐ Corn ☐ Salad ☐ Salad Dressing ☐ Rolls (1) ☐ Fat spread ☐ Applesauce, unsweetened (1 PC) ☐ Free beverage

UNIT FOODS		SNACK
# of Units	Packed	Packed
_____	☐ Roll	☐ Trail Mix
_____	☐ Pumpkin muffin	
_____	☐ Banana muffin	
_____	☐ Corn muffin	

Chicken Jambalaya

Diet D

Menu #2

Batch	1500	2000	2500	3000	3500
Rice, white, cooked and cooled	69.0	92.0	115.0	115.0	138.0
Tomato sauce	24.0	32.0	40.0	40.0	48.0
Onions, diced	8.0	10.5	13.0	13.0	16.0
Green peppers, diced	8.0	10.5	13.0	13.0	16.0
Celery. diced	7.0	9.5	12.0	12.0	14.0
Garlic, minced	1.1	1.4	1.7	1.7	2.0
Sugar	0.6	0.8	1.0	1.0	1.2
Spice mix (salt, black pepper and parsley flakes ⇒ proportion=8:1:1)	1/2 tsp	1/2 tsp	3/4 tsp	3/4 tsp	1 tsp
Tabasco sauce					
WEIGHT OF BATCH SERVING:	117.7	156.7	195.7	195.7	235.2
Batch Method: Combine rice, tomato sauce, onions, green peppers, celery, garlic, and sugar. Mix well. Portion into individual serving dishes. Add spice mix and 1-2 drops tabasco sauce to each serving. Fold to mix.					
Add to each serving:					
Egg yolk, dried	5.0	2.5	1.0	----	----
Chicken, breast meat, raw, cubed, patted dry with a paper towel	53.0	71.0	82.0	89.0	130.0
Lard	8.5	11.0	11.0	----	----
Coconut oil	8.5	11.0	12.0	27.5	36.0
Individual Method: Add cubed chicken, lard, coconut oil and egg yolk to individual portions. Mix well.					
Cooking Method: Bake uncovered in a preheated oven at 325° F for 20-25 minutes. (Convection 300° F for 15-20 minutes).					

APPENDIX II:

ADDITIONS and REVISIONS

To: Lynn Martin
From: Christine Wold
Date: September 26, 1994

Pumpkin Unit Foods:

We had several complaints during the Diet Run-In on the Pumpkin Unit Muffins. They were described as bland and tasting like cardboard. Increasing the cinnamon and/or using a pumpkin pie spice mix instead of the cinnamon, may improve the acceptability of this product. I checked to see how significant an increase in the spices would be. Doubling the amount of cinnamon would be an increase of 3 grams for the batch totaling 1494 grams of product. This is a .2% increase in the total batch size, and individual muffins would be affected by 0.1 gram which would probably not require an increase in the muffin size. Each center may want to try these suggestions and make adjustments based on regional tastes.

Also- we received our ~~potato~~ corn chips 😊

MOP Update - Chapter 5
10/24/94

Additional instructions for completing the FBI form:

Follow the standard rules for rounding when entering the number of alcoholic beverages consumed and # of unit foods eaten into the DELTA Data Management System (DMS). The initial version of the Food and Beverage Intake Record (FBI) only allows for a single digit with no decimal place entry into the Data Management System (DMS). Therefore:

These numbers should be rounded up or down to the nearest whole number when entering them into the DMS. Specifically, this means rounding numbers .4 and lower, down; and numbers .5 and higher, up to the nearest whole number. Both the exact number and the rounded number should be noted on the written form.

MOP Update - Chapter 5 - 02/22/95

DELTA MEMORANDUM

TO: **Study Coordinators and Dietitians/Diet Coordinators**

COPIES TO: Penny Kris-Etherton, Barbara Dennis and Susan Blackwell

FROM: Lynn Martin LM

DATE: February 22, 1995

SUBJECT: **Allowances for Dietary Deviations (TO BE USED ONLY IF REQUESTED BY PARTICIPANTS) for the three Fridays in Feeding Period 3 that fall during Lent**

In response to the request of three participants at Pennington, the following decisions have been made to address the needs of DELTA participants who do not want to eat meat on Fridays during Lent. **Before I review the decisions that have been made to allow for this request, LET ME EMPHASIZE AGAIN THAT THESE DEVIATIONS SHOULD ONLY BE ALLOWED IF THE PARTICIPANT INITIATES A REQUEST. THESE CHANGES ARE NOT TO BE OFFERED UNSOLICITED TO ALL PARTICIPANTS.**

IF A PARTICIPANT REQUESTS TO BE ALLOWED NOT TO EAT MEAT ON FRIDAYS DURING LENT, he/she may do the following:

1. The participant may remove the meat from the Friday lunch sandwich and not eat it. This will be recorded on the Participant Food and Beverage Intake Report (FBI) under #3; and will consequently get recorded onto a DELTA Diet Deviation Form and the Compliance Check Sheet for that participant.

2. At Friday dinner the participant may take the self-selected meal option but **MUST** take the DELTA Friday night meal as a packed meal to eat for the Saturday night dinner. This exchange of meals will be recorded as a Protocol Deviation in the Center's DELTA Log Book maintained in the kitchen at each site.

This plan should minimize deviations from the diets while making allowances for our participants religious convictions. HOWEVER, APPROPRIATE AND ACCURATE DOCUMENTATION OF THE DEVIATIONS IS REQUIRED TO INSURE THE CONTINUED INTEGRITY OF OUR STUDY.

Call Susan, Barbara, Penny, or me if you have questions or concerns about these decisions.

TABLE OF CONTENTS

CHAPTER 6: Postprandial Studies

6.0 POSTPRANDIAL STUDIES

6.0.1 Guidelines for the Preparation and Administration of the Test Meal

1. Label container with subject's name and date of preparation.

2. Set container on scale. Adjust scale to "0" g. Add 190g heavy whipping cream to container (total weight 190g).

3. Add 25g Promod to the center of container so that it doesn't stick to the sides (total weight 215g). Mix with a stirrer to prevent lumping. Use a small rubber spatula to scrape the stirrer into the container.

4. Add 30g Nestle's Quik Chocolate Syrup (or Strawberry Syrup) (total weight 245g).

5. Add 90g Breyer's Vanilla ice cream (total weight 335g).

6. Add 22g safflower oil (total weight 357g).

7. Insert beater and mix until smooth.

8. Add five (5) drops (0.2g) Lactaid. Mix again (total weight 357.2g).

9. Add calculated amount of Aquasol A. Mix.

   (Aquasol A may be added on the day of the test or 24 hours before the test, at the time the formula is prepared. If the Aquasol is added to the test meal in advance, the formula must be kept in an opaque container or put container in brown paper bag, or cardboard box, before refrigerating.)

10. Cover container with lid or Saran Wrap and refrigerate.

11. This formula is for a person with a body surface area of $2M^2$. If your participant has a body surface greater than $2M^2$, you will need to prepare extra formula by repeating steps 2 - 10.

    Body surface area is read from a Nomogram using height and weight measurements (see figure 6.2.2).

12. The amount of formula needed for the participant is calculated from the following formula: $\frac{\text{Participant's Body Surface Area}}{2} \times 357.2$

12a. The amount of Aquasol A for each participant is calculated from the following formula: $\frac{\text{Participant's Body Surface Area}}{2} \times 2$

13. Save 5g to 10g aliquot (or as much as is available--note each weight on test tube) in a stoppered, labelled test tube (see section 6.2). Freeze.

14. Serve formula with a straw. Use a squish bottle or wash bottle to wash sides of container and straw (both inside and outside). Have subject drink the rinsings.

Participant may have water or caffeine-free diet soda during the day.

Wahida Karmally, M.S., R.D., C.D.E.
Irving Center for Clinical Research
1990

6.0.2 Sampling for Fat Load Test

The fat formula steps 1-8 can be prepared in bulk the day prior to the study. On the day of the study, after Step 9, the amount of formula needed for each subject sould be weighed out -- Step 12. Three 5 to 10g aliquots must be saved -- Step 13.

General Formula:

Cream-Whipping-Heavy	190 gms
Ice Cream - 10% Fat	90 gms
Syrup Choc Flavored	30 gms
Promod	25 gms
Safflower Oil	22 gms
5 drops Lactaid	0.2 gms
Aquasol (vitamin A)	2.0 gms

Formula must be made 24 hours prior to serving so that all lactose can be converted to glucose and galactose by Lactaid.

1. Heavy cream: Tuscan Ultrapasterized Heavy Cream Grade A
   1 Tbsp (15 ml) = 50 calories
   6g fat
   3g SFA
   10mg cholesterol
   5mg Na^+
   10% fat ice cream is Breyer's Vanilla
   Syrup is Nestle's Quik - you can also use the Strawberry flavor

2. Vitamin A (Aquasol) must be added on the day of the study. Aquasol A drops .1ml has 5000 usb is manufactured by Armaur Pharmaceutical Co. Kankakee, Illinois 60901 <u>for</u> ASTRA USA Inc., Westborough, MA 01581.

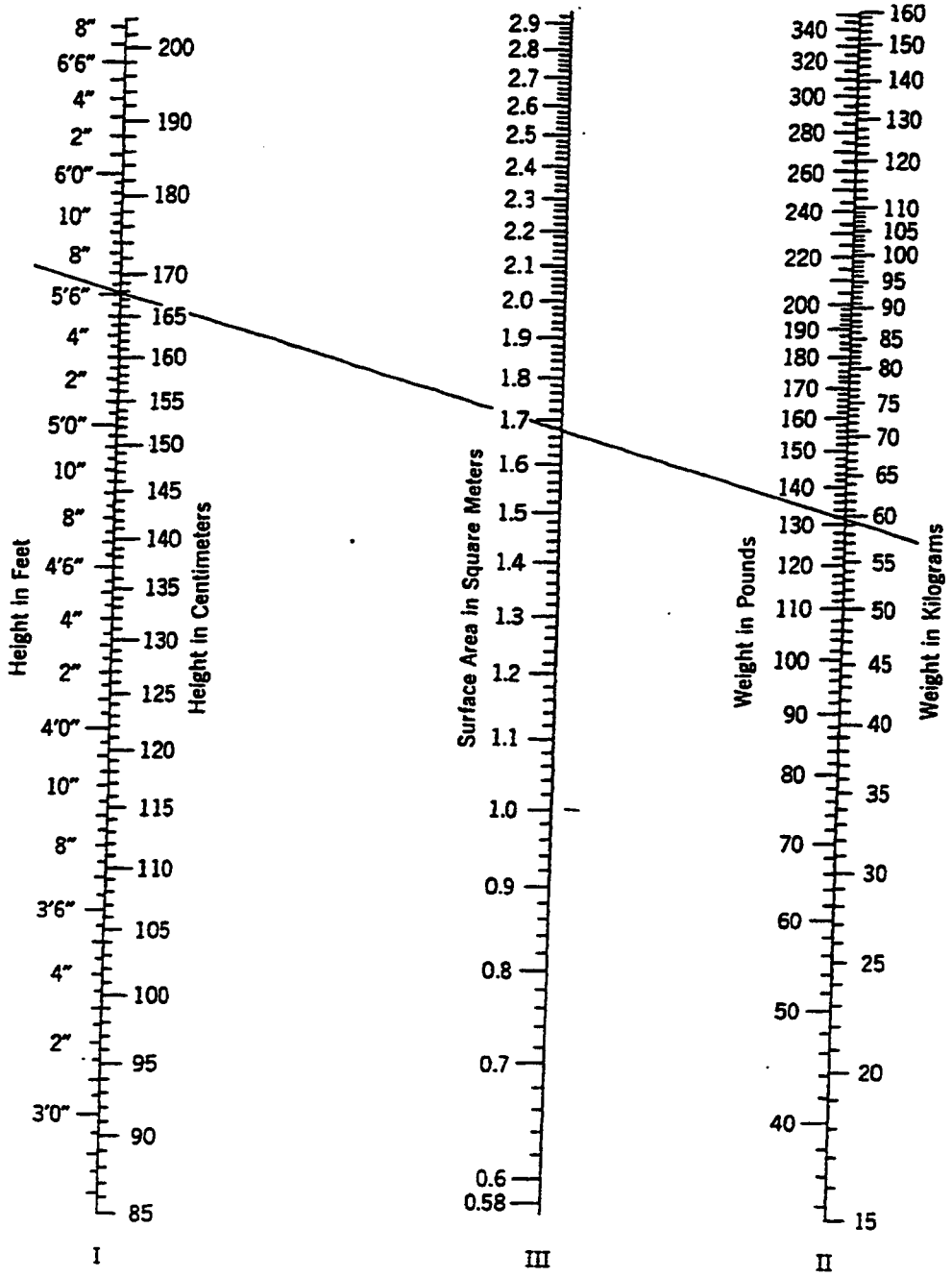
Promod is from Ross Laboratories, Columbus, OH.

Figure 6.0.2.1 Nutrient Values

Nutrient Values			
+Kilocalories	1237 kc	Protein	32.714 Gm
+Carbohydrate	47.786 Gm	+Fat	105 Gm
Fiber-Crude	0.060 Gm	+Cholesterol	300 Mg
+Saturated Fat	52.008 Gm	Oleic Fat Acid	22.811 Gm
+Linoleic FA	18.154 Gm	+Sodium	240 Mg
+Potassium	725 Mg	Magnesium	42.252 Mg
Iron	0.611 Mg	Zinc	1.630 Mg
Vitamin A	3161 IU	Vitamin D	190 IU #
Vitam. E/Total	9.899 Mg	Vitamin C	1.575 Mg
Thiamin	0.085 Mg	Riboflavin	0.455 Mg
Niacin	0.323 Mg	Vitamin B6	0.093 Mg
Folate	10.415 Ug	Vitamin B12	0.765 Ug
+Pantoth. Acid	0.931 Mg	Calcium	247 Mg
Phosphorus	402 Mg	+Tryptophan	181 Mg
+Threonine	512 Mg	+Isoleucine	623 Mg
+Leucine	994 Mg	+Lysine	814 Mg
+Methionine	227 Mg	+Cystine	130 Mg
+Phenylalanine	583 Mg	+Tyrosine	536 Mg
+Valine	764 Mg	+Histidine	281 Mg
Alcohol	0.000 Gm	Ash	1,964 Gm
+Copper	0.289 Mg #	+Manganese	0.114 Mg #
Iodine	35.120 Ug #	+Mono Fat	25.454 Gm
+Poly Fat	19.184 Gm	Caffeine	4.200 Mg
+Fluoride	57.000 Ug #	+Molybdenum	No Data #
Vitamin K	11.336 #	Selenium	0.001 Mg #
+Biotin	0.057 Ug #	+Chloride	No Data #
+Chromium	No Data #	Sugar	27.485 Gm
Fiber-Dietary	0.071 Gm	Vit. E/AT	7.542 Mg #

More than 50% of nutrient data is missing
+ Dietary Goal
Percent of KCALS from: PROT: 10% CARB: 15% FAT: 75%
Milk: 0.0 Veg: 0.0 Fruit: 0.0 Bread: 1.4 Meat: 2.5 Fat: 20.1

Figure 6.0.2.2 Nomogram for Calculating Surface Area



6.1 PREPARATION AND DELIVERY OF MEAL TEST

For the Postprandial meal test, the diets were modified, using the same foods, so that the distribution of fat, fatty acids and fiber was equalized across the 3 meals. See figure 6.1.1 for instructions on adding unit foods to Postprandial menus.

Figure 6.1.1 Adding Unit Foods to Postprandial Menus

DELTA - PROTOCOL 2

WORKSHEET FOR ADDING UNIT FOODS TO POSTPRANDIAL MENUS

General Instructions: For calorie levels of 2000 or less, use the 2000 kcal menu. For calorie levels between 2000 and 3000, use the 2000 kcal menu, and add unit foods to calibrate the meal. In order to distribute the unit foods equally across breakfast, lunch, and dinner, it will be necessary to WEIGH the unit foods. For example:

> If the target calorie level is 2100 kcal,
> weigh 1 unit and distribute 22.2% of that
> weight at breakfast, lunch, and dinner.

Use the following chart:

Calorie Increment (2000 or 3000 plus)	**Wt. of 1 Unit (grams)**	**Amt. at Each Meal (grams)**
100	_____g	22.2% = _____g
200	_____g	44.4% = _____g
300	_____g	66.7% = _____g
400	_____g	88.9% = _____g
500	_____g	111.1% = _____g
600	_____g	133.3% = _____g
700	_____g	155.5% = _____g
800	_____g	177.7% = _____g
900	X	X 2 units

Postprandial Post Meal Testing Form
(Week 6)

Form Code: PP1
Version A 5/31/94

DELTA ID: _____ Feeding Period Start Date: ____/____/____

(PP1A screen 1 of 2)

[Enter dates in format mm/dd/yy and times in format hh:mm.]

1. Period:____ **[Specify 1/2/3]**

2. Date of postprandial study:____/____/____

3. Was fasting sample collected today for main study? **YES NO**
[Circle either YES or NO. If YES, go to Q5.]

FASTING SAMPLE **[Q4 optional if using today's main study fasting sample]**

4. a. Time that fasting sample was collected:____:____ b. AM PM **[Circle response]**

c. Number of tubes for fasting sample:____

d. Code number of person drawing fasting blood:_____

5. a. Time that breakfast was started:____:____ b. AM PM **[Circle response]**

6. a. Time that breakfast was completed:____:____ b. AM PM **[Circle response]**

(PP1A screen 2 of 2)

BEFORE LUNCH SAMPLE **[4 hours after completion of breakfast]**

7. a. Time that before lunch sample was collected:____:____ b. AM PM **[Circle response]**

   c. Number of tubes for before lunch sample:____

   d. Code number of person drawing before lunch blood:_____

8. a. Time that lunch was started:____:____ b. AM PM **[Circle response]**

9. a. Time that lunch was completed:____:____ b. AM PM **[Circle response]**

BEFORE DINNER SAMPLE **[4 hours after completion of lunch]**

10. a. Time that before dinner sample was collected:____:____ b. AM PM **[Circle response]**

    c. Number of tubes for before dinner sample:____

    d. Code number of person drawing before dinner blood:_____

11. Comments? YES NO **[Circle response. If YES, enter comments below.]**

_____

_____

_____

_____

12. Code number of person completing this form:_____

NOTE PAGE

DELTA ID:_____ DATE:___/___/___

NAME:_____
first middle last

PERSONNEL CODE NUMBER _____

Postprandial Standard Fat Load Form (Week 7)

Form Code: PP2
Version A 5/31/94

DELTA ID: _____ Feeding Period Start Date: ____/____/____

(PP2A screen 1 of 2)

[Enter dates in format mm/dd/yy and times in format hh:mm.]

1. Period:____ **[Specify 1/2/3]**

2. Date of postprandial study:____/____/____

3. Was fasting sample collected today for main study? YES NO
[Circle either YES or NO. If YES, go to Q5.]

FASTING SAMPLE **[Q4 optional if using today's main study fasting sample]**

4. a. Time that fasting sample was collected:____:____ b. AM PM **[Circle response]**

c. Number of tubes for fasting sample:_____

d. Code number of person drawing fasting blood:_____

5. Weight of fat load offered to participant (gms): | | | | |

6. a. Time participant started drinking fat load:____:____ b. AM PM **[Circle response]**

Postprandial Standard Fat Load Form
(Week 7)

(PP2A screen 2 of 2)

7. a. Time participant completed drinking fat load:____:____ b. AM PM **[Circle response]**

8. Weight of any fat load remaining (gms): | | | | |

4 HOUR SAMPLE

9. a. Time that 4 hour sample was collected:____:____ b. AM PM **[Circle response]**

c. Number of tubes for 4 hour sample:____

d. Code number of person drawing 4 hour blood:_____

8 HOUR SAMPLE

10. a. Time that 8 hour sample was collected:____:____ b. AM PM **[Circle response]**

c. Number of tubes for 8 hour sample:____

d. Code number of person drawing 8 hour blood:_____

11. Comments? YES NO **[Circle response. If YES, enter comments below.]**

_____

_____

_____

_____

12. Code number of person completing this form:_____

Postprandial Standard Fat Load Form
(Week 7)

NOTE PAGE

DELTA ID:_____ DATE:___/___/___

NAME:_____
first middle last

PERSONNEL CODE NUMBER _____

Remember to recheck the body surface area of a participant who has lost or gained weight since the last feeding period BEFORE calculating the amount of formula they will need for the PPL study!

TABLE OF CONTENTS

CHAPTER SEVEN: Food Analysis

7.0 FOOD ANALYSIS PROCEDURES

DELTA Protocol I

Food Collection and Shipping

The FALCC will supply each field center with the materials and SOP for collection and shipping menus and unit foods. Food collection containers will be pre-labeled and pre-weighed. The food collection and shipping procedure should be followed exactly at each field center. Each field center will be requested to report an deviations to the FALCC, which will document such occurrences. All shipments of food will be made with either dr ice or frozen (-20°C) cold packs via overnight deliver.

Log-in

Each container of food will be weighed, and the weight will be logged into the FALCC sample database. All samples will be stored at -20°C or lower until composited.

Compositing

Each food sample (menus and unit foods) will be composited according to the appropriate Standard Operating Procedure for that sample:

Diet validation. For diet validation, each menu and each unit food for each diet (prepared in duplicate) will be individually composited.

Monitoring. Each diet cycle throughout the feeding stud, a diet cycle composites will be prepared for each diet.

Sample distribution

All composited samples will be stored at -60°C or lower:

Archive samples
Archive samples will be stored for the duration of the DELTA stud. Place five (5) samples (see Appendix C) of each composite into the archive location designated by supervisor. The dispensation of these samples requires approval by the Steering Committee.

Assay samples
Assay samples will be used for the nutrient assays specified below. An samples that remain after completion of the nutrient assays will be retained for a minimum of 3 months from the completion of Protocol I.

Reserve samples
Reserve samples are extra samples taken by FALCC to accommodate repeat assays, additional assays, etc. There will be a minimum of eight (8) reserve samples per composite. Reserve samples will be used at the discretion of the FALCC. An samples that remain after completion of the nutrient assays will be retained for a minimum of 3 months from the completion of Protocol I.

Assay Quality Control

Control samples: For each assay, a control sample (mixed diet composite) will be included with each batch of samples or at least ever 20 samples, whichever is less.

QC charts: A QC chart will be established for each assay. Prior to running samples, a minimum of 15 samples of the control material will be assayed, according to the assay SOP. The mean and +/- 2*SD and +/-3*SD will be calculated from these data and plotted as a QC chart. The value for the control material in each assay batch will then be plotted on the QC chart. If the control value falls outside the +/-3*SD limits, the assay will be rejected. The procedure will be evaluated for possible sources of error, corrected if indicated, then samples will be rerun.

Blinded samples: An internal sample numbering system will be used so that the analyst cannot decipher information about a sample from its number. In this wa, analyst bias will be minimized. The sample database maintains a link between the sample number and all information concerning that sample. The identity of samples will not be disclosed to the analysts prior to performing assays.

SOPs: FALCC will write Standard Operating Procedures (SOPs) for all nutrient assays and laboratory procedures. Each SOP will be signed and dated. Original copies will be kept in a central notebook with disk copies on file as well. Non-current SOPs will be archived.

Assays methods and validation

Total fat

Method: Total lipid will be determined gravimetrically after extraction of the diet composites with chloroform/methanol (modified AOAC 983.23, 1990; see Appendix G4).
Validation: Validation of the method will be based on acceptable recover of canola oil spiked into composited mixed diets, and acceptable results for standard reference material (NIST SRM #1548[1]). Results will also be compared to data obtained by NCL using acid hydrolysis and modified Folch assays.

[1]National Institute of Standards and Technology (NIST), Gaithersburg, MD

Cholesterol

Method: The method of R. Thompson [Thompson and Merola [USDA Nutrient Composition Laboratory (NCL), Beltsville, MD, 1992], with modifications, will be used to quantify cholesterol (see Appendix G5). The method has been adapted as follows: 1) GC analysis with temperature programming (60°C hold 1 min., then 30°C/min. to 270°C hold for 20 min.) instead of isothermal at 267°C, 2) GC split ratio decreased to 1:20, 3) injection volume decreased to 0.5 μl, 4) clohexane added before water in saponification step of sample preparation, 5) 250 μl of derivatization reagent used.

Validation: Validation of the method will be based on acceptable recover of cholesterol spiked into mixed diet composites, acceptable results for standard reference material (fortified coconut oil, NIST #1563-2), agreement of results with those obtained by Rick Thompson for EZ menus analyzed for Pilot Stud #1, and favorable comparison with independent measurements of the cholesterol content of the control sample by NCL.

Moisture

Method: Moisture in the diet composites will be determined with a microwave moisture/solids analyzer (CEM Corp.) (Appendix G6). Moisture will be measured in triplicate for each diet composite. These results will be used to calculate all assay results for the composite on a dr weight basis.

Validation: Validation of the method will be based upon favorable comparison of results by microwave drying (FALCC SOP #5007, App. G6) with those from conventional vacuum oven drying (AOAC 934.01, modified; Appendix G7), acceptable results obtained for NCL reference sample #Q93-FR-4495 used in Pilot Stud #1 assays of EZ menus, and favorable comparison of FALCC moisture data for the control sample with those obtained independently by NCL.

Fatty acids (saturated, monounsaturated, polyunsaturated, omega-3)

Method: Fatty acid composition will be determined by gas-liquid chromatograph of fatty acid methyl esters prepared from the saponified lipid extracts of diet composites (chloroform/methanol extracts, as prepared for total fat, Appendix G4) (AOCS Official Method Ce 1b-89; 1991, modified). The AOCS method has been adapted to separate and quantitate C:10 and higher fatty acid methyl esters (FAMEs) by lowering the initial temperature (from 170°C to 150°C) (see Appendix G8). The injection volume was also decreased. FAMEs will be reported as triacylglerol equivalents (TAGs) according to the classification scheme shown in. Fatty acids as TAGs will be normalized to the total fat content (determined as described above).

Validation: Validation will be based upon acceptable fatty acid recoveries from canola oil spiked into diet composites, and favorable comparison of FALCC data for the control sample with those obtained independently by NCL.

Ash

Method: Ashing will be accomplished by vacuum-drying wet diet composite samples, then heating until at 550C in a muffle furnace until completely ashed (Appendix G10).

Validation: Validation will be based upon acceptable ash content determined for NIST SRM #1548, and favorable comparison of FALCC ash data for the control sample with those obtained

independently by NCL.

Protein

Method: Protein will be determined as Kjeldahl nitrogen x 6.25. FALCC will subcontract Kjeldahl assay to the Dept. of Human Nutrition and Foods (HNF) at Virginia Tech, which has a semi-automated system. FALCC control samples (blinded) and HNF internal QC samples will be included in each assay batch.

Validation: Validation of the method will be based on acceptable results obtained for NIST SRM #1548, and favorable comparison of results for the control sample with data obtained independently by NCL.

Dietary Fiber

Method: AOAC method 991.43 will be used to measure total dietary fiber. The procedure will be modified for the assay of wet homogenized diet samples instead of ground freeze-dried samples (see Appendix K).

Validation: Validation of the method is based on acceptable results for NIST SRM #1548, and AOAC dietary fiber collaborative stud samples[2]. An modifications to the AOAC method will be validated in this wa as well.

[2]Lee et al., JAOAC Intl., **75**: 395-416, 1992.

Data to be reported

component	units
total fat	g/100g dr weight
total SFA	g/100g dr weight (as triglcerides)
total MUFA	g/100g dr weight (as triglcerides)
total PUFA	g/100g dr weight (as triglcerides)
EPA[3]	g/100g dr weight (as triglcerides)
DHA[3]	g/100g dr weight (as triglcerides)
α-L[3]	g/100g dr weight (as triglcerides)
cholesterol	mg/100g dr weight
protein	g/100g dr weight
ash	g/100g dr weight
dietary fiber	g/100g dr weight
moisture	g/100g
total weight	grams
Calculated:	
total dry weight[4]	grams
carbohydrate[5]	g/100g dr weight
total energy[6]	kcal
total fat[7]	% of total kcal
SFA[8]	% of total kcal
MUFA[9]	% of total kcal
PUFA[10]	% of total kcal

---

[3]EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; α-L: α-linolenic acid

[4]total wet weight * (1-%H_2O/100)

[5]100 - g protein/100g dry wt - g ash/100g dry wt - g total fat/100g dry wt

[6]total kcal = [g protein/100g dry wt + g CHO/100g dry wt)*4 + (g total fat/100g dry wt)*9] * total dry wt/100

[7] $\frac{\text{(g total fat/100 g dry wt)*9*total dry wt/100}}{\text{total kcal}}$ *100

[8] $\frac{\text{(g SFA/100 g dry wt)*9*total dry wt/100}}{\text{total kcal}}$ *100

[9] $\frac{\text{(g MUFA/100 g dry wt)*9*total dry wt/100}}{\text{total kcal}}$ *100

[10] $\frac{\text{(g PUFA/100 g dry wt)*9*total dry wt/100}}{\text{total kcal}}$ *100

Calculated:

component	units
EPA[11]	% of total kcal
DHA[11]	% of total kcal
α-L[11]	% of total kcal

Laboratory Quality Assurance

Instrument/equipment calibration

All balances, instruments, and equipment will be calibrated at least weekly, and more frequently if appropriate. The temperature of refrigerators and freezers is monitored daily. Balances will be calibrated using the protocol and standard weights specified in the DIET MOP.

Audit trail

Because of the large volume of samples and the complexity of processing and assaying samples, the FALCC has developed an internal database to assign numbers and keep track of data concerning an given sample. The database will maintain a link between an given whole food sample and its composited subsamples and all related data and assay results.

Storage of data

Electronic data will be stored on hard drives and floppy disk. Computers will be backed up weekly. Disk copies and hard copies of all data will be archived.

[11] $\frac{(\text{g FA/100 g dry wt})*9*\text{total dry wt}/100}{\text{total kcal}}*100$

SOP # 5011
Date: 01-08-94
Revision: 4

Food Analysis Laboratory Control Center (FALCC)
Standard Operating Procedure

TITLE: **Determination of Ash in Diet Composites**

Scope: This procedure applies to diet composites with 60 - 80% moisture content.

1. Purpose

To describe the procedure for determining the ash content of diet composites.

2. Safety

2.1 Wear powder free gloves at all times.

2.2 Use caution when operating furnace at 550°C.

3. Materials

3.1 porcelain crucibles (Coors # **60105**, Fisher # 07965-D, or equivalent)
3.2 heat-resistant ink pen
3.3 powder free gloves
3.4 long-handled metal forceps
3.5 desiccator
3.6 analytical balance (e.g. Sartorius Basic, M1-22-10 or M1-31-3, SOP# 1008), or equivalent
3.7 diet composite (SOP# 5005); (if at -60°C, thawed in refrigerator for 12-48 hours)
3.8 SOP #5028 (Use and Handling of Food Composite Samples)
3.9 stainless steel spatula
3.10 muffle furnace (550°C), Dr. Bunce - Room 201
3.11 I.B.M. personal computer and printer (e.g. M1-34-10), or equivalent, with Quattro® and file ash.wk1 installed

4. Procedure

Note: **To avoid weight from fingerprints, do not handle the crucibles with bare hands; use forceps or wear powder free gloves. Use forceps when removing crucibles from the furnace.**

Note: **Record all data on an "Ash Worksheet" (see enclosed sheet-Form F007).**

Note: **Before beginning the analysis, select an assay number from the assay number log-out book. See note in front of book for instructions.**

Note: Before beginning assay, read SOP #5028 (Use and Handling of Food Composite Samples).

4.1 If not already labeled, label each crucible with a heat-resistant ink pen for identification.

4.2 Place empty crucibles in the muffle furnace. Close the muffle furnace door.

4.3 Turn the muffle furnace switch to "on". Turn the temperature dial to 550°C (once the muffle furnace temperature reaches 550°C, the cycle light will begin to flicker).

4.4 Leave the empty crucibles in the muffle furnace at least 2 hours and up to 24 hours (e.g. overnight), timed from when the temperature reaches 550°C (takes ~ 1 hour to reach 550°C).

4.5 Turn the muffle furnace switch to "off" and crack the door.

4.6 Allow the crucibles to cool in the muffle furnace for 30 minutes.

4.7 Place the crucibles in a desiccator and allow to cool completely (~1 hour).

Note: Crucibles may be pre-ashed (steps 4.2-4.7) and stored in a desiccator until needed.

4.8 Turn on the balance. The display should read "0.00000g".

4.9 Turn on the computer. Turn on the monitor. The display should read "C:\>".

4.10 At the "C:\>" prompt, type "cd\quat". Press "Enter". At the "C:\QUAT>" prompt, type "q". Press "Enter".

4.11 When the quattro screen appears, hit the "/" key to select the quattro menu.

4.12 Move the cursor bar to "file" and press "Enter" to select the file menu.

4.13 Press "Enter" a second time to retrieve the list of files. Move the cursor bar to "Ash.wk1" and press "Enter" to open the file.

4.14 Weigh a cooled, pre-ashed crucible to the nearest 0.00001 gram.

Note: Always use the same balance throughout the entire procedure.

4.15 Record the crucible weight and the crucible number in the spreadsheet. To record the crucible weight, press the "print" key on the balance to transfer the weight value to the appropriate box in the spreadsheet.

4.16 After recording the crucible weight, press "tare" to zero out the crucible weight.

4.17 Aliquot diet composite sample following instructions in **SOP #5028**, using the rounded end of a stainless steel spatula, and weighing 2 to 2.4 grams (to the nearest 0.00001 gram) into the crucible.

Note: **Do not attempt to achieve any specific target weight. It is most important to accurately measure a weight between 2 and 2.4 grams, without prolonged exposure of the sample during weighing.**

4.18 Record the sample weight and the sample number in the spreadsheet. To record the sample weight, press the "print" key on the balance to transfer the weight value to the appropriate box in the spreadsheet.

Note: **The total weight (crucible and sample weight to the nearest 0.00001 g) is already calculated in the spreadsheet.**

4.19 Transfer the crucible to a clean, dry tray for transportation to the muffle furnace.

4.20 Repeat steps **4.14-4.19** for each sample.

4.21 Place the crucibles in the muffle furnace. Close the muffle furnace door half way.

4.22 Turn the muffle furnace switch to "on". Turn the temperature dial to 150°C (once the muffle furnace temperature reaches 150°C, the cycle light will begin to flicker).

4.23 Leave the crucibles in the muffle furnace for 45 minutes at 150°C. Make a note in bench book of exact heating time. After the 45 minutes are up, close door completely and turn temperature dial up to 550°C and leave overnight. Make a note in bench book of time temperature was turned up.

4.24 Turn the muffle furnace switch to "off" and crack the door.

4.25 Allow the crucibles to cool in the muffle furnace for 30 minutes.

4.26 Place the crucibles in a desiccator and allow to cool completely (~ 1 hour).

Note: **A grayish white powder should result after ashing. If not, notify supervisor and fill out form F015-Internal Deviation form.**

4.27 Weigh each crucible with ash residue to the nearest 0.00001 gram.

4.28 Record the weight in the spreadsheet. To record the weight, press the "print" key on the balance to transfer the weight value to the appropriate box in the spreadsheet.

4.29 Once all the data has been obtained, save the data program with a filename that corresponds to the assay number (i.e. assay # A1 has the filename A001.)

5. Storage

5.1 Discard the ash residue after weighing.

6. Calculations

6.1 Calculation of percent ash on wet basis

A = weight of crucible, (ash; grams) (step **4.28**)
B = weight of crucible, (pre-ashed; grams) (step **4.15**)
C = weight of crucible & food sample (undried; grams) (step **4.18**)

$$\% \text{ ash (wet)} = \frac{(A - B)}{(C - B)} * 100$$

Report ash as grams/100 grams wet weight.

Note: The calculations for percent ash on a wet basis are performed in the spreadsheet.

7. Reference

7.1 AOAC Official Methods of Analysis, "Ash of Sugar and Syrups Final Action", #31.012 and #31.013, 1984.

Prepared by:

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Date: _____

Approved by:

Katherine Phillips

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Date: _____

SOP # 5016
Date: 09-JUL-93
Revision: 1

Food Analysis Laboratory Control Center (FALCC)
Standard Operating Procedure

TITLE: Preparation of Fatty Acid Methyl Ester Standards

1. Purpose

To describe the preparation of the fatty acid methyl ester standards used to create a three point calibration curve for the quantitation of fatty acids in food samples by GC.

2. Safety

2.1 Read the Material Safety Data Sheet (MSDS) for iso-octane

2.2 Most chemicals are skin irritants, wear gloves, work in fume hood

3. Materials

3.1 GLC fatty acid methyl ester reference standards (e.g. from Nu Chek Prep, Inc., or Sigma)(purity $\geq$ 99%)
3.2 Iso-octane (GLC grade, e.g. Fisher - Optima® grade)
3.3 Clean glass volumetric flasks (5 and 10 mL)
3.4 Automatic pipets and tips (various sizes) (e.g. Pipetman®)
3.5 Micro Balance
3.6 Powder-free gloves
3.7 Fume hood
3.8 Nitrogen
3.9 GC autosampler vials with caps and crimper
3.10 Cryogenic marker
3.11 Standard Food Composition Table (e.g. USDA Handbook No. 8)
3.12 N-Evap evaporator (M1-16-3 or M1-34-3)

4. Procedure

Note: For each reference standard and each internal standard (C11:1, C19:0, C23:0), make up stock solutions of ca. 10 mg/ml as follows:

4.1 Standards that come in amounts of approximately 100 mg

4.1.1 Allow vial containing ca. 100 mg of standard to come to room temperature.

4.1.2 Pre-weigh (W1) a clean 10 mL volumetric flask (to nearest 0.1 mg).

4.1.3 Add 1 mL iso-octane to the vial containing the standard, swirl to mix, and quantitatively transfer to the tared volumetric flask.

4.1.4 Repeat step **4.1.3** at least 3 times.

4.1.5 Evaporate the iso-octane solution in the volumetric flask to absolute dryness under a stream of nitrogen.

4.1.6 Weigh (W2) flask and dried standards (to nearest 0.1 mg).

4.1.7 Add 8 mL of iso-octane, stopper tightly, and dissolve by shaking vigorously.

4.1.8 Make up to volume with iso-octane, stopper tightly, and mix by inverting and swirling vigorously.

4.1.9 Calculate concentration of standard stock solution:

where: SC = Concentration of Standard Stock Solution
W1 = Weight of empty Volumetric Flask
W2 = Weight of Volumetric Flask with Dried Standard
VF = Volume of Volumetric Flask

4.2 Standards that come in amounts larger than 100 mg

4.2.1 Accurately weigh (W3) out ca. 100 mg (to nearest 0.1 mg) (50 mg for C11:1 and C23:0) using a spatula (solids) or a pasteur pipet (liquids) of the standard into a clean 10 mL volumetric flask.

4.2.2 Add 8 mL of iso-octane, stopper tightly and dissolve solids by inverting and swirling vigorously.

4.2.3 Make up to volume with iso-octane, stopper tightly, and mix by inverting and swirling vigorously.

4.2.4 Calculate concentration of standard stock solution:

$$SC[mg/mL] = \frac{W3[mg]}{VF[mL]}$$

where: SC = Concentration of Standard Stock Solution
W3 = Weight of Standard

VF = Volume of Volumetric Flask

4.3 Preparation of calibration standards including internal standards

4.3.1 Calculate the expected approximate amount of each fatty acid in the final extract using a food composition table or results of previous studies with similar diets using the following equation (example see attached):

$$C[mg/mL] = \frac{CFA[\%] * F[g] * AV[mL] * 1000[mg/g]}{100[\%] * TV[mL] * V[mL]}$$

where: C = Expected concentration of fatty acid in final extract
CFA = Concentration of fatty acid in food (composition table)
F = Amount of food sample extracted (i.e. 5 g)
AV = Aliquot volume of lipid extract (i.e. 10 mL)
TV = Total volume of lipid extract (i.e. 80 mL) (see SOP# 5015)

$$SC[mg/mL] = \frac{(W2 - W1)[g] * 1000[mg/g]}{VF[mL]}$$

V = Final volume of extract (i.e. 1 mL)

4.3.2 Multiply the concentration of fatty acids in final extract by 5 to establish the upper limit of the calibration curve.

4.3.3 Calculate the amount of each GLC reference standard stock solution to be pipetted to get a calibration standard that contains the fatty acids as determined (**4.3.2**) using the following equation (example see attached):

$$V[mL] = \frac{C[mg/mL] * VF[mL]}{SC[mg/mL]}$$

where: V = Volume to be pipetted
C = Concentration of fatty acid in final volume (from **4.3.2**)
SC = Concentration of standard stock solution
VF = Volume of volumetric flask (i.e. 5 mL)

4.3.4 Pipet an amount that is close, but convenient to pipet to this calculated volume (**4.3.3**) into a 5 mL volumetric flask. Record the pipetted volume (V). Also pipet 0.5 mL of each of the three internal standard stock solutions into the flask (approximate concentration: 1.0 mg/mL C19:0 and 0.5 mg/mL C11:1 and C23:0).

Note: Since it will be necessary to evaporate some of the solvent in the volumetric flask under a slow stream of nitrogen so that the volume stays below 5 mL, pipet the long chain fatty acid

esters first and the short chain fatty acid esters last!.

4.3.5 Make up to volume with iso-octane, stopper flask tightly and mix by inverting and swirling vigorously (**Calibration Solution 1**)

4.3.6 Pipet 1.0 mL of calibration solution 1 into a second 5 mL volumetric flask

4.3.7 Calculate the volume of each of the internal standard stock solutions needed to get the same concentration of internal standards as in Calibration Solution 1.

$$ISV[mL] = \frac{(ISC[mg/mL] * V1[mL]) - (ISC[mg/mL] * V2[mL])}{ISStock[mg/mL]}$$

where: ISV = Volume of internal standard stock solution needed
ISC = Concentration of internal standard in calibration solution
V1 = Volume of volumetric flask (i.e. 5 mL)
V2 = Volume of calibration solution pipetted into flask
ISStock = Concentration of internal standard stock solution

4.3.8 Pipet this volume (ISV) into the second volumetric flask and make up to volume with iso-octane. Mix by inverting and swirling vigorously (**Calibration Solution 2**).

4.3.9 Pipet 1.0 mL of calibration solution 2 into a third 5 mL volumetric flask.

4.3.10 Repeat steps **4.3.7** and **4.3.8** and make up to volume with iso-octane and mix by inverting and swirling vigorously (**Calibration Solution 3**).

Note: The concentration of the internal standards should be identical in all three calibration solutions

4.3.11 Pipet 1.0 mL of the calibration solutions into three GC autosampler vials, cap, and label.

4.3.12 Mark volume level on outside of vial using a cryogenic marker.

5. Storage

5.1 Store the individual stock solutions (**4.1 and 4.2**) and calibration standard solutions (**4.3**), **tightly sealed,** at -20°C (up to 6 months for saturated and monounsaturated fatty acids; up to 3 months for polyunsaturated fatty acids and the calibration standards). Bring to room temperature and vortex before using.

6. Calculations

6.1 The calculations for the standard stock solutions and the internal standards in the calibration solutions are located in their respective procedure section.

6.1 For each standard, calculate the final concentration in the calibration solution 1 (**4.3**):

$$FC = \frac{SC[mg/mL] * V[mL]}{VF[mL]}$$

where: FC = Final Concentration of Each Standard in Calibration Solution
SC = Concentration of Individual Standard or Internal Standard in Stock Solution (step 4.1 or 4.2)
V = Volume of Stock Solution Added to Volumetric Flask
VF = Volume of volumetric flask

6.3 The concentration of the reference standards in calibration solution 2 and 3 are the concentrations in the calibration solution 1 divided by 5 and 25 respectively.

Prepared by:

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Approved by:

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Date: _____

SOP # 5018
Date:12-JUL-94
Revision: 2

Food Analysis Laboratory Control Center (FALCC)
Standard Operating Procedure

TITLE: Internal Standard Spikes for Fatty Acid Methyl Ester Quantitation

1. Purpose

To describe the spiking procedure for incorporating the internal standards for fatty acid methyl ester quantitation into lipid extracts.

2. Safety

2.1 Read the Material Safety Data Sheets for chloroform and the triglycerides

2.2 Since most of the chemicals are skin irritants, wear gloves and work in fume hood

3. Materials

3.1 Triglyceride internal standards (C11:1, C19:0, and C23:0) (e.g. NuChek Prep, Inc. or Sigma) (purity $\geq$ 99%)
3.2 Brinkmann Dispensette Bottletop Dispenser (5 ml) (catalog # 50-10-020-1)
3.3 Screw-cap glass bottles with Teflon® lids (250 ml)
3.4 13 x 100 mm screw-cap glass tubes (15 ml), with Teflon® lids
3.5 Powder-free gloves
3.6 Nitrogen
3.7 Micro Balance (M1-31-3 or M1-22-10)
3.8 Fume Hood
3.9 Volumetric Flask with glass stopper (1 liter)
3.10 Chloroform (Fisher, HPLC grade, catalog # C-606-4)
3.11 Stir Bar and Magnetic Stirrer
3.12 Weighing Funnels (25 x 55 mm)
3.13 Funnels of size that fit 250 ml glass bottle (one funnel for each bottle)

4. Procedure

4.1 Preparation of Triglyceride Internal Standard Working Solutions

4.1.1 Precisely (near to 0.01 mg) weigh 166.40 mg of tritricosanoin (C23:0 TG), 166.07 mg of triundecenoin (C11:1 TG) and 355.83 mg of trinonadecanoin (C19:0 TG) individually into three clean weighing funnels (record exact weight).

4.1.2 **Quantitavely**, in the fume hood, transfer weighed triglycerides into volumetric flask as follows: place carefully the weighing funnel containing C23:0 TG into the volumetric flask, the contents of weighing funnel will be emptied in volumetric flask. Wash weighing pan with at least 30 ml of chloroform, using a pasteur pipette and swirling pipette while washing. Then, pour 200 ml to 250 ml of chloroform (no pasteur pipette) to completely wash away any C23:0 TG left in weighing funnel. Remove weighing funnel. Place weighing funnel containing C19:0 TG into volumetric flask (contents of it will be emptied into volumetric flask), and proceed washing weighing funnel as explained above. Remove weighing funnel. Place weighing funnel containing C11:1 into volumetric flask (contents will not be immediately emptied into volumetric flask, C11:1 is a viscous liquid at room temperature), and wash weighing funnel as explained above. Remove weighing funnel. Wash neck of volumetric flask with 30 ml of chloroform, using a pasteur pipette and swirling pipette while doing it.

4.1.3 Take volume to one liter with chloroform, add a stir bar quickly, stopper volumetric flask and place on stir plate until triglycerides dissolve. It takes 45 to 60 minutes for C23:0 TG to dissolve completely; C11:1 and C19:0 TG dissolve faster. Some C23:0 TG may be sticking to the neck of volumetric flask; in order to incorporate this residue into solution, it is necessary to swirl or invert volumetric flask carefully, and then, stir until this residue is dissolved (notice that this is done after the 45 to 60 minute period when mostly all components are dissolved).

4.1.4 Using a funnel, **rapidly** transfer solution into one of the 250 ml screw cap glass bottles, cap volumetric flask **immediately** then cap glass bottle **fast** and tightly under nitrogen to avoid evaporation of the solvent. Repeat the same procedure for the other 3 screw cap bottles; **use a clean funnel to pour solution into each 250 ml bottle**, to avoid concentration of components in funnel as chloroform evaporates on its surface.

4.1.5 Calculate the concentrations of the solutions and record in the chemical standards book. Label the bottle with the chemical standard number, component names, concentration, date, and your initials.

4.1.6 Store bottles in the freezer at -20° C.

4.2 Preparation of Internal Standard Tubes

4.2.1 Remove working solution from freezer and let it warm up to room temperature for 60 minutes (24° to 26° C), in the lab bench. Mix solution thoroughly, by swirling or inverting bottle gently.

4.2.2 Assemble 5 ml bottle top dispenser according to manufacturers instructions. Wash assembled 5 ml bottle top dispenser with 40 ml of methanol, and then with 40 ml of chloroform. Empty chloroform from dispenser filling tube and discharge tube before connecting dispenser to reagent bottle, in order to avoid dilution of internal standard solution.

4.2.3 Remove cap from internal standard solution bottle, and replace **quickly** with the 5 ml bottle top dispenser. Dispense twice some of the solution into a waste beaker, to wash the dispenser. Set volume of dispenser to exactly 3 ml, dispense one more time into the waste beaker to get any air bubbles out, and start dispensing 3.0 ml of the triglyceride internal standard working solution (C11:1, C19:0, C23:0) into each of the 15 ml screw-cap tubes. Use up as much of the solution as permitted by dispenser filling tube, before getting any air, and discard remaining solution left in flask. Store tubes in the freezer at -20° C, tightly capped under nitrogen.

4.2.4 Clean 5 ml bottle top dispenser with 40 ml of chloroform, 40 ml of methanol, and 40 ml of distilled water. Disassemble dispenser (read manufacturers manual: "Special piston cleaning procedure") and soak in soapy water (no detergent residues should be present in soapy water, since they would clog dispenser) for 1 hour, then brush glass cylinder carefully. Rinse all parts thoroughly with tap water, distilled water and deionized water. Assemble dispenser and immerse filling tube in beaker with distilled deionized water and rinse the instrument by repeated piston operation. Disassemble dispenser, and allow parts to air dry.

5. Storage

5.1 The triglyceride internal standard working solution can be stored tightly capped in the freezer (-20°C) for up to 6 months under nitrogen.

5.2 The tubes with internal standards can be stored tightly capped in the freezer (-20°C) for up to 1 month under nitrogen. Label the racks that hold internal standard tubes with chemical book number of internal standard solution, and with an expiration date, which would be 1 month after the internal standard solution is dispensed into tubes.

6. Calculations

6.1 Calculation of internal standard concentrations in lipid extract

where: ISC = Internal Standard Concentration in Food Extract

C = Concentration of Internal Standard Working Solutions

$$ISC = \frac{C[mg/mL] * V[mL]}{FV[mL]}$$

V = Dispensed Volume of Internal Standard Stock Solution
FV = Final Volume of Derivatized Extract in GC Autosampler Vial

7.0 Notes

7.1 Use same microbalance throughout entire procedure.

7.2 If precise amounts of internal standards are weighted, no correction factor for the internal standard in the sequence file of the FAME GC method is necessary. If the amounts differ (**the ratios between internal standards still have to be the same!**),then the calculated concentrations of the internal standards must be programmed into the default sequence file of the FAME GC method (SOP #5019).

7.3 To save time and steps in the procedure, C23:0 TG and C19:0 TG (both are solid at room temperature) could be weighted in the same weighing funnel; C11:0 TG, however, is a liquid at room temperature and should be weighted in a separate weighing funnel.

7.4 C23:0 TG does not dissolve as fast as C11:1 and C19:0 TG, and when volume is taken up to 1 liter, part of C23:0 TG raises to the neck of flask adhering to it.

Prepared by:

Maria Teresa Tarrago-Trani

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Date: _____

Approved by:

Katherine Phillips

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Date: _____

SOP # 5020-2
Date: 13-SEP-93
Revision Date: 25-AUG-94

Food Analysis Laboratory Control Center (FALCC)
Standard Operating Procedure

TITLE: Saponification and Derivatization for Cholesterol Analysis

1. Purpose

To describe the procedure for preparing food extracts for cholesterol analysis by gas chromatography.

2. Safety

CAUTION: All work with the above chemicals must be done in the hood. Some are skin and eye irritants, some are respiratory irritants, and some are highly flammable. Powder-free gloves should be worn when working with these chemicals.

2.1 Read the MSDS for Isooctane, Pyrogallol, Potassium Hydroxide, Cyclohexane, Pyridine, and BSTFA.

3. Materials

3.1 Sample Preparation Tubes - 25 x 150 mm culture tube with Teflon®-lined screw cap (Corning, Pyrex®, No. 9826-25, available from Fisher, Cat# 14-933D) containing 10ml of total lipid extract and 2.5ml of dihydrocholesterol working solution (20μg/ml) (SOP #5017-1)

3.2 Derivatization Tubes - siliconized 25 x 150 mm according to SOP # 5008 (Corning, Pyrex®, No. 9826-25, available from Fisher, Cat# 14-933D)

3.3 Water bath - Precision Scientific model 186 (M1-39-5) or equivalent used at a temperature of 80-85°C

3.4 Centrifuge - Beckman J-6B centrifuge with JS-4.2 rotor and adapters for 29 mm tubes(5-22°C) or equivalent

3.5 Siphon - made using glass tubing and a stopper according to Sperry (6.2) used for removing the top layer of the saponification extraction

3.6 Syringe with glass adaptor for siphoning

3.7 Nitrogen Evaporator - N-EVAP™ Analytical Evaporator, model 111 (M1-16-3 or M1-34-3) or equivalent used at a temperature of 59-61°C

3.8 Solvent dispensers - Repipet® II, 20ml capacity (Labindustries No. 9020, Fisher Cat# 13-687-62C)

3.9 Rocker device for extraction - Thermolyne Vari-Mix platform mixer (M1-8-4) (Barnstead/Thermolyne No. 48725, or equivalent)

3.10 Vortex® mixer

3.11 Adjustable automatic pipets (e.g. 5ml and 1ml Pipetman®, or 10 ml Rainin EDP Plus™ Motorized Microliter Pipette)

3.12 Standard solutions (SOP# 5017):

- 20μg/ml Dihydrocholesterol Internal Standard Solution
- 20μg/ml Cholesterol Working Solution
- 1.28g/ml Potassium Hydroxide Solution
- Ethanol/Pyrogallol solution (3% w/v)
- Derivatization Reagent (1:1 Pyridine and BSTFA w/ 1% TMCS)
- Calibration standard (50μg/ml Dihydrocholesterol, 100μg/ml Cholesterol)

3.13 Cyclohexane(HPLC grade)

3.14 Deionized water

3.15 Pasteur pipets and pipet bulb

3.16 Crimp seal vials, wide mouth (Fisher Cat# 03-340-8F) or equivalent

3.17 Limited volume inserts, 200μl, polyspring (Fisher Cat# 03-375-3A)

3.18 Crimper

3.19 Cryogenic marker

3.20 Powder-free gloves

4. Procedure

4.1 Label clean, dry sample preparation tubes with the sample # of the composite. Obtain 10 ml of lipid extract from total lipid assay (SOP# 5015) for each sample tube containing 2.5ml of 20μg/ml dihydrocholesterol internal standard solution(SOP# 5017). Tubes can be stored in explosion proof freezer up to 3 months. Make sure tubes are capped tightly, and stored in a stable rack in the freezer. The day of the assay, remove the tubes from the freezer, and continue with step **4.2**. Thawing is unnecessary.

4.2 Turn on the N-EVAP™ water heater by turning the switch to the right. Allow the water to heat to 60°C. Turn on the water bath used for saponification (**3.3**) and allow the water to heat up to 85°C (it will take about three hours to reach temperature). Monitor temperature with a thermometer.

4.3 Place the tubes in the N-EVAP™ evaporator. Lower the needles to about 1cm above the surface of the liquid, and turn on the gas so that the solutions bubbles slightly. Lower the tubes into the 60°C water bath and evaporate to dryness.

4.4 Remove the tubes from the N-EVAP™. Prime the Repipet® dispenser for Ethanol /Pyrogallol solution and inspect the solution. If the solution is no longer colorless, discard and prepare a new solution (SOP# 5017-1). Working in the hood, add 8ml of the Ethanol /Pyrogallol solution using a Repipet® dispenser to each tube. Pipet 0.5ml KOH solution to each tube. Cap the tubes **tightly**, and mix using the Vortex® mixer for ten seconds.

4.5 Place all of the tubes into a test tube rack and place the rack into an 85° C water bath for eight minutes (the bottom half of each tube should be immersed in the water). Do

not allow the temperature to drop below 80° C (monitor temperature with a thermometer). Closing the water bath with the cover is necessary to maintain temperature. During the eight minute heating, place another rack tightly over the top so that all of the tubes are covered, remove the racks, and shake the tubes vigorously for a few seconds after one, two, four, and six minutes of the heating period, inverting the tubes in the process.

4.6 After the heating period, run cool tap water over the bottoms of the tubes before removing the caps (opening the hot tubes can result in splattering).

4.7 Remove the caps, add 20ml of Cyclohexane, then 12ml deionized water (using the Repipet® dispensers) to each tube. Recap the tubes and place them on the Vari-Mix® rocker for five minutes at maximum speed.

4.8 Centrifuge the tubes at 500 rpm for 5 - 10 minutes. This will speed up the separation process.

4.9 Place the tubes back into a test tube rack. Remove the cap from one of the tubes. Place a clean siphon in the mouth of the tube, and adjust the level of the suction tube so that it is about 2cm above the black phase (this prevents contamination of the top layer when siphoning). Label the siliconized derivatization tubes. Place the derivatization tubes under the drain tube of the siphon to receive the top (cyclohexane) layer of the corresponding sample preparation tube. Pull out the plunger on the syringe, and loosely fit the glass adaptor (attached to the syringe) into the second hole in the stopper. Push air into the test tube until liquid begins to flow into the derivatization tube, and quickly remove the syringe adaptor from the stopper. Let the siphon transfer the top layer. A small portion of the clear, cyclohexane layer will remain on top of the black phase in the sample preparation tube. Repeat this step for all of the tubes. **DO NOT TRANSFER ANY OF THE BLACK AQUEOUS PHASE.**

4.10 Using the Rainin EDP pipette, accurately pipet 5.0ml of cholesterol working solution into a siliconized derivatization tube containing 2.5ml of dihydrocholesterol working solution, label Con-Cal. Place this tube in the N-EVAP™ and evaporate to dryness. This sample is treated just like the other samples for the remaining assay procedure.

4.11 Evaporate the cyclohexane completely (to dryness) from the derivatization tubes in the N-EVAP™ using a gentle stream of nitrogen and a water temperature of 60°C, as described in step **4.3**.

4.12 Prepare the derivatization reagent according to **SOP# 5017**. The reagent must be prepared fresh for each assay.

4.13 In the hood, accurately pipet 250μl of the Derivatization Reagent into one of the derivatization tubes, recap the tube, and mix on a Vortex® mixer for ten seconds. It is not necessary to try to get residue off of the walls of the tube, because the cyclohexane condenses on the walls of the tube while in the N-EVAP®, washing any

residue to the bottom of the tube. Discard any remaining reagent.

4.14 Using a pasteur pipet and pipet bulb, transfer all of the solution from the test tube into a 200μl limited volume insert. Place the full insert into a crimp-seal vial, and seal the vial with the crimper. Using a cryogenic marker, label the vial with the sample number, expiration date, initials, and mark the level of the liquid.

4.15 Repeat steps **4.13** and **4.14** for each of the test tubes.

4.16 Empty the remaining saponification mixtures (black phase) into a waste bottle. Rinse both the empty sample preparation tubes and the derivatization tubes twice with a small amount of water, cap, vortex, and empty the rinse into the appropriate waste bottle.

4.15 Chromatograph samples within 4 weeks (SOP# 5022).

5. Storage

5.1 The crimp-seal vials can be stored in an explosion-proof refrigerator (2°-8° C), for up to 4 weeks, until they can be chromatographed.

5.2 A bottle of derivatization reagent can be used for only one assay, due to contamination with moisture from the air. If there is any reagent remaining in the bottle after the assay, discard it in the appropriate waste bottle. Rinse the reagent bottle twice with distilled water, and discard the rinse in the appropriate waste bottle. The reagent bottle can be cleaned and reused.

5.3 The Ethanol/Pyrogallol in the repipet dispenser can be stored in the hood. The stock solution should be capped or bubbled with nitrogen, and stored at room temperature. Both solutions can be stored until they turn brown, at which time they must be discarded.

5.4 Store the KOH solution at room temperature in a screw top bottle.

5.5 The DI water and the cyclohexane in the repipet dispensers can be stored in the hood. The stock cyclohexane should be stored under the hood.

6. Reference(s)

6.1 Thompson, Raymond H. Jr. and George V. Merola Quantitation of Cholesterol, USDA-NCL (1993)

6.2 Sperry, W. M. (1963) J. Lipid Res. **4**

Prepared by:

Robert Harris

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Date: _____

Approved by:

Katherine Phillips

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Date: _____

SOP # 5024
Date: 7-JUL-94
Revision: 2

Food Analysis Laboratory Control Center (FALCC)
Standard Operating Procedure

TITLE: Gravimetric Determination of Total Lipid in Foods

1. Purpose

To describe the procedure for gravimetric determination of total lipid content in composited, homogenized food samples.

2. Safety

CAUTION: Wear **100% nitrile gloves** at all times when working with chloroform

2.1 Read the material safety data sheet (MSDS) for chloroform.

2.2 Read the material safety data sheet (MSDS) for methanol.

2.3 Make sure a waste bottle for the chloroform and methanol is available in the hood.

2.4 Dispense chloroform in the hood.

3. Materials

3.1 Lipid extract in 500 mL polypropylene centrifuge bottle (SOP #5015)
3.2 100% nitrile gloves (powder free)
3.3 Waste bottle for chloroform, methanol, water, food mixture
3.4 Sartorius Balance (M1-31-3 or M1-22-10), or equivalent
3.5 2 ml glass pipet (Fisher # 13-676-26B), or equivalent
3.6 Sodder iron, 1 mm diameter, 37-50 mm long
3.7 Polypropylene tubing plug for 1/16 ID tubing (Value Plastics, Inc. #PIP210-6)
3.8 Hamilton MicroLab 910 with 25 ml Hamilton gas tight syringe
3.9 N-Evap analytical evaporator (M1-16-3/M1-34-3) with water bath at 60° C
3.10 30-40 ml thick walled glass centrifuge tubes (Corex® #8445-30)
3.11 Metal test tube rack (to withstand 121°C)
3.12 Crucible tongs with tygon tubing on tips
3.13 Fume Hood
3.14 Drying Oven at 101°C ± 2°C (M1-8-1), or equivalent
3.15 Desiccator with desiccant (M1-39-6), or equivalent
3.16 SOP #1003a & 1003b, Calibration and Use of Hamilton, respectively

3.17 Tweezers
3.18 30 mL Qorpak jar, or small glass beaker
3.19 Cryogenic marker
3.20 Two 13.2 x 18 inch Teri-wipers® (Fisher # 15-235-60), or equivalent
3.21 **50 mL glass beaker (not thick-walled)**

4. Procedure

CAUTION: Handle glass tubes with clean 100% nitrile gloves, or with clean rubber-tipped crucible tongs at all times.

4.1 Number the 30 mL glass tubes with corresponding sample number and place them in a metal test tube rack.

4.2 Place the rack of tubes in the drying oven (101°C ± 2°C) for 30 min.

4.3 **Read and follow SOP #1003a and 1003b for proper use of the Hamilton MicroLab 910**, for syringe preparation and priming procedure and calibration. Make sure that the tubing is not kinked; if it is, then the tubing should be changed as instructed in the manual.

4.4 Transfer the rack of glass tubes to the desiccator and allow them to cool for 1 hour. The manufacture does not recommended any item above 50°C be placed on the desiccator surface, so the shelf should be covered with two 13.2 x 18 inch teri-wipers.

4.5 Clean and fill the N-Evap water bath with fresh distilled water every other day if used daily, or before each use if used less frequently. Set at 60°C to equilibrate.

4.6 Using the rubber-tipped crucible tongs or a gloved hand, weigh the glass tube using a clean, dry, tared 50 mL beaker to hold the tube on the balance, and record the weight on the "Total Lipid Worksheet" (Form #F008, copy attached).

4.7 Insert the tubing plug into the tip end of a clean 2.0 ml glass pipet (i.e. one pipet per sample). It should be a snug fit, but the plug will not go into the tip entirely.

4.8 Carefully follow "Use of the Hamilton", SOP #1003b, steps 4.1-4.9, to prime the Hamilton with, in order: water, methanol, and chloroform.

4.9 Remove the centrifuge bottle from the 22-25°C water bath located in the Gyrotory shaker incubator (step 4.12 in SOP #5015).

4.10 Using a clean 2.0 ml plugged pipet (step 4.7), carefully penetrate the methanol-food layer in the centrifuge bottle with the tip. Once the tip has reached the chloroform layer, insert the sodder iron into the pipet and push the plug out of the tip of the pipet. The pipet tip **must** remain in the **chloroform layer**.

4.11 Carefully follow "Use of the Hamilton", SOP #1003b, steps 4.10-4.25. These steps will provide instructions on pipetting 20 ml of the chloroform layer into a dry, pre-weighed 30 ml glass tube, and explain how to rinse the instrument between samples. (Put SOP #5024 off to the side while pipetting the samples).

4.12 Place the tubes on the N-Evap analytical evaporator. The luer lock needle should be lowered to just inside the glass tube, not touching sample solution.

4.13 Lower the tubes into a 60°C water bath, and evaporate to dryness under a stream of nitrogen (approx. 60 min.). Make sure gas flow does not cause liquid to splash out of tubes.

Note: Periodically check the tubes during evaporation. The luer lock needles should be lowered to within 4 cm of the solvent level throughout the drying process to ensure optimal drying.

4.14 While the samples evaporate, retrieve the tubing plugs from the centrifuge bottles with a pair of tweezers and place in a Qorpak jar, or small beaker.

4.15 Rinse the tubing plugs in chloroform, then methanol. Then repeat the rinsing in reverse order to remove any lipids.

4.16 Use distilled water as a final rinse, then allow the plugs to dry.

4.17 Place the evaporated samples into a clean metal test tube rack and heat them in a 101°C ± 2°C oven for 30 minutes. Place directly into desiccator and cool for a minimum of **1 hour**.

4.18 Weigh each tube to the nearest 0.1 mg using the same beaker (step **4.6**) to hold the tubes on the balance, taring beaker between tube weighings.

4.19 Record all values on the "Total Lipid Worksheet" (Form # F008)

4.20 Carefully place all waste in the chloroform-methanol waste bottle in the hood.

5. Storage

5.1 The dried sample in glass tube may be covered with foil, and stored at room temperature in the hood, protected from contamination by dust, etc., (maximum of 24 hr) if time does not allow for oven drying (Step 4.17).

6. Calculations

6.1. Calculate the amount of lipid (extractable material), on a wet weight basis:

$$Lipid[g/100g wet weight] = \frac{(W2 - W1) * 80 * 100}{AV * SW}$$

W2 = Weight of glass tube with dried extract (grams)
W1 = Weight of empty dried glass tube (grams)
80 = Total chloroform volume (mls) in the centrifuge bottle
AV = Aliquot volume (mls) dispensed into 30 ml glass tube (i.e. 20 mL)
SW = Sample Weight (grams)

7. Reference(s)

7.1 AOAC 1990. Fats in Foods. Chloroform-Methanol Extraction Method #983.23, In *Official Methods of Analysis*, 15th ed. K. Helfrich (Ed), 1100, Assoc. Off. Anal. Chem. Arlington, Va.

7.2 Bligh E.G. and Dyer W.J., 1959. A Rapid Method of Total Lipid Extraction and Purification. Can. J. Biochem. Physiol. 37:911-917.

Prepared by:

Jennifer Boyle

_____

Date: _____

Approved by:

Katherine Phillips

_____

Date: _____

SOP #5026
Date: 12-JUL-93
Revision: New

Food Analysis Laboratory Control Center (FALCC)
Standard Operating Procedure

TITLE: Cholesterol Determination in Mixed Diets

1. Purpose

To describe the procedure of quantitation of cholesterol in homogenized composites of mixed diets.

2. Safety

No particular safety precautions have to be taken in addition to those outlined in the various SOPs required for this procedure.

3. Materials

3.1 SOP#5015 - Extraction of Lipids from Food with Chloroform-Methanol
3.2 SOP#5020 - Saponification and Derivatization for Cholesterol Analysis
3.3 SOP#5022 - GC Method for Cholesterol Analysis
3.4 Digital DECpc 433dxLP Computer (M1-29-5)
3.5 Hewlett Packard Laser Jet IIIp (M1-34-7)
3.6 Quattro Pro for Windows® spreadsheet program

4. Procedure

4.1 Extract sterols according to SOP#5015, "Extraction of Lipids from Food with Chloroform-Methanol".

4.2 If new calibration standards or internal standards have to be prepared, proceed with SOP # 5017, if not, continue with **4.3**.

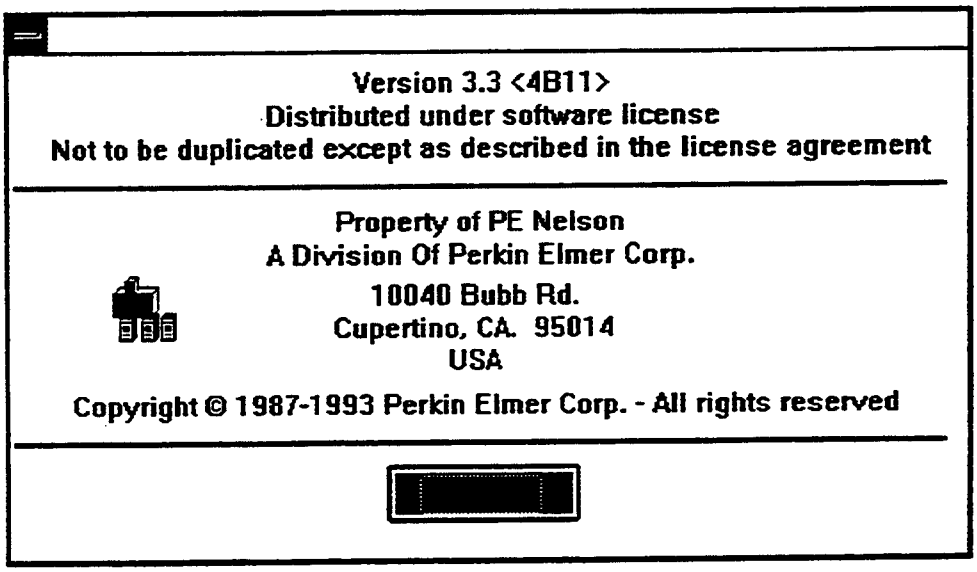
4.3 Saponify and Derivatize cholesterol according to SOP#5020, "Saponification and Derivatization for Cholesterol Analysis".

4.3 Quantify the cholesterol according to SOP#5022, "GC Method for Cholesterol Analysis"

4.4 All data files of GC chromatograms are stored in the form of ASCII files as well as Turbochrom application files. The filename of both, the Turbochrom® data file and the ASCII file is the sample number. However, the Turbochrom® data files have the appendices

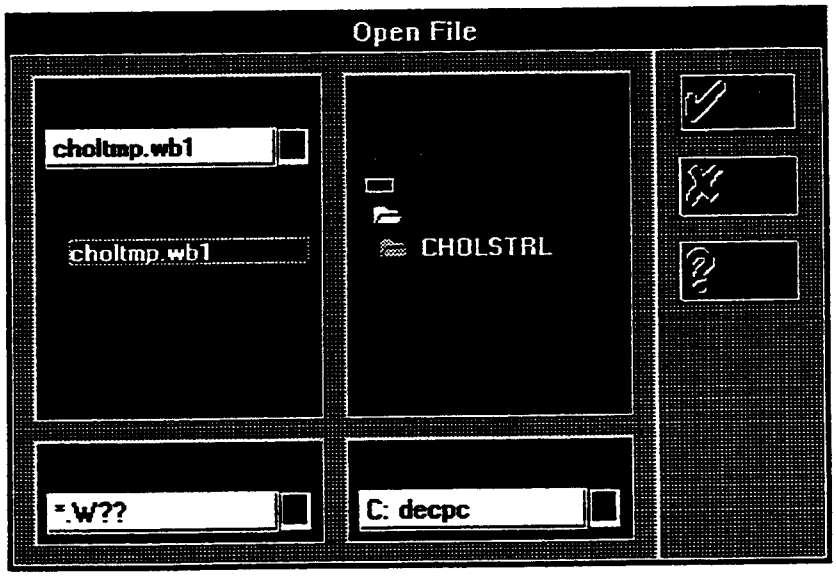
"RAW" and "RST" for the raw data and the result data file respectively, while the ASCII file carries the appendix "TX0" or "TX1". Retrieve the ASCII file with "Quattro Pro for Windows".

4.4.1 Turn on the computer by pressing the ⊘ button on the right-front of the computer. The Microsoft Windows® screen will appear followed by:

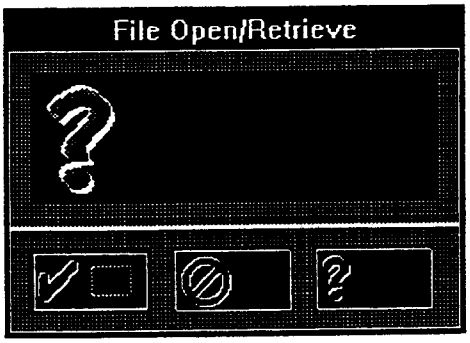


4.4.2 Using the mouse, move the pointer to the *Continue* bar and press the left mouse button. Double-click on the "Quattro Programs" icon and the quattro submenu will open. Double-click on "Quattro Pro for Windows®" and the application will open a blank spreadsheet.

4.4.3 Click on *File*, then *Open*. The following screen will appear:

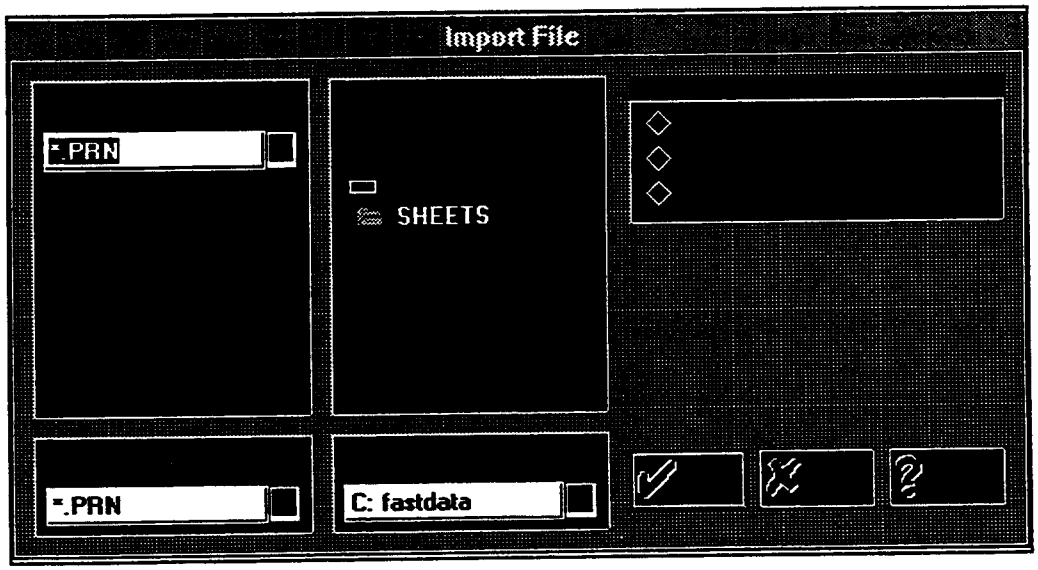


Under *Directories*, double click on *C:*, then *2700*, then *CHOLSTRL*. Under *File Name*, double click on *CHOLTEMP.WB1*, which will bring up the template for the cholesterol quantitation. The following screen will appear;



Click on the *Yes* box. This prevents the template from being overwritten.

4.4.4 Make sure that the highlighted box is in the top-right corner (cell A1). Click on *Tools* and then *Import*, and the following screen will appear:



4.4.5 Type: **.tx?*. Then, using the mouse, click the diamond next to *Comma and "Delimited File* under *Option*, then click on *OK*. All the ASCII files created by Turbochrom® that are in the subdirectory *\cholstrl* will be displayed under *File Name*. Scroll down the list and highlight the file that is to be processed. Then click on the *OK* button. The ASCII file will be retrieved into the spreadsheet.

4.5 To analyze and sort the ASCII file, click on the *Analyze* button in the middle of the spreadsheet. This macro will organize the spreadsheet into a readable format. When the program asks for the weight of the food composite that was used for the lipid extraction, type in the weight, including the decimal point (obtained from total lipid data), and press the ENTER key. When the program asks for the assay number, type in "CL" followed by the

assay number (e.g. CL001), and press the ENTER key.

4.6 The *Print Preview* screen will then appear. Click on the button with the blue wrench at the top of the screen. Move the pointer to the beginning of the footer box. Type in the name of the file followed by .WB1 (i.e. VT301VT.WB1). The entire footer will then look like this: *VT301VT.WB1 / FORM F012 / 19-JUL-93 / page 1 of 1.* Click on the print button (looks like a laser printer with a piece of paper coming out of the top), then click on the *Close* button.

4.7 The report will then print. The final report should look like this:

F012
Date: 19-JUL-93
Revision: New

Cholesterol Analysis Report

Date: 7/16/93 03:33 PM
Analyst: JMC
SOP#: 5026
Assay#: CL003
QA/QC _____
Sample Name: Continuous Calibration
Sample Weight: 5.00001 g

CHOLESTEROL ANALYSIS REPORT

Peak #	Component Name	Ret Time [min]	Peak Area [uV-sec]	BL Type	Cholesterol UG	Adjusted Amount	Cholesterol mg/100g wet
5	Cholesterol	22.16	133087.45	BB	41.06	328.51	6.57
6	Dihydrocholesterol	22.45	153068.74	BB	0	0	
			286156.19			328.51	

4.8 When the spreadsheet comes back onto the screen the prompt in the top-left corner will read, "Press ENTER to erase the Macro and continue..." Press the ENTER key. This erases the *Analyze* program from the spreadsheet, which saves disk space.

4.9 To save the spreadsheet, click on *File*, then *Save As...* Type the file name followed by .WB1 (e.g. VT301VT.WB1). Click on *File*, then *Close*.

4.10 Repeat steps **4.4.3** through **4.9** for each sample in the assay.

4.11 When all of the worksheets have been completed, obtain the sample information from QA/QC, and make out the final report.

4.11.1 In Quattro Pro for Windows®, click on *File* then *Open*. Highlight the file *C:\2700\CHOLSTRL\F025.WB1*.

4.11.2 Type in the dates, the analyst's initials, the assay#, and the %moisture. Fill in the rest of the spreadsheet with the data from the worksheets. Each composite that is run in one assay will have two sample numbers. Assign the sample numbers, that have the same sample description, to the columns

Sample #1 and *Sample #2*. The Cholesterol (mg/100g wet) for sample #1 should be listed under column 1, likewise the Cholesterol (mg/100g wet) for sample #2 should be listed under column 2. The computer will calculate the mean of the two and the Cholesterol (mg/100g dry).

4.11.3 Click on *File* then *Print*. Click on the *Preview* box. The document will then be presented as it will appear on the page. Click on the blue wrench button at the top of the screen, move the pointer to the beginning of the footer text box, and click the left mouse button. Type in the assay number followed by .WB1 (i.e. CL001.WB1). The footer should then look like this:

CL001.WB1 / FORM F025 / 26-JUL-93 / page 1 of 1

Change only the assay number in the footer. The date in the footer is the date that the form format was approved. Click on the *OK* button.

4.11.4 Click on the *Print* button then the *Close* button. Press the *Home* key on the keyboard, and save the file by clicking on *File, Save As...* Type in the assay number followed by .WB1 (i.e. CL001.WB1), then click on *OK*.

4.12 The assay request form, worksheets, and final report should be turned in to the QA/QC Officer, for processing.

5. References

Figures were obtained from Turbochrom® 3 and Quattro Pro for Windows® software.

Prepared by:

James Cooke

Date: _____

Approved by:

Katherine Phillips

Date: _____

SOP # 5029
DATE: 12-NOV-93
Revision: 2

Food Analysis Laboratory Control Center (FALCC)
Standard Operating Procedure

TITLE: Preparation of Diet Cycle Composites

Scope: Procedure applies to total food weight of 8 - 36 kilograms.

1. Purpose

To describe the procedure for preparing diet cycle composites of daily menus.

2. Safety

2.1 Read SOP # 1022 on use of the 25 L Robot Coupe® Batch Processor.
2.2 Wear ear muffs while Robot Coupe® is running.
2.3 Do not handle the Robot Coupe® blade unprotected. Always wear Kevlar® gloves when handling the blades.
2.4 Always transport the blades in a second container, i.e. Robot Coupe® bowl or other deep container.
2.5 Do not open lid of Robot Coupe® until blades have come to a complete stop.

3. Materials

3.1 25 L Robot Coupe® batch processor (M1-39-8, SOP # 1022)
3.2 foods to be composited
3.3 12 X 1" stainless steel spatula (Fisher #14-375-57), or equivalent
3.4 freezers (-20 and -60°C)
3.5 refrigerator (2-8°C)
3.6 fume hood
3.7 thermometer
3.8 straight sided, 30 mL Qorpak glass sample jars with Teflon® lined screw caps (Fisher #03-320-7A), or equivalent
3.9 permanent, cryogenic marker (Fisher #13-382-52), or equivalent
3.10 Teri Wipes (Fisher #15-235-60)
3.11 timer (showing seconds)
3.12 powder-free gloves
3.13 tape
3.14 rectangular pan 10" X 18", filled with ice
3.15 8 quart stainless steel bowls (2)
3.16 6 quart stainless steel bowl
3.17 10" stainless steel wire whisk
3.18 siliconized Rubbermaid® spatula (SOP # 5008)

3.19 100 mL siliconized tri-cornered polypropylene beakers (Fisher # 02-593-50C), or equivalent
3.20 12" stainless steel scoopula
3.21 small plastic pan for placement under jars while pouring aliquots
3.22 600 mL glass beaker or equivalent deep bucket
3.23 analytical balance (M1-39-9, Fisher # 01-913-317), or equivalent
3.24 Synco 2001 Computer (M1-33-5), or equivalent
3.25 Hewlett Packard Laser Jet IIIp printer (M1-39-10), or equivalent
3.26 Plastic bucket for leftover food composite
3.27 Ear muffs
3.28 Kevlar® gloves
3.29 Dish soap
3.30 Robot Coupe model R-6 batch processor (M1-7-2, SOP # 1005)

4. Procedure

Note: This Standard Operating Procedure encompasses two days.

Note: Avoid touching anything that may come into contact with the composite. Wear powder free gloves throughout the procedure.

Day 1

4.1 Label the sample jars.

4.1.1 Turn on the computer by pressing the "on" button on the surge protector box just to the left of the keyboard. The Windows Program Manager screen will appear.

4.1.2 Using the mouse, double click with the left button, on the *Sample Log-in* icon. A sub menu will open. Double click on *Sample Log-in* and the sample log-in main menu will appear.

4.1.3 Using the down arrow key, highlight the *Composite Log-In* block. This block will be the second block in the series. Hit *Enter*.

4.1.4 A screen will appear in which you will enter the date and description of the food to be composited. If not already present, enter the date. The date is in the format MM/DD/YY. Hit *Enter*.

4.1.5 For the description of the diet cycle, type in the number of containers of food samples for the cycle (i.e. "8 Menus") and the diet cycle number (e.g. "Diet Cycle 2"). Hit *Enter*.

4.1.6 A screen will appear in which you will enter the *Source Sample Number* of the food to be composited. The source sample numbers will be on the containers holding the food to be composited. These numbers will always

begin with VT and will be followed by 5 numbers or letters (VTXXXXX). Enter the numbers as the range of the 8 menus (e.g. VT304PA - VT304PH). **Carefully** review the entered range and make **sure** it is correct. Hit *Enter*, then *Esc*.

Note: If an incorrect source sample number is entered, alert the person in charge of the database. The database will need to be edited.

4.1.7 A screen will appear which asks the number of samples that will be generated. Enter *22* (this is the number of jars that will be filled for each composite; each jar must have an individual number). Hit *Enter*.

Note: If an incorrect number is entered when asked for the number of samples that will be generated, alert the person in charge of the database. The database will need to be edited.

4.1.8 A screen will appear which asks if you are ready to print. Hit *Y* for yes. Hit *Enter*.

4.1.9 A screen will appear which asks how many copies of the Composite Report Form (F009), to print. Enter *3* (one copy will be for the sample log-in notebook, one copy will be for the compositing notebook, and the third copy will be given to Katherine Phillips). Hit *Enter*. Three copies of Form F009 will then be printed. On this form will be the 22 sample numbers which will be used to label the 22 jars used for the composite, and the composite number.

4.1.10 Record the composite number on Form F029.

Note: The jars **must** be labeled with consecutive numbers in the order in which they will be aliquotted. Jar #1, the first jar aliquotted, will receive the first sample number generated by the Computer's Sample Log-in process. Jar #2, the second jar aliquotted, will receive the second sample number generated, and so on.

4.1.11 Using a **PERMANENT CRYOGENIC MARKER**, clearly write the composite number (from form F009), the sample number, and date on top of the lid of each jar. Zeros should be written ∅ to avoid confusion with the letter "O".

4.2 Thaw the samples

4.2.1 If samples were kept in the freezer (-20°C), continue with **4.2.2**, if samples were received today and are to be composited tomorrow, continue with **4.2.3**.

4.2.2 If samples were kept in the freezer (-20°C), remove the containers from the freezer. Place the containers on a bench surface at least 4"

away from each other. Let the containers sit out at room temperature for four (4) hours. Continue with **4.2.4**

4.2.3 If samples were received today and are to be composited tomorrow, log the samples into the data base (SOP#5027). Inspect all containers for cracks that could cause leakage. In case a container is cracked, weigh a stainless steel bowl, record the weight on Form # F029, and place the container in the bowl. In order to inspect the samples, randomly open two containers. If the food samples in those two containers are still solidly frozen, let the containers sit out at room temperature for four (4) hours, then continue with **4.2.4**. If the samples are not solidly frozen but some thawed liquid is visible, do not let the containers sit out at room temperature but continue with **4.2.4**.

4.2.4 Estimate the weight of the food, by weighing the eight containers. Record the weights on Form F029. Also weigh an empty container of the same kind and subtract eight times this empty weight from the total weight of the food containers, and record on Form F029.

Note: If the estimated weight of the food exceeds 18 kg, the diet cycle must be composited in two batches.

4.2.6 Place the containers in the refrigerator (2-8°C) overnight (up to 24 hours) at least 4" away from each other.

Day 2

4.3 Composite the daily menus together

4.3.1 On the day of compositing, remove containers with the food to be composited (8 or 4 depending on the weight estimate **4.2.4**) from refrigerator and place them on the bench surface. The containers should not be stacked and be at least 4" apart.

Note: Begin transferring samples to the Robot Coupe® bowl (**4.3.4**) after 60 minutes, thus the containers will sit out at room temperature before compositing for at least 90 but no more than 150 minutes.

4.3.2 Prepare station for compositing. Fill the rectangular pan with ice. Twenty-two (22) jars of composite sample will be aliquotted for each diet. Set out the previously labeled sample jars (**4.1.11**) with sample numbers in consecutive order.

4.3.3 Clean and assemble the Robot Coupe® according to SOP # 1022. Test-run the Robot Coupe® by briefly pushing the *pulse* button. If the Robot Coupe® does not respond, push in the black covered button on the back of the Robot Coupe® assembly.

4.3.4 QUANTITATIVELY and CAREFULLY transfer ALL of the food from the containers into the Robot Coupe® bowl.

Note: Diet cycles comprise eight (8) containers of food. The purpose of the following steps is to determine the total weight of the food in the eight containers. The final weight will be determined by weighing the food in batches in a stainless steel bowl and adding those weights together. The weight limitation for each stainless steel bowl of food is 3.5 to 4.5 kg. If the weight limitation of 3.5 to 4.5 kg has been reached and the container is not completely empty, the remaining contents can be included in the next bowl being weighed.

4.3.4.1 Using a Teri Wipe, wipe off any moisture that has accumulated on the outside of the first container. Remove the labels from the lid of the plastic container and affix it to the Compositing Worksheet, Form F029. Also affix any other labels adhering to the container to Form F029 (i.e. label tape with noted deviations from total menu).

4.3.4.2 Using the stainless steel spatula cut the composite in the containers into smaller pieces (~3 - 4" across).

4.3.4.3 Tare the stainless steel bowl on the 5 K balance.

4.3.4.4 Using the stainless steel spatula, carefully transfer between 3.5 and 4.5 kilograms of the composite from several containers into the stainless steel bowl. **Do not lose any of the composite. If loss occurs, notify supervisor. Complete an Internal Deviation From SOP Form (F015) and file it with the records associated with the diet.**

4.3.4.5 Using the stainless steel spatula, thoroughly scrape all food residues from the emptied containers. Place the side of the spatula against the edge of the container and completely scrape the entire surface. By scraping in this way, you should be able to remove any food residue that is adhering to the sides of the container. Place all residue into the stainless steel bowl. **DO NOT** lose any food on spatula, countertop, etc. Place the spatula, handle side down, in a 600 ml glass beaker.

4.3.4.6 Using the siliconized Rubbermaid® spatula, completely scrape any remaining food residue or droplets from the emptied containers into the stainless steel bowl. Place the spatula, handle side down, in a 600 ml glass beaker.

4.3.4.7 Record the food weight on Form F029.

4.3.4.8 Carefully, as to not to loose any food, transfer the food from the stainless steel bowl into the Robot Coupe® bowl.

4.3.4.9 Decrease the bulk of the food in the Robot Coupe® bowl. Close the lid, of the Robot Coupe®, switch on the power and blend the food by pushing and holding the *pulse* button for two (2) seconds. Repeat this twice for a total blending of (three times two) six (6) seconds.

Note: It is not important that the stainless steel bowl is completely free of any food residue until after the last container of the diet cycle is transferred from the stainless steel bowl into the Robot Coupe®.

4.3.4.10 Repeat **4.3.4.3** through **4.3.4.9** for all containers of the cycle. If the estimated weight of the food is above 18 kg (**4.2.5**), repeat **4.3.4.3** through **4.3.4.9** for only four 4 containers.

Calculate the total *composite weight* as the sum of the individual weights recorded in step 4.3.4.7.

4.3.4.11 After the last container of the diet cycle is emptied and weighed, QUANTITATIVELY and CAREFULLY transfer ALL of the food from the stainless steel bowl into the bowl of the Robot Coupe®. Using the siliconized Rubbermaid® spatula, thoroughly scrape all food residues or droplets from the stainless steel bowl into the bowl of the Robot Coupe®. **Do not lose any of the composite. If loss occurs, notify supervisor. Complete deviation from SOP form # F015 and file with the records associated with the diet.** Place the spatula, handle side down, in a 600 mL glass beaker.

4.3.4.12 Using a Teri Wipe, wipe out the stainless steel bowl (this is to insure that any food residue which does remain, will not be incorporated into the composite when the bowl is used in steps **4.4** and **4.5**).

4.3.4.13 Scrape down the sides of the Robot Coupe® bowl using a stainless steel spatula. Gently tap the spatula on the edge of the Robot Coupe® bowl and scrape off any residuals with the siliconized Rubbermaid® spatula. Place the spatula, handle side down, in a 600 mL glass beaker.

4.3.4.14 Measure the temperature of the food, being careful not to touch the blades or the bowl with the thermometer. Record the temperature of the food on Form F029. Use the siliconized Rubbermaid® spatula to scrape any food material off the thermometer. Gently tap the spatula on the edge of the Robot Coupe® bowl. Place the spatula, handle side down, and the thermometer, bulb side up, in a 600 mL glass beaker.

4.3.5 Composite the food.

4.3.5.1 Blend the food by pushing in and holding down the pulse button for two (2) seconds. Wait five (5) seconds, then repeat two (2) times for a total blending of (three times two) six (6) seconds .

4.3.5.2 After opening the lid, use the siliconized Rubbermaid® spatula to scrape any food material adhering to the lid, back into the Robot Coupe® bowl. Gently tap the spatula on the edge of the Robot Coupe® bowl. Place the spatula, handle side down, in a 600 mL glass beaker.

4.3.5.3 Scrape down the sides of the Robot Coupe® bowl using a stainless steel spatula. Gently tap the spatula on the edge of the Robot Coupe® bowl and scrape off any residuals with the siliconized Rubbermaid® spatula. Place the spatula, handle side down, in a 600 ml glass beaker.

4.3.5.4 Using the stainless steel scoopula, stir the composite and dislodge any large particles that might be stuck underneath the upper blade. Use the siliconized Rubbermaid® spatula to scrape any food material off the scoopula. Gently tap the spatula on the edge of the Robot Coupe® bowl. Place the scoopula and the spatula, handle side down, in a 600 ml glass beaker.

4.3.5.5 Close the lid and blend the composite for 15 seconds by switching the *Run* switch to *On* for 15 seconds. At the end of the 15 seconds switch the *Run* switch to *Off*.

4.3.5.6 After opening the lid, use the siliconized Rubbermaid® spatula to scrape any food material adhering to the lid, back into the Robot Coupe® bowl. Scrape the residue off the siliconized spatula on the side of the Robot Coupe® bowl. Gently tap the spatula on the edge of the Robot Coupe® bowl. Place the spatula, handle side down, in a 600 ml glass beaker.

4.3.5.7 Scrape down the sides of the Robot Coupe® bowl using a stainless steel spatula. Gently tap the spatula on the edge of the Robot Coupe® bowl and scrape off any residuals with the siliconized Rubbermaid® spatula. Place the spatula, handle side down, in a 600 ml glass beaker.

4.3.5.8 Using the stainless steel scoopula, stir the composite and dislodge any large particles that might be stuck underneath the upper blade. Use the siliconized Rubbermaid® spatula to scrape any food material off the scoopula. Gently tap the spatula on the edge of the Robot Coupe® bowl. Place the scoopula and the spatula, handle side down, in a 600 ml glass beaker.

4.3.5.9 Close the lid and blend the composite for 30 seconds by switching the *Run* switch to *On* for 30 seconds. At the end of the 30 seconds switch the *Run* switch to *Off*.

4.3.5.10 After opening the lid, use the siliconized Rubbermaid® spatula to scrape any food material adhering to the lid, back into the Robot Coupe® bowl. Gently tap the spatula on the edge of the Robot Coupe® bowl. Place the spatula, handle side down, in a 600 mL glass beaker.

4.3.5.11 Scrape down the sides of the Robot Coupe® bowl using a stainless steel spatula. Gently tap the spatula on the edge of the Robot Coupe® bowl and scrape off any residuals with the siliconized Rubbermaid® spatula. Place the spatula, handle side down, in a 600 mL glass beaker.

4.3.5.12 Using the stainless steel scoopula, stir the composite and dislodge any large particles that might be stuck underneath the upper blade. Use the siliconized Rubbermaid® spatula to scrape any food material off the scoopula. Gently tap the spatula on the edge of the Robot Coupe® bowl. Place the scoopula and the spatula, handle side down, in a 600 ml glass beaker.

4.3.5.13 Close the lid and blend the composite a second time for 30 seconds by switching the *Run* switch to *On* for 30 seconds. At the end of the 30 seconds switch the *Run* switch to *Off*.

4.3.5.14 After opening the lid, use the siliconized Rubbermaid® spatula to scrape any food material adhering to the lid, back into the Robot Coupe® bowl. Gently tap the spatula on the edge of the Robot Coupe® bowl. Place the spatula, handle side down, in a 600 mL glass beaker.

4.3.5.15 Scrape down the sides of the Robot Coupe® bowl using a stainless steel spatula. Gently tap the spatula on the edge of the Robot Coupe® bowl and scrape off any residuals with the siliconized Rubbermaid® spatula. Place the spatula, handle side

down, in a 600 mL glass beaker.

4.3.5.16 Measure the temperature of the food, being careful not to touch the blades or the bowl with the thermometer. Record the temperature of the food on Form F029. Use the siliconized Rubbermaid® spatula to scrape any food material off the thermometer. Gently tap the spatula on the edge of the Robot Coupe® bowl. Place the spatula, handle side down, and the thermometer, bulb side up. in a 600 mL glass beaker.

4.3.5.17 Close the lid and blend the composite a third time for 30 seconds by switching the *Run* switch to *On* for 30 seconds. At the end of the 30 seconds switch the *Run* switch to *Off*.

4.3.5.18 After opening the lid, use the siliconized Rubbermaid® spatula to scrape any food material adhering to the lid, back into the Robot Coupe® bowl. Gently tap the spatula on the edge of the Robot Coupe® bowl. Place the spatula, handle side down, in a 600 mL glass beaker.

4.3.5.19 Scrape down the sides of the Robot Coupe® bowl using a stainless steel spatula. Gently tap the spatula on the edge of the Robot Coupe® bowl and scrape off any residuals with the siliconized Rubbermaid® spatula. Place the spatula, handle side down, in a 600 mL glass beaker.

4.3.5.20 Close the lid and blend the composite a fourth time for 30 seconds by switching the *Run* switch to *On* for 30 seconds. At the end of the 30 seconds switch the *Run* switch to *Off*.

4.3.5.21 After opening the lid, use the siliconized Rubbermaid® spatula to scrape any food material adhering to the lid, back into the Robot Coupe® bowl. Gently tap the spatula on the edge of the Robot Coupe® bowl. Place the spatula, handle side down, in a 600 ml glass beaker.

4.3.5.22 Scrape down the sides of the Robot Coupe® bowl using a stainless steel spatula. Gently tap the spatula on the edge of the Robot Coupe® bowl and scrape off any residuals with the siliconized Rubbermaid® spatula. Place the spatula, handle side down, in a 600 ml glass beaker.

4.3.5.23 Measure the temperature of the food, being careful not to touch the blades or the bowl with the thermometer. Record the temperature of the food on Form F029. Use the siliconized Rubbermaid® spatula to scrape any food material off the thermometer. Gently tap the spatula on the edge of the Robot Coupe® bowl. Place the spatula, handle side down, and the thermometer, bulb side up, in a 600 mL glass beaker.

4.3.6 At this point, the composite should have a uniform, homogeneous appearance and consistency. Using the large, stainless steel scoopula, stir the composite thoroughly and inspect for icy lumps, larger pieces of food, or any inconsistencies with the typical composite appearance. **NOTIFY SUPERVISOR IF ANYTHING LOOKS UNUSUAL. If the supervisor decides that another run is needed, record reason and resulting action on deviation form F015).** Tap the scoopula on the edge of the Robot Coupe® bowl. Place the scoopula, handle side down, in a 600 ml glass beaker.

4.3.7 Measure the temperature of the composite being careful not to touch the blades or the bowl with the thermometer. Record the temperature on Form F029. Place the thermometer, bulb side up, in a 600 ml glass beaker. **The temperature of the composite must be between 15 - 25 °C at this point. Notify supervisor if composite is not in the proper temperature range. If the composite is too cold (i.e. below 15°C), blend the composite for consecutive sets of 30 seconds until the composite temperature is in the desired range. Monitor and record composite temperature after every blending. Composites which are too warm (i.e. above 25°C), will be handled individually.**

4.3.6 Close the lid, and blend the composite by switching the run switch to "On" for 15 seconds. **IMMEDIATELY PROCEED TO STEP 4.4.**

Note: No scraping is required in this step.

4.4 **Quickly** pour an aliquot of the composite from the Robot Coupe® bowl into the 8 quart stainless steel bowl that was used in steps **4.3.4.3 - 4.3.4.11.** If the estimated sample weight (**4.2.5**) was above 18 kg, continue with **4.5**, if all the food has been composited, continue with **4.12**.

4.5 Tare the bowl of the 6 L Robot Coupe. Pour approximately 1 kg of the composite from the stainless steel bowl into the Robot Coupe bowl. Record the weight of the aliquot on Form F029. Cover the Robot Coupe bowl and place it in the refrigerator.

4.6 Repeat steps **4.3** through **4.4** for the remaining 4 containers of food.

4.7 Calculate the aliquot amount of the second composite necessary to achieve the proper weight ratio of the two composites.

$$Aliquot\ Weight\ 2 = \frac{Aliqot\ Weight\ 1 * Composite\ Weight\ 2}{Composite\ Weight\ 1}$$

Note: *Composite weight(s)* are the weights referred to in step **4.3.4.10**.

4.8 Pour the calculated aliquot amount of composite number 2 into a second stainless steel bowl. Transfer **QUANTITATIVELY** and **CAREFULLY** **ALL** of the aliquot into the 6 L Robot Coupe bowl.

4.9 Set the speed setting on the Robot Coupe to 3500 rpm. Blend the two composites for 45 seconds by turning on the power switch. Measure and record the temperature. **If the composite is too cold (i.e. below 15°C), blend the composite for consecutive sets of 30 seconds until the composite temperature is in the desired range. Monitor and record composite temperature after every blending. Composites which are too warm (i.e. above 25°C), will be handled individually.**

4.10 Set the speed setting on the Robot Coupe to 3500 rpm. Blend the composite for 15 seconds by turning on the power switch. **IMMEDIATELY PROCEED TO STEP 4.11.**

4.11 Quickly pour the composite from the Robot Coupe bowl into the 6 quart stainless steel bowl.

4.12 Aliquot the composite into sample jars

4.12.1 Place the first four (consecutive sample numbers) sample jars from **4.1.11** on the edge of the work space.

4.12.2 Using the stainless steel whisk, stir the composite in the following manner: start stirring at the outer edge of the bowl and work towards the center and then back out again in a smooth, circular motion. Repeat this stirring pattern for a minimum of 15 seconds. Make sure the whisk is touching the bottom of the bowl to insure complete mixing of the composite. Try not to incorporate air while stirring. Proceed immediately to step **4.12.3.**

4.12.3 Using a gloved hand, grasp a siliconized, tri-cornered, polypropylene beaker by one of it's corners at the lip. Quickly dip an aliquot of the composite into the beaker. While holding the jar over the small plastic pan, pour the composite into the prepared sample jars (step **4.12.1**). Fill the jars to approximately two-thirds capacity. There should be enough composite in the

beaker to do at least four jars. **Do not hold the jars over the bowl containing the composite while pouring.** Set the beaker aside (this beaker will not be used again). **DO NOT POUR THE COMPOSITE REMAINING IN THE BEAKER BACK INTO THE BOWL CONTAINING THE COMPOSITE.**

4.12.4 Make sure there is no food residue around the threads or on the outside of the jars. Clean the jars with a Teri Wipe if necessary. Cap each sample jar with the appropriately numbered lid, and place the jars in consecutive order on ice in the previously prepared pan (step **4.3.2**).

4.12.5 Measure the temperature of the composite being careful not to touch the bottom of the bowl. Record the temperature of the composite on Form F029. Place the thermometer, bulb side up, in a 600 ml glass beaker.

Note: When the composite reaches 20°C, begin monitoring time. **The composite must not be allowed to remain at 20 - 25°C for more than 30 minutes.**

4.12.6 Place the next four sample jars (consecutive sample numbers) on the edge of the work space.

4.12.7 Using the stainless steel whisk , stir the composite in the following manner: start stirring at the outer edge of the bowl and work towards the center and then back out again in a smooth circular motion. Repeat this stirring pattern for a minimum of 15 seconds. Make sure the whisk is touching the bottom of the bowl to insure complete mixing of the composite. Try not to incorporate air while stirring. Proceed immediately to step **4.12.8.**

4.12.8 Using a gloved hand, grasp a **clean**, siliconized, tri-cornered, polypropylene beaker by one of it's corners at the lip. Quickly dip an aliquot of the composite into the beaker. Pour the composite into the prepared sample jars (step **4.12.6**). Fill the jars to approximately two-thirds capacity. There should be enough composite in the beaker to do at least four jars. **Do not hold the jars over the bowl containing the composite while pouring. DO NOT POUR THE COMPOSITE REMAINING IN THE BEAKER BACK INTO THE BOWL CONTAINING THE COMPOSITE.** Set the beaker aside.

4.12.9 Make sure there is no food residue around the threads or on the outside of the jars. Clean the jars with a Teri Wipe if necessary. Cap each sample jar with the appropriately numbered lid and place the jars on ice in the previously prepared pan (step **4.3.2**). Keep samples in the order in which they were aliquotted. The samples must be numbered consecutively.

4.12.10 Measure the temperature of the composite after the 12th and the 22nd jar, being careful not to touch the bottom of the bowl. Record the temperature of the composite on Form F029. Place the thermometer,

bulb side up, in a 600 ml glass beaker. When the composite reaches 20°C, begin monitoring time. **The composite must not be allowed to remain at 20 - 25°C for more than 30 minutes.**

4.12.11 Continue in this way until all 22 samples have been aliquotted.

4.13 Clean the Robot Coupe® bowl and blade

4.13.1 Turn off the power switch on the Robot Coupe®. Unplug the power cord of the Robot Coupe®. Remove the lid according to SOP # 1022.

4.13.2 Pour leftover food composite out of the Robot Coupe® bowl into a bucket. Scrape the Robot Coupe® bowl with a spatula. Discard the food by pouring it into the sink with running warm water.

4.13.3 Disassemble the bowl and blades according to SOP # 1022. Leave the blades in the bowl while carrying it to the sink for safety reasons. Wear Kevlar® gloves when cleaning the blades.

4.13.4 If more diet cycle composites are to be made, clean the Robot Coupe® bowl, lid and blades in the sink under running warm water. After the last composite for the day wash Robot Coupe® bowl, lid and blades with warm soapy water and rinse them thoroughly.

4.13.5 Use Kevlar® gloves when drying the blades and Robot Coupe® bowl with Teri wipes.

4.13.6 Clean the blade shaft of the Robot Coupe® with warm water and dry with Teri wipes.

5. Storage

5.1 Make sure jars are tightly sealed. Store the sample jars at -60°C in the appropriate freezer location in the original boxes in which the samples jars were shipped. Samples that will be assayed the same day or the following day, will be placed into the refrigerator (2-8°C).

5.2 Record the storage location of the jars in the sample database.

5.2.1 Return to the sample log-in main menu (steps **4.1.1-4.1.2**). Use the down arrow key to highlight *Editing*. Hit *Enter*.

5.2.2 Highlight *Composite Storage Location*. Hit *Enter*.

5.2.3 A screen will appear which asks for the 22 individual *Composite Sample Numbers*. This screen will be accompanied by a pop-up screen which has all of the available freezer locations. Use the down arrow keys to highlight the

proper location and hit *Enter*.

Note: The screen will then pause, and the entries will disappear. The screen will then be ready for another entry of source sample number and storage location. Continue to enter the information in this way until all entries have been made.

5.2.4 When all entries have been made, hit *Esc* to exit the editing portion of the program and return to the editing menu.

5.3 Record the source sample weight in the sample database.

5.3.1 Use the down arrow keys to highlight *Original Sample Net Weight*. Hit *Enter*.

5.3.2 A screen will appear which asks for the *Source Sample Number*. Enter the first number of the range of source sample numbers entered in **4.1.6.** Hit *Enter*.

5.3.3 A screen will then appear which asks for the *Original Sample Net Weight*. Enter the weight. Hit *Enter*.

Note: The screen will then return to the *Source Sample Number* side for entry of another sample number and weight. Continue to enter the information in this way until all entries have been made.

5.3.4 When all entries have been made, hit *Esc* to exit the editing portion of the program and return to the editing menu.

5.3.5 Use the down arrow keys to highlight *Quit*. Hit *Enter*. The program will then return to the Windows Program Manager screen.

5.4 Combine the Composite Worksheet Form F029, the Composite Report Form F009, and the Internal Deviation From SOP Form F015, in that order, and file the package in the Compositing Notebook.

Prepared by:

Ingolf Gruen

_____

Date: _____

Approved by:

Katherine Phillips

_____

Date: _____

SOP # 5010
Date: 9-JUL-93
Revision: 1

Food Analysis Laboratory Control Center (FALCC)
Standard Operating Procedure

Title: Fatty Acid Methylation

1. Purpose

To describe the procedure for methylating fatty acids.

2. Safety

2.1 All parts of this procedure are to be performed in the hood.
2.2 Wear rubber gloves and a lab coat at all times.
2.3 iso-octane and BF_3/Methanol are eye and skin irritant and may be fatal if swallowed.
2.4 Read MSDS's for iso-octane and BF_3/Methanol.

3. Materials

3.1 10 mL aliquots of total lipid extract (SOP#5015)
3.2 15 mL glass culture tubes and Teflon screw top caps with and without internal standards (SOP # 5018)
3.3 Nitrogen
3.4 N-EVAP™ Analytical Evaporator (M1-16-3) or (M1-34-3)
3.5 0.5N NaOH (Methanolic)
3.6 Waterbath/hot plate (90°C to 100°C - boiling)
3.7 Thermometer
3.8 2mL vials of BF_3/Methanol reagent (Cat# 3-3020, Supelco)
3.9 LabLine™ waterbath (M1-8-6) (30°C to 40°C)
3.10 Saturated NaCl aqueous solution
3.11 2mL crimp seal vials and Teflon caps
3.12 Crimp sealer
3.13 Cryogenic marker
3.14 Iso-octane (GLC-grade, e.g. Fisher Optima grade)
3.15 Disposable glass pipettes

4. Procedure

4.1 Label all tubes and crimp seal vials with the sample number.

4.2 Obtain 10mL from the total lipid extract (SOP # 5015) in the culture tubes containing the internal standards (SOP # 5018).

4.3 Evaporate the solvent with a stream of dry nitrogen gas in the N-Evap™ analytical evaporator at 40 C.

4.4 Add 1.5mL 0.5N NaOH to each tube, blanket tubes with nitrogen, cap tightly, vortex for 5 seconds, and heat in a boiling waterbath for 7 minutes.

4.5 Cool the culture tubes to room temperature for about 15 minutes, then slowly add 2mL BF_3/Methanol reagent.

4.6 Blanket the tube with nitrogen, cap tightly, invert the tube to mix, vortex for 5 seconds, and heat in a boiling waterbath for 30 minutes.

4.7 Cool the culture tubes to 30°C-40°C in the Labline™ waterbath for at least 15 minutes, then add 2mL of iso-octane.

4.8 Blanket the tube with nitrogen, cap tightly, and vortex for 30 seconds.

4.9 Immediately add 5mL of a saturated NaCl solution.

4.10 Blanket the tube with nitrogen, cap tightly, invert the tube to mix, and vortex for 5 seconds.

4.11 Let the culture tubes cool to room temperature for at least 15 minutes. Allow the iso-octane (upper) layer to separate from the aqueous (lower) layer. The layers will separate in approximately 15 minutes.

4.12 Using a pasteur pipette, transfer the iso-octane layer to a clean, dry culture tube, blanket the new tube with nitrogen gas, and cap tightly. Dispose pasteur pipette.

4.13 Add an additional 2mL of iso-octane to the aqueous layer.

4.14 Blanket the tube with nitrogen, cap tightly, and vortex for 30 seconds.

4.15 Allow the iso-octane (upper) layer to separate from the aqueous (lower) layer. The layers will separate in approximately 15 minutes.

4.16 Using a clean pasteur pipette, transfer the new iso-octane layer to the same culture tube as the first iso-octane layer and concentrate the extracts to approximately 1mL with a **very slow** stream of dry nitrogen. Dispose pasteur pipette.

4.17 Pipet the iso-octane extract into a crimp seal vial using a clean pasteur pipett. Blanket with nitrogen, cap, and seal the vial with the crimp sealer.

4.18 The GC analysis (SOP # 5019) of the extracts can be done at a convenient time.

5. Storage

5.1 After receiving the aliquot of the total lipid extract, the tubes with the extract can be stored in a freezer at -20°C for up to one week, capped under nitrogen.

5.2 Store the crimp seal vials in the freezer (-20°C), until GC analysis.

6. Reference

6.1 American Oil Chemist Society, 1991. Official Methods of the American Oil Chemist Society, A.O.C.S. Official Method Ce 1b-89, "Fatty Acid Composition by GLC", A.O.C.S. Champaign, IL.

Prepared by:

Maria Teresa Tarrago-Trani

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Date _____

Approved by:

Katherine Phillips

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Date _____

SOP # 5015
Date: 7-JUL-94
Revision: 3

Food Analysis Laboratory Control Center (FALCC)
Standard Operating Procedure

TITLE: Extraction of Lipids from Foods with Chloroform-Methanol

1. Purpose

To describe the procedure for quantitatively extracting total lipids from *composited* food samples.

2. Safety

CAUTION: Wear **100% nitrile gloves** at all times when working with chloroform.

2.1 Read the material safety data sheet (MSDS) for chloroform.

2.2 Read the material safety data sheet (MSDS) for methanol.

2.3 Make sure a waste bottle for the chloroform and methanol is available in the hood.

2.4 Dispense chloroform in the hood.

3. Materials

3.1 Food sample (*composited*), SOP #5005 or #5029
3.2 Fume Hood
3.3 Stainless steel or Teflon® spatula (9-10 inches long)
3.4 500 mL **silicon coated** (SOP#5008) polypropylene centrifuge bottles (2 per sample)
3.5 Teflon® tape
3.6 Chloroform - ACS Certified, or equivalent
3.7 Methanol - HPLC Certified, or equivalent
3.8 0.5 M Sodium Acetate Solution (64.04 g of NaAc/L dH_2O)
3.9 4 Adjustable Repipettors/Dispensers for dispensing $CHCl_3$, MeOH, NaAc & H_2O
3.10 Timer
3.11 100% Nitrile Gloves (powder free)
3.12 25 position orbital shaker by New Brunswick Scientific Co., Inc. (M1-34-4), or equivalent
3.13 Beckman J-6B Centrifuge (4-22°C), or equivalent
3.14 Sartorius Balance (M1-31-3 or M1-22-10), or equivalent
3.15 Waste bottle for chloroform and methanol
3.16 Multi-bottle rack to aid in the transportation of centrifuge bottles (e.g. Fisher #14-

785-1)

3.17 Tub of water equilibrated to 23-25°C in a 25°C Gyrotory shaker incubator (M1-39-11), or equivalent

4. Procedure

4.1 Read and follow SOP #5028 for proper handling and aliquotting of food composite samples.

4.2 Precisely, (to the nearest 0.1 mg) weigh 4.9 - 5.1g of well mixed sample (see SOP #5028 into a **500 ml siliconized polypropylene centrifuge bottle**. Record the weight and sample number in the "Total Lipid Worksheet" (Form # F008, copy attached)

4.3 Based on the moisture content (SOP #5007,or equivalent) of the sample, add enough 0.5 M Na acetate solution so that the total volume of H_2O + Na Acetate equals 32 ml.

Example: 5 g*74.87% = 3.74 ml / 32.0 mL-3.7 mL = 28.3 ml 0.5 M Na acetate

4.4 Prime the repipettors before each use by dispensing 1 pump of liquid into a waste beaker before addition to an actual sample. Make sure dispensing tubes are completely filled; if not, re-prime until they are. Add 80 ml of MeOH and 40 ml of $CHCl_3$ to the sample (the ratio of methanol:chloroform:water will be 2:1:0.8 v/v/v).

4.5 Cap the centrifuge bottle and place it on the orbital shaker for **2 hr. at 325 rpm**.

4.6 Prepare gravimetric tubes according to SOP #5024.

4.7 Add 40 ml of chloroform and shake **(300 rpm)** for an additional **30 min**.

4.8 Switch on Gyrotory shaker incubator, place tub with water (T at about 25°C) in incubator, set control thermostat at 25°C and safety thermostat at 30°C. Leave a thermometer in the water and check that the temperature is between 22-25°C after 30 minutes.

4.9 Add 40 ml of H_2O and shake **(275 rpm)** for an additional **30 min**.

4.10 Centrifuge at **1473 x g** (2300 rpm in the Beckman J-6B) at temperatures above 4°C but not exceeding 22°C for **10 min** to clarify the bottom layer. Set brake speed at "5".

4.11 Place the bottles into a multi-bottle rack and carefully transport them to the fume hood. Try not to disturbe the layers.

4.12 Make sure the bottles sit at 25°C for **15 minutes** in the Gyrotory incubator by using a thermometer in the water bath to monitor the temperature of the water. Start timing 15 min. when water temperature reaches 23-25°C, after the addition of the bottles. This will ensure total separation and allow the samples to equilibrate to the set dispensing temperature.

5. Storage

5.1 AFTER Step 4.11, the centrifuge bottle containing the sample can be tightly capped, sealed with Teflon® tape and stored undisturbed at room temperature for up to 24 hrs. **Extracts must be equilibrated to 25°C (step 4.12) before dispensing.**

Note: If layers are not completely separated after storage or if particles are floating in the lower layer, make sure to document observations in the laboratory notebook and notify supervisor immediately.

6. Calculations

6.1 Utilization of chloroform extract: refer to corresponding SOP(s) for gravimetric lipid determination (SOP # 5024), cholesterol (SOP # 5020), and fatty acid methyl esters (SOP # 5010).

7. Reference(s)

7.1 AOAC 1990. Fats in Foods. Chloroform-Methanol Extraction Method #983.23, In *Official Methods of Analysis*, 15th ed. K. Helfrich (Ed), 1100, Assoc. Off. Anal. Chem. Arlington, Va.

7.2 Bligh E.G. and Dyer W.J., 1959. A Rapid Method of Total Lipid Extraction and Purification. Can. J. Biochem. Physiol. 37:911-917.

Prepared by: Jennifer Boyle

Date: _____

Approved by: Katherine Phillips

Date: _____

SOP # 5017-1
Original Date: 12-JUL-93
Revision: 25-AUG-94

Food Analysis Laboratory Control Center (FALCC)
Standard Operating Procedure

TITLE: Preparation of Cholesterol Calibration Standards

1. Purpose

To describe the procedure for preparing the calibration standards for cholesterol assay.

2. Safety

CAUTION: Pyridine and BSTFA are toxic, wear gloves and work in the hood when using these two chemicals. Pyridine and Isooctane are highly flammable. Work in the hood and stay away from ignition sources. Powder-free gloves should be on worn when working with these chemicals.

2.1 Read the MSDS for BSTFA [bis(trimethylsilyl)trifluoroacetamide], Pyridine, Isooctane, Potassium Hydroxide, and Pyrogallol.

2.2 Know the location of the eye wash and the safety shower.

2.3 Wear powder-free gloves when working with any of the above chemicals.

3. Materials

3.1 Standard Coconut Oil (National Institute of Standards and Technology Reference Material # 1563-1)
3.2 Standard Fortified Coconut Oil (National Institute of Standards and Technology Reference Material # 1563-2).
3.3 Standard cholesterol (99.8±0.1% purity, SRM 911b National Institute of Standards and Technology, U.S. Department of Commerce).
3.4 Dihydrocholesterol >99% (3β-hydroxy-5α-cholestane or 5α-cholestanol) Nu-Check Prep Cat# CHS-1
3.5 200-proof, absolute Ethanol (Biochemistry Dept. Stock room).
3.6 Isooctane (Fisher Optima™ grade).
3.7 Pyridine ACS Reagent (Silation Grade Alltech Cat# 2753050).
3.8 Bis (trimethylsilyl) trifluoroacetamide with 1% trimethylchlorosilane (BSTFA w/ 1% TMCS) Alltech Cat# 2144100
3.9 Pyrogallol ACS Reagent (Sigma Cat# P2923)

3.10 Potassium Hydroxide 85% (Fisher Cat# P251-500)
3.11 Weighing Funnel (25 x 55 mm)
3.12 Clean glass volumetric flasks (2L, 1L, 500 ml, 250 ml, 100 ml)
3.13 Glass storage bottles (amber and clear) with Teflon® lined screw caps
3.14 Pasteur pipettes and pipette bulbs
3.15 Micro Balance (M1-31-3 or M1-22-10)
3.16 Vortex® mixer
3.17 N-EVAP® Analytical Evaporator, model 111 (M1-16-3 or M1-34-3 or equivalent)
3.18 Nitrogen
3.19 GC autosampler vials
3.20 Crimper (for crimp-seal vials)
3.21 Crimp seal vials, wide mouth (Fisher Cat# 03-340-8F) or equivalent
3.22 Limited volume inserts, 200μl, polyspring (Fisher Cat# 03-375-3A)or equivalent
3.23 Teflon® coated spatula
3.24 Automatic pipettes (5 ml and 1 ml Pipetman®, or 10 ml Rainin EDP Plus Motorized Microliter Pipette)
3.25 Fume Hood
3.26 Powder-free gloves
3.27 Cryogenic marker
3.28 Sonicator
3.29 Siliconized (SOP# 5008) and un-siliconized derivatization tubes (Pyrex 25 x 150 mm)
3.30 Desiccator
3.31 Brinkman Dispensette-Bottletop Dispensers (100 ml & 5 ml) (catalog # 50-10-060-0 and # 50-10-020-1 respectively)

4. Procedure

Note 1: **All standard solutions must be logged into the Chemical Standards book.**

Note 2: **The same balance must be used throughout the whole procedure.**

Note on Standard Solutions: The exact concentration of any solution is very critical to the GC analysis of cholesterol. Record all weights and calculate the concentrations of any solution and inform the GC operator of the concentrations so corrections can be made in the GC analysis.

4.1 Preparation of cholesterol stock solution (0.2mg/ml)

4.1.1 Rinse the clean glassware to be used (weighing funnel, one liter volumetric flask, amber storage bottle) with 200-proof, absolute ethanol and allow to dry **COMPLETELY**.

4.1.2 Using a Teflon® coated spatula and a micro balance, accurately weigh 200mg (to the nearest 1mg) of the standard cholesterol into a weighing funnel. Record the exact weight in grams in the chemical standards book.

4.1.3 Transfer the cholesterol into a one liter volumetric flask. Rinse the weighing funnel several times with 200-proof, absolute ethanol, pour wash into the volumetric flask.

4.1.4 Fill the volumetric flask half-full with ethanol, and mix thoroughly. Fill the flask to volume with 200-proof, absolute ethanol, cap, mix well using a stirring bar.

4.1.5 Quickly, to avoid evaporation, transfer solution to nine all-glass, 125 ml amber bottles. The exact volume in each bottle does not matter as long as all bottles contain more than 100 ml of solution.

4.1.6 Before storing the cholesterol and the cholesterol standard solution, blow nitrogen into the top of each bottle for 10 seconds, then quickly cap and store in a refrigerator (2-8°C). Calculate the final concentration of cholesterol in the solution ($\approx$0.2mg/ml). Label the bottles with the chemical standards book number, expiration date, analyst initials and precise concentration.

4.2 Preparation of cholesterol working solution (20μg/ml)

4.2.1 Rinse the clean glassware to be used (100 ml volumetric flask, storage bottle) with 200-proof, absolute ethanol and allow to dry **COMPLETELY**.

4.2.2 Accurately pipet 100 ml of the 0.2mg/ml cholesterol stock solution into a 1000 ml volumetric flask using the Brinkman 100 ml bottletop dispenser.

4.2.3 Fill the flask half-full with 200-proof, absolute ethanol, and mix thoroughly. Fill to volume with ethanol, cap immediately and mix well.

4.2.4 Quickly, to avoid evaporation, divide the solution among nine all-glass 125 ml bottles. Beforestoring the cholesterol working solution, blow some nitrogen into the top of the bottle for 10 seconds, then quickly cap and store in explosion-proof refrigerator (2-8°C). Calculate the final concentration of cholesterol ($\approx$20μg/ml). Label the bottles with the chemical standards book number, expiration date, analyst initials and precise concentration.

4.3 Preparation of Dihydrocholesterol Internal Standard Solution (20μg/ml)

4.3.1 Rinse the clean glassware to be used (2000 ml volumetric flask, weighing funnel, storage bottles) with 200-proof, absolute ethanol and allow to dry **COMPLETELY**.

4.3.2 Accurately weigh 40 mg of dihydrocholesterol (to the nearest 1 mg) in a weighing funnel. Record exact weight in grams in chemical standards book.

4.3.3 Add the dihydrocholesterol to the volumetric flask. Rinse the funnel with 200-proof, absolute ethanol several times into the flask. Fill the flask half-full with ethanol, and mix thoroughly. Fill to volume with ethanol, cap, mix well and, quickly (to avoid evaporation) transfer solution to eight all-glass 250 ml bottles. It is not critical to have exactly 250 ml in each bottle. Any amount between 225 and 250 ml is sufficient. Any excess solution may be discarded.

4.3.4 Before storing the dihydrocholesterol internal standard solution, blow some nitrogen

into the top of each bottle for 10 seconds, then quickly cap and store in an explosion-proof refrigerator (2-8°C). Calculate the final concentration of dihydrocholesterol in the internal standard solution ($\approx 20\mu g/ml$). Label the bottles with the chemical standards book number, expiration date, analyst initials and precise concentration.

4.4 Validation of cholesterol stock and working solutions, and dihydrocholesterol internal standard solution.

4.4.1 Newly prepared cholesterol stock and working solutions, and dihydrocholesterol internal standard solution should be validated using Coconut oil and Fortified Coconut oil Reference Standards (NIST #1563-1 and NIST #1563-2) before using them in any other assays. Please refer to SOP# 5037-0 for validation of such solutions.

4.5 Preparation of Internal Standard Tubes

4.5.1 Remove dihydrocholesterol internal standard solution ($\approx 20\mu g/ml$) from refrigerator. Allow it to warm up to room temperature (24° to 26° C) for 60 minutes in the laboratory bench. Mix the dihydrocholesterol working solution thoroughly by gently swirling the bottle.

4.5.2 Assemble the 5 ml bottletop dispenser in accordance with the manufacture's instructions. Prime the dispenser with 40-50 ml of 200 proof, absolute ethanol. Dry the filling tube and discharge tube to prevent dilution of the internal standard solution. Attach the dispenser to the bottle of dihydrocholesterol working solution.

4.5.3 Make sure the dispenser is set to dispense 2.5 ml. Prime the dispenser by delivering the first two solution aliquots into a waste beaker. Begin dispensing 2.5 ml of the solution into the 25 x 150 mm Pyrex tubes. Fill as many tubes as possible (approximately 80 tubes). Discard any remaining solution. Blow nitrogen over the tubes and cap. Store the tubes in the refrigerator (2-8°C). Label the tubes with the chemical standard book number, expiration date, analyst initials and concentration of the solution.

4.6 Preparation of Derivatization Reagent

Note: **Prepare new reagent before every assay.**

4.6.1 Accurately pipet 2 ml of pyridine and 2 ml of BSTFA into a small (5 ml) glass container that has been cleaned, rinsed and **completely dried.**

4.6.2 Cap the container with nitrogen, and swirl gently and thoroughly, to mix. Cap both the pyridine and the BSTFA with nitrogen. Store the pyridine in the hood. Store the BSTFA in the explosion-proof refrigerator.

4.7 Preparation of Calibration Curve Standards

4.7.1 Obtain tubes containing dihydrocholesterol internal standard solution ($\approx$20μg/ml) (step **4.4**). Label the tubes: Blank, 25μg, 50μg, 100μg, and 200μg, 300μg, and 400μg. Turn on the N-EVAP evaporator by flipping the switch on the bottom right, to the right, and heat the water to 60°C. Monitor temperature with thermometer.

4.7.2 Accurately pipet using a Rainin EDP Plus Motorized Microliter Pipette the following amounts of the given cholesterol solution into the tubes containing the dihydrocholesterol internal standard solution (step **4.6.1**):

Blank - 0 ml
25μg - 1.25 ml (20μg/ml Cholesterol Working Solution)
50μg - 2.50 ml (20μg/ml Cholesterol Working Solution)
100μg - 5.00 ml (20μg/ml Cholesterol Working Solution)
200μg - 1.00 ml (0.2mg/ml Cholesterol Stock Solution)
300μg - 1.50 ml (0.2mg/ml Cholesterol Stock Solution)
400μg - 2.00 ml (0.2mg/ml Cholesterol Stock Solution)

4.7.3 Place each of the tubes in an N-EVAP. Lower the needles to about 1cm above the surface of the solution, and turn on the gas so that the solution bubbles slightly. Evaporate the solvents in the tube to complete dryness.

4.7.4 In the hood, accurately pipet 250μl of the Derivatization Solution into one of the tubes, recap the tube, and mix on a Vortex® mixer.

4.7.5 Using a clean pasteur pipet and pipet bulb, transfer all of the solution from the test tube into a 200μl limited volume insert. Place the full insert into a crimp-seal vial, and seal the vial. Label the vial with the sample number, date, initials, and mark the level of the liquid.

4.7.6 Repeat steps **4.7.4** and **4.7.5** for each of the test tubes.

4.7.7 Each sample batch run on the GC will require a continuous calibration standard. This standard should be prepared like the 100μg calibration curve standard (**4.7.2**). Prepare one during each assay (SOP# 5020-2).

4.7.8 Cap the cholesterol solutions and store in an explosion-proof refrigerator. Store the crimp-seal vials containing the calibration curve solutions in an explosion-proof refrigerator (2-8°C) for up to 4 weeks.

4.8 Preparation of Reference Standards

Note: **Wear clean powder-free gloves for this preparation.**

4.8.1 Rinse the clean glassware to be used (weighing funnel, 250 ml volumetric flask, glass storage bottle) with 200-proof, absolute ethanol and allow to dry **COMPLETELY**.

4.8.2 Fill the sonicator with warm water (25-30°C) and sonicate an ampule of Standard Coconut Oil (NIST #1563-1) for 5-10 minutes (until the sample is completely melted). Wipe outside of ampule with clean kimwipe.

4.8.3 Mix contents of ampule and then break the ampule. Immediately weigh out 1.5g (to the nearest 1mg) of oil into a weighing funnel, using a glass pipette. (The coconut oil oxidizes in the presence of air, so the weighing and the transfer should be done without delay. Once the oil is dissolved in isooctane, the oxidation process is negligible.) Record exact weight in chemical standards book.

4.8.4 Immediately transfer the coconut oil to the 250 ml volumetric flask using repeated rinses with isooctane. Fill the flask half-full with isooctane, and mix the solution thoroughly. Fill to volume with isooctane, cap and mix thoroughly.

4.8.5 Quickly transfer the solution to a glass storage bottle, cap under nitrogen, and store in an explosion-proof refrigerator (2-8°C). Calculate the final concentration of coconut oil ($\approx$6mg/ml). Label bottle with chemical standardsbook number, expiration date, analyst initials, and concentration of solution

4.8.6 Repeat steps **4.8.1** through **4.8.5** using Standard Fortified Coconut Oil (NIST #1563-2).

4.9 Preparation of Ethanol/Pyrogallol Solution

4.9.1 Rinse the clean glassware to be used (weighing bottle, one liter volumetric flask, glass storage bottle) with 200-proof, absolute ethanol and allow to dry **COMPLETELY**.

4.9.2 Using a Teflon® coated spatula and a micro balance, weigh approximately 30g of pyrogallol into a glass weighing bottle. Record exact weight in chemical standards book.

4.9.3 Transfer the pyrogallol into a one liter volumetric flask. Rinse the weighing bottle several times with ethanol and pour into the volumetric flask.

4.9.4 Fill the volumetric flask half-full with ethanol, and mix thoroughly. Fill the flask to volume with ethanol, and transfer to an all-glass storage bottle.

4.9.5 Before storing the Ethanol/Pyrogallol solution, blow some nitrogen into the top of the bottle for 20 seconds, then quickly cap. Pyrogallol reacts with air, forming a brown solution, so it may be necessary to occasionally bubble nitrogen through the solution to remove any dissolved oxygen. Store the Ethanol/Pyrogallol solution in the solvent storage cabinet, under the hood. The final concentration of Pyrogallol is approximately 30mg/ml, or 3% w/v. Label the bottle with chemical standards book number, date of preparation, analyst initials and precise concentration. This solution should be discarded when it turns brown, and fresh solution should be prepared.

4.10 Preparation of Potassium Hydroxide Solution

4.10.1 Rinse the clean glassware to be used (100 ml glass storage bottle with a glass stopper, 50 ml graduated cylinder) with deionized water and allow to dry.

4.10.2 Weigh approximately 60g of 85% potassium hydroxide (KOH) into a 100 ml glass storage bottle.

4.10.3 Using a graduated cylinder, measure 40 ml of deionized water, and pour into the storage bottle. Seal the bottle with the glass stopper, and mix by swirling.

4.10.4 Cap the KOH and store at room temperature in a desiccator. Store the KOH solution at room temperature. The final concentration of KOH is approximately 1.28g/ml. Label the bottle with chemical standards book number, date of preparation, analyst initials and precise concentration.

5. Storage

5.1 All of the solutions used for this assay (except for the Calibration Curve Standards **4.7** and the Potassium Hydroxide Solution **4.10**) should be capped under nitrogen before storage. The storage conditions of the chemicals and solutions are listed in their respective preparation procedure.

6. Reference(s)

6.1 Thompson, Raymond H. Jr. and George V. Merola Quantitation of Cholesterol, USDA-NCL (1993)

Prepared by:

Robert Harris

_____

Date: _____

Approved by:

Katherine Phillips

_____

Date: _____

SOP # 5019
Date: 09-JUL-93
Revision: New

Food Analysis Laboratory Control Center (FALCC)
Standard Operating Procedure

TITLE: GC Method for Fatty Acid Methyl Ester Analysis

1. Purpose

To describe the procedure for programming the GC using Turbochom 3, and the GC control panel.

2. Safety

CAUTION: The Gas Chromatograph uses highly pressurized Hydrogen as a fuel source for the flame ionization detector (FID). Hydrogen, when mixed with air is highly explosive, make sure that the Hydrogen is turned off when the flame is not lit.

2.1 Read the material Safety Data Sheet (MSDS) for iso-octane.

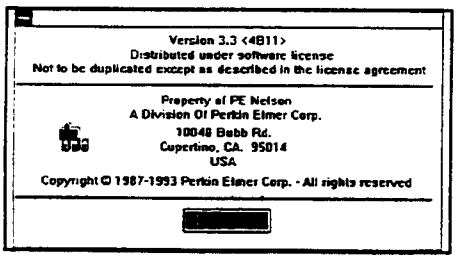
2.2 Do not touch the injector, it is extremely hot.

3. Materials

3.1 Gas Chromatograph - Perkin Elmer Autosystem GC with Autosampler (Instrument B, M1-34-5) (Column: Stabilwax®, 30 m, 0.25 mm ID, 0.1 μm df - Restek # 10608)
3.2 Digital DECpc 433dxLP Computer (M1-29-5)
3.3 Turbochom 3 software system for data aquisition and analysis
3.4 Hewlett Packard Laser Jet IIIp
3.5 Fatty acid methyl ester samples (see SOP# 5010), blank - iso-octane, continuous calibration standard - AOCS Rapeseed Oil Reference Mix (Matreya Catalog # 1083)

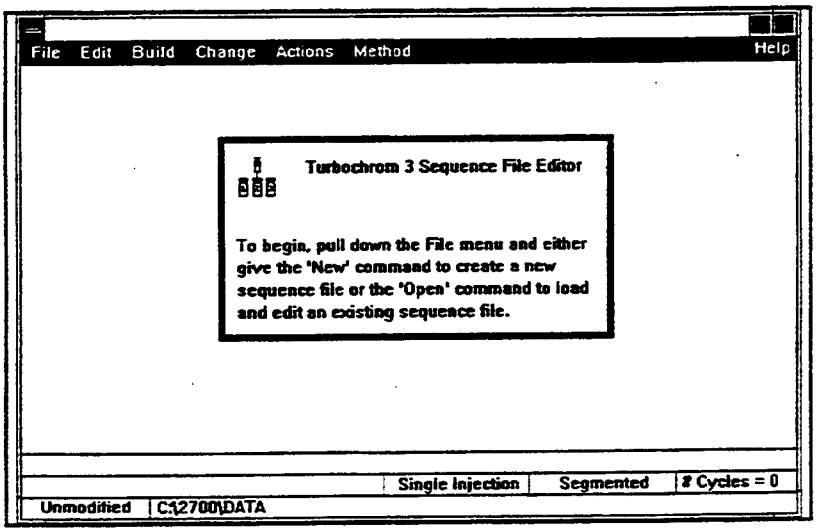
4. Procedure

4.1 Turn on the computer by pressing the ⊘ button on the right-front of the computer. The Microsoft Windows® screen will appear followed by:

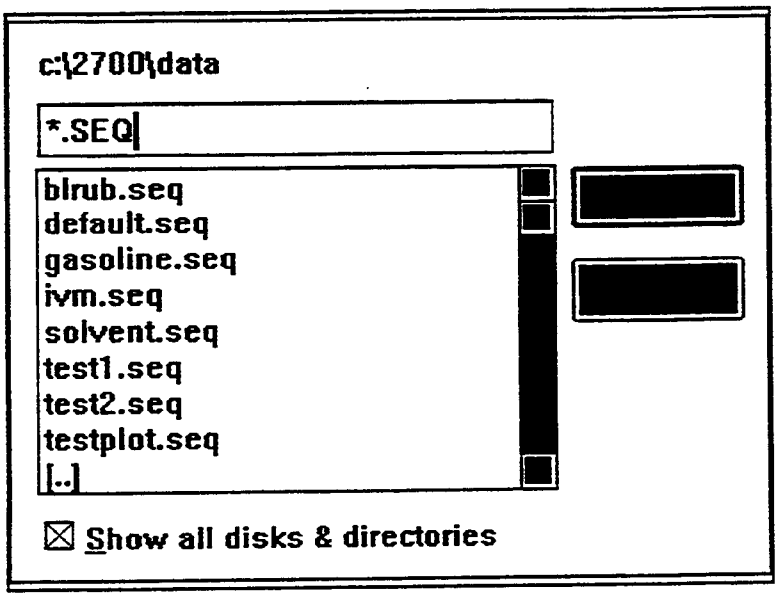


4.2 Using the mouse, move the pointer to the *Continue* bar and press the left mouse button. The Turbochom 3 Main Menu Bar will appear at the bottom of the screen.

4.3 Move the pointer to *Editors* and press the left mouse button. Click on *Sequence*, and the *sequence editor* will appear.

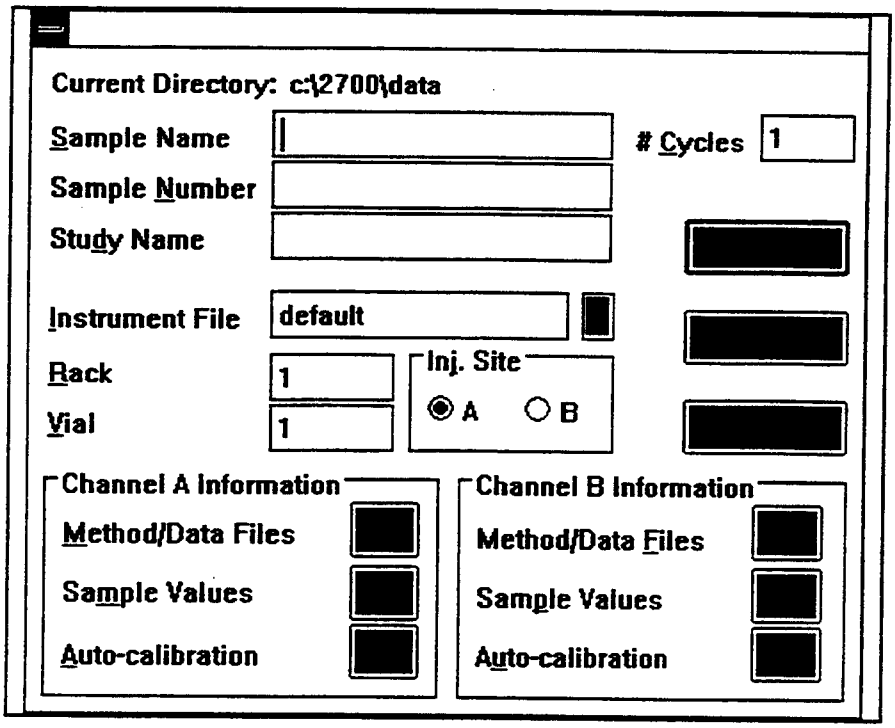


4.4 Click on *File*, then *Open*, and the following screen appears:



Double click on *[..]*, then on *[fame]*. Highlight *default.seq* by clicking on it and then click on *OK*. The editor loads the default sequence file and returns to the *sequence editor* (**4.3**) screen.

4.5 Click on *Build*, then on *Append New Cycles*, and the *Add Sequence Information* Screen will appear:



The cursor blinks in the *Sample Name* box. Type in the name of the sample for the first sample in the batch (e.g. VT301VT). The *Sample Number* box should read 3. Move the cursor to the *Study Name* box, click and type "FAMEASSAY" followed by the assay number as assigned (e.g. FAMEASSAY001). Move to the *Vial* box and type in the number that the vial will be placed under on the autosampler rack (since slots 1 and 2 are reserved for a blank (isooctane) and a continuous calibration sample, 3 is the first available slot).

4.6 Click on the *Add* box. The Sample Number will then change to 4.

4.7 Type in the sample name for the next sample in the batch, and change the *vial number* to 4 or the next consecutive number. Click the *Add* box.

Note: While the sample numbers will change automatically, the sample names and vial numbers have to be changed manually.

4.8 Repeat **4.7** for all the samples in the run. Click on the *Quit* box to exit the sequence editor after the last sample has been added.

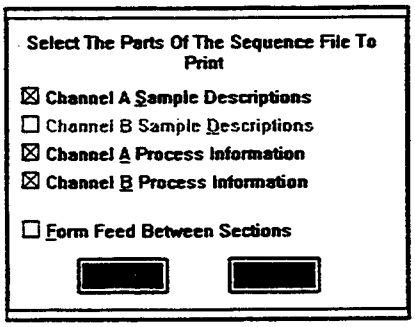
4.9 It is necessary to edit the sequence in the sequence spreadsheet. To view the spreadsheet, click on *Edit* in the *Sequence Editor* Screen, then *Channel A*. The spreadsheet can be viewed and edited using the mouse or arrow keys. Change all of the entries in the *DATA* column to the path followed by the name of the sample in that row (e.g. c:\2700\fame\VT301VT). The entries in the *DATA* column for the blank and the continuing calibration (Con-Cal) samples

should be BLNK and CCAL respectively followed by the assay number as assigned (e.g. c:\2700\fame\BLNK001). Check all other entries for the correct information that were just entered for all the samples.

4.10 After editing the spreadsheet, click the *minus sign* box in the left corner above the O of *Options* and click on *Close*.

4.11 Save the file by clicking *File*, then *Save As...* **(Caution: Do not use the *save* command since it will overwrite the default file)** Type in the assay number (as assigned, e.g. FA001) as the filename of the sequence, including the correct path (the path will always be **C:\2700\fame**\FA#.seq). Click on *OK*. (In case of a warning screen, do not overwrite the old sequence file! Check at once with a supervisor on the correct assay number.)

4.12 Print the sequence by clicking on *File*, then *Print*. The following screen will appear:



Since only the box labeled; *"Channel A Sample Description,"* and *"Channel A process Info"* should be marked by an X, click the other boxes so that the X disappears. Then click the *OK* box. Wait for the printout. Cut and paste the sequence printout into the GC-B Log Book.

4.13 Exit the sequence editor by clicking on the *minus sign* box in the top left-hand corner of the screen then click *Close*.

4.14 On the GC control panel press *DET TEMP* (white button), then punch in 200 (black buttons), and press *ENTER* (yellow button). This will allow the detector block to heat up for lighting the detector.

4.15 Remove the blank (isooctane), the continuous calibration standard and the samples to be run (SOP# 5010), from the freezer, and place them in their respective vial holders in the GC as determined by the sequence file.

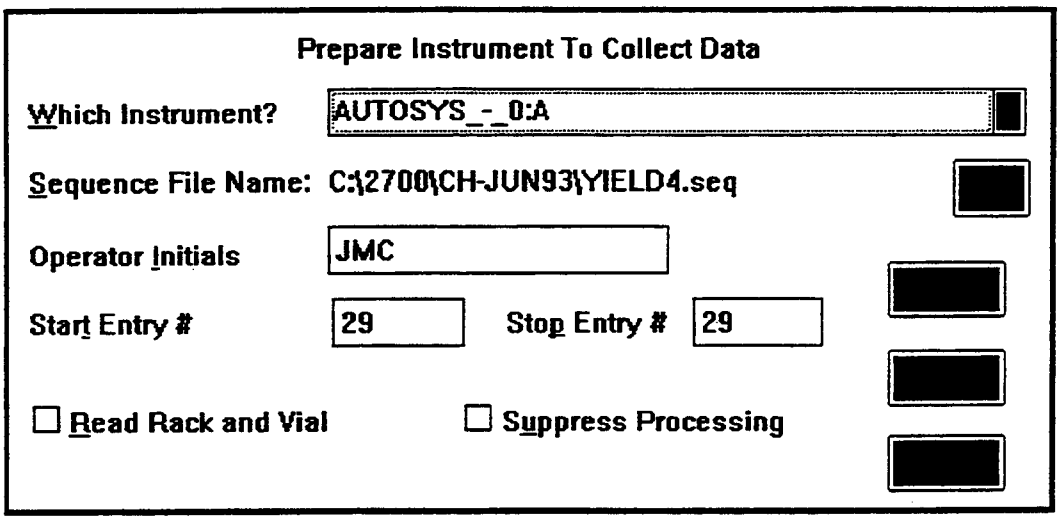
4.16 Open the hydrogen and air gas tanks designated for this GC (instrument B). On the GC, open the split, air and hydrogen valves by turning the valve knobs (towards the rear end) counter-clockwise to "ON". Check the carrier gas pressure (19.9-20.1 psi) by pressing the *carrier gas* (white) button on the front panel.

4.17 Press *Auto Zero* (white) and light the Flame Ionization Detector by opening the lid on the right top of the GC, removing the ignitor, closing the air valve, pressing the ignitor on top of

the detector, and slowly opening the air valve. A popping sound should be heard, and the value on the front panel should jump to several mV. The value should then drop down to 0.5 to 1 mV, and remain there. Open the air valve completely. If the value drops below 0.05 mV, the detector failed to ignite and the procedure has to be repeated.

4.18 Make sure that wash vials 1 and 2 (in the center of the autosampler tray) are filled with iso-octane, and that waste vials 1 and 2 are empty.

4.19 From the Turbochrom 3 main menu bar (**4.2**), click on *Acquire*, then click *Download*. The following screen will appear:



Highlight *AUTOSYS_-_0;B* by clicking on the ↓ key. Click on the ⇒ button next to the Sequence File Name, double click on *[fame]* and highlight the sequence file that was created in steps **4.3** through **4.11**. Click on *OK*. Type in the Operator Initials, and click on the *Verify* button. The sequence will then be dowloaded to the GC. From the *Data Aquisition* screen, click *Inst*, then *Start*.

4.20 Make sure the printer is turned on and paper is loaded into the paper tray.

4.21 SHUTDOWN procedure: After the last sample run has been completed, in the *Data Aquisition* Screen of the *Aquire* menu click on *Comm* (for Communication). Click *Release Control*, highlight *AUTOSYS_-_0;B* and click OK. Then click on the *minus sign* box in the top left-hand corner of the screen and click *Close*. On the GC, turn off the split, air and hydrogen knob valves, and turn off the air and hydrogen tanks (**not the Helium tank!**). On the GC control panel, press the *DET TEMP* button and press *OFF* (black button) then *Enter*. Then press *INJ TEMP*, type in 100 and *Enter*, then press *OVEN TEMP*, punch in 50 and press *ENTER*.. The GC is now shut down and the computer and printer can be turned off as soon as all chromatograms are printed.

4.22 Remove the caps of the crimp-seal vials using the decapper. Reseal the vials with new caps and mark the level of the liquid in the vial. Store the vials in a freezer.

5. Calculations

5.1 The results consist of a chromatogram and a report and will be printed automatically by the computer. The *Adjusted Amounts* are μg of fatty acid methyl esters per sample. In addition, the program generates an ASCII file that will be used for further quantitation (SOP#5025). Punch holes in the reports and place them in the fatty acid data notebooks.

6. Reference(s)

6.1 PE Nelson Turbochrom 3 User's Guide 1992

6.2 All figures were obtained directly from the Turbochrome 3 software (version 3.3)

Prepared by:

Ingolf Gruen

_____

Date: _____

Approved by:

Katherine Phillipis

_____

Date: _____

SOP # 5022
Date: 12-JUL-93
Revision: New

Food Analysis Laboratory Control Center (FALCC)
Standard Operating Procedure

TITLE: GC Method for Cholesterol Analysis

1. Purpose

To describe the procedure for programming the GC using Turbochom 3, and the GC control panel.

2. Safety

CAUTION: The Gas Chromatograph uses highly pressurized Hydrogen as a fuel source for the flame ionization detector (FID). Hydrogen, when mixed with air is highly explosive, make sure that the Hydrogen is turned off when the flame is not lit.

2.1 Read the Material Safety Data Sheet (MSDS) for pyridine and BSTFA.

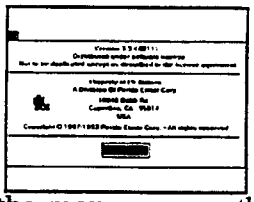
2.2 Do not touch the injector or detector, they are extremely hot.

3. Materials

3.1 Gas Chromatograph - Perkin Elmer Autosystem GC with Autosampler (Instrument A, M1-34-5) (Column: DB-5 fused silica column, 60m, 0.25mm ID, 0.1μm film, J&W Scientific Cat # 122-5061)
3.2 Digital DECpc 433dxLP Computer (M1-29-5)
3.3 Turbochom 3 software system for data acquisition and analysis
3.4 Hewlett Packard Laser Jet IIIp (M1-34-7)
3.5 Cholesterol samples (see SOP# 5020), derivitization reagent blank, continuous calibration standard (see SOP# 5017)

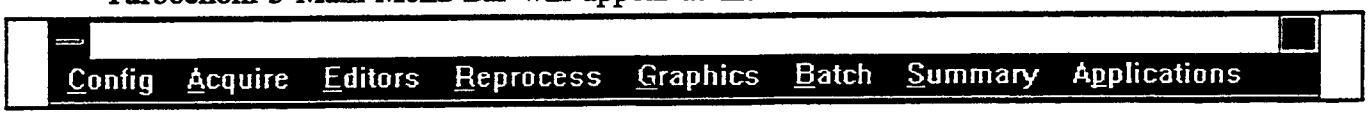
4. Procedure

4.1 Turn on the computer by pressing the ⊘ button on the right-front of the computer. The Microsoft Windows® screen will appear followed by:

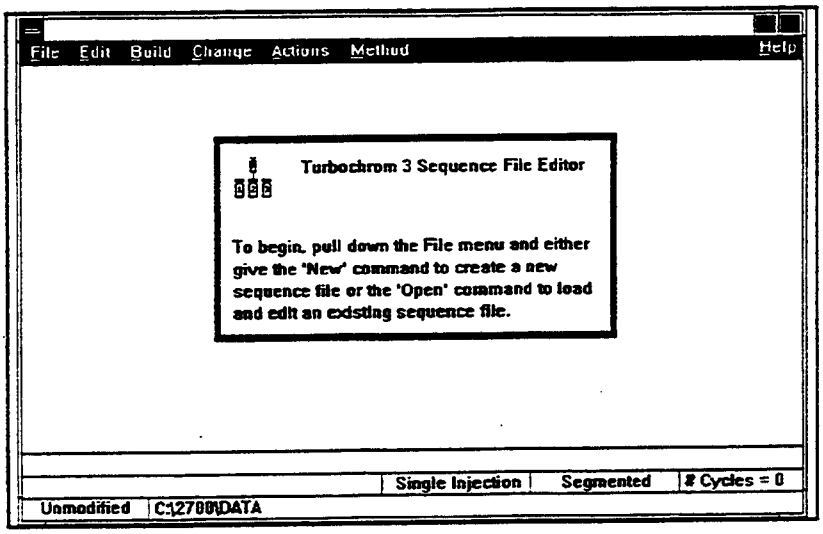


4.2 Using the mouse, move the pointer to the *Continue* bar and press the left mouse button. The

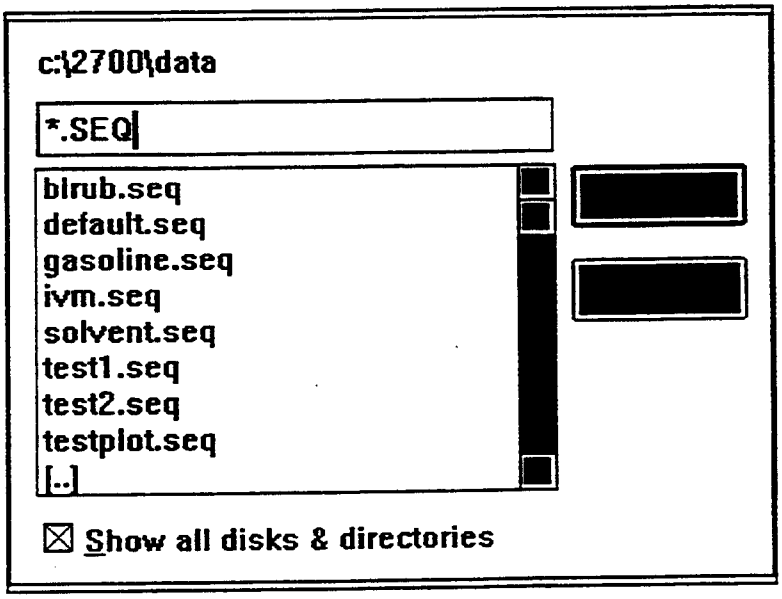
Turbochom 3 Main Menu Bar will appear at the bottom of the screen.



4.3 Move the pointer to *Editors* and press the left mouse button. Click on *Sequence*, and the *sequence editor* will appear.

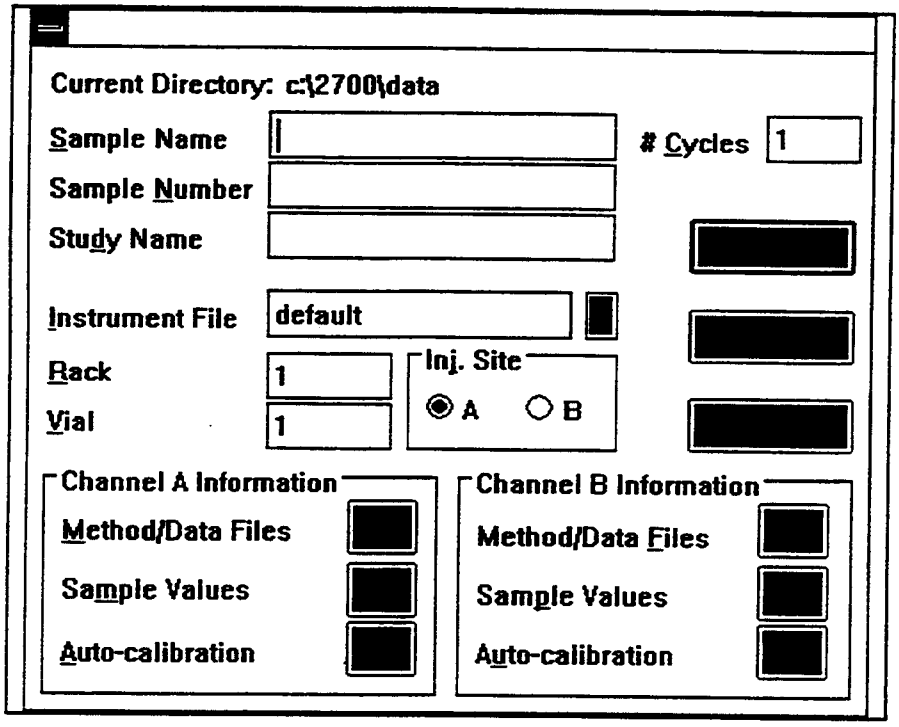


4.4 Click on *File*, then *Open*, and the following screen appears:



Double click on *[..]*, then on *[cholstrl]*. Highlight *default.seq* by clicking on it and then click on *OK*. The editor loads the default sequence file and returns to the *sequence editor* (**4.3**) screen.

4.5 Click on *Build*, then on *Append New Cycles*, and the *Add Sequence Information* Screen will appear:



The cursor blinks in the *Sample Name* box. Type in the name of the sample for the first sample in the batch (e.g. VT301VT). The *Sample Number* box should read 3. Move the cursor to the *Study Name* box, click and type "CHOLESTEROL" followed by the assay number as assigned (e.g. CHOLESTEROL001). Move to the *Vial* box and type in the number that the vial will be placed under on the autosampler rack (since slots 1 and 2 are reserved for a blank (pyridine/BSTFA) and a continuous calibration sample, 3 is the first available slot).

4.6 Click on the *Add* box. The Sample Number will then change to 4.

4.7 Type in the sample name for the next sample in the run, and change the *vial number* to 4 or the next consecutive number. Click the *Add* box.

Note: While the sample numbers change automatically, the sample names and vial numbers must be changed manually.

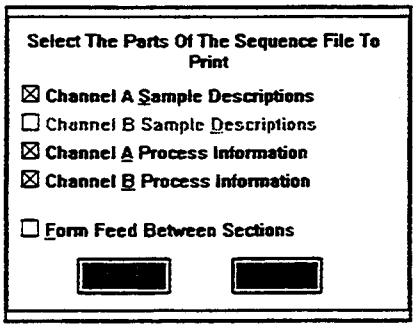
4.8 Repeat **4.7** for all the samples in the run. Click on the *Quit* box to exit the sequence editor after the last sample has been added.

4.9 It is necessary to edit the sequence in the sequence spreadsheet. To view the spreadsheet, click on *Edit* in the *Sequence Editor* Screen, then *Channel A*. The spreadsheet can be viewed and edited using the mouse or arrow keys. Change all of the entries in the *DATA* column to the path followed by the name of the sample in that row (e.g. c:\2700\cholstrl\VT301VT). The entries in the *DATA* column for the blank and the continuing calibration (Con-Cal) samples should be BLNK and CCAL respectively followed by the assay number as assigned (e.g. c:\2700\cholstrl\ BLNK001). Check all other entries in the spreadsheet for errors.

4.10 After editing the spreadsheet, click the *minus sign* box in the left corner above the O of *Options* and click on *Close*.

4.11 Save the file by clicking *File*, then *Save As...* **(Caution: Do not use the *save* command since it will overwrite the default file)**. Type in the assay number (as assigned) as the filename of the sequence, including the correct path (e.g. c:\2700\CHOLSTRL\CL001.seq). Click on *OK*. (In case of a warning screen, do not overwrite the old sequence file! Check at once with a supervisor on the correct assay number.)

4.12 Print the sequence by clicking on *File*, then *Print*. The following screen will appear:



Since only the boxes labeled, *"Channel A Sample Description,"* and *"Channel A Process Information,"* should be marked with an X, click the other boxes so that the X disappears. Then click the *OK* box. Wait for the printout. Cut and paste the sequence printout into the GC-A Log Book.

4.13 Exit the sequence editor by clicking on the *minus sign* box in the top left-hand corner of the screen then click *Close*.

4.14 On the GC control panel press *DET TEMP* (white button), then press *RESET OVEN* (yellow button), punch in *300* (black buttons), and press *ENTER* (yellow button). This will allow the detector block to heat up for lighting.

4.15 Remove the blank (pyridine/BSTFA), the continuous calibration standard and the samples to be run (SOP# 5020), from the refrigerator, and place them in their respective vial holders in the GC as determined by the sequence file.

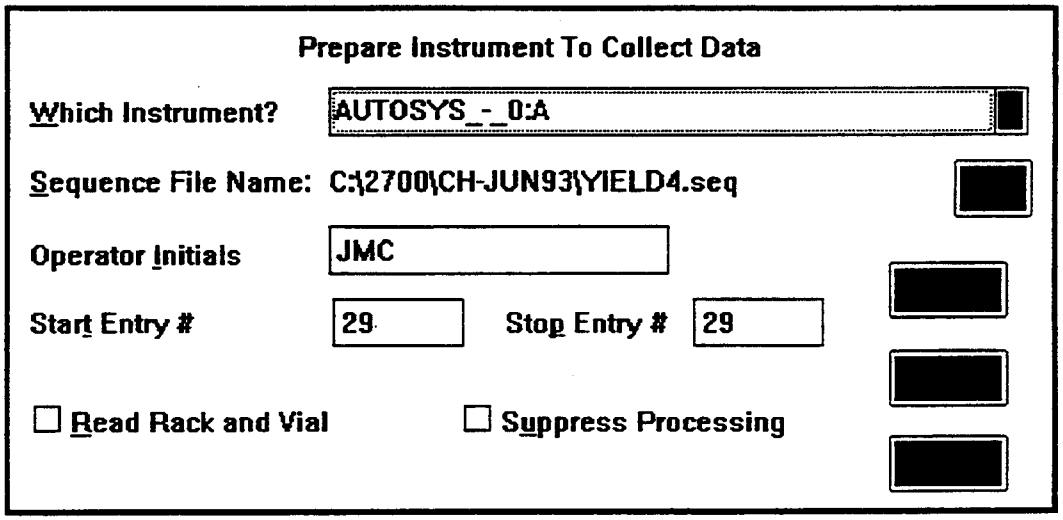
4.16 Open the hydrogen and air gas tanks designated for this GC (instrument A). On the GC, open the split, air and hydrogen valves by turning the valve knobs (towards the back of the

instrument) counter-clockwise to "ON". Check the carrier gas pressure (39.9-40.1 psi) by pressing the *carrier gas* (white) button on the front panel.

4.17 Press *Auto Zero* (white) and light the Flame Ionization Detector by opening the lid on the right top of the GC, removing the ignitor, closing the air valve, pressing the ignitor on top of the detector, and slowly opening the air valve. A popping sound should be heard, and the value on the front panel will jump to several mV. The value should then drop down to 0.5 to 1 mV, and remain there. Open the air valve completely. If the value drops below 0.05 mV, the detector failed to ignite and this step must be repeated.

4.18 Make sure that wash vials 1 and 2 (in the center of the autosampler tray) are filled with 200 proof-absolute ethanol, and that waste vials 1 and 2 are empty.

4.19 From the Turbochrom 3 main menu bar (**4.2**), click on *Acquire*, then click *Download*. The following screen will appear:



Highlight *AUTOSYS_-_0;A* by clicking on the ↓ key. Click on the ⇒ button next to the Sequence File Name, double click on *[cholstrl]* and highlight the sequence file that was created in steps **4.3** through **4.11**. Click on *OK*. Type in the Operator Initials, and click on the *OK* button. The sequence will then be downloaded to the GC. From the *Data Acquisition* screen, click *Inst*, then *Start*. Highlight *AUTOSYS_-_0;A*, then click on *OK*.

4.20 Make sure the printer is turned on and paper is loaded into the paper tray.

4.21 SHUTDOWN procedure: After the last sample run has been completed, in the *Acquire* menu click on *Comm* (for Communication). Click *Release Control*, highlight *AUTOSYS_-_0;A* and click *OK*. Then click on the *minus sign* box in the top left-hand corner of the screen and click *Close*. On the GC, close the split, air and hydrogen valves, and turn off the air and hydrogen tanks (**not the Helium tank!**). On the GC control panel, press the *DET TEMP* button and press *OFF* (black button) then *Enter*. Then press *INJ TEMP,* type in *100* and *Enter*, then press *OVEN TEMP*, type in *60* and press *ENTER*. The GC is now shut down and the computer and printer can be turned off as soon as all chromatograms are printed.

4.22 Remove the caps of the crimp-seal vials using the decapper. Reseal the vials with new caps and mark the level of the liquid in the vial. Store the vials in an explosion-proof refrigerator.

Note: If at any time an error message appears on the screen, notify supervisor immediately.

5. Calculations

5.1 The results consist of a chromatogram and a report and will be printed automatically by the computer. The *Adjusted Amounts* are μg of cholesterol per sample. In addition, the program generates an ASCII file that will be used for further quantitation (SOP#5026). Punch holes in the reports and place them in the cholesterol data notebooks.

6. Reference(s)

6.1 PE Nelson Turbochrom 3 User's Guide 1992

6.2 All figures were obtained directly from the Turbochrome 3 software (version 3.3)

Prepared by:

James Cooke

_____

Date: _____

Approved by:

Katherine Phillips

_____

Date: _____

SOP #5025
Date: 15-OCT-93
Revision: 2

Food Analysis Laboratory Control Center (FALCC)
Standard Operating Procedure

TITLE: Fatty Acid Determination in Mixed Diets

1. Purpose

To describe the procedure of quantitation of fatty acids in composites of mixed diets.

2. Safety

2.1 No special safety measures are necessary beyond those outlined in the various SOPs required for this procedure.

3. Materials

3.1 SOP#5015 - Extraction of Lipids from Food with Chloroform-Methanol
3.2 SOP#5016 - Preparation of Fatty Acid Methyl Ester Standards
3.3 SOP#5018 - Internal Standard Spikes for Fatty Acid Methyl Ester Quantitation
3.4 SOP#5010 - Fatty Acid Methylation
3.5 SOP#5019 - GC Method for Fatty Acid Methyl Ester Analysis
3.6 Digital DECpc 433dxLP Computer (M1-29-5)
3.7 Hewlett Packard Laser Jet IIIp (M1-34-7)
3.8 "QuattroPro for Windows®" software program

4. Procedure

Note: Read all SOPs mentioned in section **3. Materials**

4.1 Extract lipids according to SOP # 5015, "Extraction of Lipids from Food with Chloroform-Methanol".

4.2 If new calibration standards have to be prepared, proceed with SOP # 5016, if not, continue with **4.3**

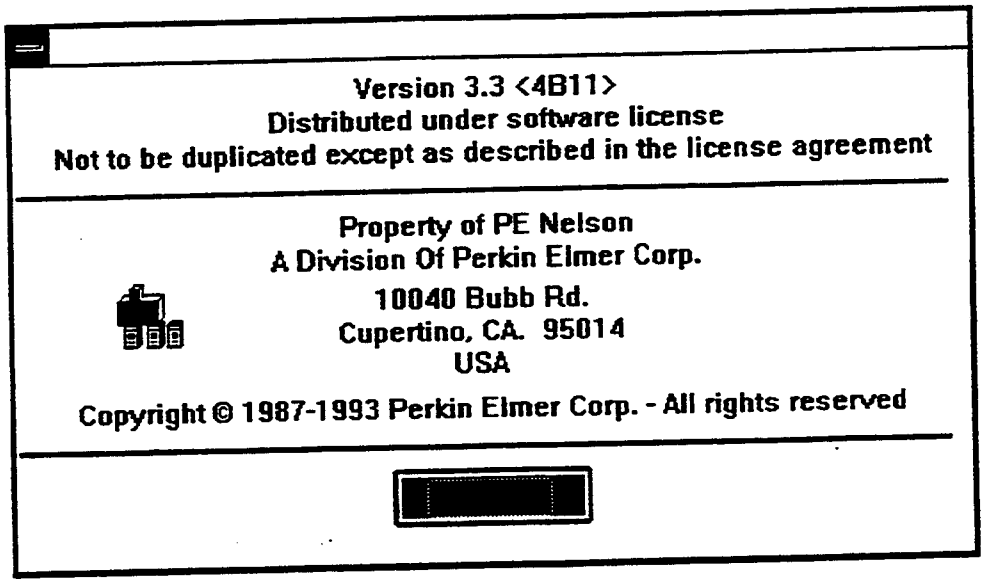
4.3 If new internal standard working solutions or internal standard tubes have to be prepared, proceed with SOP # 5018, if not, continue with **4.4**

4.4 Methylate fatty acids according to SOP # 5010, "Fatty Acid Methylation".

4.5 Quantitate the fatty acid methyl esters according to SOP # 5019, "GC Method for Fatty Acid Methyl Ester Analysis"

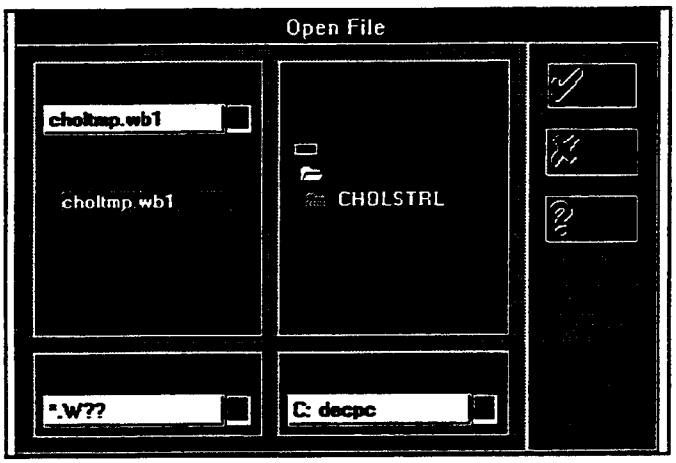
4.6 All data files of GC chromatograms are stored in the form of ASCII files as well as Turbochrom® application files. The filename of both, the Turbochrom® data file and the ASCII file is the sample number. However, the Turbochrom® data files have the appendices "RAW" and "RST" for the raw data and the result data file respectively, while the ASCII file carries the appendix "TX0". Retrieve the ASCII file with "Quattro®Pro for Windows".

4.6.1 Turn on the computer by pressing the ⊘ button on the right-front of the computer. The Microsoft Windows® screen will appear followed by:

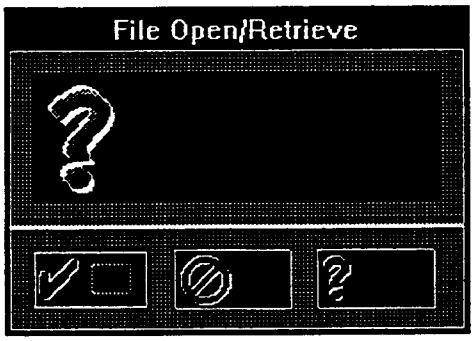


4.6.2 Using the mouse, move the pointer to the *Continue* bar and press the left mouse button. Double-click on the "Quattro Programs" icon and the quattro submenu will open. Double-click on "Quattro®Pro for Windows" and the application will open a blank spreadsheet.

4.6.3 Click on *File*, then *Open*. The following screen will appear:

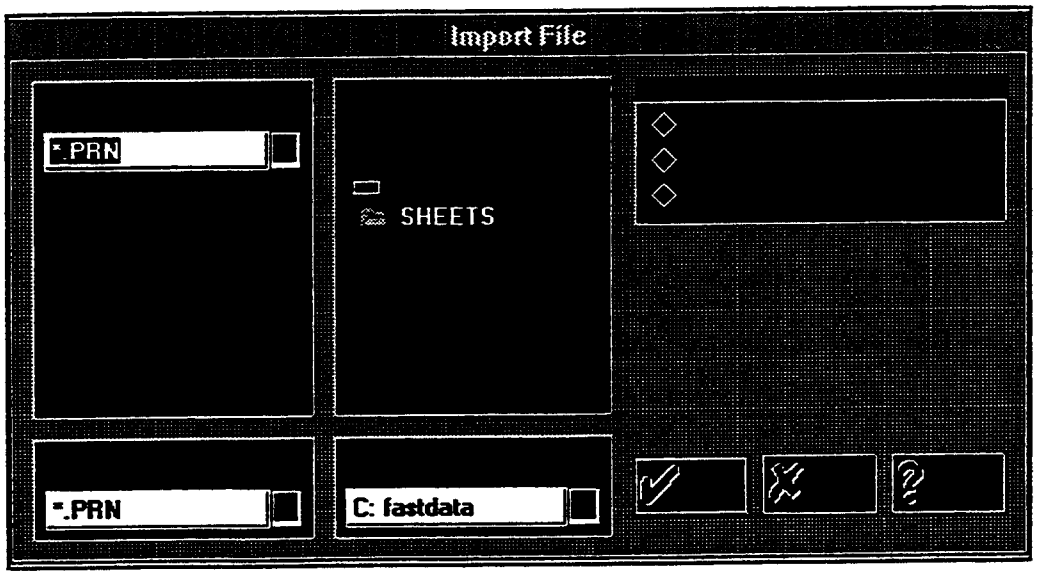


Under *Directories*, double click on *C:*, then *2700*, then *FAME*. Under *File Name*, double click on *FAMETEM2.WB1*, which will bring up the template for the fatty acid quantitation. The following screen will appear;



Click on the *Yes* box. This prevents the template from being overwritten.

4.6.4 Make sure that the highlighted box is in the top-right corner (cell A1). Click on *Tools* and then *Import*, and the following screen will appear:



4.6.5 Type: *.tx0 (zero not O). Then, using the mouse, click the diamond next to *Comma and "Delimited File* under *Option*, then click on *OK*. All the ASCII files created by Turbochrom® that are in the subdirectory *\fame* will be displayed under *File Name*. Scroll down the list and highlight the file that is to be processed. Then click on the *OK* button. The ASCII file will be retrieved into the spreadsheet.

4.7 To analyze and sort the ASCII file, click on the *Analyze* button in the middle of the spreadsheet. This macro will organize the spreadsheet into a readable format and print a result file. When the program asks for the sample number, type in the sample number (e.g. VT302VT), and press the ENTER key.

4.8 When the program asks for the weight of the food composite that was used for the lipid extraction, type in the weight (including the decimal points), and press the ENTER key.

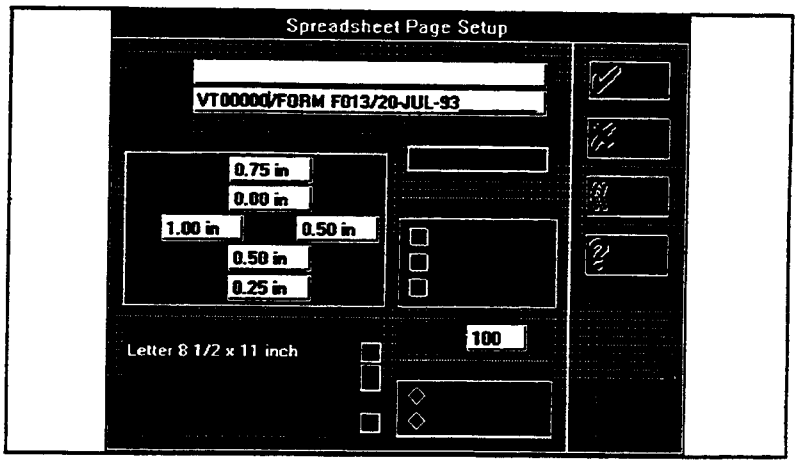
4.9 When the program asks for the percent moisture of the food composite, type in the percent moisture and press the ENTER key.

4.10 When the program asks for the percent lipid (dry) of the food composite, type in the percent lipid on a dry weight basis and press the ENTER key.

The program will automatically print the spreadsheet.

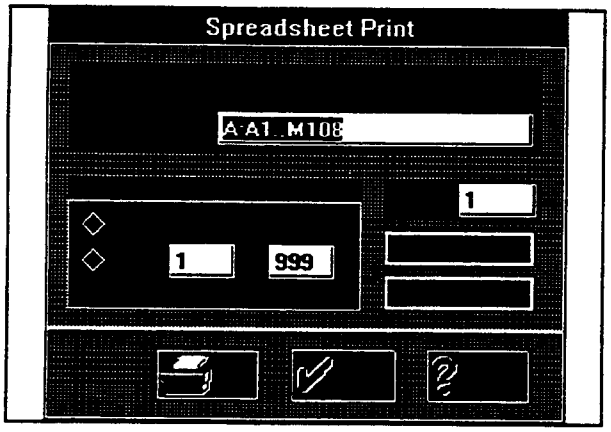
4.11 After about twenty seconds the printing screen will disappear and the program will display "*Hit ENTER to erase macro and continue.....*". Hitting *Enter* will erase the macro, which will save disk space.

4.12 Then click on *File* and then on *Page Setup*, and the following screen will appear.



4.13 With the mouse, move the cursor into the *Footer* field, and replace the five zeroes after the VT with the sample number (eg. 302VT). Then click on *OK*, which will return to the previous screen.

4.14 Then click on *File* and then on *Print* and the following screen will appear.



In *Print Blocks*, change *M* to *J*, and then click on the *print* button and wait until the printing screen disappears.

4.15 Click on *File*, then on *Save as...* . Type in the sample number with the 3 digit extension "wb1" (e.g. VT302VT.wb1), and click on *OK*.

4.16 Click on *File*, then on *Close*, which will return to the blank spreadsheet in **4.6.2**. Now the next sample can be processed by opening the "fametem2.wb1" spreadsheet again (**4.6.3**).

4.17 For the continuous calibration file, in the blank spreadsheet (**4.6.2**) click on *File*, then on *Open*, then on *f021.wb1* and *OK*. Like in **4.6.3** the program will prompt *Open for read only*. Clicking on the *Yes* box will open the continuous calibration template.

4.18 Take the area values for the fatty acids, specified in the template, from the original Turbochrom® printout of the continuous calibration sample and type them into the

spreadsheet under *Area*. The *Area%* will change accordingly.

4.19 In order to print this file, click on *File*, then on *Page Setup*, and the same screen as in **4.12** will appear. In the *Footer* field change the entry "CCAL000" to the assay number (e.g. CCAL008) and click on *OK*.

4.20 Then click on *File* and then on *Print* and the same screen as in **4.14** will appear. Click on the *print* button to print the spreadsheet. Wait until the printing screen disappears.

4.21 In order to save this file, click on *File*, then on *Save as...* . Type in "CCAL" with the assay number and the 3 digit extension "wb1" (e.g. CCAL008.wb1), and click on *OK*.

4.22 Click on *File*, and *Exit* to leave the Quattro® program.

4.23 Give the continuous calibration printout and the second sample printout (with the footer showing the sample number) to the QA/QC officer. File the first sample printout (with the footer showing VT00000) together with the Turbochrom® printout into the "Diet Validation Samples, FA....- FA...." folder.

5. References

5.1 All figures were obtained from the QuattroPro for Windows® program.

Prepared by:

Ingolf Gruen

_____

Date: _____

Approved by:

Katherine Phillips

_____

Date: _____

SOP # 5028
Date: 4-NOV-93
Revision: 3

Food Analysis Laboratory Control Center (FALCC)
Standard Operating Procedure

Title: Use and Handling of Food Composite Samples

1. Purpose

To describe the procedure for handling food composite samples for analysis.

2. Safety

No special safety measures are necessary.

3. Materials

3.1 Composited food samples (see SOPs # 5005, 5029)
3.2 stainless steel spatula with rectangular end (for stirring, aliquotting)
3.3 water bath **M1-8-6** (set at 30°C)
3.4 refrigerator (2-4°C)
3.5 paper towels

4. Procedure

4.1 The analyst will hand either an assay request form (Form # F010 - which was received from his/her supervisor), or a note requesting samples for research use and specifying the day when the assay is to be run, to the QA/QC officer.

4.2 Have samples pulled by the QA/QC officer, his/her supervisor or a person designated by the QA/QC officer. Samples can be pulled in three ways:

NOTE: The method (A, B, or C described below) used to pull a sample for an assay should be noted in the bench book entry for that assay.

Method A

4.2.1 If the assay is to be done the same day or the day after the samples are composited, place the samples designated for the assay in the refrigerator (2-4°C) instead of in the freezer (-60°C). Samples must be assayed within 24 hours of compositing or else placed in the -60°C freezer. Continue with **4.3**.

Method B

4.2.2 Samples that are stored in the freezer (-60°C) can be thawed in two different ways:

4.2.2.1 Place the samples on a tray in a refrigerator (2-4°C). The samples must not touch each other and cannot be stacked. Samples must thaw in the refrigerator for **at least 18 hours** prior to analysis and have to be used for analysis **within 35 hours**. Continue with **4.3**.

Method C (alternate)

4.2.2.2 Place samples (maximum of 5 at one time) in the 30°C water bath for 20 minutes, so that 2/3 to ¾ of the jar is submersed. Do not place the samples directly on the bottom of the water bath, but elevate them with e.g. a vial rack. Stir each sample after ten minutes. After 20 minutes remove jars from water bath. **Make sure no water gets into the composite by carefully drying the jars and seams with paper towels before opening.** Continue with **4.6**.

4.3 The QA/QC officer (or whoever pulled samples) will notify the analyst when (time of the day) samples were removed from the freezer, and where they were placed.

4.4 At the discretion of the analyst, samples are removed from the refrigerator for the analysis, according to the time limits specified in step **4.2.2.1**.

4.5 Place jars on lab bench for 20 minutes and allow to come to room temperature.

4.6 To remove an aliquot of sample from the jar, unscrew the lid of the jar. Hold the jar at a 30 to 45 degree angle, and stir the diet composite in a circular motion for a minimum of 30 seconds with the rectangular end of a clean, dry stainless steel spatula. While stirring, lift the composite up from the bottom of the jar to the top to insure complete mixing. **Do not lose any of the jar's contents while stirring. If loss occurs, notify supervisor.** Scrape the sides of the jar and scrape off the spatula on the inner edge of the jar. Immediately take aliquot for analysis. The purpose of this step is to insure the thawed composite is homogenous prior to sampling.

4.7 If more than one aliquot is to be taken from the jar, repeat step **4.6** for each aliquot.

5. Storage

5.1 After removal of samples from the freezer for analysis, store tightly capped samples in the refrigerator at 2-4°C (maximum of 35 hours).

5.2 Samples that were not frozen, but were placed into the refrigerator after compositing must be assayed within 24 hours.

5.3 Clearly mark sample as "used" and place remainder of sample back into the freezer (-60°C) (latest after ≤ 35 hours from removal from -60°C) according to instructions from person in charge of QA/QC, unless instructed otherwise by supervisor.

Prepared by:

Ingolf Gruen

_____

Date: _____

Approved by:

Katherine Phillips

_____

Date _____

Food Analysis Laboratory Control Center (FALCC)
Standard Operating Procedure

TITLE: Determination of Moisture in Composited Mixed Diets by Microwave Drying

1. Purpose

To describe the procedure for determining the moisture content of composited mixed diets using the CEM LabWave 9000™ Moisture/Solids Analyzer.

2. Safety

No special safety precautions are necessary.

3. Materials

3.1 CEM LabWave 9000™ (M1-28-4)
3.2 diet composite (SOP #5005)
3.3 glass fiber sample pads (CEM# 200150)
3.4 stainless steel spatula with rectangular end
3.5 CEM stainless steel spatula
3.6 Star NX-1020 color printer
3.7 Fat-free gloves
3.8 Teri wipes (Fisher #15-235-60), or equivalent

4. Procedure

4.1 Thaw diet composite according to SOP# 5028

4.2 Turn on the CEM LabWave 9000™ by pressing any key on the keyboard. Turn on the printer and make sure that the "On Line" light is green. If it is not, press the "On Line" key.

4.3 Press the "F4" key until the 'MAIN MENU' screen appears. From this screen, press the "F2" key to activate the Program/Edit/Run screen. Press the "down" arrow key to select the "Warm Up" program, and press the "Enter" key. This program should be run if the instrument has not been in operation for an hour or more. Open the door, lift the draft shield, and make sure that the balance tripod is clear. Press the "Tare" key, wait for the beep, and press the "Start/Stop" key to begin the warm up.

4.4 After the five minute warm up has finished, press the "F4" key three times to get

back to the 'Select Programs' screen. Press the down-arrow key until the word 'FOOD' appears on the highlighted line, and press "Enter".

4.5 When the 'STANDARD' screen appears, press the "0," and type in the sample number by moving the flashing cursor to the desired position and pressing the number keys. If there is a letter in the sample number, press the number key directly below the letter several times until the letter appears (ex: press the "1" key three times for a "B"). Press "Enter" when the sample number is correct.

4.6 Open the door and place two clean glass fiber sample pads on the balance pan under the box with a forceps or dry gloves (oil from fingers can affect the weight of the pads). Close the door and press the "Tare" key.

4.7 When the machine beeps, open the door and place the two sample pads (one on top of the other) onto a clean, dry lab bench.

4.8 Aliquot sample according to SOP #5028.

Proceed <u>immediately</u> to step 4.8.

4.8 Using the CEM stainless steel spatula, quckly spread between 2 and 2.5 grams of the sample thinly over the entire (rough) surface of the top pad. A thin layer of food is necessary to completely dry the sample and prevent the sample from burning or charring. Lift the top pad, turn it over, and place it on the bottom pad, making a sandwich.

4.9 Immediately place the sample sandwich on the balance pan, close the door and press the "Start/Stop" key. Since evaporative losses between spreading the sample on the pad and starting the CEM will bias results speed is of essence. Wash the spatula with warm water and dry it thoroughly with a paper towel.

4.10 After the five minute cycle the results should appear on the screen and they should print out on the printer. If the results do not print, make sure that the printer is on and the "On Line" light is green. Press the "F4" key to return to the 'STANDARD' screen,and press the "2" key to reprint the report.

4.11 Open the door, and remove the glass fiber sample pads. Carefully separate the pads and examine the dry sample. If there are any signs of browning or charring, note this result in the printout, and rerun the sample (i.e. press the "F4" key and repeat steps **4.4** through **4.11**).

4.12 Press the "F4" key to return to the Main Menu.

4.13 Fill out the bottom of the assay request form (F010), and return it with the printed results to the QA/QC officer.

Prepared by:

_____

Date: _____

Approved by:

_____

Date: _____

Memorandum

TO: All DELTA Field Centers
cc: Kent Stewart
Katherine Phillips
Barbara Dennis - DELTA Coordinating Center
Nancy Van Heel
Abir Farhat
Marlene Windhauser
Rebecca Chen

FROM: Karen Richardson
Food Analysis Laboratory Control Center

DATE: October 25, 1994

RE: Prior Notification of Shipping Coolers

As a final clarification, it is necessary for each center to notify the FALCC prior to packing the cooler for shipment. This is specifically stated in the SOP supplied by us, SOP 1027-0, on page 8. There are several possible reasons why the centers would not be able to ship on a given day:

1. Severe weather at either the origin or destination, that could cause delays with Federal Express. (This has happened often enough, especially in the winter.)
2. Too many shipments being received at the same time, making it difficult to log-in all shipments in a timely manner. (We receive shipments from seven centers!)
3. Several employees at the FALCC being on leave at the same time (as can happen during the holidays).

If a center were to pack and seal a cooler prior to calling us, and we informed that center we could not take the shipment on that date, then they would have to go to the trouble of unpacking the cooler, with a potential waste of dry ice, etc.

Please call prior to packing your coolers. If everything is OK for you to ship, then you can notify of shipment during the same call. **If you are calling to see if it is OK to ship, but are unsure if you will actually ship on that day, you will need to contact us again to notify us of the actual shipping.**

You can contact us by phone at (703) 231-4361 (ask for Karen or Holly if available); by E-Mail at FALCC@VTVM1.CC.VT.EDU; or by FAX at (703) 231-9070 (again, please address to Karen or Holly).

As a final point, we are directly connected to the Fed Ex computer system so that we can track a cooler shipment, **but we cannot track a cooler if we are unaware that it has been sent!**

Your cooperation in this matter would be greatly appreciated.

Thanks! Karen J. Richardson

TABLE OF CONTENTS

CHAPTER EIGHT: Clinical Laboratory Protocol

CLINICAL LABORATORY MEASURES

I. OVERVIEW

A. Introduction

The Delta Study will examine the relationship between the levels of various substances in the blood as well as genes found in blood cells, and diets containing various combinations of fats, carbohydrates, etc. The blood samples will be extensively analyzed initially; storage samples will have further studies performed as new hypotheses and assays come forth. Because the blood samples collected are the foundation for all these tests, both present and future, the proper collection, processing, and storage of these samples is a crucial phase of the entire study. If the blood sample is not correctly drawn and processed, the laboratory results may not be precise or valid. Thus, the research assistant/research nurses who perform the blood drawing and sample processing must be well-trained, competent at drawing and processing the blood, and highly conscientious about the quality of their work.

The DELTA Study requires this considerable effort to standardize blood collection, processing, and storage, since it involves four U.S. field centers. This protocol is necessitated by the importance of all sample collection procedures being feasible and of high quality in order to meet all the investigators' needs. This protocol is derived, in part, from that developed by Russell Tracy, Ph.D., for the Cardiovascular Health Study, for which the University of Vermont serves as central laboratory.

B. Laboratory Organization

The laboratories involved in the protocol will consist of those affiliated with the field centers, including Columbia University (CU), Louisiana State University (LSU), Pennsylvania State University/The Mary Imogene Bassett Research Institute (MIBH), and the University of Minnesota (UM), as well as the central coagulation laboratory at the University of Vermont (UV) (Russell Tracy, Ph.D.).

II. SAMPLE COLLECTION -- AN OVERVIEW.

A. Overview.

Table 1 (see Appendix I for tables) provides a summary of the samples to be collected for endpoint analysis. DELTA Study requires collection of approximately 41 ml of blood three times at the end of each diet phase. In addition participants will be asked to collect a first morning urine each of the last two weeks of each diet phase. Some participants may be asked to participate in postprandial studies which involve additional collection of 14 to 34 mL of blood on each of those days. Since the study depends on the voluntary participation of subjects, both initially and in follow-up, every effort must be made to make the entire procedure as easy and painless as possible, both for the participants and for the field center personnel.

The endpoint package contains a serum and urine for routine endpoint tests. These are outlined in Table 2. Sample requirements and disposition of samples for special tests are outlined in Table 3. Sites of analysis are given in Table 4.

For the endpoint package, any participants who are concerned about the volume of bloo should be reassured that the total amount of blood is 1/15th of the volume given in a typical Red Cross blood collection (450 ml). Each endpoint package should be collected after an 8-hour fast with sample collection occurring between 7-10 a.m.

The processing requirements of for all samples except the postprandial studies are shown in Table 5. The collection and processing of the samples for the postprandial studies are given in Table 6.

B. Blood Collection Trays and Tubes.

1. Blood Collection Tray.

Blood collection trays are prepared in advance for the following day. Each tray is stocked with a full supply of blood-drawing equipment for three to four participants and holds individual blood collection racks for each participant in an ice bath. Several racks are prepared to hold various plastic tubes and vials for the final serum and plasma aliquots. The blood collection tube rack and aliquot tube rack are prelabeled with the appropriate code numbers for the participants drawn that day.

2. Blood Collection Rack.

The collection tray itself is made of hard plastic which is unbreakable and can be easily cleaned. The tray has 10 individual compartments which will be filled with the following supplies:

21 g butterfly needles with luer adapter
alcohol swabs
Band-Aids
Gauze
Tourniquets (2)
Vacutainer holders
Needle/Sharps container
Smelling salts
Timer/Stop watch
Scissors
Adhesive tape
Styrofoam ice bath filled approximately
10 minutes before draw
Pencils/Pens
Latex gloves
Test tube racks (2)

A separate rack contains the necessary draw tubes, etc. for each participant. The tubes are arranged according to the priority of the draw. This rack will fit into the blood collection tray. The blood collection tubes are pre-labeled with identification numbers.

3. Description of blood collection tubes for endpoint package.

Tubes 1 and 6 are a 10 ml siliconized yellow/black stopper tube. This tube contains thrombin so that the blood clots to form serum in 5 minutes. Aliquots are coded red. This serum is used for the lipid profiles, glucose, insulin, and apoproteins.

Tube 2 is a 4.5 ml blue-top tube containing 0.5 ml of 3.8% sodium citrate. It is crucial that this tube remain at room temperature. After centrifugation, plasma is aliquoted into green-coded aliquots. The plasma will be used for Factor VII coagulation assay.
This tube will be used to provide seven 3-uL aliquots for platelet activation in week 7.

Tube 3 is also a 4.5 ml blue-stopper tube identical to Tube 3. It is different in the way it is processed. Instead of room temperature, this tube must be placed on ice after filling. Plasma from this tube is aliquoted into blue-coated aliquots and will be used for fibrinogen assay and PAI-1 assay.

Tube 4, identified as a Diatube, is a 5-ml blue top tube with a red label. It contains special stabilizers. It should be kept cold prior to the blood draw. It is recommended that it be chilled on ice at least 15 min before use. This tube will be collected on weeks 5 and 6 only. It must be placed on ice immediately after collection. The plasma from this tube is filtered and the filtered plasma is will be used for measurement of beta-thromboglobulin. These tubes may be purchased from the University of Vermont (Russell Tracy) or directly from American Bioproducts Company (5 Century Dr., Parsippany, NJ 07054; Ph. 800 222-2624)

Tube 5 is a 7.0 ml purple stopper tube. This tube requires mixing after filling and placement on ice. After centrifugation, plasma is aliquotted into yellow-coded cryovials (0.5 ml). The plasma will be used for ancillary and supplemental studies.

Each draw tube is assigned a color-coated aliquot system. There is one type of aliquot used: 0.5 ml cryovials. The cryovials have colored caps.

4. Priority of tubes.

A total of approximately 41 ml will be drawn from each participant in five tubes at each endpoint package. The tubes are drawn in priority order.

The tubes are numbered 1-6. 1-2 and 6 are in a room temperature rack. Tubes 3, and 5 are kept on ice.

5. Forms.

a. Purpose:

The purpose of the blood collection form is to facilitate the collection of plasma and serum samples from participants. This collection must be done in a rapid and efficient manner, with maximum protection for the participant. In addition, the process must facilitate the monitoring of phlebotomy and other quality assurance parameters as well.

Note: All forms are to be completed in ink.

b. Description:

There are two parts of the Phlebotomy Processing Form associated with blood drawing. The top half is the participant/phlebotomy question about fasting status. If the person has eaten less than eight hours earlier, he/she should be rescheduled on an alternate day, within three days of the initially scheduled date.

The bottom half deals with phlebotomy and processing of samples.

These forms have the following purposes: 1) Assure the most efficient and safest possible venipuncture for participants. 2) Allow the monitoring of the quality of the above procedures. 3) Allow more efficient processing of the samples at central laboratories. 4) Provide information critical to the interpretation of assay results.

III. PROCESSING THE BLOOD SAMPLES

A. General:

Processing should be done immediately following venipuncture. Personal protective equipment (non-permeable lab coats, double gloves with at least one Latex pair, spatter shield) must be worn. Flowchart is included at the end of this section to diagram this process (Figure 2).

B. Immediate Processing of Endpoint Samples:

Upon reaching the blood processing station, remove the blood tube drawing rack and ice bath containing tubes from the blood collection tray. The rack should contain three tubes: #1, #2, #6. The ice bath should contain three tubes: #3, #4, #5. All tubes should be processed within 30 minutes of phlebotomy. The corresponding aliquot racks (two per patient) should be ready. Rack #1 (with color codes blue, clear and purple) should be placed in an ice bath. Rack #2 (with color codes red and green) remains at room temperature.

C. Centrifugation:

Tubes #3, #4, and #5 are centrifuged at 4°C for 10-20 minutes at 3,000 g. The centrifuge may need to be precooled to 4°C. 4.5 ml, 5 ml, and 7 ml balance tubes are required for tubes #3, #4 and #5. Simultaneously, tubes #1,#2 and #6 are centrifuged at room temperature for 10-20 minutes at 3,000 g. A 4.5-ml balance tube may be required for tube #2. Once centrifuged, the maximum time allowed before aliquoting is 10 minutes. While these tubes are spinning, the blood collection tray can be restocked with tube rack and blood collection tubes, ice, and forms for the next participant. Recheck labels on the two aliquot racks. Perform any necessary clean-up.

D. Aliquots:

Allow the centrifuge(s) to come to a complete stop. Remove tubes from the refrigerated centrifuge and place in an ice bath, being careful not to shake the tubes. Remove tubes from the room temperature centrifuge. Place in tube rack at room temperature. Assess the plasma. Mark o. the Phlebotomy Processing Form if lipemic (L), icteric (I), hemolyzed (H), clotted (C). Follow the

outline on the Phlebotomy Processing Form for aliquoting the samples. Checkmark on the form if the aliquot is completed, and mark any different volumes. Be careful not to disturb the other white cell layers. If plasma is a lower than expected volume, use one less aliquot rather than disturb the red cells or buffy coat. Do not have any red cells in the aliquots. Use a new pipette tip for each tube type. Recap aliquots after each draw tube has been pipetted.

		ALIQUOT
Tube #5	Use 500 uL MLA Cryovials (6) - 0.5 mL plasma/cryovial	Rack 1 (on ice) Lavender code
Tube #3	Use 500 uL MLA Cryovials (4) - 0.5 mL plasma/cryovial	Rack 1 (on ice) Blue code
Tube #2	Use 500 uL MLA Cryovials (4) - 0.5 mL/cryovial	Rack 2 (room temp.) Green code
Tube #1	Use 500 uL MLA pipette Cryovials (7) - 0.5 mL serum/cryovial	Rack 1 (on ice) Red code
Tube #6	Use 500 uL MLA pipette Cryovials (7) - 0.5 mL serum/cryovial	Rack 1 (on ice) Red code
Tube #4	Transfer 1 ml of plasma into a 3-ml syringe that has an Acrodisc attached. Gently push the plasma through the filter into the cryovial - placing one half in each of 2 cryovials.	Rack 1 (on ice) Clear code

Checkmark on the Phlebotomy Processing Form if the aliquot is complete, then mark any different volumes. Original blood collection tubes can be properly disposed of in biohazard waste bags.

E. Freezing:

Upon completion of the processing steps, aliquots must be frozen within 10 minutes. Green aliquots (4 cryovials) at room temperature are added to the rack at 4°C. That rack is removed from the ice bath and placed upright in the freezer at -80°C for at least ½ hour (preferably until the end of the day). Make sure the aliquots are not wet when they are placed in the freezer. If the -80°C freezer is not immediately available, the aliquots can be placed on dry ice or snap frozen in liquid nitrogen/methanol mixture.

F. Completed Forms:

The completed Phlebotomy Processing Form can be set aside in the daily work folder. Originals are filed at each field center; the copy will be sent to the University of Vermont when samples are shipped.

G. End of the Day Procedures:

Frozen aliquots and racks are packaged with prelabeled freezer box. One box contains the aliquots from a single endpoint series on each patient (30) aliquots per total). The boxes containing aliquots are stored at -80°C freezer by date. The copy of the Phlebotomy Processing Form is kept in a file to be included with the shipment of samples. Restock blood collection tray with samples. Label with next day's participants. Blood draw tubes, aliquots, forms. Arrange draw tubes and aliquots in their proper racks. Wipe down all work areas with 10% Clorox solution.

H. Summary of Processing Time Limitations:

On blood drawn prior to processing:

1) Serum 10 ml - 40 minutes;
2) Citrate 4.5 ml - room temperature - 30 minutes;
3) Citrate 4.5 ml - 4°C - 30 minutes;
4) Diatube 5.0 ml - 4°C - 30 minutes;
5) EDTA 7.0 ml - 4°C - 30 minutes;
6) Serum 10 ml - 40 minutes;

Once centrifuged, maximum time before aliquoting is 20 minutes; after aliquoting all samples, freeze within 30 minutes.

IV. URINE COLLECTION

Urine samples will be collected the mornings of two scheduled blood draws per diet period (weeks 6,7). Participants should be provided a plastic bottle (labeled with the subject ID number) the day before with instructions (See Appendix II) to collect the first urine sample when they get up the next morning. This sample should be brought to the lab when they arrive for the drawing of their blood sample. Note the receipt of the sample on the Urine Collection Form (Appendix II).

Before aliquoting, mix the sample by swirling the bottle. Remove the cap. Aliquot 2 ml in each of five 2-ml cryovials prelabeled with the subject ID. Place yellow caps on these samples and freeze at -80°C.

V. POSTPRANDIAL BLOOD COLLECTION AND PROCESSING

The processing of samples for the postprandial studies is summarized in Table 6.

A. Regular diet

When the subject returns before lunch and before dinner, one 7-ml red top tube is drawn. This tube should sit at room temperature for at least 45 minutes to allow the blood to clot. The tube is then centrifuged for 30 minutes at 4°C at 3000xg.

Four 0.5 ml aliquots of serum are placed into four cryovials labeled with the subject ID and coded for the appropriate time point for this postprandial study. Each vial is capped with a re cap and frozen at -80°C.

B. Fat load

Each subject will return at 4 and 8 hours after consuming the fat load. Two tubes of blood are drawn at each time - a 7-ml red top tube (serum) and a 7-ml purple top tube (EDTA plasma) and a 4.5-ml blue top (citrated plasma). The purple top tube must be protected from light to prevent degradation of these compounds; if they will not be processed immediately, they should be wrapped in foil.

Red top tube

The red top tube should be allowed to sit at room temperature for 45 minutes to clot. The tube is then centrifuged for 30 minutes at 4°C at 3000xg.

Four 0.5 ml aliquots of serum are placed into four cryovials labeled with the subject ID, and coded for the appropriate time point for this postprandial study. Each vial is capped with a red cap and frozen at -80°C.

Purple top tube

The purple top tube should be placed in an ice bath immediately after drawing and kept at 4°C until centrifugation. Centrifugation is for 30 min at 4°C at 3000 x g. Two 1-ml aliquots of plasma are transferred to 3.7-ml amber vials (Fisher Scientific cat.# 03-339-23B). A gentle stream of nitrogen is layered on top of the plasma and the vials are capped and frozen at -80°C.

It is vital that the plasma be protected from light. If the transfer is not immediate to the amber vials, the plasma tubes should be wrapped in aluminum foil until transfer. Transfer should occur with room lights dimmed.

Blue top tube

The blue top tube should be kept at room temperature. Centrifugation is for 30 min at room temperature. Two 0.5-ml aliquots should be transferred to cryovials with green caps. These aliquots should be placed at -80°C immediately after separation from red cells.

C. Sample Preparation for Retinyl Ester Determination

Blood to be used for retinyl ester determination will be drawn in to EDTA containing tubes (lavender top) wrapped with aluminum foil to protect the sample from light. Isolation of plasma can be done following the usual centrifugation protocol for lavender top tubes. When plasma is transferred into aliquot vials, the room should be dim. Plasma to be used for determination of retinyl ester levels is stored under nitrogen at -70°C until assayed.

The aliquot vials are from Fisher Scientific, Cat # 03-339-23B. They have a capacity of 3.7 mls, are 15 x 45 mm, and have rubber-lined closures. A pack of 144 vials is $39.00. For 25 subjects, keep 2 samples per fat load test (4 and 8 hrs.) x 3 diets = 150 vials. Each center will need 2 packs of vials.

D. Retinyl ester determination

Plasma retinol and retinyl ester (retinyl palmitate, retinyl stearate, retinyl linoleate and retinyl myristate) levels will be measured by reverse phase high-performance liquid chromatography (HPLC). This procedure employs an internal standard technique for the calculation of retinal and retinyl ester levels. The within-assay and between-assay coefficients of variation of retinol and retinyl ester determination are less than 7 percent.

For retinoid determinations, 100 μl serum or plasma will be denatured by addition of 100 μl ethanol containing internal standard retinyl acetate, and the retinoids will be extracted into hexane. The hexane extract will be backwashed with H_2O, evaporated to dryness under a gentle stream of nitrogen, and redissolved in benzene for injection onto the HPLC column. Chromatography will be carried out on a 4.6 x 25 mm Beckman 5 μUltrasphere ODS column using 70% acetonitrile-15% methanol as solvent. Flow rate will be 2.0 ml/min. Retinol and retinyl esters will be detected by absorbance at 325 mm. Levels will be determined from a standard curve relating integrated peak area ratios of the retinoid of interest and the internal standard retinyl acetate to mass ratios of the two compounds. Standard curves will be constructed using authentic renitinol, retinyl plamintate, retinyl stearate, retinyl oleate, retinyl linoletate and retinyl myristate which will be obtained either commercially (retinyl palmitate and retinol) or in the laboratory of Dr. William Blaner.

All HPLC procedures will be carried out on equipment obtained from Waters Associates. This includes 2 Model 6000 pumps, a WISP Model 710 Autosampler, a Model 7230 System Controlled, a Model 730 Data Module, and a Model 480 variable wavelength UV detector. This HPLC system is fully automated and is regularly used to determine serum and tissue retinoid levels.

VI. STORAGE OF ALIQUOTS

Blood samples sent to Field Lipid Laboratories and central laboratories will be stored at -80°C until analyzed.

Numerous additional aliquots are to be stored at the Field Centers for later analyses, as yet not proposed. The rationales for using the Field Centers for storage:

1. This reduces the risk of loss of endpoint samples, since some samples would be retained by the Field Centers,

2. The laboratories analyzing these samples are as yet unknown, and they could be shipped directly at the time needed.

Each Field Center should identify a -80°C freezer which is protected from thawing through a reliable alarm system or back-up power system. The loss of aliquots through thawing may result in exclusion of that center's samples from important analyses.

A software program will be developed for the purpose of inventory control. The maintenance of this inventory is the responsibility of the field centers. All samples collected, stored,

and shipped shall be accounted for on this program. Training on the use of the program will be included in the training sessions.

VII. SHIPPING AND RECEIVING SAMPLES

A. General:

Samples are stored at the field centers at -80°C and shipped to the various laboratories according to the schedule in Table 7.

B. Packaging for shipping:

The samples to be shipped are removed from boxes stored at -80°C and packaged in bags for each subject and be accompanied by a shipping list that identifies the samples being sent.

The styrofoam mailer is lined with absorbent material; i.e., paper towels. Approximately half the dry ice is placed on the bottom of the mailer. Check the ID number against the transmittal form. Carefully place the zip-lock bags, each containing the samples from a single subject, in the mailer. Remaining dry ice (6 pounds total) is placed on the sample. The top of the styrofoam is sealed up with tape. It is closed with the outer cardboard sheath. The paperwork is placed on top of the styrofoam before the outer sheath is closed by tape or strap. Fix shipping labels. Place the entire box in the refrigerator if pick-up is not immediate (samples should not be on dry ice for more than 24 hours). Note: The number of samples per box may vary depending on the size of the container used. We recommend using your judgment and 6 pounds of dry ice.

C. Sample Shipping Checklist:

Styrofoam mailing container with outer cardboard sleeve
- Absorbent material
- Zip-lock bag
- Packaging tape
- Dry ice (6 pounds per shipment)
- Labels (provided by carrier)
- Completed Phlebotomy Processing Form
- Blood sample transmittal form

D. Shipping and receiving communications:

Details of which samples are to be shipped to each center and the specific addresses for these samples will be provided at the time the samples are to be shipped.

Samples received at central laboratories will be preceded by a FAX of the transmittal form. Upon receipt of the shipment, the transmittal form is checked against the fax, to assure that no confusion has occurred and to allow for telephone verification if there are any lingering doubts. The samples are removed from the package and immediately transferred to a -80°C freezer for later analyses. Any problems in the shipment in terms of thawing, breakage, etc. should be noted.

Samples are thawed prior to analyses per protocol, either at room temperature or in a warming bath.

VIII. QUALITY CONTROL PROCEDURES

A. Overview

To assure validity of the results in this study requires rigid adherence to collection, labeling, and shipping protocols. To ensure that these are well understood, a training session will be held to provide training and experience before the beginning of the project. Adherence to these protocols, including periodic reviews and retraining, are the responsibility of each field center.

In addition, other quality control measures will be taken to assess the integrity of the stored samples once they are removed from storage for analysis in addition to individual laboratory quality control programs that are in place for each analyte.

B. Sample Integrity

Aliquots of samples will be frozen upright at -80°C before being placed in boxes for long-term storage and shipping. Upon removal from freezers for shipping or analysis, the sample should be inspected to for evidence of thawing and leaking. If the liquid is no longer frozen (with top of frozen layer being horizontal in the vial) in the bottom of the vial or if there is evidence that the sample has leaked, based on less than the original volume remaining in the vial, the sample will be discarded and replaced by another sample that is acceptable.

Upon receipt at local or central laboratories, samples will be inspected upon unpacking to assure that they remained frozen in transit. Any sample showing evidence of being thawed will be noted. If transportation to the local laboratory occurs immediately upon removal from the -80°C freezer (within three hours) the sample needs to be kept frozen. Any sample that showed evidence of thawing but remained cold during transport may be analyzed for lipids and lipoproteins, but not for coagulation factors. Analysis should occur within three days of receipt of these samples. Any sample that leaked during transport will not be analyzed.

Any sample that has been identified as thawed will not be included in the data analysis if the analytical results are greater than 3SD (biologic plus analytical) from the average of the other three in that set of four.

Expected biologic plus analytical SD for triplicate analysis (Clin. Chem. 36,209, 1990)

	1 SD
Cholesterol	5%
Triglyceride	13%
HDL-chol	7%
Apo A-1	10%
Apo B-100	11%

Sample identity will also be verified based on packing lists and FAXed inventory lists. Any shipment that shows a discrepancy between the packing list and the actual contents may be sent back to the originating center to correct if the discrepancy cannot be resolved by a phone call.

C. Analytical Quality Control Procedures

All analytical runs will include appropriate quality control samples to assure the validity of the run. Quality control samples will be analyzed at specific intervals with each run as specified by the manual of operation for the laboratory performing the analysis. If the quality control materials do not meet the criteria set by the laboratory's manual of operation, the run must be rejected and repeated in total.

D. Longitudinal Control Procedures

To be able to assess comparability of analyses in earlier years and later years for this study, samples other than diet study samples, will be used as not to deplete the archival stores of study subject samples.

Three volunteers were recruited from each center to provide a series of "endpoint" samples which that were treated and aliquoted as a modified endpoint set to provide six sets of vials for each of the core analytes. See Protocol 1.

All field center laboratories will, in addition, participate in the Lipid Standardization Program of the Centers for Disease Control, which provides some control for longitudinal samples of lipoprotein profiles.

IX. DATA RECORDING AND TRANSFER

A hard copy of all results will be retained by all laboratories for the duration of the study. Values from the field center lipid laboratories and the MIBH Lipid Laboratory will be submitted to each field center by FAX, regular mail, or electronically, using predesigned forms. The UV will send hard copy plus data entered on floppy disk.

X. TRAINING PROCEDURES

The purpose of training is to provide standardized methodology for venipuncture and blood processing for the field centers. Standardization of procedures is important for the quality of blood samples from participants

A. Training and Certification.

Field center technician training and certification was carried out as part of Protocol I. A field center coordinator, designated at each field center, is responsible to maintaining the level of competence of field center personnel in the standard procedures. Personnel who participated in the initial training program were certified. Recertification of field technicians takes place prior to each blood-drawing year or when new technicians are enrolled. This training is the responsibility of the field center coordinator.

B. New protocols

New protocols, involving techniques not used in the last protocol, will be introduced by a video prepared by the University of Vermont. These will include the method for filtering the plasma from the Diatube for beta-thromboglobulin assays and the methods for treating platelets for the studies involving flow cytometric analysis.

APPENDIX I

Table 1 Overview of Endpoint Sample Collection

Once per week to give 3 sets per diet period				Twice per diet period	Twice per subject	Once per diet period		
Endpoint package similar to Protocol 1 with six tubes of blood (41 mL)				Urine first AM	Buffy Coat	Platelets Special Prep	Postprandial Regular diet**	Postprandial Fat load**
							12 pm & 5 pm	@ 4 h & 8 h
Serum 2x10 mL	Citrated plasma 2x4.5 mL	Diatube plasma* 5 mL	EDTA plasma 7 mL	Urine		Platelets special	Serum** 2 x 7 mL	2 x 7 mL EDTA*** 2 x 7 mL Serum** 2 x 4.5 mL Citrate
14 vials	4 vials (4°) 4 vials (r.t.)	2 vials	6 vials	5 vials	3 buffys	7 tubes	2 x 4 vials	2 x 2 vials EDTA*** 2 x 4 vials serum 2 x 3 vials citrate

* Week 5 and 6 only

** Assumes that postprandial studies will take place on the same day as the fasting endpoint sample from that week. One tube of blood of each type will be drawn at each of the two scheduled times and aliquoted to give one set of vials. Since this is done twice in the same day, the need for two samples or vials is indicated as 2 x each tube or vial type.

*** Must be protected from light; stored under nitrogen.

Table 2 **Disposition of samples for testing and archiving**
Endpoint tests

	Per week per diet period						Per person
	serum	citrated plasma 4°	citrated plasma r.t.	Diatube plasma	EDTA plasma	urine	buffy
Tests							
Lipid profile, glucose	2						
Insulin	1						
Factor VII			1				
Fibrinogen		1					
PAI-1		1					
Apo E genotype							1
LDL size					2		
beta-thromboglobulin				1			
VLDL-chol	1						
HDL-subfractions	2						
Apo A/B	1						
Total used for testing	**7**	**2**	**1**	**1**	**2**	**0**	**1**
Remainder for archives	**7**	**2**	**3**	**1**	**4**	**5**	**5**
Possible tests from archives							
Microalbuminuria						1	
CRP	1						
Uric acid	1						
Lp(a)	1						

Table 3 Disposition of samples for testing and archiving
Special tests

Postprandial (Regular diet)	Serum (12 pm & 5 pm)		
Triglycerides, glucose	1		
Insulin	1		
Total for testing	**2**		
Total for archives	**2**		
Postprandial (Fat load)	Serum (4 & 8 h)	EDTA plasma (4 & 8 h)	Citrated plasma (4 & 8 h)
Triglycerides, glucose	1		
Insulin	1		
Retinyl palmitate		1	
Total for tests	**2**	**1**	**0**
Total for archives	**2**	**1**	**3**

Platelets

7 tubes per diet; all will be analyzed; no archives

Table 4 Summary of Locations for Laboratory Testing and Test Volume

TEST	SITE	TEST SCHEDULE tests/diet phase	timing (week #)
Lipid Profile	Local Lab	3	5,6,7
Glucose	Local Lab	3	5,6,7
Insulin	MIB	3	5,6,7
Factor VII	Vermont	3	5,6,7
Fibrinogen	Vermont	3	5,6,7
PAI-1	Vermont	3	5,6,7
Apo E	MIB	once per subject	
LDL Size	Pennington	2	6,7
Beta Thromboglobulin	Vermont	2	5,6
VLDL-C	MIB	2	6,7
HDL Subfractions	Columbia	2	6,7
Apo A-1 and Apo B-100	Columbia	3	5,6,7
C-reactive Protein	Vermont	3	5,6,7
Uric Acid	Local Lab	3	5,6,7
Microalbuminuria	MIB	2	6,7
Lipoprotein (a)	MIB	3	5,6,7
Platelets by Flow Cytometry	Vermont	1	7
Post-prandial standard fat load*			
Triglyceride	Local Lab	1	6 or 7
Glucose	Local Lab	1	6 or 7
Insulin	MIB	1	6 or 7
Retinyl ester	Columbia	1	6 or 7
Post-prandial post meal testing*			
Triglyceride	Local Lab	1	6 or 7
Glucose	Local Lab	1	6 or 7
Insulin	MIB	1	6 or 7

**will be carried out on same day that fasting samples are collected for other testing for that week. In that way the fasting results for triglyceride, glucose and insulin will be available for comparison.*

Table 5 Sample processing scheme for endpoint samples

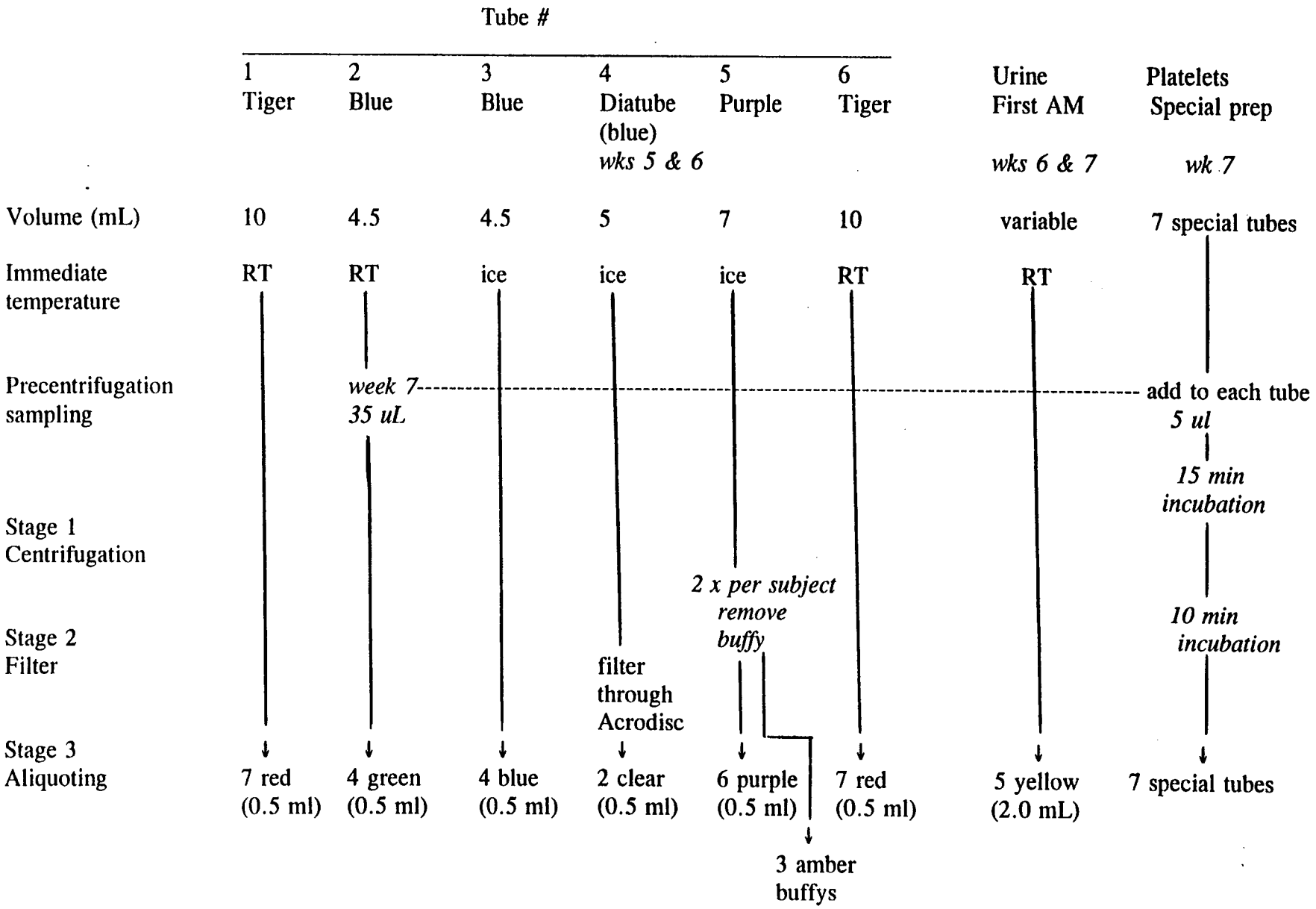


Table 6 Sample processing scheme for postprandial studies

	7-ml red top *Standard meal study* *or* *Fat load study*	7-mL purple top *Fat load study*	4.5-ml blue top *Fat load study*
Initial Temp.	RT	Ice, immediately	RT
Time 0	Allow to clot at room temperature for 45 - 60 min ↓ ↓ ↓ ↓	Protect from light. Centrifuge immediately** at 4° for 30 min at 3000 x g ↓ Transfer in subdued light* into two (2) 3.7-ml amber vials. ↓ Store under nitrogen. Freeze immediately at -80°	Centrifuge immediately at room temperature for for 20 min at 3000 x g ↓ Aliquot into three (3) 0.5-ml cryovials with green caps ↓ Freeze immediately at at -80°
1h	Centrifuge 15 min at 3000 x g. ↓ Prepare four (4) 0.5 mL aliquots. Use red cap cryovials. ↓ Freeze at - 80°C		

** Time 0 is draw time and refers to immediately before lunch and supper for standard meal study and 4 and 8 hours post fat load for the fat load study.*

*** It is vital that plasma be protected from light. If the transfer is not immediate to the amber vials, the tubes should be wrapped in foil or placed in a light-tight container until transfer.*

Table 7 Shipping schedule

Date End of	Ship samples
Period 1	Ship all platelet samples to U of Vt.
Period 2	Ship all platelet samples to U of Vt. Ship buffys (one amber capped vial per subject) to MIBH
Period 3	Ship all platelet samples to U of Vt.
	Ship all endpoint samples to appropriate labs (This distribution list will be prepared in Spring 1995 to reflect the final set of endpoints)
	Ship longitudinal control samples to appropriate labs (This distribution list will be prepared in Spring 1995)

APPENDIX II

Instructions
DELTA Phlebotomy Processing Form (PPR)

Use black ball-point pen and press firmly to insure that all information recorded can be read on both the original and the copy.

Print legibly and be sure to complete all information requested. Information from this form will be keyed into the Data Management System.

Initial and date all changes. Do not use liquid correction fluid.

Record date in mm/dd/yy format using lead zeros for single digit numbers.

Upon completion of the PPR Form, the original remains at the Field Center and the copy is sent to the Central Hematology Lab.

Additional specific information for completing this form can be found in the Manual of Operations Laboratory Procedures Chapter 8 page 8 - 8 and will be addressed in the Laboratory Training Video.

DELTA

Phlebotomy Processing Form--Endpoint Samples

Form Code: PPR
Version B 9/15/94

DATE: _____ PHLEBOTOMIST ID: _____ PARTICIPANT ID: _____

TIME: _____(Should be 6-10 AM) SAMPLE ID: _____

PARTICIPANT QUESTIONS:

How many hours since you last had anything to eat or drink, other than water? (SHOULD BE > 8 HOURS) NUMBER OF HOURS: _____

How many hours since you last drank any alcohol? (SHOULD BE > 48 HOURS) NUMBER OF HOURS: _____

VENIPUNCTURE:

Venipuncture time elapsed: _____ minutes

Time elapsed until tourniquet released: _____ (2 min optimum)

Quality of venipuncture:

☐ Clean ☐ Traumatic (*PLEASE SPECIFY*) _____

A. Vein collapse D. Multiple sticks
B. Hematoma E. Excessive duration of draw
C. Vein hard to get F. Leakage at venipuncture site

ner problems with venipuncture: _____

ALIQUOTS:

	PHLEBOTOMY			PROCESSING			
TUBE	IF TUBE FILLED, INDICATE (✓), IF NOT SPECIFY VOLUME			L,H,I,C*	NO. OF ALIQUOTES	COLOR CODE	RACK
#1 Serum (yel/blk)	10 mL	RT	( ) _____	_____	_____(7)	Red	1,ice
#2 Citrate (blue)	4.5 mL	RT	( ) _____	_____	_____(4)	Green	2,RT
#3 Citrate (blue)	4.5 mL	ice	( ) _____	_____	_____(4)	Blue	1,ice
#4 Diatube (blue) (pre-chilled tube)	5 mL	ice	( ) _____	_____	_____(2) (filtered)	Clear	1,ice
#5 EDTA (lav.)	7 mL	ice	( ) _____	_____	_____(6)	Lavender	1,ice
#6 Serum (yel/blk)	10 mL	RT	( ) _____	_____	_____(7)	Red	1,ice

*Note if sample is lipemic (L), hemolyzed (H), icteric (I), or clotted (C)

SAMPLE PROCESSING:

Problems with sample processing: _____

Problems with centrifugation: _____

CENTRAL LAB USE ONLY

CEIVED DATE: _____ TIME: _____ FROZEN: _____

COMMENT: _____

White Copy - Field Center
Canary Copy - Central Hematology Lab

Instructions
DELTA Urine Collection Form (UCF)

For each participant, the first section of the UCF will be completed during Week 6 and the second section will be completed during Week 7 of each feeding period. This form will be keyed into the DELTA DMS. Use black ink, initial all changes, do not use liquid correction fluid, and follow all other general instructions for completing DELTA forms. Additional information on Urine Collection can be found on page 8 - 8 in the Laboratory Procedures (Chapter 8) of the Manual of Operations.

Verify that the collection is a FIRST MORNING CATCH. If so, proceed with completion of the form. If not, give the participant a clean container and instruct them to bring a first morning catch tomorrow.

Record the participant's DELTA ID.

Record the start date of the current feeding period.

Question 1. Record the number of the current feeding period (1,2, or 3).

WEEK 6

Question 2. Record the date of the Week 6 urine collection in the mm/dd/yy format using zeros to complete all spaces.

Question 3. Record the number of aliquots frozen.

Question 4. Record the personnel code number of the DELTA staff member completing this section of the form.

WEEK 7

Question 5. Record the date of the Week 7 urine collection in the mm/dd/yy format using zeros to complete all spaces.

Question 6. Record the number of aliquots frozen.

Question 7. Record the personnel code number of the DELTA staff member completing this section of the form.

Urine Collection Form
(Weeks 6 and 7)

Form Code: UCF
Version A 10/04/94

IMPORTANT: Before completing this form, verify that the participant has followed the instructions for Overnight Urine Collection. If the participant has not followed the instructions, give them a clean container and ask them to repeat the collection.

DELTA ID: _____ ***Feeding Period Start Date:*** ____/____/____

1. PERIOD:____ [Specify 1/2/3]

WEEK 6

2. Enter date and time of **last** urination before collection (from Urine Collection bottle):

   2a. Date ____/____/____ **[mm/dd/yy]** 2b. Time ____:____ **[hh:mm]** 2c. ____ **[AM/PM]**

3. Enter date and time of **first morning urine** (from Urine Collection bottle):

   3a. Date ____/____/____ **[mm/dd/yy]** 3b. Time ____:____ **[hh:mm]** 3c. ____ **[AM/PM]**

4. Number of aliquots frozen:____ (should be 5)

5. Code number of person completing form:_____

WEEK 7

6. Enter date and time of **last** urination before collection (from Urine Collection bottle):

   6a. Date ____/____/____ **[mm/dd/yy]** 6b. Time ____:____ **[hh:mm]** 6c. ____ **[AM/PM]**

7. Enter date and time of **first morning urine** (from Urine Collection bottle):

   7a. Date ____/____/____ **[mm/dd/yy]** 7b. Time ____:____ **[hh:mm]** 7c. ____ **[AM/PM]**

8. Number of aliquots frozen:____ (should be 5)

9. Code number of person completing form:_____

Postprandial Post Meal Study Form
(Week 6)

Form Code: PPM
Version A 11/3/94

DELTA ID: _____ Feeding Period Start Date: ___ / ___ / ___

1. Period: _____ (Specify 1, 2, 3)

2. Date of post meal study (mm / dd / yy): ___ / ___ / ___

FASTING AM SAMPLE

3. Collected as part of endpoint series ☐ Yes ☐ No

If no,

4. Number of aliquots frozen (red cap) _____ (Should be 4)

BEFORE LUNCH SAMPLE

5. Number of aliquots frozen (red cap) _____ (Should be 4)

BEFORE DINNER SAMPLE

6. Number of aliquots frozen (red cap) _____ (Should be 4)

7. Comments: YES _____

_____

_____

_____

Postprandial Fat Load Study Form
(Week 7)

Form Code: PPF
Version A 11/3/94

DELTA ID: _____ Feeding Period Start Date: ___ / ___ / ___

1. Period: _____ (Specify 1, 2, 3)

2. Date of FAT LOAD STUDY (mm / dd / yy): ___ / ___ / ___

FASTING AM SAMPLE

3. Collected as part of endpoint series ☐ Yes ☐ No

If no,

4. Number of aliquots frozen (red cap) _____ (4)

5. Number of aliquots frozen (green cap) _____ (3)

6. Number of aliquots frozen (amber cap) _____ (2) Under N_2? Yes ☐

4 HOUR SAMPLE

7. Number of aliquots frozen (red cap) _____ (4)

8. Number of aliquots frozen (green cap) _____ (3)

9. Number of aliquots frozen (amber cap) _____ (2) Under N_2? Yes ☐

Postprandial Fat Load Study Form
(Week 7)

8 HOUR SAMPLE

10. Number of aliquots frozen (red cap) _____ (4)

11. Number of aliquots frozen (green cap) _____ (3)

12. Number of aliquots frozen (amber cap) _____ (2) Under N_2? Yes ☐

13. Comments: YES _____

_____

_____

_____

APPENDIX III

APPENDIX III Supplies for DELTA 2

Standard supplies for Blood collection

21 g butterfly needles with luer adapter
alcohol swabs
Band-Aids
Gauze
Tourniquets (2)
Vacutainer holders
Needle/Sharps container
Smelling salts
Timer/Stop watch
Scissors
Adhesive tape
Styrofoam ice bath filled approximately
10 minutes before draw
Pencils/Pens
Latex gloves
Test tube racks (2)
Phlebotomy Processing Form
Have available on phlebotomy cart:
basin
cold cloth
tube mixer
biohazard containers
needle/sharps container
paper towels

Vacutainers

Vacutainers for endpoints

10-ml	Yellow/Black
4.5 ml	Blue
7-ml	Purple
5-ml	Diatube H (American Bioproducts, #0556: available from the

University of Vermont or from American Bioproducts Company, 5 Century Drive, Parsippany, NJ 07054; Ph 800-222 2624)

Vacutainers for postprandial studies

7-ml	red
7-ml	purple
4.5-ml	blue

Supplies for processing Diatube

Filters	VWR #28144-590 Gelman Acrodisk #4520
Syringes, 3cc	VWR #BD309585 (Becton Dickenson)
Transfer pipets	VWR #14670-205

Supplies for processing platelets

3 ul pipetter	Recommend multidispense unit like Rainin EDP series
100 ul pipetter	that allows a large sample to be taken into the pipette and dispensed as smaller, programmed aliquots. 25 ul unit that can dispense 7 3-ul aliquots. (Rainin E2-25 $295; tips RT-20, $43.50/1000 tip) 1000 ul unit that dispenses 7 100-ul aliquots (Rainin E2-1000 $295: tips RT-200, $44.50/1000 tips) Rainin Instrument Co., Mack Rd., Woburn, MA 01801 800-472-4646 or 617-935-3050

Supplies for collecting urine

Bottle (250 ml plastic)
Transfer pipets (2 mL)

Supplies for sample processing

Pipette: MLA 500
Pipette tips
Pasteur pipette with bulbs
Ice bath/ice
Latex gloves
Lab mat
Kimwipes
Biohazard waste bags
Clorox (10% in wash bottle)
Pen/pencils
Study participant aliquot racks (2) with prelabeled aliquots
Phlebotomy Processing Form (page 1 completed)

Freezer

Refrigerator

Centrifuges -- room temperature and 4°C

Balance tube

Thermometer

Zip-lock bag

Lab coats, gloves, face shields

Cryovials for storage (per participant)

0.5-ml vials for routine endpoints

126 red top for endpoints
36 green tops
36 blue tops
12 clear top
54 purple tops
6 amber tops

2.0-ml vials for urine storage

30 yellow tops

0.5-ml vials for postprandial studies

Post meal studies
24 red tops

Fat load studies
24 red tops
18 green tops

3.7-ml vials for postprandial fat load study

12 vials (Fisher Scientific, cat.# 03-339-23B 3.7-ml

APPENDIX IV

APPENDIX IV: DETAILED PROTOCOL FOR BLOOD COLLECTION

A. Preparation for Specimen Collection:

Preparation for specimen collection is done in the following manner:

Early morning, prior to arrival of any participants:

1) Check to make sure that blood collection tray is properly equipped. Every item on the checklist must be ready before proceeding.

2) Check that each vacutainer tube is properly labeled with the appropriate participant number and number 1-4 (baseline) or 1-5 (endpoint) in order of draw. A sheet of numbered labels will be provided for each participant.

3) Check that the sample processing station is properly equipped. Every item on the checklist must be ready and in its proper position.

4) Check that each sample aliquot tube is labeled with its appropriate participant identification number and in its proper rack.

5) Check that the participant Phlebotomy Processing Forms are labeled and included with the blood collection tray.

6) Perform quality control check on refrigerator temperature (refrigerator temperature log).

7) Perform quality control check on freezer temperature (freezer temperature log).

8) Make sure the phlebotomy area is tidy and stocked with extra smelling salts, basin, disposable wash clothes, and that the blood mixer is functional.

Approximately 10 minutes before scheduled participant arrival:

1) Fill styrofoam bath 3/4 full with crushed ice.

At participant arrival:

1) Check that the ID number on the tubes matches the participant ID.

B. Venipuncture

1. Precautions for handling blood specimens:

In accordance with OSHA regulations on blood borne pathogens (see appendix for the complete OSHA regulations), the following laboratory safety protocol for field-center laboratories is recommended: Use of non-permeable lab coats, Latex gloves, and face shields when handling any blood in any situation where splashes, spray, splatter, or droplets of blood may be generated and eye, nose, mouth contamination can be reasonably anticipated. Follow universal precautions when handling any blood products. Contaminated needles and sharps shall be immediately placed in a puncture-resistant, leak-proof container. Never re-cap or break needles. Hepatitis B vaccine should be offered to all technicians handling blood.

2. Phlebotomy area:

The blood drawing area should take place in an isolated room or participants should be separated by room dividers. The room should be equipped with all the necessary blood drawing supplies. A separate counter or work cart should be equipped with all the materials and vials that are used for blood handling and processing. The centrifuge, refrigerator, and freezer should be near by.

3. Participant preparation:

Informed consent must be obtained before drawing blood. This procedure is followed to ensure that the subjects understand the purpose of blood drawing and the possible complications of venipuncture. A standard informed consent has been prepared for this study. With regard to laboratory procedures, the consent statement informs study subjects that there is a small risk of bruising at the spot on the arm where the blood is taken and that about 2 tablespoons of blood are drawn. The consent statement also informs study subjects that they will be contacted if clinically important test results are abnormal.

4. Participant phlebotomy question:

Minimal fasting time required for testing is 8 hours (optimum is 12 hours), and all samples for endpoint package should be collected between 6-10 a.m. Record the time of last food and the time of blood drawing. If the participant is not fasting, the blood sample will not be drawn and the subject should be rescheduled for phlebotomy within the next three days. Similarly, if the collection time is outside the 6-10 a.m. period, the subject should be rescheduled for phlebotomy.

5. General:

Blood drawing is standardized for the sitting position. The venipuncture performed with the 21-gauge butterfly needle with 12 inches of plastic tubing between the venipuncture site and the blood collection tubes. The butterfly is a small, thin-walled needle which minimizes trauma to the skin and vein. The use of 12 inches of tube allows tubes to be changed without any movement of the needle in the vein. If the participant is concerned about the venipuncture, he/she may be reassured to know that such care is taken. The participant should be given enough time to feel comfortable both before and after the blood collection. In many cases, the most memorable part of the experience for the participant will be the contact with the person who draws the blood and their general attitude and competence. If the participant is nervous or excited, the technician briefly describes the procedure; e.g., "I am going to be drawing about 2 tablespoons of blood. This blood will be used for tests for lipids and cholesterol and blood clotting factors."

HANDLING PARTICIPANTS WHO ARE EXTREMELY APPREHENSIVE ABOUT HAVING BLOOD DRAWN.

Do not under any circumstances force a participant to have blood drawn. It may help to explain to the participant that the blood drawing is designed to be as nearly painless as possible. It is sometimes best to let the participant go on with another part of the examination. It may also be helpful to have the participant relax in the blood drawing chair just so the phlebotomist can check the veins in the participant's arms, without actually drawing blood.

6. Venipuncture procedure: Wear Latex gloves and lab coat

a) Arrange draw tubes in order of draw on the table top within easy reach. Assemble butterfly apparatus and vacutainer holders, gauze, and alcohol prep prior to tourniquet application.

b) Apply tourniquet to either arm.

c) Examine participant's arms for the best site for venipuncture. Release tourniquet.

d) Cleanse venipuncture site. Prepare area by wiping with alcohol swab in a circular motion from center to periphery. Allow area to dry.

e) Reapply tourniquet and start timer.

f) Grasp the participant's arms firmly using your thumb to draw the skin taut. This anchors the vein. The thumb should be one or two inches below the venipuncture site.

g) With the needle bevel upward, enter the vein with a smooth continuous motion.

h) Make sure the participant's arms are in a flat or dominant position while maintaining the tube below the site when the needle is in the vein. It might be helpful to have the participant make a fist with the opposite hand and place it under the elbow for support.

i) Grasp the sheath of the needle holder and push the tube forward until the butt end of the needle punctures the stopper, exposing the full length of the needle.

j) Note the blood flow into the first collection tube. If blood is flowing freely, the butterfly needle can be taped to the participant's arm for the duration of the study. If the blood flow is very slow, the needle may not be positioned correctly.

k) Remove the tourniquet at two minutes. Note the time on the PP form. Once the draw has started, do not change the position of the tube until it is withdrawn from the needle. If blood flow ceases after the tourniquet is removed, it may be reapplied for another two minutes, but this is noted on the Phlebotomy Processing Form.

l) Keep a constant, slight amount of pressure (in the direction of the needle) on the end of the tube (especially tubes #1 and #2). This prevents release of the shut-off valve and stopping of blood flow, do not bring pressure or reintroduce pressure after completion of the draw.

m) Fill each vacutainer tube as completely as possible; i.e., until the vacuum is exhausted and the blood flow ceases. If a vacutainer tube fills only partially, remove the vacutainer and attach another without removing the needle from the vein. As each tube is filled, mix by gently inverting before placing tube on the mixer. (See Section Blood Mixing During Venipuncture.)

n) When the blood flow ceases, remove the tube from the holder. Shut off valve recovers the point, stopping blood flow until the next tube is inserted (if necessary).

o) Average venipuncture time is 3-6 minutes, but any difficulties may increase this time to 10 or 15 minutes.

7. Removing the Needle:

To remove the needle, lightly place clean gauze over the venipuncture site. Remove the needle quickly and immediately apply pressure to the site with a gauze pad. Discard needle into puncture-proof sharps container. Have the participant hold the gauze pad firmly for 2 minutes to prevent a hematoma. Remove tube from the blood mixer and place on ice (#4,#5) and at room temperature (#1,2,3).

8. Bandaging the Arm:

a) Under Normal Conditions:

1) Set the gauze pad down over the site, continuing mild pressure.

2) Apply an adhesive or gauze bandage over the veni puncture site after making sure that blood flow is stopped.

3) Tell the patient to leave the bandage on for at least 15 minutes.

b) If the patient continues to bleed:

1) Apply pressure on the site with a gauze pad. Keep the arm elevated until the bleeding stops.

2) Wrap a gauze bandage tightly around the arm over the pad.

3) Tell the patient to leave the bandage on for at least 15 minutes.

9. Procedures for difficult draw and complications of blood drawing.

If a blood sample is not forthcoming, the following manipulations may be helpful.

a) If there is a sucking sound, turn the needle slightly or lift the holder in an effort to move the bevel away from the wall of the vein.

b) If no blood appears, move needle slightly in hope of entering vein. Do not probe. If not successful, release tourniquet and remove needle. A second attempt can be made on the other arm.

c) Loosen the tourniquet. It may have been applied too tightly, thereby stopping the blood flow. Apply the tourniquet loosely. If the tourniquet is the Velcro type, quickly release and press back together. Be sure, however, that the tourniquet remains on for no longer than 2 minutes at a time.

d) The phlebotomist should not attempt to venipuncture more than twice.

e) Reassure the participant that the inability to obtain a clean venipuncture is not any sign of a medical problem on his/her part.

f) If venipuncture is unsuccessful, the participant must be rescheduled at a later date with a different field center phlebotomist.

10. Syringe Technique for Venipuncture

Collection of blood sample using syringes may also be used if the phlebotomist, upon examination of the participant's veins, feels that sample will be difficult to obtain by vacutainer method. Blood collection using syringes should be used only if the phlebotomist anticipates a difficult draw (vein collapse, small veins, etc.).

11. Blood Mixing During Venipuncture:

Each tube should be treated as follows:

a) Serum (#1,6) - Invert once, place on rack at room temperature.

b) Citrate (#2) on mixer for 30 seconds then place on rack at room temperature.

c) Citrate (#3) on mixer for 30 seconds then place in ice bath.

d) Diatube (#4) on mixer for 30 seconds then place in ice bath.

d) EDTA (#5) on mixer for 30 seconds then place in ice.

12) Precautions:

a) When a participant feels faint or looks faint following the blood drawing:

1) Have the participant remain in a chair, if necessary, have him/her place head between knees.

2) Provide the person with a basin if he/she feels nausea.

3) Have the person stay reclined until the color returns and he/she feels better.

4) Place a cold wet cloth on the back of the person's neck.

5) If the person faints, use smelling salts to revive by crushing the ampule and waving it under the person's nose for a few seconds.

6) If the person continues to feel sick, contact the medical staff member who will advise you on further action.

13. Completing the Blood Draw Procedure:

a) Dispose of the needle and tubing:

Dispose of needle and tubing in the appropriate biohazard needle/sharps containers. (It may be necessary to use Hemostats to remove tubing from vacutainer holders.) Complete the first page of the Phlebotomy Processing Form. Clean up the venipuncture area (if necessary). Bring blood collection tray to the processing area with the filled vacutainer tubes.

TABLE OF CONTENTS

CHAPTER NINE: Data Management

9.1 GENERAL INSTRUCTIONS FOR FILLING OUT FORMS

9.1.1 Background

The Dietary Effects on Lipoprotein and Thrombogenic Activity (DELTA) study will use paper forms to collect most data. Data from these paper forms will then be entered at a later date either into a computer-assisted data management system (DMS) located at each field center and the food analysis laboratory or into non-standard format files at the central lipid and hematology laboratories.

The purpose of this section is to provide instructions for completing these paper forms. It should be read carefully prior to working with any forms. The form-specific instructions should then be read before filling out each form.

9.1.2 Form Structure

Most paper forms in DELTA are similar in design to the forms layout of the computer screens used for data entry. The paper forms are organized by pages, but the forms in the data management system are organized by computer screens. This may cause some problems in locating an item on a paper form and the corresponding item on the computer screen, however, the items are listed in the same order.

The forms are structured with a header of the form title, form code, form version number, and date of form version across the top of each page. The first page begins with the key data fields of participant DELTA ID number and a visit date. The form code, form version number, participant DELTA ID number, and visit date comprise a unique set of data which identify an individual form type for a participant at a certain point in time on the study database.

Following these header and key data items on the forms are the questions for the participants. Instructions to the interviewer are in brackets ("[]") on most forms.

An example of a typical "first page" is presented in Figure 1.

Figure 1: Example of DELTA Form - First Page

Participant Weekly Monitoring

Form Code: PWM
Version B 5/10/94

DELTA ID: _____

Monday's DATE: ____/____/____
[THIS WEEK] (mm/dd/yy)

1. Code Number of personnel completing this form: _____

BLOOD DRAW
[Complete numbers 2-4 during weeks 5, 6, 7.]

2. Period **1 2 3** **[Circle correct number]**

3. Week **5 6 7** **[Circle correct number]**

4. Date of blood draw ____/____/____
mm/dd/yy

WEIGHT [THIS week]
[Participants are weighed before dinner, without shoes or coats.]

5. a. Date of first weekly weight: _____
(mm/dd/yy)

First weekly weight, either in lbs or kg:

b. lbs: _____ or c. kg: _____

d. Current calorie level: **1500 2000 2500 3000 3500** **[Circle correct number]**

6. a. Date of second weekly weight: _____
(mm/dd/yy)

Second weekly weight, either in lbs or kg:

b. lbs: _____ or c. kg: _____

d. Current calorie level: **1500 2000 2500 3000 3500** **[Circle correct number]**

9.1.3 General Instructions for Completing and Correcting Items on the Forms

All items fall into three main categories: (1) fill in the space, (2) multiple choice, and (3) narrative description. Techniques for completing each of these types of items, as well as making corrections, are described below. A general rule is to record information only in the spaces provided (except for some error corrections).

9.1.3.1 Fill in the Space: Recording Information

This category is similar to filling in boxes with numeric or alphabetic information of a pre-specified length and within a range of valid values. Examples include participant's last name, date of birth, or sitting blood pressure measurements. The maximum length of the field is not apparent on the paper form, however, a maximum field length is indicated on the computer screens. The instructions below correspond to the entry in the data management system.

When alphabetic information is required, enter the response beginning in the leftmost space using capital letters. Punctuation may be included.

Example: If the participant's last name were O'Reilly, it should be entered as follows:

Last Name: O'REILLY___

If the response contains more characters than there are spaces, begin with the first character and enter as many characters as there are spaces.

Example: If the subject's last name were Hobgoodnotting, it should be entered as follows:

Last Name: HOBGOODNOTTI

Whenever numerical responses are required, enter the number so that the last digit appears in the rightmost space. Enter leading zeroes where necessary to fill all spaces. (This does not apply to the address section or to any item which combines alphabetic and numeric information. Such items should be treated as alphabetic.)

Example: If the participant's diastolic blood pressure were 96, it should be entered as:

Diastolic: 096

When dates are recorded, slashes ("/") are required as the separator characters for month, day, and year. The format to be used to record dates is indicated under the spaces as month/day/year. Use leading zeros within each date unit (month or day or year) so that each space is filled.

Example: Data collected on April 3, 1993 would be entered as:

Today's Date: 04/03/93

DELTA usually records time using a 12-hour clock, with AM or PM indicated separately. Colons (":") are used as the separator character for hours and minutes. The format to be used is indicated under the space. Use leading zeros within each time unit (hour or minute) so that each space is filled. Note that midnight is recorded as 12:00 AM, and noon is recorded as 12:00 PM.

Example: A time of fasting determination of 8:05 in the morning is entered as:

a. Time: 08:05 b. AM (A) or PM (P): A

9.1.3.2 Fill in the Spaces: Correcting Mistakes

If a number or letter is entered incorrectly on the paper form, mark through the incorrect entry with an "X". Code the correct entry clearly above the original incorrect entry. Record the initials of the person correcting the mistake and the date of correction next to the correction.

For example, if the participant's systolic blood pressure were actually 130, but was incorrectly recorded as 139, put an "X" through the 9 and write a 0 above the correction. Place your initials and date of correction beside the corrected entry.

If a mistake is made, corrected, and then it is discovered that the correction is incorrect (the value in the example above should be 132), make a second correction by placing an "X" through the handwritten 0 and writing a 2 beside the correction. Again, place your initials and date of correction beside the corrected entry.

9.1.3.3 Fill in the Spaces: Unknown or Inapplicable Information

If an item of this type (alphabetic or numeric) *does not apply* to this participant, leave the space blank. For example, if the participant does not have a work phone number, that item is left blank.

If the item *does apply*, but the response is unknown, mark through the space with *two* horizontal lines (equal signs "=").

For example, the participant was asked date of birth but does not recall the month and day. The question does apply because it can verify the age of the participant for stratification by age in data analyses. In this case, the response would look like "==/==/40". The same response would be entered on the computer screen.

9.1.3.4 Multiple Choice: Recording Information

In this type of question several alternatives are given for the answer, each having a corresponding letter or number. When it is decided which alternative is most appropriate, circle the corresponding letter in the space provided. Always circle *one letter only* unless otherwise specified.

Example: How many years has it been since your last cigarette?
A Less than 1 year
B 1 year or more

In the above example, for a response of 1 year or more, circle response B. Enter B as the response on the computer screen.

For questions with YES/NO responses, circle either YES or NO.

Example: Do you now smoke cigarettes? YES NO

In the above example, if the participant does not now smoke cigarettes, circle response NO. Enter N for NO as the response on the computer screen.

9.1.3.5 Multiple Choice: Correcting Mistakes

If a response is coded incorrectly, mark through the incorrectly coded response with an "X" and circle the correct response.

Example: How many years has it been since your last cigarette?
A Less than 1 year
B 1 year or more

The actual response is A, but B was circled incorrectly. Put an "X" through the circled response B and circle the corrected response A. Place your initials and date of correction next to the corrected entry.

If a mistake is made, corrected, and then it is discovered that the correction is incorrect (correct response is actually B in the example above), make a second correction by placing an "X" through the circled response A and writing and circling the corrected response B. Place your initials and date of correction next to the corrected entry.

To record the correct response on the computer screen of the data management system, enter the correct response in the space provided.

9.1.3.6 Narrative Description

On forms where narrative descriptions or comments are requested, space is provided to write this information. The person who made the hand-written comments should review the descriptions for accuracy and legibility. Abbreviations should be avoided. These narrative

descriptions are entered in fixed length fields or open-ended note logs in the data management system (see instructions on the data management system for details).

Responses to fixed length items on the form, such as medication name or type or physical activity, is entered in BLOCK CAPITAL LETTERS in the data management system.

9.1.4 Completing Key Data Information

The following guidelines should be observed in completing the key data information following the "header" located at the top of the first page on all forms:

9.1.4.1 DELTA ID

Apply the pre-printed ID label, or write in the participant's 6 character ID number. DELTA 2 utilizes a different sequence numbering for screening (pre-randomization) and post-randomization labels. Screening (pre-randomization) labels have the following sequence: Column 1 contains a letter identifying the field center, column 2 contains the current protocol number 2, and columns 3-6 contain a 4-digit number from 0100-0499 if the applicant is eligible following Telephone Screening Visit (TSV), or 0500-1599 if the applicant is not eligible following TSV. Post-randomization ID's follow the same format: Column 1 contains a letter identifying the field center, column 2 contains the current protocol number 2, and columns 3-6 contain a 4-digit number from 0001-0035.

The letters for each field center are as follows:

C	Columbia
L	Louisiana
M	Minnesota
P	Penn State

9.1.4.2 Date

Today's Date: Fill in the date of the participant's visit to the field center. Enter the date as month/day/year using zeros to fill in all spaces. The participant's visit date is also referred to as Monday's Date on the Participant Weekly Monitoring Form and as Date Blood Drawn on the Lipid Profile Forms.

9.1.5 Skip Patterns ("Go to" Spaces)

Skip patterns occur in many multiple choice type items. Here, if a certain response is selected, it is necessary to skip over one or more items to the next applicable item. This is indicated by a "go to" instructions on the form. If the response is selected which necessitates a skip, the next item to be asked is indicated in the instructions on the form. If the other response is selected, always proceed to the next item unless otherwise directed.

Occasionally, a skip pattern will occur in a fill-in type item. In those instances, specific instructions are provided on the form. Again, if the skip criteria are not satisfied, continue with th next item.

9.1.6 Security of Data

DELTA paper forms contain confidential participant name and information and are to be stored in a secured filing cabinet or room. The file cabinet or room containing the paper forms should be a reasonable distance from the computer system and diskettes to minimize loss of data in case of a disaster.

Only personnel who are authorized to enter data on the computerized data management system should be given the Access Code and Password for the DMS to prevent unauthorized entry. If unauthorized persons have obtained the Access Code or Password, change these using the procedures described in the instructions for using the data management system.

Backup copies of all data files and diskettes should be maintained to avoid loss of data. The work and backup diskettes should be stored in a secured location, as far as conveniently feasible from the microcomputer, to reduce the risk of data loss.

The Coordinating Center can restore data that has been received, but the centers must ensure that at least one of their copies of currently entered data (paper forms, files on diskettes or the computer system) will survive an occurrence of data loss such as computer theft, hard disk crash, automatic sprinklers, or coffee spills.

9.1.6.1 Columbia

Completed forms awaiting data entry are held in Steve Holleran's office, along with the backup tapes and any printed material containing DELTA Data. Mr. Holleran's office is part of a larger complex of rooms (a kind of office within an office within an office). There are three doors between his office and the outer hall, and all three are securely locked when no one is in them. The data is exported on a regular basis from a computer of DELTA. The computer is in a small room with a locked door and it is used only by DELTA personnel.

9.1.6.2 Minnesota

The University of Minnesota DELTA is housed within the Division of Epidemiology, Moos Tower location. All areas occupied by DELTA (offices, labs, storerooms, kitchen and dining room) have separate keying to limit access to only those individuals who work for the DELTA study and who have been assigned keys. The DELTA computer system is located in an office occupied by the study research assistant and research dietitian. The computer is locked when not in use. Computer keys are in the possession of the study coordinator, research assistant and research dietitian only and are kept in non-accessible locations. Completed forms are stored in the computer office in a locked file cabinet. Forms are data entered in a timely manner. Data is exported biweekly according to DELTA CSCC protocol. Backup of the data is performed with each export. Backup tapes are stored in locked files. Each DELTA participant has a chart in which forms are "filed" upon completion of data entry. Charts and any forms and/or copies are locked in metal file cabinets with DELTA offices.

9.1.6.3 Penn State

The DELTA computer system is set up in a room by itself, and the room is accessible only by the Study Coordinator and the two data entry personnel. The passwords to access DELTA files are kept confidential by these three individuals. All subject forms that are data entered are handled only by the Study Coordinator, who does the necessary transcription and then forwards them to the data entry personnel. All subject records and forms are kept in the DELTA computer room, in locked file cabinets.

9.1.6.4 Pennington Biomedical Research Center

The DELTA computer system is located in the Subject Recruiter's office and is locked when not in use. The key to the computer is in the possession of the Subject Recruiter. Forms are data entered in a timely manner by designated personnel. Phlebotomy processing forms are stored in a locked file cabinet in the Assistant Laboratory Director's office. All other forms are stored in Medical Records which is kept locked and accessible only to designated personnel.

9.2 SET-UP OF THE DMS

9.2.1 Installation

The computers are shipped to the field centers with the DELTA data management system software installed. In addition, word processing, electronic mail, and utility programs are installed for your use on the DELTA Study. Instructions to set up the computer and user's guides for the software are shipped with the computers.

During the study, it is possible that the CSCC may release an upgrade to the DMS due to a form version change, a new feature or report, or a problem fix. Instructions to install the upgrade will be distributed with the software upgrade.

Important notice: If you plan to install other software on the computer, please notify the Coordinating Center prior to your installation. Some software could cause interference with the functioning of the DMS.

9.2.2 Preparing Diskettes and Backup Tapes

Refer to section 3.2 on Export and Tape Backup of the DELTA DMS User's Guide for information on the preparation of diskettes to transfer data to the CSCC. You are requested to purchase and use new, pre-formatted high density 3.5 inch diskettes for the export.

Tapes for backup files must be formatted and prepared for use by the DMS. Refer to section 6 on Backup Tape Preparation in the DELTA DMS User's Guide to format a tape for use.

9.2.3 Beginning to Use the DMS

The DMS was tested at the Coordinating Center prior to installation on each computer. Each time you turn on or reset your computer, several diagnostics are run to check for any problems or

viruses. Following a successful start-up of the computer, a menu will be displayed to select the DMS or other software.

Refer to the DELTA DMS User's Guide for directions once you are in the DMS.

9.2.4 Reporting Problems

At any time during the start-up diagnostics or within the DMS that you receive an error message which informs you to call the Coordinating Center, *call us at once* to report the problem.

The contact at the CSCC is Nancy Anderson at 919-962-3052.

At the time of a problem call to the CSCC, a problem log will be completed and forwarded to the appropriate person for attention.

9.3 DATA CHECKING AND REPORTS

9.3.1 Display CXI

The CXI inventory form provides the number of each form type entered in the DMS. (See section 2.6 on Display CXI in the DELTA DMS User's Guide for more information.) The CXI inventory form can be viewed from the ID screen or from within any form screen. Either the inventory for a participant is shown if you have entered an ID, or an inventory of the entire database is shown if you have not entered an ID.

9.3.2 Search through Form Types

The Accept command has been renamed the Search command in the DMS to define more adequately the function of the command. You can use the Search command to scroll through every form type for a participant if you have entered an ID, or to scroll through every occurrence of a form type if you have not entered an ID.

9.3.3 Export Report

When you prepare the export file to send to the Coordinating Center, two reports are produced. The first report lists the key fields of each record exported, separately for each form type. The second report provides a summary of the number of records added, changed, and deleted for each form type.

A paper copy of the export report will be sent via FAX to DELTA Central Receiving at the CSCC on the day you prepare your export file on diskette. This will notify us that a diskette was prepared and mailed.

9.3.5 Basal Energy Expenditure Report

From the Reports option of the Main Menu, you can calculate the Harris-Benedict equations for basal energy expenditure (BEE) for the run-in phase and for randomization for the participants.

Once the BEE is known for a participant, total calories is calculated for three physical activity level of moderate activity, light activity, or sedentary to determine the calorie level to assign the participant for feeding.

9.4 DATA TRANSFER

9.4.1 Shipping Preparations and Schedule

Once every two weeks the field centers will run the DMS export facility of the DMS to send data to the Coordinating Center on diskette. There are no exceptions for Protocol 2, see shipping schedule posted on page 13 of this chapter. Refer to the DELTA DMS User's Guide for instructions on how to prepare a transfer diskette for shipment. It is important that the diskettes be prepared on the dates indicated and that CSCC receive your data in a timely fashion. The schedule of diskette mailing dates for protocol 2 in 1994-95 is on the following page.

The CSCC expects to receive a diskette from each center within two working days of the shipping dates. All shipping dates fall on Friday, so diskettes are to arrive at the CSCC on Tuesday of the following week. If for some reason you have entered no data for a particular shipment interval, the CSCC needs to be informed so that we will not expect a diskette from you. *Even if you have no data to send and your diskette is empty, please run the DELTA DMS export procedure on the mailing date and send us a copy of your summary.* Each centers will send to the CSCC via FAX a paper copy of the export summary report on the scheduled Friday mailing date to inform the CSCC on whether or not to expect a diskette.

9.4.2 Mailing Procedures

Use a sturdy foam-lined diskette mailer to send your diskette to the CSCC. Send the diskette on the scheduled Friday date for *two working day delivery* to the CSCC for arrival the following Tuesday. Again, use new, formatted 3.5 inch high density diskettes to export files to the CSCC.

Mailers should be addressed as follows:

DELTA Central Receiving
CSCC
Suite 203, NationsBank Plaza
137 E. Franklin Street
Chapel Hill, NC 27514

Send a paper copy of the export summary report via FAX to DELTA Central Receiving at the CSCC on the day you prepare your export file on diskette. This will notify us that a diskette was prepared and mailed.

DELTA Study
1994-95 DELTA Protocol 2 Diskette Shipping Dates

1994	Oct	21
	Nov	4
		18
	Dec	2
		16
		30
1995	Jan	13
		27
	Feb	10
		24
	Mar	10
		24
	Apr	7
		21
	May	5
		19
	Jun	2
		16
		30
	Jul	14
		28
	Aug	11
		25
	Sep	8
		22
	Oct	6

9.5 IN HOUSE DATA PROCESSING PROCEDURES

The Dietary Effects on Lipoprotein and Thrombogenic Activity (DELTA) study will receive most data from the field centers and central agencies on diskettes prepared from the EXPORT utility of the DELTA data management system (DMS). Diskettes will be sent on scheduled shipping dates once every two weeks. The files will contain all data records added or changed in the centers' database since the last data transfer.

9.5.1 Receipt and Processing of Data

1. File copies of the export summary reports sent via FAX to the Coordinating Center for notification of the shipment of a diskette. Record the center name, diskette filename, and date of receipt on the reports, and file these reports in the processing log. This report provides counts of all the data on the transfer by form type and transaction type (add, change, delete) and the run data of the export procedure.

2. On the day of receipt of the diskettes, record the information from the diskettes in the processing log. A file containing the export summary report received via FAX will accompany the diskette.

3. Within one working day of receipt of the diskettes, check the diskette for read errors. If errors are detected, contact the field center by telephone or E-MAIL to resolve the problem. Maintain written reports of any correspondence with the centers.

4. If a diskette for a center has not been received after one day of the scheduled receipt date, contact the field center by telephone or E-MAIL to inquire on the status of the data transfer. If the diskette has not been sent, ask the center personnel to send the diskette for next day arrival at the Coordinating Center. If the diskette has been delayed in shipping, ask the center personnel to check on the location of the diskette. If the diskette has been lost, have the center personnel contact Brian Stewart at the Coordinating Center for instructions on creating a replacement diskette.

5. An acknowledgement of receipt of the diskette at the Coordinating Center will be sent to the field center or sending agency within one working day of receipt.

6. When all errors are resolved the files are ready to upload to the Study data base.

9.5.2 Data Transfer and Processing of Non-Standard Format Files

The central hemostasis laboratory will send non-standard format files on diskette to the Coordinating Center at the end of period 3. These diskettes ***will not be*** processed by DTIMPORT but through special programming procedures by the systems programmers as the Coordinating center to include these data on the study database.

1. Record the information in the processing log from the diskettes on the day of receipt of the diskette.

2. An acknowledgement of receipt of the diskette at the Coordinating Center will be sent to the field center or sending agency within one working day of receipt.

3. Forward the diskette to the systems programming staff at the coordinating center for processing.

9.5.3 Paper Forms Sent to the Coordinating Center

1. In the future, some data may be sent to the Coordinating Center on paper forms. Copies of the paper forms are retained at the field centers, and the original paper forms are sent in batches to the Coordinating Center. A shipping list will accompany the batch of forms identifying the contents in the batch.

2. Coordinating Center personnel will key data from the paper forms usually in the order of receipt. These data will be keyed within five working days of receipt or by the next scheduled arrival of paper forms.

3. A 100% verification (re-keying) will be performed on laboratory data. Verification is preferably done by a different person, or on different days if keyed by the same person. Verification will be done within three working days of the data entry. The person keying these data will work with the research staff to resolve any discrepancies.

4. After data are keyed and verified, data are prepared for processing by the EXPOR utility of the DMS. The data will be transferred to the study database following the procedures in section I.B. on processing data from the DMS.

5. DELTA paper forms contain confidential participant name and information and are to be stored in a secured filing cabinet or room. The file cabinet or room containing the paper forms should be a reasonable distance from the computer system and diskettes to minimize loss of data in case of a disaster.

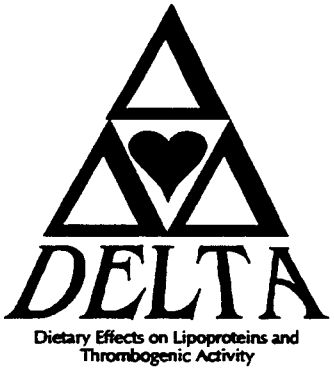
Only personnel who are authorized to enter data on the computerized data entry system should be given the Access Code and Password for the DMS to prevent unauthorized entry. If unauthorized persons have obtained the Access Code or Password, change these using the procedures described in the instructions for using the data entry system.

Backup copies of all data files and diskettes should be maintained to avoid loss of data. The original and backup diskettes should be stored in a secured location, as far as conveniently feasible from the microcomputer, to reduce the risk of data loss.

The Coordinating Center can restore data that has been received, but the centers must ensure that at least one of their copies of currently entered data (paper forms, files on diskettes or the computer system) will survive an occurrence of data loss such as computer theft, hard disk crash, automatic sprinklers, or coffee spills.

DELTA Protocol 2
Data Management System
Users Guide

Version 1.0



Collaborative Studies Coordinating Center
University of North Carolina at Chapel Hill

Table of Contents

1 Introduction

This Users Guide contains instructions pertinent to the operation of version 1.0 of the DELTA Protocol 2 Data Management System [DMS]. When running an earlier version, please refer to the instructions provided with that version. If your Protocol 2 DMS is a later version than this guide, please refer to the update memos and any available addenda to this guide for complete instructions. In the event of significant changes to the DMS, a complete updated Users Guide will accompany the update.

1.1 Overview of Data Collection

In the course of performing a study, data for a number of participants must be collected at various times for later analysis. These data items are organized into groups of logically related information called **forms** or form types. Each form is then assigned a brief mnemonic code for easy reference, i.e. "EV1" for Eligibility Visit 1, "TSV" for Telephone Screening Visit, etc.

Though undesirable, it is sometimes necessary to change the content of a form during the course of a study. To allow for such changes, we assign a **version** letter to each form. The initial version is "A", and subsequent versions follow alphabetically. Thus, "TSVA" refers to "Telephone Screening Visit Form, Version A."

Since each form must be collected one or more times for each study participant, extra information is included to uniquely identify each recorded instance, or **record**, of a form. These identifiers, or **key fields**, include Sub ect ID (**subjid**), Time Point (**timept**) and Form Sequence (**formseq**). The Sub ect ID is a unique code assigned to the participant. The Time Point is the date of collection of the form. If more than one record is collected for a participant at a given time point, a unique Form Sequence number must be assigned to each record.

Data items which appear on a form typically consist of demographic information, questions and measurements. For simplicity, we refer to all data items as questions and assign a **question number** to each item. Typical question numbers may include both letters and numbers, e.g. 1, 2, 3a, 3b, etc.

Data items are initially collected on paper forms. Then, the data on the paper forms is entered (or **keyed**) into an electronic database for statistical analysis.

A database consists of tables of data, arranged into fields and records. Each table (form) can store many records (instances of a form), each containing a set of values for every field (question) in the table.

Each table in the database must have a unique name for identification, as must each field in a table. We assign each table's name to be the form and version of the source of its data. We assign each field's name to be the name of its table and the question number of the source of its data. Hence, the table containing data for form TSV, version A, is named TSVA and contains fields named TSVA1, TSVA2, TSVA3A, TSVA3B, etc.

Each record in a table is uniquely identified by its set of key fields. Thus, no two records in a table may have the same set of key field values (sub id, timept, and formseq).

1.2 DMS Function

The DELTA Data Management System [DMS] is a set of programs which manage data collected in the DELTA field centers. The DMS uses the FoxPro data base management system for screen display, data editing and storage.

The DMS provides several ma or functions:

Data Entry: Allows data to be keyed, edited and updated.

Data Transfer: Allows data to be sent to the DELTA Coordinating Center for inclusion in a consolidated database.

Reports: Provides counts of records entered by form type, counts of unverified records by form type, and basal energy expenditure for a participant.

1.3 Starting the DMS

To run the DMS, type DELTA from the C:\ prompt. A menu will be displayed:

```
     Select one of the following

F1   run DELTA DMS (Protocol 2)
F2   run WordPerfect 6.0 for DOS
F3   run pcAnywhere (modem program)
F4   run DaVinci Email
F5   Call CSCC to swap mail messages
F6   run DELTA DMS (Protocol 1)

ESC  Exit to DOS
```

Press F1 to start the Protocol 2 Data Management System.
Press F6 to start the Protocol 1 Data Management System.

1.4 User Interface Standards

The DMS uses a combination of menus and function key commands to control its actions.

1.4.1 Keyboard and Mouse

The DMS uses the keyboard in a conventional way:

- The typewriter keys are used to type numbers, letters and symbols.

- The cursor control arrow keys highlight menu bar options. Once the cursor is on a menu option, the ENTER key either performs the action or brings up a submenu. The left and right

arrow keys move within a field. The down arrow key and TAB move the cursor to the next field. The up arrow key and SHIFT+TAB move the cursor to the previous field. The Home and End keys move to the beginning and end of a field respectively. PAGE UP and PAGE DOWN move to the previous and next screens in a form. CTRL+PAGE UP and CTRL+PAGE DOWN move to the previous and next record.

- Tapping ALT moves the cursor to the menu bar. Tapping ALT again or pressing ESC returns the cursor to the data entry window.

- Most menu options have shortcut keys which are a combination of the ALT key and a letter, usually the highlighted letter. In this guide these are written as ALT+*letter*, for example ALT+E. To use the shortcut hold down the ALT key and simultaneously press the letter. Specific shortcut keys will be described when the menus are discussed.

- Most submenu options (the lists displayed after you choose a menu item) have shortcut keys which are usually a combination of the CTRL key and a letter. To initiate an action with a shortcut key hold down the CTRL key and simultaneously press the letter.

- F1 (function key 1) is the help key.

- F2 (function key 2) is the field duplication key.

- F3 (function key 3) is the list display key.

Menu items can be selected using the mouse. To select an item, move the pointer to the item and press the left mouse button once.

1.4.2 Menus

Most screens in the DMS have horizontal menu bars on the first line. These menus list the options available from the screen. To move the cursor to the menu, tap the ALT key. Once the cursor is on the menu bar, there are two ways to select an option:

Use the left and right arrow keys to move the highlighted bar to the desired option and press ENTER, or

Type the highlighted letter of the desired option.

Some menu options have further choices which are displayed in a pull-down list when the option is selected. Use the up and down arrow keys to move the bar to the desired option or type the highlighted letter.

Shortcut keys have been defined for some menu and submenu options. To use a shortcut key, press the key combination while the cursor is in the data entry section of the screen. The cursor does not have to be on the menu. Shortcut keys for menu options are ALT and the highlighted letter of the options. Most shortcut keys for submenu options are a combination of the CTRL key and a letter. The shortcut keys are displayed on the pulldown lists. Exceptions

to these rules are the help key (F1), the field duplication key (F2), the list display key (F3) and the movement keys (PAGE UP, PAGE DOWN, CTRL+PAGE UP, CTRL+PAGE DOWN).

Under some conditions menu options are unavailable. For example if a user does not have delete privileges, the Delete option is not available. Unavailable options are not highlighted and cannot be selected.

1.4.3 Lists

Some fields, for example the form field on the ID screen, can be selected from master lists. When the cursor is on the form field, a list of all available form types can be displayed using F3. Use the arrow, page up and page down keys to move the highlighted bar through the list. To select an item, place the highlighted bar on the item and press ENTER. The item under the bar will be put in the field.

1.4.4 Information and Warning Messages

Messages from the DMS are of two types. The first displays a message and tells you to press a key to continue:

```
Insert blank EXPORT disk in Drive B.  ESC to Exit
Press any key to continue....
```

These usually appear when you must perform an action, like inserting a diskette. The message remains on screen until you press a key. The other type of message is used when no user action is required:

```
Login failure.  Please retry.
```

It will disappear from the screen after a few seconds. However, you can make it disappear instantly by pressing a key.

1.5 Desktop, User IDs and Passwords

The password screen is the first DMS screen displayed:

```
Help  Desktop  Quit

        Enter your USER ID  > ***


                    Login Screen
```

At the top of the screen the menu bar lists three options:

Help presents a list from which you can select a DMS topic.

Desktop contains several features which are not vital to the DMS but which were included for your convenience. A pull-down menu is displayed when you choose Desktop:

Quit will exit the DMS.

```
Toggle Time     CTRL+T
Calendar/Diary  CTRL+D
Calculator      CTRL+C
Color Picker    CTRL+P
ASCII Chart     CTRL+A
Puzzle          CTRL+Z
```

The only selection that affects the DMS is the color picker. The color picker lets you define colors for the DMS. See Section 5 for a description of all the desktop utilities.

To use DMS functions other than Desktop utilities, you must enter a valid ID and password at the Login screen. These are assigned by using the System Administration utility described later. IDs are three characters long. Passwords are at least three and at most eight characters long and are not shown on the screen. If you enter an invalid ID or password, an error message is displayed:

```
Login failure.  Please retry.
```

The cursor returns to the ID field for re-entry. After 3 failures, the DMS exits automatically.

To exit the DMS from this screen, select **Quit** from the menu.

1.6 Timeout

At many field centers the DMS is started in the morning and not shut down until evening. This presents a security problem because a computer left unattended could be used to look at confidential data. To prevent this the DMS has a **timeout** feature. If no keys are pressed for 10 minutes, a message is displayed:

```
System timing out in 30 seconds.
```

Audible ticks count down from 30.

Press ALT to reset the clock and continue work. Note that it may take several rapid ALT key presses to reset the clock.

If you fail to press ALT before the 30 seconds expire, the system "times out". The Password screen is displayed with a message that the system has timed out. You must reenter your ID and password to resume using the DMS.

If an ID and password are not entered within 30 seconds, a screen saver will put a random pattern on the screen. Press any key to stop the screen saver and return to the Password screen.

If a timeout occurs during data entry before a record is saved, changes to the record are saved. After logging in, the DMS displays a message indicating which record was active when the timeout occurred:

```
System timed out
Changes to active form were saved

Last active form was
 Subject ID:  C20000
 Form Name:   LBA
 Version:     A
 Time Point:  01/01/95
 Form Seq #:  00

(The DUP key [F2] has been loaded with these values)
─────────────────────────────────────────────────────
                Press any key to continue....
```

From the ID screen, press the Dup Key (F2) for each key field to return to the saved record.

2 Data Entry

After you enter a valid ID and password, the DMS Main menu is displayed:

Select **Data Entry** to add and modify participant data. Select **Utilities** to run support programs such as the password program and the export and import programs. Select **Reports** to run report programs such as the Basal Energy Expenditure report. **Exit** returns to the Password screen. **Quit** exits the DMS.

The highlighted letters of each option indicate which letter, in combination with the `ALT` key, comprises the shortcut. The shortcut key for Help is `F1`.

Press `ALT+D` to start the Data Entry System.

2.1 ID Screen

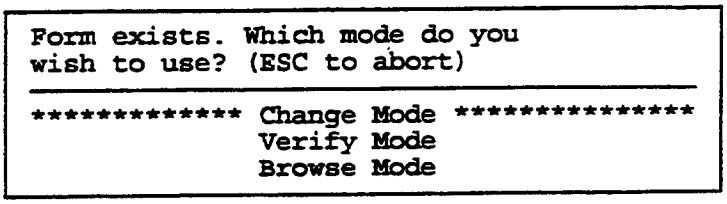
The ID screen is the first screen shown:

```
 Help  CXI  Search  Exit  Quit
┌──────────────────────────────────────────────────────────────┐
│          Subject ID: ******                                  │
│          Form Name:  ***                                     │
│          Version:    *                                       │
│          Time Point:  **/**/**                               │
│          Form Seq #:  00                                     │
│                                                              │
│Input subject ID   *   Press Tab to skip                      │
└──────────────────────────────────────────────────────────────┘
```

Entry mode, e.g. add, change or browse, is determined by which key fields you enter, which records exist in the database, and some form-specific rules.

If you enter **all** key fields and a record with the specified keys **does not** exist in the database, the mode is **Add**. An empty record is displayed for data entry. If you do not have Add privileges and are trying to add a record, you are informed that you cannot add records and are returned to the ID screen.

If you enter **all** key fields and the specified record **does** exist in the database, you are notified that the record exists and are asked to choose **Change**, **Verify**, or **Browse** mode.



The first line may read "Form is UNVERIFIED" or "Form is VERIFIED" if the form type requires verification.

If you enter only **some** of the fields on the ID screen, the system searches for a record which matches those keys, ignoring the keys which you left blank. If a match is found, you are notified that a matching record was found and are asked to choose **Change**, **Verify**, or **Browse** mode.

The following table summarizes the mode the data entry system assumes when certain fields are entered on the ID screen:

Fields Entered	Found in DB?	Mode
All Fields	No	Add
All Fields	Yes	Choice of Change, Verify or Browse
Some Fields	Yes	Choice of Change, Verify or Browse
Some Fields	No	Disallowed, return to ID Screen

To summarize, to add a new record for a participant you must complete the ID, form and time point fields. (Sequence number defaults to 00.) To change records you can enter all or some of the fields on the ID screen.

As you enter the ID, it is checked (or **edited**) by the DMS for validity. If it is not a valid DELTA ID, an error message is displayed and the cursor remains in the field. If it is valid, the cursor moves to the form field.

FORM can be entered in one of two ways. You can type a form abbreviation into the field, in which case the default or current version is automatically chosen and displayed in the version field. Or you can press the F3 key to display a list of all valid form types. Then TAB into the scrolling list of forms, position the highlighted bar on the desired form and press ENTER. The form and version are plugged into their respective fields. Note that this second method is the only way to choose a version other than the default.

Next, the cursor enters the time point field. Enter the date the record was collected. Edits check that you have entered a valid date.

To add a record with a form sequence number other than the default of 00 (i.e. this is the second record entered on the same date for the same sub ect), you must change the sequence number field **first**. When the screen is initially displayed, use SHIFT+TAB to go to the sequence field. Enter the appropriate sequence. Then fill in all other relevant fields.

You can leave the ID screen by one of two methods. If you have entered all fields, the requested record is automatically displayed when the last field is filled. However, if you want to leave the data entry session or return to the main menu, you must use the menu or a shortcut key to leave the ID screen.

The menu options are:

Search: display a record which most closely matches those fields entered.

Exit: leave the ID screen and return to the Main Menu.

Quit: leave the DMS.

2.2 Add / Browse Menu

When a record is displayed in Add or Browse mode, the top portion of the screen shows the key fields for the record. The cursor is on the first data field of the record. A menu bar fills the first line of the screen. The Add and Browse menus are identical with three exceptions: **Permanently Missing** applies only in Add mode; **Delete** and **Key Field Change** apply only in Browse mode. Some options on the Browse menu may not be highlighted. This means that they are not available. For example if you do not have delete privileges, **Delete** is not highlighted. If you do not have change privileges, **Save** is not highlighted.

Add Menu:

Browse Menu:

2.3 Field, Screen and Form Movement

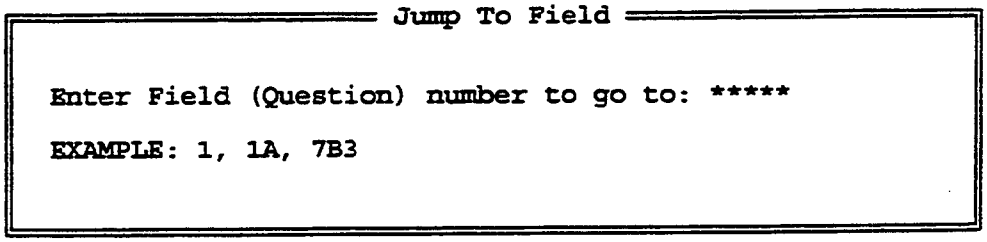
In Add mode you will usually enter fields in sequence. However in both Browse and Add modes you can move through fields, screens and records using the menus or shortcut keys. The Move option of the menu bar lists the available options:

Next Field	TAB
Prev Field	BACKTAB
Next Screen	PGDN
Prev Screen	PGUP
Next Form	CTRL+PGDN
Prev Form	CTRL+PGUP
Jump to Field	CTRL+J
Switch Paths	CTRL+W

Most of the options are self-explanatory. TAB and BACKTAB (SHIFT+TAB) move to the next and previous field respectively. PAGE UP and PAGE DOWN move to the next and previous

screen of a form. CTRL+PAGE UP and CTRL+PAGE DOWN move to the next and previous records in the current search order. If you go past the last record in the current search order the first record will be shown again.

Jump to Field allows you to move to a specific question on the form. Selecting this option brings up a menu in which you enter the question number to which you want to go:



If you enter an invalid number the message 'Field not found' will be displayed in the window. Enter another field or press ESC to return to the data screen.

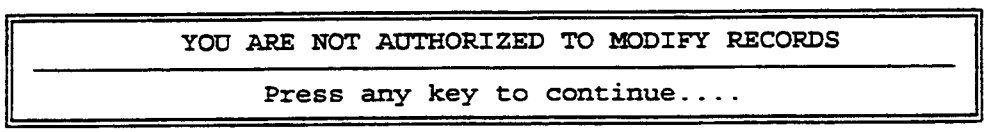
Jump to Field allows you to go to skipped fields, permitting you to view screens which may have been skipped entirely. However you cannot enter values in these skipped fields. **Jump to field** will not let you bypass a **must enter** (mandatory) field. If you enter a field after a must enter field which is blank, the cursor instead stops at the must enter field.

Switch paths allows you to control the order in which records are presented when you select **Next Form** and **Previous Form**. The default order is by ID. With this path, the next record for the current ID is shown when you press **Next Form**. In Form order, the next record for the current form type is shown when you press **Next Form**. Using form order, for example, you could view all EV1 records which have been entered. Selecting **Switch Paths** toggles the path. Note that the path has no effect in Add mode since after saving or canceling a record, the ID screen is always displayed.

2.4 Edits

Each data field that you enter has an associated trio of status bytes which stores additional information about the field, such as whether the field is empty, missing, or contains an out-of-range value. The **Problem** option on the Add and Browse menus gives you a way to provide this additional information.

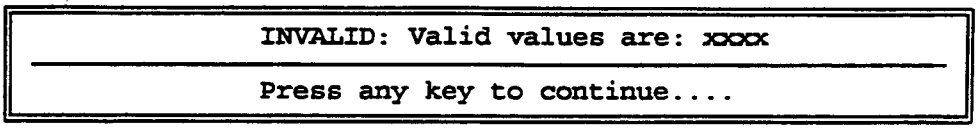
As you enter data values into a record, they are edited. If you do not have modify privileges, you will be alerted:



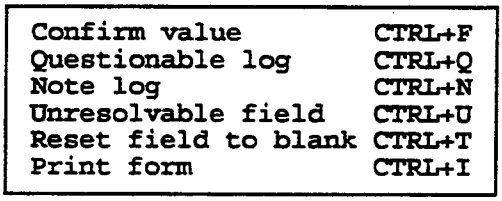
and the field's prior value (blank or otherwise) is restored.

If a value fails an edit, for example if it is out of range or inconsistent with other values, an

error window alerts you and gives the valid range:



Press any key to clear the error and return to the field. If you made a keying mistake, retype the value. However, if the value is correct you must confirm it. Use the Problem menu to do this:

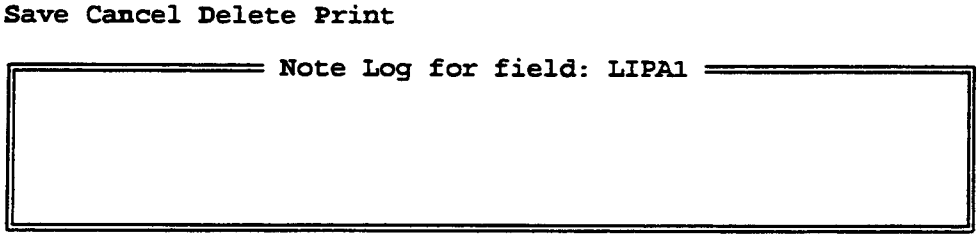


Choose **Confirm** to confirm that an out of range value is accurate by setting the first status byte to 'C'.

Use **Unresolvable** when a value cannot be collected or when the value you did collect is suspicious and should not be used in analysis. **Unresolvable** sets the first status byte to 'U' and, if the field is blank, fills the field with equal signs (==). Note that you can set a field to Unresolvable by keying the equal signs into the field rather than using the Problem menu.

Reset removes the value in the field and sets the first status byte back to 'E' (empty).

Questionable Log and **Note Log** allow you to comment on a field to explain a response or to enter a response that will not fit into the field. Choosing either option opens a window in which you can type comments.



If a note log or questionable log has already been entered for the field, it is shown in the window. You can add to the end of the existing text. The window is empty if no log exists for the field.

There are four options on the menu bar. Choose **Delete** to delete the log displayed. To leave the log window without saving changes, select **Cancel**. Choose **Save** to save changes and return to the record. To print the log, choose **Print**.

Note and questionable logs function similarly but are used in different contexts. Use a questionable log when a value cannot be entered in a field, for example when the field is too

short to hold the value. Use a note log to comment on a value, for example to explain an 'other' response.

Adding a note or questionable log set the third status byte to N for Note logs, Q for questionable logs or B for both.

When you start the DMS, the status bytes appear on the screen. To remove the status bytes from the screen, choose the Display option from the menu. The Display submenu appears, as follows:

Choosé Disp SB. Or use the shortcut key CTRL+B. Once the status bytes are displayed choose the same option to turn off the display.

Only the first status byte of the three bytes associated with a field is displayed beside the field. When the cursor is on the field, all three status bytes appear in the upper right hand corner of the screen. The middle byte indicates the verification status of the field: 'E' (Empty) indicates that the field does not require verification, 'N' (Not Verified) indicates unverified, 'V' (Verified) indicates verified. The last byte indicates whether a note log ('N'), a questionable log ('Q'), both ('B'), or neither ('E' - Empty) exist for the field.

2.5 Skips

Some fields are answered conditionally. That is, a certain response to one field can cause subsequent fields to be unnecessary or irrelevant. In the DMS these fields are skipped. After a response is entered into the trigger field, the cursor skips ahead to the next relevant field. This field might be on the same screen or several screens ahead.

You cannot move to a skipped field using the Next Field or Prev Field keys. The only way to move to a skipped field is by using the Jump to Field option on the Move submenu. Once the cursor is positioned on a skipped field, you cannot enter a value into the field.

The status bytes of skipped fields are changed to indicate the fields were skipped. The status byte values remain the same but are changed from upper to lower case.

2.6 CXI (Inventory) Display

The DMS maintains an inventory of records entered for each participant. This inventory form is called the CXI. It can be displayed from the ID screen or when a data entry record is on the screen.

From the ID screen, to show the CXI choose **CXI** from the menu (ALT+C). If you have entered an ID, the inventory for that participant is shown. If you have not entered an ID, an inventory of the entire database is shown.

To show the CXI for the current participant from a data screen, choose **Display** from the menu. From the submenu select **CXI Display**. Or use the shortcut key CTRL+C.

The CXI Display lists the number of records entered for each form type in the database. Where applicable, a count of unverified records for the form type is also displayed.

2.7 Permanently Missing Forms

If you are unable to collect an entire record of required data for a participant, enter the record into the DMS and set it to permanently missing. This tells the Coordinating Center staff that you will never be able to get the information so they will not ask you about it.

A record can be set to permanently missing only in Add mode. To set a record to permanently missing, choose **Perm. Miss.** from the Add menu. You are prompted to confirm the permanently missing:

```
Are you sure you want to set the form to
permanently missing?
──────────────────────────────────────────
********** Yes **********
           No
```

If you have already entered data into some fields and then decide to set the record to permanently missing, the fields will be blanked. You are prompted to confirm again:

```
Form is not empty.  Do you still want to
set the form to permanently missing?
──────────────────────────────────────────
********** Yes **********
           No
```

When a record is set to permanently missing, the first status bytes for all fields are set to 'M'. When browsing the database and a permanently missing record is shown, a message informs you that the record is permanently missing. You cannot add data to any field.

Note that permanently missing forms must be verified if the form type requires verification. See section 2.13, Verification.

2.8 Delete

To delete a record, select **Delete** from the Browse Menu. There is no shortcut key for delete. You will be prompted to confirm the delete:

```
Are you sure? Y/N
```

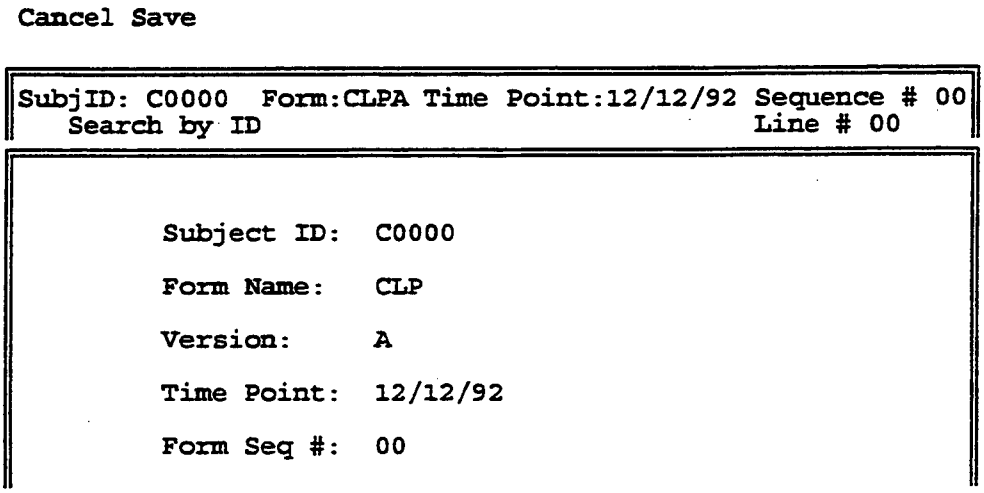
Type 'Y' to delete the record or 'N' to return to the screen. After you delete a record, the ID screen is redisplayed.

If you do not have delete privileges, the Delete option on the menu will not be highlighted. Delete privileges are granted via the system administrator utility.

2.9 Key Field Change

When a record is displayed for modification, most fields can be changed by simply entering a new value. However to change the key fields, the fields which identify the record, you must use **KF Chg** from the menu. With this option you can change the ID, the time point and the sequence number.

A screen similar to the ID screen will be shown:



Type in a new value for the field(s) you want to change.

If there is a record with the new keys in the database, the key field change is not accepted. When you are satisfied with the new values, go to the menu and select **Save**. To cancel the change, go to the menu and select **Cancel**.

The window will close and the record, with the new key fields, will be displayed. The Delete option is no longer highlighted. Deletes are not permitted after a key field change.

If you choose **Cancel** from the Browse menu, the key field changes will be lost.

If you do not have Change privileges **KFChg** on the Browse menu will not be highlighted.

2.10 Print Form

If you want a hard copy of a record, from the Browse or Add menu select **Problem Menu**. Choose **Print Form**. The following messages will be displayed:

```
Formatting printout - EV1A - Form as of 04/07/93 18:00:19
```

```
Printing - EV1A - Form as of 04/07/93 18:00:19
```

The record with the data values you have entered will be printed.

2.11 Help

Help is an option on most of the primary menus in the DMS. It can be selected from the menu or by pressing F1. **Help** from the Add or Browse menu lists a submenu with three options: field, screen or general. Select the type you want. If you choose **General** a list of topics is presented. If you choose **Field** or **Screen** information specific to the current field or screen is presented. Once a screen is shown you can view related topics or select from a list of all help topics.

To return from help, press ESC or select **Exit** from the menu.

2.12 Save and Cancel

A record is automatically saved when you:

are in Add mode and enter the last field on a record; or

use CTRL+PAGE UP or CTRL+PAGE DOWN to go to another record.

In the second case you are prompted:

```
Form was modified.  Do you want to save
the changes?
---------------------------------------
 ********** YES **********
            No
```

in case you made changes inadvertently.

You can also save a record manually by selecting **Save** from the Add or Browse menu. There are some situations in which you must manually save a record:

- When the response to a trigger field causes all remaining fields on a record to be skipped, you get the message

```
Can't move forward from current field
```

because there is no field for the cursor to move to.

- If you are in Add mode and must use an option from the problem menu (such as Confirm or Unresolvable) on the last field of a record, you get the message

```
This is the last screen of the form
```

- If you are in change mode and want to return to the ID screen. Note that if you change the last field of the record in change mode, the record is not automatically saved.

In any of these cases, select **Save** to save the record and return to the ID screen.

Save is not available when you are in Browse Only mode. See section 2.1 for a description of Browse Only mode.

If you have entered incorrect information and want to cancel all changes, choose **Cancel** from the menu. The ID screen will be displayed.

2.13 Verifying Records

Certain forms, e.g. laboratory data, must be **verified** by keying the record a second time.

Use the **CXI Display** (section 2.6 above) to locate unverified records by form type.

CXI_RPT.TXT [Read Only]

CXI DISPLAY

Record Counts for Entire Database
by Form Type

Form	# Records	# Unverified
APEB	2	0
DPOB	1	n/a
EV1B	1	n/a
EV2B	1	n/a
LBAA	0	0
LBBA	1	1

Use cursor keys to scroll. Press ESC to exit

ID Screen

Note that forms with no records (e.g. LBAA) will always show 0 unverified records. If at least one record has been entered, the report will show "n/a" if the record does not require verification (e.g. EV1B), or the actual number of unverified records (e.g. APEB, LBBA).

If you wish to verify all records of a certain form type, you should key only the form type on the ID screen and select **Search** from the menu. If you wish to verify all records for a particular participant, you should key only the sub ect ID and select **Search**. In either case, when asked to choose Change, Verify or Browse mode, select **Verify**. If any matching unverified records exist, the first is displayed for verification; otherwise, the system responds

```
No unverified records exist for [Form/ID] ****
Returning to ID screen
                Press any key to continue....
```

In verify mode, the record is displayed with its data items empty. You must re-key each field on the form in order. As you enter each field, the DMS compares your newly keyed value to the original value; if the values do not match, you are prompted

```
Does not match old value: 1

Accept OLD or NEW or RETRY ? <O/N/R>
```

Choose **Old** if the old value is correct, **New** if the new value is correct, or **Retry** to type in a different value if neither is correct. If you select **New**, your new value will be edited (ust as values are in Add or Change mode) and you may see an edit failure message. As in Add or Change mode, select **Confirm** from the Problem menu if your new value is indeed correct.

As you verify each field, the second character of its status byte vector will change from 'N' (Not verified) to 'V' (Verified). Any fields which do not require verification, e.g. "Comments? [Y/N]" fields, have 'E' (Empty) in this status byte position and will be skipped automatically in verify mode. Once you have verified the last field of the record, you will see the message

```
SubjID: C20000   Form:  APEB   T | Can't move forward from current field |
Verifying by ID             Veri |                                        |
```

At this point, use the **Save** menu option to save the record, or use CTRL+PAGE DOWN to save the record and move to the next unverified record for the current form type or sub ect ID. Once you have verified all records for the specified form type or sub ect ID, the system will respond

```
No more unverified records exist for [Form/ID] *****
Returning to ID Screen
                Press any key to continue....
```

If a permanently missing record requires verification, it will be displayed in turn as you navigate through the unverified records using CTRL+PAGE DOWN. To verify these records, select the option **Verify permanently missing form** from the **Problem** menu.

A record is fully verified once all of its fields have been verified. Typically, a record which

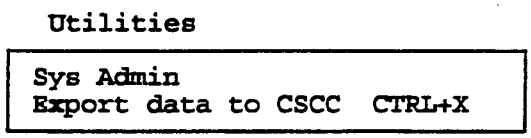
requires verification is excluded from analysis until it has been fully verified. Thus, if you save a record before verifying all of its fields, you must return to the record in verify mode and verif the remaining fields before the entire record is considered verified.

In DELTA, some laboratory records end with a number of empty fields. Normally, each of these empty fields would have to be verified by pressing ENTER for each field. To simplify verification of these records, we have included a feature in the **Problem** menu called **Set Rest of form to unresolvable** (shortcut key CTRL+R). After verifying the record's last field containing data, select this feature to set all remaining empty fields to verified unresolvable. Note that the "Comments? [Y/N]" field is not affected by this feature in Verify mode since it does not require verification.

Once a record has been fully verified, you may need to change the values in a few fields. After changing a field's values, those fields and the entire record become unverified. If you must change a large number of values, you may find it easier to change all necessary values and then revisit the records in Verify mode to verify those changed fields. If you are only changing a few values, you may verify each field individually while in Change mode. After changing the field, move the cursor back to the field and select **Verify Field** (shortcut CTRL+V) from the **Problem** menu. Then, re-key the field's value. As in verify mode, if the values do not match you will be prompted to select the old value, the new value, or to re-key. Once the value has been verified (by selecting Old or New), the system will once again behave as it normally does in Change mode.

3 Utilities

The utilities are programs separate from the data entry functions of the DMS but they affect how it runs or which act on the data entered using the DMS. The utilities are run by selecting **Utilities** from the main menu. A submenu lists the available options:

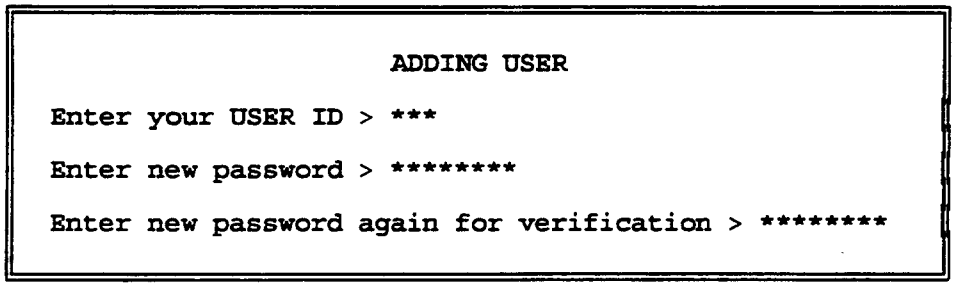


3.1 System Administration

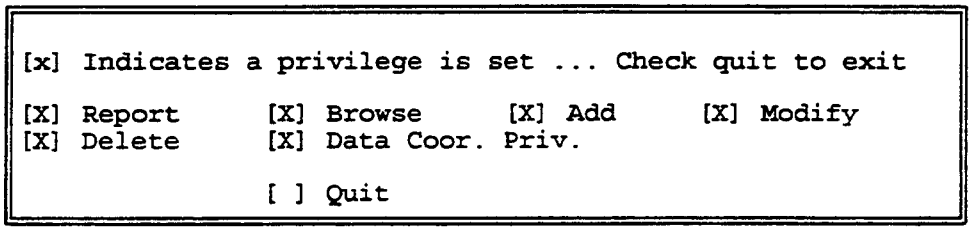
To use the DMS you must log in using an ID and password. You use the password utility to assign IDs, passwords and privileges to users. Generally this system administration task is assigned to a single person called the data coordinator.

To run the password utility. choose **Sys Admin** from the Utility submenu. Another submenu is shown giving four choices:

To add new IDs and passwords, choose **Add User**:



Enter the user's login ID and password. Enter the password again for verification. Choose which privileges the user is allowed:



Use the TAB key to navigate through the options. Use the Space Bar add or remove an 'X' in the box. An 'X' in the box allows a privileges.

This screen is also used when you select 'Set Privileges' to change what a user is allowed to do in the DMS.

You can remove users from the system with **Delete User** and can change a user's password with **Change Passwords**.

There is a hierarchy of IDs which determines who is allowed to add and modify users and privileges. The Coordinating Center is on the first level, the data coordinator is on the second level and all other users are on the third level. A user can modify user IDs which are on a higher level. Thus the Coordinating Center can modify all users and the data coordinator can modify users on the third level. Users on the third level can only change their passwords.

The DMS is shipped with a default Data Coordinator ID and password. You can add your own data coordinator ID by creating a new ID and assigning that ID data coordinator privileges.

3.2 Export and Tape Backup

Once every two weeks data entered at the field centers will be sent to the Coordinating Center on diskette. The first step of the export is a backup to tape and the second is the creation of files to be sent to the CSCC. You will need one of the two backup tapes for the backup and a blank, formatted high density 3.5 inch diskette for the export.

To start the export, choose **Export Files to CSCC** from the **Utilities** menu.

The tape backup alternately uses one of the two backup tapes each week. As the first step in the export process you will be asked to insert the correct tape:

```
    Tape Backup of DMS Files
 Please insert backup tape #1, then press ENTER
 (or press ESC to abort export)
```

The system checks to make sure you have inserted the correct tape:

```
Checking name of tape in tape drive.  Please wait...
```

If the tape has not been formatted or prepared for use by the DMS, you will be notified and the backup will not continue. See section 6 for instructions on formatting and preparing tapes for use.

If you have inserted the wrong tape, you are notified and returned to the main menu. If the correct tape is in, the backup to tape proceeds. The files are first compressed and the compressed file is put on the tape.

After the tape backup, the export begins. You will be asked to insert a blank diskette in drive B for the transfer files. Use a formatted 3.5 inch high density diskette. Press a key when you have done so.

Two reports are produced. One lists the key fields of each record exported and is produced separately for each form type. The second is a summary which gives the number of adds, changes and deletes for each form type. You are asked whether you want to print the summary report. These reports are also copied onto the diskette.

The export files are compressed into a single file which is copied onto the diskette. The export file is named X000000n.zip, where X is the field center initial and n is the export file number. A message tells you the name of the file produced.

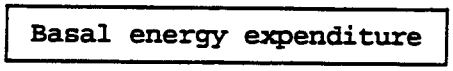
Please label the transfer diskette with this file name using the following format:

DELTA Export
Data Transfer
File Number X000000n
Date:

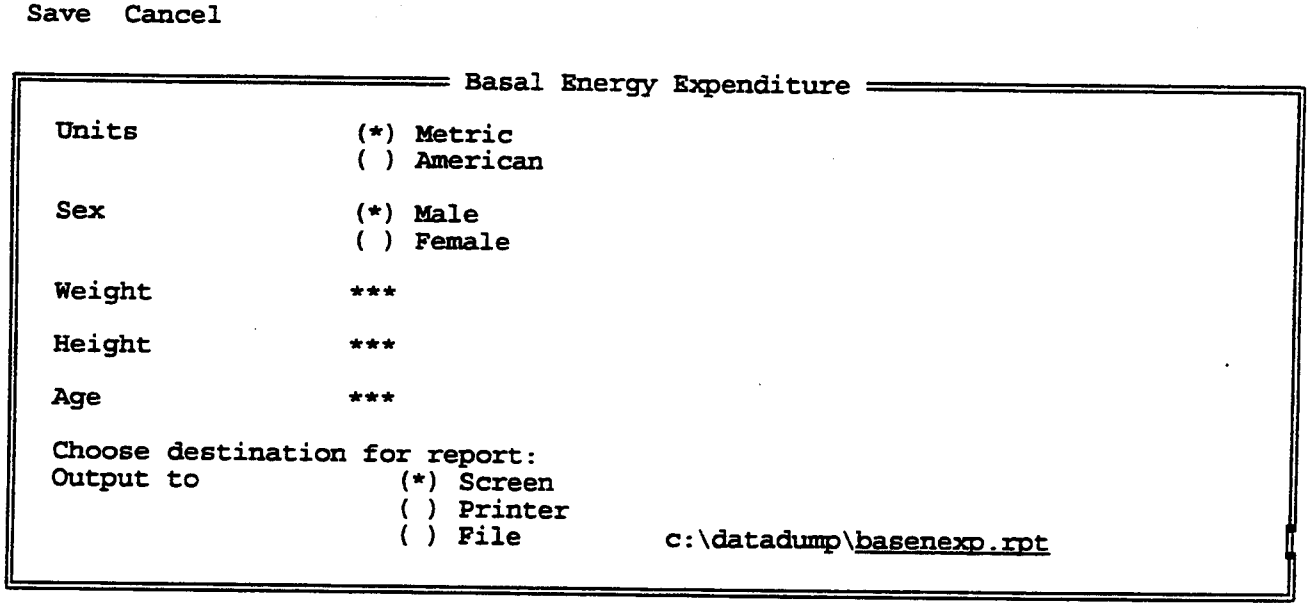
NOTE: If you get a fatal error during export, restart the export process using blank diskettes. You can later reformat any diskettes written to during the failed export process.

4 Reports

From the Main Menu choose **Reports** to display a list of reports available in the DMS:



Most reports require information such as sub ect ID, a date range and an output destination. The information screen is similar for all reports:



A single option can be selected from vertical lists (those preceded by parentheses ( )). To select an option, use the TAB or cursor control arrow keys to move to the desired option. Press the Space Bar or ENTER to select the option. This put a dot in the parentheses.

From horizontal lists (none are shown on above screen but would be preceded by brackets [ ]) multiple options can be chosen. Move to the desired options an use the Space Bar or ENTER key to select. This puts an X in the brackets.

Some options require additional input, like a file name or date range. The cursor will move to the required field automatically.

If you choose 'file' as an output destination, you must enter a file name. Notice that the file name is preceded by c:\datadump\, which means the file will be written to drive C:, directory \DATADUMP. If you choose printer, make sure the printer is turned on.

From this screen you can choose the **Save** or **Cancel** menu options displayed at the top of the screen to produce the report or cancel and return to the Main Menu.

5 Desktop

On the Password Screen Menu is a Desktop option which contains some useful and fun programs which are not really part of the DMS but which might be useful. The Desktop submenu has these options:

Toggle Time	CTRL+T
Calendar/Diary	CTRL+D
Calculator	CTRL+C
Color Picker	CTRL+P
ASCII Chart	CTRL+A
Puzzle	CTRL+Z

Color picker is the only one which has an effect on the DMS. The others are completely separate from the DMS.

5.1 Color Picker

The DMS comes with preset colors. However you can modify the colors using the color picker. The process is complicated so if you want to do this, please contact the Coordinating Center for help.

5.2 Time, Calendar/Diary, Calculator, ASCII chart, Puzzle

Time toggles off and on the display of the current time in the upper right hand corner of the screen.

The calendar/diary presents a calendar of the current month and lets you record notes associated with a particular day:

Calendar/Diary
December 1992

Su	Mo	Tu	We	Th	Fr	Sa
		1	2	3	4	5
6	7	8	9	10	11	12
13	14	15	16	17	18	19
20	21	22	23	24	25	26
27	28	29	30	31		

< - M > < M > < - Y > < Y -

The calculator displays calculator which performs simple arithmetic. Use the mouse to 'press' the keys and compute sums, differences, etc.

The ASCII chart shows the ASCII characters for keyboard keys.

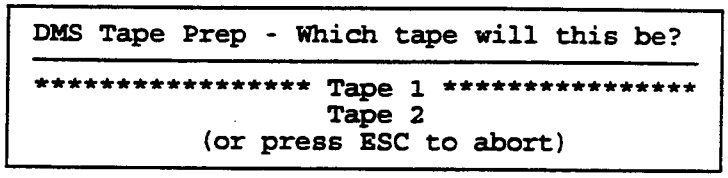
The puzzle is a game in which you try to arrange the numbered blocks in ascending order by sliding a block into the open hole. Use the mouse to select and move a block.

6 Backup Tape Preparation

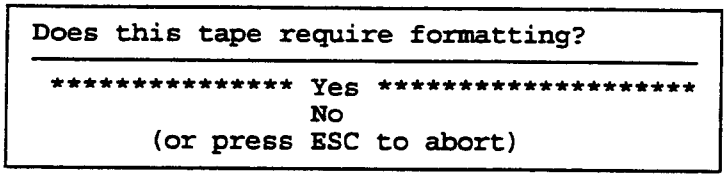
Before tapes can be used to backup files they must be formatted and prepared for use by the DMS.

From the main menu select **Utilities** and from the **Utilities** pulldown menu select **Prepare a tape for DMS**:

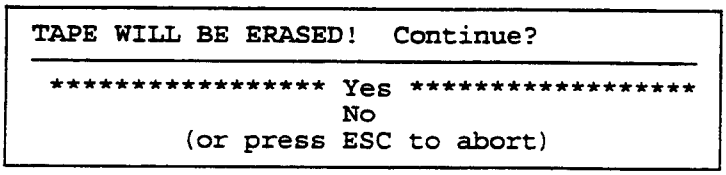
You will be asked which tape you are preparing. Remember that the DELTA DMS uses a cycle of two tapes, each one on alternate weeks.



Select which tape you are preparing. You will then be asked whether you want to format the tape. Generally only new tapes require formatting. However old tapes may be formatted to remove existing information.



If you respond Yes, you will be warned:



You get the message:

```
Formatting Tape (This will take about 1 hour.)
   Tape format started at 3:23 pm, Monday, August 2, 1993
```

Formatting takes about one hour during which time you cannot do anything else on the pc. After the format is complete you will see the messages:

```
Preparing TAPE2 for DELTA DMS use. Please wait...
```

Old tapes have already been formatted and only require preparation for DMS use. Respond **No** when asked if the tape need formatting. You will get the messages:

```
Erasing tape (this will take about 2 minutes.)
     Tape erase started at 12:51 pm, Monday, August 2, 1993


Creating first volume on tape (this will take about 2 minutes.)
     Tape backup started at 12:52 pm, Monday, August 2, 1993
```

When these processes are finished, the main menu will be displayed. The tape is now ready for use. For instructions on using the tapes see section 3.2 Export.

7 Remote User Service (via pc-Anywhere)

Sometimes the CSCC must call the field center computers to diagnose or repair a problem. To allow the CSCC computer to control the field center computer we use a product called pc-Anywhere. To use pc-Anywhere, select F4: Run Remote Software from the DELTA menu.

The first screen gives a menu with several options. Select the first Begin Host Operation. Press ENTER.

The second screen gives another menu from which you select the first Wait For A Connection. Press ENTER.

The pc-Anywhere Connection status screen appears with the message

```
Initializing modem...
```

and then with the message

```
Waiting for connection...
```

The CSCC can then call your pc to fix problems.

While we are working do not type anything unless instructed to do so.

When we have finished with the remote service session, we will reboot your computer. The reboot process will return you machine to its state prior to running pc-Anywhere.

8 E-mail

The DELTA sites are communicating with each other and with the Coordinating Center using an e-mail package called DaVinci mail. Please refer to the software user guide for assistance.

9 WordPerfect 6.0 for DOS

To use WordPerfect, type DELTA from the C:\> prompt. Then, press F2 to run WordPerfect.

Appendix A Keys Used in the Data Management System

System Wide	
Help	F1
Dup Buffer	F2
Display List	F3

Password Screen	
Desktop	ALT + K
Time	CTRL + T
Diary	CTRL + D
Calculator	CTRL + C
Color Picker	CTRL + P
ASCII Chart	CTRL + A
Puzzle	CTRL + Z
Quit	ALT + Q

Main Menu	
Data Entry	ALT + D
Utilities	ALT + U
Reports	ALT + R
Exit	ALT + X
Quit	ALT + Q

ID Screen	
CXI Display	ALT + C
Search	ALT + S
Exit	ALT + X
Quit	ALT + Q

Browse/Add	
Move	ALT + M
Next Field	TAB (or UPARROW)
Prev Field	SHIFT + TAB (DOWN)
Next Screen	PAGE DOWN
Prev Screen	PAGE UP
Next Record	CTRL + PAGE DOWN
Prev Record	CTRL + PAGE UP
Next Line	CTRL + RIGHTARROW
Prev Line	CTRL + LEFTARROW
Jump to Field	CTRL + J
Switch Paths	CTRL + W
Problem	ALT + R
Confirm	CTRL + F
Quest. Log	CTRL + Q
Note Log	**CTRL + N**
Unresolvable	CTRL + U
Verify perm mis	CTRL + O
Set rest to unres	CTRL + R
Verify field	CTRL + V
Reset to blank	CTRL + T
Print form	CTRL + I
Display	ALT + I
Status Bytes	CTRL + B
CXI Display	CTRL + C
Save	ALT + S
Cancel	**ALT + N**
Delete	------
Perm. Missing	ALT + P
KF Change	ALT + K

Dietary Effects on Lipoproteins and
Thrombogenic Activity

Publications and Presentations Policy

(Adopted by the DELTA Steering committee: 9-13-94)

Table of Contents

I. Policy Objectives

The objectives of the editorial policy for publications, abstracts and presentations are:

A. To assure and expedite orderly and timely presentation to the scientific community of all pertinent data resulting from DELTA;

B. To have accurate and scientifically sound abstracts, presentations and papers from DELTA and its collaborating investigators;

C. To assure that all investigators, collaborators and other health professionals, have the opportunity to participate and be recognized in study-wide presentations and the preparation of DELTA papers;

D. To assure that abstracts, press releases, interviews, presentations and publications are accurate and objective, and do not compromise the collaborative trial and the acceptance of its results;

E. To insure that it is the responsibility of all authors for the contents of all abstracts, presentations and papers.

II. Definitions

A. Primary End Point Publications, Abstracts and Presentations = Category 1

Primary End Point publications, abstracts and presentations are those reporting results dealing with the main hypotheses of the randomized controlled feeding trial.

B. Secondary Publications, Abstracts and Presentations = Category 2 (Hypotheses, Food Assay Data, Design of the Diets, and Recruitment)

Category 2 publications, abstracts and presentations are all others relating to the above topics, reporting overall results from the national collaborative feeding trial and its overall common data set.

C. Other Hypotheses and Methodological Publications, Abstracts and Presentations = Category 3

Other publications, abstracts and presentations are those not encompassed by the above two categories; but use data from the study database maintained at the Coordinating Center. This category also includes single center and ancillary studies. The Coordinating Center will not provide data entry systems for data collected for ancillary studies, nor does it expect to participate in the analysis of ancillary study data.

III. Statement of Policy

To minimize the possibility that published materials may be based on faulty data or that flawed conclusions are drawn from the data, it is the policy of the DELTA Publications and Presentations Committee (the P&P Committee) that all definitions, criteria, and data criteria used in all Category 1 and 2 DELTA papers be submitted to the P&P Committee including review by the Coordinating Center and the NHLBI Program Administrator, to verify that they are accurate and consistent with those used in other DELTA documents and papers.

A. Primary Endpoint and Priority Hypotheses Papers and Presentations (Category 1 & 2)

1. Category 1 and Category 2 papers and presentations of DELTA are to be identified and prioritized by the P&P Committee based on suggestions from staff members of any participating center. For each report, a writing group leader and a writing group (from among the professional staff of all centers) is to be appointed to prepare the paper within a stated time limit. The P&P Committee will periodically review the work of all writing groups; aiding and encouraging them as appropriate; revising their membership or reconstituting them when indicated (with written notification and right of appeal).

2. It is the intent that selection of writing group members be equitable and fair to all groups and individuals participating in this collaborative program, including encouragement of participation by younger professionals and students.

3. There will be multiple authors on all Category 1 papers; with authors designated as writing for the Steering Committee of the DELTA Study. Any PI not listed as an author will be listed in the credits. The author list of all Category 1 papers will include a representative from each center. Each abstract will list a smaller number of authors and full authorship will be noted on the final publication or presentation.

4. For Category 2 papers and presentations, names of members of the writing group are to be listed as authors in behalf of the DELTA Steering Committee. The leader of the writing group, with the concurrence of its other members, is to determine the order of authorship. A major criterion for this determination is to be the effort and contribution made by members of the writing group in preparation of the report.

5. The central role in coordinating the statistical analyses of Category 1 and Category 2 papers will be the responsibility of the DELTA Coordinating Center. The final analyses and data verification of all Category 1 and 2 papers will be completed at the Coordinating Center. Closed data files will be provided to the field centers after the final results papers have

been written. (See Appendix A for additional information related to collaborative data analysis.)

6. A credit list **of all** major committees, units, and centers, with their members, is to appear in each Category 1 and Category 2 paper, printed as a footnote at the end of the article. This credit list will be prepared by the DELTA Coordinating Center and will be updated periodically to account for staff changes. The Principal Investigators are responsible for the make-up of the list at each site.

7. All requests for reprints of Category 1 and Category 2 papers are to be directed to the DELTA Coordinating Center.

8. DELTA as a whole needs to keep track of all contributions and come up with a listing and an acknowledgment.

B. Other Papers and Presentations (Category 3)

1. Papers and presentations being developed based on special data sets collected on DELTA participants by centers involved in substudies or ancillary studies are to be identified by the P&P Committee from suggestions from the individual center. In general, the writing group to prepare such a report is to consist of individuals designated by the participating center(s). The authorship of such a report is to be designated in the usual manner for a scientific report with the order of names appearing after the title to be decided upon by the participating center(s). The P&P Committee can act as a referee, if requested, to help resolve the order of names of authors. In addition to a statement of authorship, such a paper is to have a clear statement that this work is a substudy or ancillary study of DELTA and the support from NHLBI is to be acknowledged.

2. At the end of the list of authors of the paper an asterisk is to appear, for a footnote designating that this work was performed as part of DELTA, as a substudy, an ancillary study, or an analysis of local data. Where appropriate, a listing of participating centers and investigators who are not authors is to be included. This decision is to be made by the participating centers and is to be refereed by the P&P Committee.

3. DELTA centers are encouraged to write papers on local data and experience. A local paper dealing with a matter of a secondary paper should be prepared only after the parallel secondary paper, based on multicenter data, has been published or has been officially accepted for publication. The authorship of a local paper is to be decided at the discretion of the Principal Investigator of the respective center.

4. All substudy, ancillary study, and local manuscripts must be reviewed and approved by the P&P Committee and reviewed by the NHLBI Program

Administrator before submission for publication. On the front page of the article, the manuscripts should give a clear reference to DELTA and the collaborative nature of the Program, including the grant number with the NHLBI.

5. All requests for reprints of publications related to ancillary and local studies are to be directed to the Principal Investigator of the field center or her/his designee.

C. Procedures for Identification of Category 1 and Category 2 Papers

1. At periodic intervals, the P&P Committee is to distribute to all DELTA centers a listing of titles and publication status of all DELTA-related publications.

2. Additional Category 1 and Category 2 papers are to be identified by the P&P Committee from proposals by all DELTA staff.

D. Identification of Writing Groups, Selection of Writing Group Leaders, and Work of Writing Groups

1. As soon as a Category 1 or Category 2 paper has been identified and approved by the P&P Committee, the committee will also identify a writing group leader. The leader is to communicate with all centers to determine those qualified to participate as members of a writing group for that paper.

2. The P&P Committee is to select, from the submitted list of nominees, the membership of the writing group for that paper. (See Appendix B for specific membership lists.)

3. The following steps should be followed in the preparation of the manuscript.

   a. Contact each writing group member to consider the specific charge of the P&P Committee, and to

   b. Prepare a comprehensive outline of the manuscript.

   c. Draft, in conjunction with the Coordinating Center, dummy tables which members consider appropriate for writing the manuscript.

   d. All comments with respect to the charge of the P&P Committee and copies of drafted dummy tables should be sent to the leader of the writing group.

   e. The leader is to collate comments and dummy tables, solicit opinions of the writing group members, and when a final decision

is reached, is to submit the dummy tables (or data requests) to the Coordinating Center with copies to the Chairperson of the P&P Committee.

4. The leader of the writing group should send copies of all correspondence, including dummy tables, to the Coordinating Center and to the Chairperson of the P&P Committee. It is the responsibility of the Coordinating Center to identify areas of overlap of proposed dummy tables or data requests between writing groups, as well as to provide writing groups with relevant dummy tables requested by other writing groups. If a problem of overlap cannot be resolved by the leaders of the respective writing groups, the issue is to be resolved by the P&P Committee as the final arbitrator.

5. Members of each DELTA writing group should participate actively in the writing of the paper assigned to that group. The input from every member of the writing group is essential. The leader has the responsibility to obtain input from every member of his/her group. If any member of the writing group does not respond to the leader's request or **does not contribute** to the writing of the paper, the leader must take immediate action, through the P&P Committee, to replace that individual. The writing group member has the right to receive written notice of this action and to appeal to the P&P Committee.

   It is the responsibility of the leader of the writing group to approve the final version of the paper before its submission to the P&P Committee. <u>All members of the writing group should have seen the final draft before its submission to the P&P Committee and the NHLBI Program Administrator</u>.

6. If in the judgement of the P&P Committee, a writing group is not working well and there is an unjustifiable delay in writing the paper assigned to it, the P&P Committee is empowered to change either the leader or the entire membership, to expedite the writing of that particular paper. Affected members of the writing group are to be informed in writing of this action and have the right of appeal to the Steering Committee.

E. Preparation and Submission of Papers for Publication

1. Prior to submission for publication, a copy of the complete paper, in final form, is to be submitted to the P&P Committee for review and approval.

2. Clearance and approval is required for all DELTA publications prior to their submission for publication. Clearance is defined as all members of the P&P Committee having received a copy of the paper, and approval is defined as a positive vote of acceptance from a simple majority of the

Committee members. Included here are all Category 1, Category 2, and 3 papers.

3. All review and clearance functions of the P&P Committee and the NHLBI Program Administrator are to be done judiciously and expeditiously and solely to help fulfill the Policy Objectives set forth, and are in no sense censorship functions.

4. All ancillary and local publications are to include an acknowledgement substantially as follows:

The research upon which this publication is based was performed pursuant to grant 1-U01-HL49649-01 with the National Institutes of Health, Department of Health and Human Services and support from. . .

F. Clearance of Abstracts and Presentations

1. The DELTA Coordinating Center, in conjunction with the P&P Committee, is to maintain a current list of all relevant meetings and their deadlines for submission of abstracts. Abstracts of papers for presentations are to be prepared only if the paper on the same issue is in preparation.

2. The P&P Committee should approve the abstract topic and focus prior to preparation. Author/Authors should notify the Coordinating. Center and Publications Committee of plans for abstract submission and include in the information:

   a. Name and date of meeting
   b. Authors
   c. Topic
   d. Nature of data needed and analyses

3. All such publications, abstracts and presentations must be approved by the P&P Committee before they are submitted to any national and/or international organizations. This should be done by a sending copy at least 3 days prior to submission. Comments on the abstract/presentation should be sent to the lead author and chairperson of the P&P Committee. A revised abstract should be circulated for final approval. Approval can be done by fax or conference call. The chairperson has the authority to deny submission of the abstract/presentation if it is judged to be problematic. In the absence of the chairperson, the Principal Investigator of the Coordinating Center will make this decision.

   If the abstract is accepted for presentation or publication, the P&P Committee and *the* Coordinating Center are to be notified. The text or slides of the presentation must be submitted for approval by the P&P Committee prior to the presentation.

It is permissible to submit previously cleared abstracts to other meetings; copies should be sent to the Coordinating Center for inclusion in the listings of publications and presentations.

4. In the case of all DELTA papers, a member of the writing group responsible for that paper, as selected by the writing group, is to be responsible for the presentation of the paper on behalf of the authors (to be determined) and the DELTA Research Group.

5. Once a paper has been presented at a scientific meeting, the tables used should be available to DELTA professional staff and can be used by them at other scientific meetings. However, such subsequent presentations may not appear in any published form unless and until the data in the original paper are published.

6. In the case of papers scheduled for presentation to organizations issuing press releases, the presenter may submit the text of the presentation after it has been approved by the P&P Committee and the NHLBI Program Administrator for release to the press. If the presentation is based on a manuscript not yet accepted for publication in a peer review journal, a sentence must be included on the front page indicating the preliminary nature of the results.

G. Preparation and Submission of Abstracts

1. All abstracts must be approved by the Committee before they are submitted to any national and/or international organizations.

2. The P&P Committee is to be responsible for assuring that all abstracts are also submitted in timely fashion to the NHLBI Program Administrator for review.

3. The Coordinating Center shall receive copies of abstracts, tables, charts, and figures from the presenter and shall then reproduce and distribute them to other interested DELTA investigators. Copies of slides distributed to field center Principal Investigators and the Program Administrator shall be limited to Category 1 and 2 presentations. All Category 1 and 2 slides shall conform to a standard format with respect to use of the logo and the DELTA study name.

5. The leader of a writing group for a paper based on an ancillary or local study to be presented is responsible for the submission of the complete text and visual aids (including tables and graphs) of his/her presentation to the Publications and Presentations Committee for review and approval by the P&P Committee prior to the date of that particular presentation. Unless the complete text and visual aids of a particular paper are approved by the Publications and Presentations Committee, it shall not be presented even though its abstract may have been approved for presentation.

H. Invitations to DELTA for Presentation of Papers

The DELTA Research Group welcomes opportunities to participate and present reports at national and international scientific meetings. When an invitation is received by a member of the DELTA Research Group, DELTA policies with regard to abstracts, presentations (and publications) are to be followed.

1. Any published DELTA data may be presented without the approval of the P&P Committee. When a personal invitation to an investigator to make a presentation of unpublished results is received, notification of this invitation is to be sent to the P&P Committee. Such presentations should include acknowledgement of all collaborating DELTA units. The P&P Committee and the Coordinating Center will keep an up-to-date comprehensive list of all publications on DELTA.

2. All presentations in response to such invitations are to be based on previously published DELTA reports unless approved beforehand by the P&P Committee. The text of any presentation of unpublished DELTA data must be reviewed and approved by the P&P Committee prior to the date of presentation.

3. When an invitation involves more than one investigator, or if it comes to the Chairperson of the Steering Committee or the Chairperson of the P&P Committee requesting a representative of DELTA, then the Publications and Presentations Committee is to be notified in order to decide who is to represent the study.

4. Requests received by Principal Investigators or their staff to present or discuss at local meetings any previously published DELTA data need IIO prior clearance by the P&P Committee and should be encouraged to accept such invitations. It is requested that Principal Investigators receiving such requests notify the Publications and Presentations Committee so that the P&P Committee can keep records of these presentations. Any publication of such presentations must be approved by the P&P Committee before publication.

I. Use of DELTA Data for Theses by Graduate Student

1. All requests for use of DELTA data by students are to be reviewed by the P&P Committee.

2. It is assumed that the student requesting use of DELTA data is associated with the Program through the DELTA investigator who is acting as the student's "sponsor" with regard to the data.

3. DELTA data may not be used by students if the data relate to a DELTA Category 1 or 2 paper in progress or if the P&P Committee deem the data necessary for a future Category 1 or 2 paper.

4. If the P&P Committee recommends approval of the use of the requested data, a writing group is to be established and is to have the student as leader.

5. The writing group is to take no action regarding the paper until the student has completed and defended the thesis provided this occurs in a reasonable length of time. The student's sponsor is to report the student's progress to the P&P Committee at least annually.

6. The following must be included in the completed thesis:

   a. A statement acknowledging DELTA for use of the data, and

   b. A statement indicating that opinions, ideas, and interpretations included in the thesis are those of the student alone and not those of the DELTA investigators or the NHLBI Program Administrator.

7. When the thesis has been completed as determined by the sponsor, the entire writing group is to proceed to prepare the paper(s) for publication.

8. The standard DELTA publication policy is to apply to any material published from the thesis.

9. DELTA reserves the right to proceed with preparing a paper for publication on the thesis topic if, in the view of the P&P Committee and the student's sponsor, the student has not made reasonable progress in completing the thesis.

SAMPLE

DELTA MANUSCRIPT PROPOSAL FORM

For Administrative Use

Manuscript #: 109 Date Rec'd: 06 24 94 (mm dd yy) Date Approved: 07 07 94 (mm dd yy)

Category #: 3 Priority #: ☐

1. **Title:** Menu Validation in the DELTA Study: The Use of Total Nutrient Weights to Determine Sources of Variances and Errors

2. **Writing Group (List person with lead responsibility first):**

Stewart, Kent K. _____ Dennis, Barbara H.
Phillips, Katherine M. _____ _____
Champagne, Catherine M. _____ _____

3. **Timeline (List month and year):**

Date of initiation 07 / 94

Date of projected completion 01 / 95

4. **Rationale:** Given the costs of menu development and validation, it is important to understand the sources of variance and error in menu composition so that the appropriate actions may be taken to improve the feeding study.

5. **Main Hypothesis:** The use of total nutrient weights per menu (e.g. total grams of fat per menu) can be useful in assessing the sources of variance and error in the menu validation.

6. **Data (variables, source, inclusions/exclusions):**
Calculations of menu composition and FALCC assay values for menu contents.

Appendix A

Coordinating Center Plan for Collaborative Data Analysis

Definition: data analysis - Any extraction of information from the collaborative study database.

Purpose: To respond to data analysis needs for both manuscript preparation efforts as well as interim data analysis needs such as protocol planning and monitoring efforts.

1. Interim data analysis

Analyses required for protocol planning and monitoring: Requests for data analyses will emanate from the DELTA Subcommittees (Diet, Lab, Protocol, etc.) When particular information needs to be obtained from the collaborative database, the Subcommittee's chair will be responsible for appointing a short term task-team of 2, 3, or 4 persons charged with obtaining that information. The Subcommittee may have multiple task-teams acting to meet multiple specific needs for information. Each task-team will be responsible for formulating an analysis plan and for reporting the results to the originating subcommittee. In order to work quickly and effectively, the task-team must remain small (2-4 persons). The task-team can be small because the Subcommittee, with its broader spectrum of expertise, retains oversight. In this way, expertise among the DELTA investigators can be fully utilized while maintaining efficient working group size. The assignment of persons to task-teams will vary depending on availability and expertise needs. However, one of the members of the task-team must be a statistician from our Coordinating Center (either Dr. Paul Stewart or Ms. Nancy Anderson). This linkage is necessary for efficient use of the collaborative database and the statistical computing unit provided by the coordinating center.

2. Analyses required for publications and presentations

Primary results papers: The primary results papers for the various protocols will be identified by the Steering Committee. For each of these papers, the Publications Subcommittee will designate a small writing group. For example, a representative from each Field Center, the Coordinating Center and the Program Office. The writing group would not necessarily be the authors. In any case, the writing group would be responsible for formulating an analysis plan, for interpreting the results, and for writing the first draft of the paper. Authorship on the papers will be decided according to policies established for the DELTA study. All analyses for the main results papers would be carried out by the coordinating center under the supervision of the chief statistician (Dr. Paul Stewart.)

Other papers and presentations. Writing groups for other papers and presentations will similarly be responsible for formulating analysis plans, interpreting results, and writing the papers. Proposals for such papers and presentations will be submitted to the Publications Committee in a standard format that includes a description of the analysis, hypotheses, data that will be used, and proposed writing group. (A completed sample form is appended.) The

Publications Subcommittee may suggest additional writing group members if essential expertise is not represented. Provision of resources for statistical computation and analysis will vary. The Coordinating Center will make collaborative data sets available to the field centers as they are completed. "Complete" means the Coordinating Center has received all the data it expects from the field centers and all queries have been resolved. Any DELTA investigator may use these data sets as well as Coordinating Center resources to generate analyses, but abstracts, presentations and publications are subject to the review and approval policies of the DELTA Steering Committee. Data analysis for ancillary studies will be the responsibility of the principal investigator. The Coordinating Center does not expect to provide data entry systems for ancillary studies nor does it expect to participate in the analysis of such data.

General procedures: All papers and presentations will follow these steps:

Step 1. Topic is identified. either by Steering Committee or by an individual investigator.

Step 2. The Publications Subcommittee approves the proposal and assigns it a category (i.e., main results, secondary, presentation) and a priority. The priorities will determine the order in which analyses are carried out in the Coordinating Center.

Step 3. Writing groups working with a Coordinating Center statistician work as a team to finalize and submit request(s) for statistical computing.

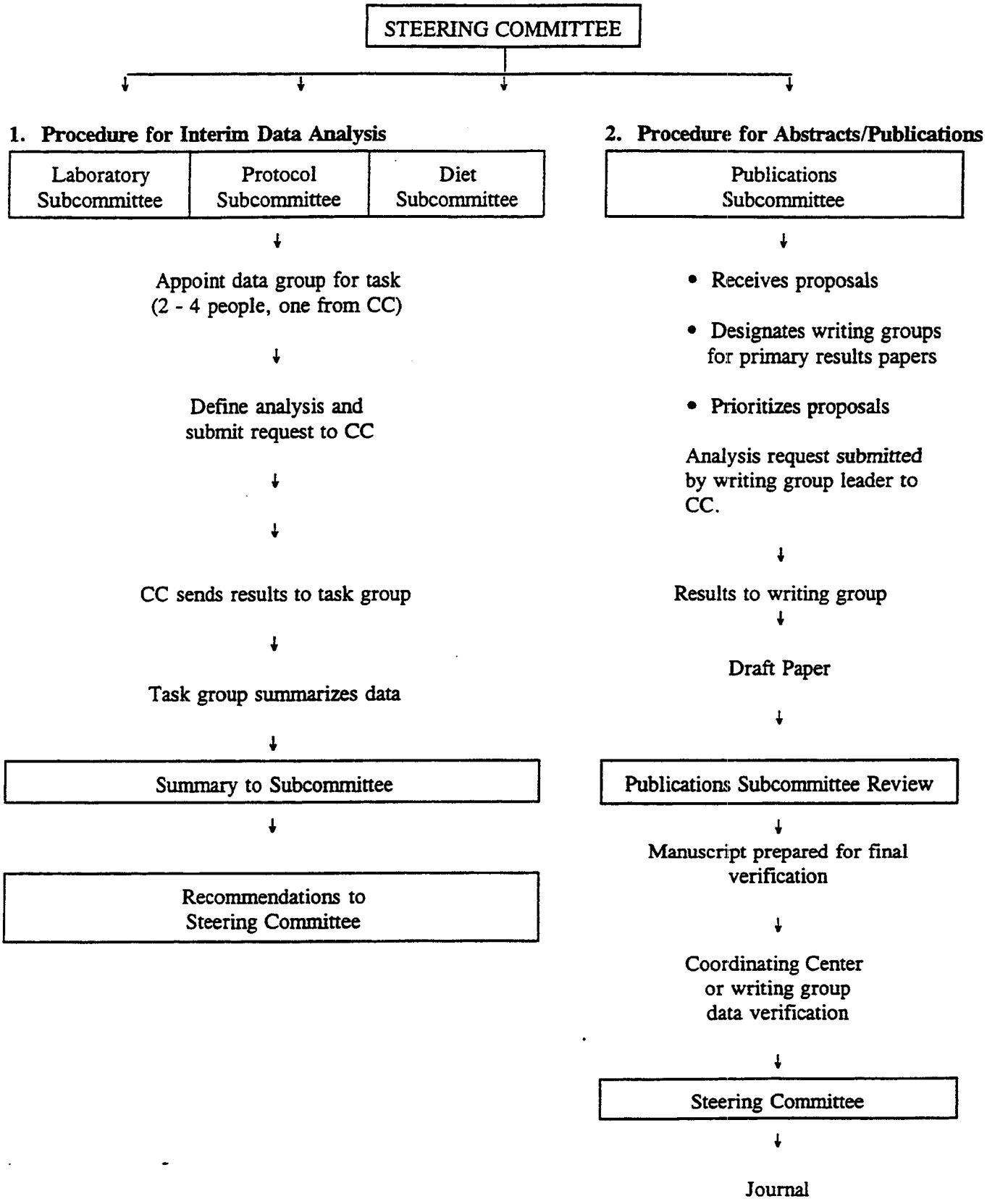
Step 4. Completed draft of paper is sent to the Publications Subcommittee. Paper receives scientific and statistical review.

Step 5. The paper is revised subject to additional analyses as necessary.

Step 6. All data reported in the final draft of primary results papers will be verified by the coordinating center, prior to submission of the manuscript to a journal.

Step 7. Steering Committee approves manuscript for submission to journal.

Step 8. The corresponding author will be responsible for communicating with the journal, checking galley proofs, obtaining author's signatures.


STEERING COMMITTEE
1. Procedure for Interim Data Analysis
Laboratory Subcommittee
Protocol Subcommittee
Diet Subcommittee
Appoint data group for task (2 - 4 people, one from CC)
Define analysis and submit request to CC
CC sends results to task group
Task group summarizes data
Summary to Subcommittee
Recommendations to Steering Committee
2. Procedure for Abstracts/Publications
Publications Subcommittee
• Receives proposals
• Designates writing groups for primary results papers
• Prioritizes proposals
Analysis request submitted by writing group leader to CC.
Results to writing group
Draft Paper
Publications Subcommittee Review
Manuscript prepared for final verification
Coordinating Center or writing group data verification
Steering Committee
Journal

Appendix B

DELTA Writing Group Membership Lists
(Subject to change by committee decision.)

MS#100. Category 1
Topic: The Design and Implementation of Multicenter Feeding Trials: The DELTA Program
Writing Group Leader: Thomas Pearson
Members: Barbara Dennis, Abby Ershow

MS#101. Category 1
Topic: Lipid responses by gender, age/menopausal status, and race
Writing group leader: Henry Ginsberg
Members: Penny Kris-Etherton, Michael Lefevre, Paul Stewart, Sekhar Ramakrishnan, Patricia Elmer, Abby Ershow, Roberta Reed

MS#102. Category 1
Topic: Hemostasis
Writing group leader: Patricia Elmer
Members: Russell Tracy, Thomas Pearson, Aaron Folsom, Michael Lefevre, Henry Ginsberg, Paul Stewart, Penny Kris-Etherton, Abby Ershow, Sekhar Ramakrishnan

MS#103. Category 1
Topic: Interactions: Saturated Fatty Acids and Dietary Cholesterol
Writing group leader: Penny Kris-Etherton
Members: Patricia Elmer, Russell Tracy, Thomas Pearson, Aaron Folsom, Michael Lefevre, Henry Ginsberg, Paul Stewart, Abby Ershow

MS#104. Category 1
Topic: Diet responsiveness and predictors of variability in response to diet
Writing group leader: Michael Lefevre
Members: Henry Ginsberg, Penny Kris-Etherton, Sekhar Ramakrishnan, Paul Stewart, Janice Derr, Roberta Reed, Patricia Elmer, Abby Ershow, David Gordon

MS#105. Category 2
Topic: Recruitment of Participants
Writing group leader: Patricia Elmer
Members: Nancy Anderson, Michael Lefevre, Nancy Van Heel, Wahida Karmally, Satya Jonnalagadda

MS#106. Category 2
Topic: Design of Experimental Diets
Writing group leader: Barbara Dennis
Members: Cathy Champagne, Kent Stewart, Marlene Windhauser, Penny Kris-

Etherton, Wahida Karmally, Nancy Van Heel

MS#107. Category 3
Topic: Effects of Menstrual Cycle on Lipids
Writing group leader: Roberta Reed
Members: Thomas Pearson, Paul Stewart, Penny Kris-Etherton

MS#108. Category 3
Topic: Simplified Gravimetric Determination of Total Fat in Food Composites Using Chloroform-Methanol Extraction
Writing group leader: Katherine Phillips
Members: Kent Stewart and Other FALCC Staff Members

MS#109.

MS#110. Category 3
Topic: Energy intake - Estimated and Actual
Writing group leader: Satya Jonnalagadda
Members: Helen Smicklas-Wright, Penny Kris-Etherton, D.C. Mitchell, K. Meaker, K. Fritz, Wahida Karmally, Abby Ershow, Barbara Dennis

MS#111. Category 3
Topic: Mixed Diet Quality Control Material
Writing group leader: Gary Beecher
Members: Joanne Holden, W.R. Wolf, Abby Ershow, Barbara Dennis

MS#112. Category 3
Topic: Issues of Weight Maintenance
Writing group leader: Wahida Karmally
Members: Susan Raatz, Nancy Van Heel, Sekhar Ramakrishnan

MS#113. Category 3
Topic: Role of Fat Blends in Experimental Diets
Writing group leader: Penny Kris-Etherton
Members: Satya Jonnalagadda, Marlene Windhauser

MS#114. Category 3
Topic: Comparison of Nutrient Databases
Writing group leader: Abby Ershow
Members: Cathy Champagne, Barbara Dennis, Penny Kris-Etherton, Wahida Karmally, Satya Jonnalagadda, Kent Stewart, Janice Derr, member from Minnesota

MS#115.

MS#116. Category 3
Topic: Predictive Equations for Diet Response
Writing group leader: Barbara Dennis
Members: Paul Stewart, Penny Kris-Etherton

MS#117.

MS#118. Category 2
Topic: Predictors of Lipid Response
Writing Group Leader: Thomas Pearson
Members: Paul Stewart

MS#119. Category 2
Topic: HDL Metabolism: Subfractions
Writing Group Leader: Paul Roheim

MS#120. Category 2
Topic: LDL Metabolism: Subfractions
Writing Group Leader: Michael Lefevre

MS#121. Category 2
Topic: Effects of Menstrual Cycle on Hemostatic Factors
Writing Group Leader: Thomas Pearson

MS#200. Category 1
Topic: DELTA 2: Design
Writing Group Leader:

MS#201. Category 1
Topic: DELTA 2: Lipid Responses
Writing Group Leader:

MS#202. Category 1
Topic: DELTA 2: Hemostasis
Writing Group Leader:

DELTA MANUSCRIPT LIST

MS #	Category	Priority #	Topic	Leader	CC Statistician	Current Status
100	1	1	The Design & Implementation of Multicenter Feeding Trials: The DELTA Program	Pearson	P. Stewart	Draft manuscript in preparation.
101	1	1	DELTA 1: Lipid responses by gender, age/menopausal status, and race	Ginsberg	P. Stewart	Final manuscript in preparation.
102	1	1	DELTA 1: Hemostasis	Elmer	P. Stewart	Draft manuscript in preparation.
103	1	1	Interactions of hemostasis and lipids	Kris-Etherton	P. Stewart	Abstract submitted to AHA for 11/95; draft manuscript in preparation.
104	1	1	Relationship Between ApoE Genotype and Lipid Response to Dietary Saturated Fat	Lefevre	P. Stewart	Draft manuscript in preparation.
105	2	1	DELTA 1: Recruitment	Elmer	Anderson	Draft manuscript in preparation.
106	2	1	Design of Experimental Diets	Dennis	Anderson	Final manuscript in preparation.
107	3	1	Effects of menstrual cycle on lipids	Reed	P. Stewart	Final manuscript in preparation.
108	3		Simplified gravimetric determination... methanol extraction	Phillips		Final manuscript in preparation; planned submission to JAOAC on 9/1/95
109						
110	3	3	Energy intake: Estimated and actual	Jonnalagadda		Writing group identified; abstract deferred.

DELTA MANUSCRIPT LIST

MS #	Category	Priority #	Topic	Leader	CC Statistician	Current Status
111	3	3	Mixed diet quality control material	Beecher		Writing group identified; manuscript in preparation.
112	3	3	Issues of Weight Maintenance	Karmally		Writing group identified; ? written proposal.
113	2	2	Interactions: SFA and d Cholesterol	Kris-Etherton	P. Stewart	Written proposal in progress.
114	3	3	Comparison of Nutrient Databases w/Assay Values	Ershow		Writing group identified; proposal submitted.
115						
116	3	3	Predictive Equations for diet response	Dennis	P. Stewart	Proposal in progress; partial writing group identified.
117						
118	2	2	Predictors of Lipid Response	Pearson	P. Stewart	Written proposal form needs to be submitted for approval.
119	2	3	HDL Metabolism: Subfractions	Roheim		Written proposal form needs to be submitted for approval and writing group needs to be identified.
120	2	3	LDL Metabolism: Subfractions	Lefevre		Written proposal form needs to be submitted for approval & writing group needs to be identified.
121	2	2	Effects of Menstrual Cycle on Hemostatic Factors	Pearson		Written proposal form needs to be submitted for approval & writing group needs to be identified.
200	1		DELTA 2: Design			
201	1		DELTA 2: Lipid responses		P. Stewart	
202	1		DELTA 2: Hemostasis		P. Stewart	

APPENDIX A

INFORMED CONSENT ISSUES

A.1 INFORMED CONSENT ISSUES

A.1 Reasons for Problems in Obtaining Informed Consent

Many trials are unable to achieve their recruitment goals because the investigators fail to obtain informed ocnsent from patients who pass (or would pass) screening examinations. This failure may occur for the following reasons.

1. Culture and tradition of the country, or the investigator's own background, make it emotionally dificult for the investigator to ask patients for their informed consent. The easiest way to resolve this problem is to have someone else ask the patients. This person should be more comfortable with the process and could be another investigator, a nurse, the trial coordinator, or a special resource person (e.g., a translator). For DELTA, it is necessary to limit participants to those who can read and understand English.

2. The investigator who asks for informed consent may be rather stiff or brusque in describing the trial and in asking for the patient's informed conset. In this case, the investigator should spend more time with each patient and not rush the process.

GUIDELINES FOR OBTAINED INFORMED CONSENT

(Taken from Spilker, Bert and Cramer, Joyce A., *Patient Recruitment in Clinical Trials*. New York: Raven Press, 1992.)

A.2 OFFERING PAYMENTS TO RECRUIT VOLUNTEERS OR PATIENTS

A.2.1 Offering Money to Recruit Volunteers

Because volunteers receive no medical benefit from participation in a dietary trial, and are often subjected to severe restrictions in daily living, it is reasonable to pay them for their participation. This is standard practice by my many academicians and is accepted as both ethical and appropriate. Money should not be given only to those volunteers who complete an entire trial because this places undue pressure on volunteers to remain in a trial from which they might rather withdraw. Money given to volunteers should be prorated based on the extent of their participation unless there are legal requirements to give all volunteers the entire sum, even for partial participation. The amount of money offered cannot be so excessive that volunteers would feel strong pressure to remain in a clinical trial from which they would rather withdraw.

A.2.2 Basing Payments on Reasons for Leaving a Trial

Subjects may leave a diet study for several reasons. The rigid control of diet over an extended period places severe constraints on daily living. Subjects have given over control of a significant part of their lives to the investigator and study personnel. Inability to comply with these restrictions may make them ineligible to continue with the study. Categories of noncompliance include willful departures from the study diet, severe illness, extended absences for business or personal emergencies. The decision to withdraw a subject involuntarily from the study is made by the principal investigator after discussion with the DELTA Steering Committee. Payment to the subject for partial participation is at the discretion of the local field center. If a subject withdraws voluntarily from the study, it is important for the investigator to determine if and to what extent the diet contributed to the dropout. If any link, however remote, is found with the treatment, the situation should be categorized as diet related. Such instances would require full payment to the subject, who provided important data on adverse effects leading to discontinuation of the diet. Not only should the patient or volunteer not feel that he or she disappointed the investigator by stopping the protocol, but each person should be encouraged to report his/her reasons for leaving the study as fully as possible. Subjects who feel vaguely uncomfortable should be encouraged to express this effect rather than deny any problem when asking to withdraw.

Although a subject may not have completed a study for personal reasons, he or she should be given partial payment based on the proportion of the protocol fulfilled. It would be unfair not to provide compensation for activities that might have taken time and effort even if the data cannot be used in study outcome analyses.

A.3 USING COERCIVE TACTICS TO RECRUIT PATIENTS

Any time there is a superior-subservient relationship between two individuals, it is imposible to ensure that coercion was not felt by the person in the subservient position, even if coercion was not overtly used in the recruitment procss. Three examples of this relationship in clinical research are (1) an investigator recruiting his or her staff or students, (2) a professor recruiting his or her students, and (3) a pharmaceutical company conducting a clinical trial and recruiting its junior level employees.

Such groups cannot provide a true informed consent if the volunteers believe that refusal to enroll may result in being fired, may affect their career prospects, or influence thier course grades.

A.4 ETHICAL GUIDELINES FOR RERUITMENT OF HEALTHY VOLUNTEERS

1. Use of an informed consent that inclues standard elements. The most important element that differs between the vast majority of volunteers and most patients involves the statement that the research is unlikely to be of any direct personal benefit to the volunteer. Similarly, some patients are unlikely to benefit from their participation in a trial.

2. Normal volunteers should not be enrolled in clinical trials where there is perceptible risk. The definition and application of the term risk raises numerous other issues (e.g., how is perceptible risk measured and how much risk is excessive). The balance should be determined by considering the benefits to be gained by society as a whole versus the risks to the individual.

3. Volunteers should not be sought where a dependency relationship exists.

4. Volunteers sought from any organized group should be contacted after officials of that organization have been informed and have fully discussed the issues with those who will conduct the trial.

5. Self-experimentation or experimentation on colleagues or friends of the investigator should be subjected to the same professional and ethical reviews as for other more conventional clinical trials.

6. Issues of liability for injury should be considered and discussed for both negligent causes. It shold be determined who is responsible for payment and how payment would be determined. Numerous options and proposals exist in this area (e.g., see Macrae et. al., 1989).

CONSENT FORM
The Multicenter Study of Diet and Lipoproteins in Humans (Protocol 2)

You are invited to be in a research study of the effect of different diets on blood cholesterol, fat levels and the clotting activity of blood. You were selected as a possible participant for the following reasons: 1) you do not have any significant health conditions; 2) you are between the ages of 21 and 68 and 3) you have a blood profile that meets the study criteria (triglyceride level above the 70th percentile; HDL-cholesterol below the 30th percentile and/or blood insulin level above the 70th percentile). While this type of blood profile does not represent a significant abnormality, it may mean that you have a higher risk of developing heart disease and diabetes. Please read this form carefully and ask any questions you may have before agreeing to take part in the study.

This study is being conducted by the University of Minnesota Division of Epidemiology.

Background Information:

The purpose of this study is to determine how diets containing different kinds and amounts of fat and carbohydrate affect blood lipids and blood clotting factors. The information gained from this study will help us develop better nutritional guidelines for preventing heart disease.

If you agree to be in this study, you will be fed three different test diets for periods of seven weeks each. All your meals and snacks will be provided during the feeding periods, and you will be required to eat ONLY food provided by the study. You will come to the study feeding center at Moos Tower to eat breakfast and dinner Monday through Friday. On those days you will pick up packaged containers of lunch and a snack to eat away from the feeding center. Weekend meals will be packaged for you to take home. All of the test diets will consist of standard foods. No experimental foods will be included. If you use alcohol, you will be required to limit your intake to 5 drinks per week. Caffeine-containing beverages will be limited to no more than 5 per day.

During the feeding periods you will be weighed daily. Your caloric intake will be adjusted so that you neither gain nor lose weight. Your blood will be drawn during weeks 5, 6 and 7 of all three feeding periods. Two to four tablespoons of fasted blood will be drawn each week. You will be asked to complete an overnight urine collection during weeks 6 and 7 of each feeding period.

You will also be invited to take part in the post-prandial aspect of the DELTA study. The post-prandial study will look at the effect of diet on levels of blood fats, cholesterol and insulin following the eating of meals. For the post-prandial aspect of DELTA, on one day during week 6, subjects will come to the feeding center for all three meals. The week 6 fasted blood will be drawn before breakfast. Additionally, before both lunch and dinner, two teaspoons of blood will be drawn. On one day during week 7, subjects will have the usual fasted blood drawn and will then be given a milkshake to consume. Subjects will not eat any additional food, and will drink only non-caloric, non-caffeine drinks during the following eight hours. Two teaspoons of blood will be drawn after both four and eight hours. This post-prandial part of DELTA will be done during weeks 6 and 7 of each feeding period.

There will be breaks of four to six weeks between feeding periods. During the breaks you will be able to eat any foods you choose and you will not need to come to the feeding center.

Risks and Benefits of the Study:

The study has minimal risks. The blood drawing may cause bruising or fainting. Infection is a remote possibility. Every effort will be made to minimize these risks by using well-trained and experienced individuals to draw blood.

A benefit of participation is that you will receive free, nutritious food. You will also receive the results from the blood analyses. If you take part in the post-prandial study, you will receive a payment of $150 after completing each of the first two 7-week feeding periods and an additional $500 after completing the third and last feeding period. If you do not take part in the post-prandial study, you will receive $100 after completing each of the first two 7-week feeding periods and an additional $400 after completing the third and last feeding period.

Alternatives to Participating in This Study:

Because your blood lipid values are not optimal, you may wish to see your physician for treatment instead of joining this study. However, the level of fat in the study diets is similar to or less than that typically eaten by most Americans. It is very unlikely that seven weeks on any of the study diets will significantly affect your health. Also, if you are overweight, losing weight may be beneficial for your health. You may wish to join a weight loss program rather than take part in this study. You will not be allowed to lose weight while you are in this study.

Compensation:

In the event that this research activity results in an injury, treatment will be available, including first aid, emergency treatment and follow-up care as needed. Payment for any such treatment must be provided by you or your third party payor, if any (such as health insurance, Medicare, etc.)

Confidentiality:

The records of this study will be kept private. All data will be kept in locked files and identifiable by ID number. Any reports we publish will not include information that will make it possible to identify you as a subject.

Voluntary Nature of the Study:

Your decision whether or not to participate will not affect your current or future relations with the University of Minnesota. If you decide to participate, you are free to withdraw at any time without affecting this relationship. If you choose to withdraw from the study, you will be paid only for the diet periods you have fully completed.

Contacts and Questions:

The researchers conducting this study are Drs. Patricia Elmer, Joanne Slavin and Aaron Folsom, M.D. You may ask any questions you have now. If you have questions later, you may contact them at (612) 624-1818.

You will be given a copy of this form to keep for your records.

Statement of Consent:

I have read the above information. I have asked questions and have received answers. I consent to participate in the study.

Signature_____ Date_____

Signature of Study Coordinator_____ Date_____

Pennington Biomedical Research Center
LOUISIANA STATE UNIVERSITY

Informed Consent

DIETARY EFFECTS ON LIPOPROTEINS AND THROMBOGENIC ACTIVITY (DELTA)

I _____, voluntarily consent to participate in the research study entitled "The Dietary Effects on Lipoproteins and Thrombogenic Activity." This research program is the first multi-center, controlled feeding study on the effects of diet on coronary heart disease risk factors. The Pennington Bimedical Research Center is one of four centers in the United States praticipating in this controlled feeding study.

Coronary heart disease remains the leading cause of death in Americans. The development of blockages in blood vessels caused by high blood cholesterol along with abnormal blood clotting can severely affect heart function. It is important to determine what diet changes have a beneficial effect on blood cholesterol and blood clotting in order to better understand how to control and prevent this disease. Controlled feeding studies are needed to scientifically determine what dietary changes may be most beneficial.

I understand that this study will last about nine months and will consist of three seven-week diet periods separated by breaks lasting between four to six weeks. I understand that by volunteering to participate in this study I am agreeing to eat diets provided by the Pennington Biomedical Research Center (PBRC). I will eat only foods and beverages provided by PBRC during these three seven-week periods of the study. I agree not to consume any foods or beverages (other than water and approved diet soft drinks) from outside sources, with the exception of dinner on Saturday which will be considered a free-choice meal. The diets will be made up of wholesome foods that will have adequate calories, vitamins, minerals, and other nutrients in order to maintain my current weight and health.

I understand that I will be fed three separate diets, differing in the total amount of fat and fat type during the course of this study. A different diet will be tested during each of the three seven-week diet periods. I agree to participate in interviews and questionnaires in order to assess my compliance to the diets.

6400 Perkins Road. Baton Rouge. Louisiana 70808-4124 ♦ Phone: (504) 767-0880. Fax: (504) 765-2525

I understand that I will be weighed twice a week to monitor my weight. I agree to eat ten meals per week at PBRC, consisting of breakfast and dinner each weekday, Monday through Friday. I understand that strict adherence to the diet schedule is required and that this may interfere with social activities such as dining in restaurants. Even though menus are carefully planned to include as much variety as possible, I am aware that the food selection will be restricted compared to what is available in the supermarket. This limited selection may result in some boredom with the diets over the course of the study.

I am willing to have a blood sample taken from my arm once a week during the last three weeks of each seven-week diet period. This means that I agree to have my blood sampled nine times over the course of this nine month study. The amount of blood taken each time will be limited to about three tablespoons. Possible risks associated with obtaining blood samples include discomfort as the needle is inserted into the vein in the arm. There may be some local bruising or swelling at the point of entry of the needle and in rare cases, a blood clot or bleeding or irritation of the vein at the site may occur.

I am willing to provide complete 24-hour urine collections once a week during the last three weeks of each seven-week diet period. This means that I agree to collect and provide 24-hour urines nine times over the course of this nine month study.

Foods will be prepared according to accepted standards of sanitation and provisions will be made to ensure the safety of the foods which are packed for me to take home. However, it is possible that contamination during shipping, storage or preparation could go undetected and result in food-borne illness. Every effort will be made to guard against this occurring. I understand that upon receipt of the packaged meals, I am responsible for refrigerating them to protect against food-borne illness.

I understand that all meals, procedures and assessments will be provided at no expense to me. I will be paid a total of $900 after completing all phases of the study. I will be paid in increments of $100 after the first seven weeks, $300 after the second seven weeks, and $500 upon completion of the study.

I understand that through my participation in this study I will be contributing to the body of knowledge in biomedical science. Strict confidentiality concerning both my participation and my medical records will be maintained. The Principal Investigators, Co-Investigators and Clinical Trials staff may review my records at any time to look at, or analyze data, or to insure my compliance with the study and its procedures. I have been informed that the results

of the study may be published but that my privacy will be protected and I will not be identified by name.

I understand that as a volunteer in this study I am free to withdraw at any time. I also understand that my participation may be terminated if I am found not to be compliant with the study requirements. I understand that I will be compensated financially only for the phases of the study that I have successfully completed.

The study has been explained to me in detail and I have been given an opportunity to ask questions of Dr. Michael Lefevre and the staff of PBRC Clinical Trials. I understand the nature and purpose of the study and of the various tests and their potential risks. I understand that I may contact Dr. Lefevre at (504) 765-2569. I may contact the staff of Clinical Trials at (504) 765-2672 for questions or scheduling problems at any time during the course of the study.

I have been informed that if I believe that I have sustained injury as a result of participating in this research study, I may contact Dr. Lefevre or the Clinical Trials staff so that I can review the matter and identify the medical resources which may be available to me. I understand that PBRC is a research facility only and would not be a source of medical treatment.

I have thoroughly read the above and understand what is involve in participating in this controlled diet study.

_____ _____

Signature of Research Volunteer Date

_____

Social Security Number

_____

Witness

_____

Michael Lefevre, Ph.D.
Co-Principal Investigator

_____

Donna H. Ryan, M.D. or
Study Medical Monitor

INFORMED CONSENT FORM FOR CLINICAL RESEARCH STUDY
The Pennsylvania State University

Title of Project: Dietary Effects on Lipoproteins and Thrombogenic Activity

Principal Investigator: Dr. Penny M. Kris-Etherton

Other Investigators: Thomas A. Pearson, Janice A. Derr, C. Channa Reddy, Helen Wright, Edward W. Mills, Madeleine J. Sigman-Grant, Andrea M. Mastro, Russell P. Tracy, Roberta G. Reed

This is to certify that I, have been given the following information with respect to my participation as a volunteer in a program of investigation under the supervision of Dr. Penny M. Kris-Etherton.

1. Purpose of the study:

You are invited to participate in a research study of the effects of diets with differing fat and carbohydrate levels on your blood cholesterol and fat levels and on the clotting activity of your blood. Dr. Kris-Etherton and her associates hope to learn more about the way the dietary fat affects the amount of cholesterol and fat in your blood; and the way that fat in the diet affects the way the blood clots. All of this information is important because of the link between diet, blood cholesterol, blood clotting, and the chance of having a heart attack or stroke. You were selected as a possible participant in this study because you do not have any significant diseases, but you do have some abnormalities of fat and glucose metabolism that could increase your risk for developing atherosclerosis (hardening of the arteries) and adult-type diabetes. Specifically, although your total blood cholesterol level is within the average range for someone your age, your blood level of triglyceride (blood fat) may be higher than is considered best for your health. Additionally, your HDL cholesterol (the good cholesterol fraction) may be lower than is considered optimal. Higher triglycerides and lower HDL cholesterol may increase the risk for atherosclerosis, heart attacks and strokes. Finally, your blood insulin level (the hormone that controls blood sugar levels) may not be optimal either. This could mean that you are at risk for developing diabetes.

In this study, we will be comparing the effects of diets containing different amounts of fat and carbohydrate: one diet will contain the amount of saturated fat (16% of total calories) that is usually eaten by Americans while the other two diets will have the amount recommended by the American Heart Association for all Americans (8% of total calories). The two diets that are lower in saturated fat will differ in that, one will have carbohydrate replacing the saturated fat while the other will use monounsaturated fat (the type present in olive oil) to replace the saturated fat. The

diets will all contain the same amounts of protein, polyunsaturated fat and cholesterol.

The idea behind this study is that reducing the amount of saturated fat in the diet will reduce the level of cholesterol and fat in the blood, and will make the blood less likely to clot. Heart attacks are the major cause of death in the United States, and we know that both the level of cholesterol and fat in the blood as well as the likelihood that the blood will clot contribute to an individual's risk for developing coronary artery disease (narrowing of the blood vessels supplying the heart with oxygen). Coronary heart disease leads to heart attacks. However, there is a controversy among nutrition experts concerning the best way to reduce the amount of saturated fat in the diet; some want to replace the saturated fat with carbohydrates (breads, most vegetables, pasta) while other believe that monounsaturated fat would be the best replacement for the saturated fat. This study will determine which diet is best for individuals like you who may be at increased risk for heart attacks and diabetes.

2. Procedures to be followed:

If you decide to participate, you will agree to eat only foods that we provide for you (except for one dinner on the weekend) during three 7-week periods. You will come to the PSU DELTA Dining Room for lunch and dinner and eat your meals there Monday through Friday. We will give you packed snacks and breakfasts to eat at home. On Friday evenings, you will be given coolers containing weekend breakfasts, lunches, one or two dinners, and snacks. There will be breaks of 4-6 weeks between the first and second, and between the second and third diet periods.

We will weigh you daily and you will provide the staff dietitians with information about your one weekend meal as well as alcohol intake (limited to five drinks per week) and any non-study foods you may have eaten.

During the final three weeks of the study (weeks 5, 6, & 7), you will come to our offices on one day of each week for blood tests. All blood samples will be obtained after a 10-hour overnight fast.

The amount of blood drawn at a single visit will be about one ounce (two tablespoons). Each visit for blood sampling should take no more than 20 minutes.

Successful completion of this study depends on the excellent cooperation of the participants. If, during the study, you cannot eat the foods provided and/or eat other foods, you will be asked to leave the study.

3. Discomforts and risks:

The diets to be used in these studies are eaten by significant numbers of typical Americans. Because you were recruited on the basis of abnormalities in lipid and/or

glucose metabolism, the average American diet is not optimal for you. However, we do not believe that 7 weeks on the average American diet will significantly affect your health. The other two diets that we will feed you are within the guidelines for diets to be used in people who have abnormalities in lipid and/or glucose metabolism.

You may be overweight at the start of the study. We will attempt to maintain your weight throughout the study and will weigh you daily. Because it may be beneficial to your health to lose weight, we will offer you advice on how to lose weight at the conclusion of the study. You will not, however, be allowed to lose weight during the study. You may choose to join a weight loss program elsewhere at this time, rather than joining our diet study.

The risks involved with blood drawing include some local pain and bruising from venipuncture: we will use well trained and experienced phlebotomists. Blood sampling can also cause light-headedness and dizziness; we will watch for this and if it occurs, we can alleviate symptoms by having you lie flat with your feet raised.

4. a. Benefits to me:

You will be fed a nutritionally adequate diet for 3 seven-week periods. During this time, you will receive all your food. You will learn the principles of good nutrition practices. At the end of the study, you will be taught the principles of the best blood cholesterol lowering diet for you. You may also participate in a nutrition program to lose weight.

b. Benefits to society:

This study is designed to identify the most efficacious diet therapy for the treatment of dyslipidemia and insulin resistance, disorders that affect about 30% of our population.

5. Alternative procedures which could be utilized:

As noted above, you have several alternatives to entering our study. You might wish to see your physician for treatment of your elevated level of triglycerides or reduced level of HDL cholesterol. You might wish to join some weight loss program rather than enter our study (during which your weight will be steady).

6. Time duration of the procedures and study:

Screening is scheduled for July, August, and September.

Two screening visits are required. Blood will be collected both times. Other measurements taken include height, weight, and blood pressure. We also will

measure your waist and hip circumference.

The Run-In is scheduled for 4 days sometime between September 12-25, 1994. During this time, you will be fed the test diets that will be used during the three feeding periods.

Feeding Period 1-September 30 - November 11
Feeding Period 2-January 6 - February 24
Feeding Period 3-March 31 - May 19
(Study procedures are described above).

7. **Statement of confidentiality:**

All records associated with my participation in the study will be subject to the usual confidentiality standards applicable to medical records, and in the event of any publication resulting from the research no personally identifiable information will be disclosed.

Signature of Participant

Signature of Investigator

Date

7/5/94
Date

IRB approval date _____
Approval expiration date _____

Columbia Presbyterian Medical Center

Consent to participate in a Research Study:

The purpose of this consent form is to provide you with the information you need to consider in deciding whether to participate in this research study.

Study title: "Comparison of the efficacy of diets high in monounsaturated fat, carbohydrates on lipid metabolism and hemostasis in individuals with insulin resistance and/or dyslipidemia."

IRB # 6065

Principal Investigator: Henry N. Ginsberg, Professor of Medicine

Study Purpose:

You are invited to participate in a research study of the effects of diets with differing fat and carbohydrate levels on your blood cholesterol and fat levels and on the clotting activity of your blood. Dr. Ginsberg and his associates hope to learn more about the way the diet you eat affects the amount of cholesterol and fat in your blood and the way the blood clots. All of this information is important because of the link between diet, blood cholesterol, blood clotting, and the chance of having a heart attack or stroke. You were selected as a possible participant in this study because you do not have any significant diseases, but you do have some abnormalities in the way your body uses fat and glucose that could increase your risk for developing atherosclerosis (hardening of the arteries) and adult-type diabetes mellitus. Specifically, although your total blood cholesterol level is within the average range for someone your age, either your blood level of triglyceride (blood fat) is higher than is considered best for your health or your HDL cholesterol (the good cholesterol fraction) is lower than is considered optimal. Higher triglycerides and lower HDL cholesterol may increase the risk for atherosclerosis, heart attacks and strokes. Finally, your blood insulin level (the chemical that control blood sugar levels) may not be optimal either. This could mean that you are at risk for developing diabetes.

In this study, we will be comparing the effects of diets containing different amounts of fat and carbohydrate: one diet will have the amounts of saturated fats (16% of total calories) that is usually eaten by Americans, while the other two diets will have the amount of saturated fat that is recommended by the American Heart Association for all Americans (9% of total calories). The two diets that are low in saturated fat will differ in that one will use carbohydrate to replace the saturated fat while the other will use monounsaturated fat to replace the saturated fat. The diets will all contain the same amounts of protein, polyunsaturated fat and cholesterol.

The idea behind this study is that reducing the amount of saturated fat in the diet will reduce the level of cholesterol and fat in the blood, and will make the blood less likely to clot. Heart attacks are the major cause of death in the United States, and we know that both the level of cholesterol and saturated fat in the blood, as well as the likelihood that the blood will clot, contribute to an individual's risk for developing coronary artery disease (narrowing of the blood vessels supplying the heart with oxygen). Coronary artery disease leads to heart attacks. However, there is a controversy among nutrition experts concerning the best way to reduce the amount of saturated fat in the diet; some want to replace the saturated fats with

carbohydrates (breads, most vegetables, pasta) while others believe that monounsaturated fats (the type present in olive oil) would be the best replacement for the saturated fats. This study will determine which diet is best for individuals like you who may be at increased risk for heart attacks and diabetes.

Study Procdures:

If you decide to participate, you will agree to eat only foods that we provide for you (except for one dinner on the weekend) during three 7-week periods. You will come to the Bard Hall cafeteria for lunch and dinner and eat your meals there Monday through Friday. We will give you packed snacks and breakfasts to eat at home. On Friday evenings you will be given packages containing weekend breakfasts, lunches, one dinner and snacks. There will be breaks of 4-7 weeks between the first and second, and between the second and third diet periods.

We will weigh you twice weekly and you will provide the staff dieticians with information about your one weekend meal as well as alcohol intake (limited to five drinks per week) and any non-study foods you may have eaten.

During the final three weeks of the study (weeks 5,6,7) you will come to our offices on one day of each week for blood tests. All blood samples will be obtained after a 12 hour overnight fast. In week 6 you will also come to our offices just before lunch and just before supper for additional blood tests. In week 7 you will drink a milk/cream shake that contains about half the calories, and all of the fat you would normally eat during the day. You will return to our offices 4 and 8 hours after you finish the drink for additional blood tests. You will not eat anything from the time you finish the drink until after the 8 hour blood test. Also in weeks 6 and 7 you will bring a urine sample with you when you come for the fasting blood test. You will collect the urine when you wake up in the morning.

The amount of blood drawn at a single visit for just the fasting blood tests will be about one ounce (two tablespoons). Approximately half of that amount will be drawn at each of the visits before lunch and dinner and at each of the visits 4 and 8 hours after you drink the milk/cream shake. Each visit for blood sampling should take no more than 20 minutes.

Successful completion of this study depends on the excellent cooperation of the participants. If, during the study, you cannot eat the foods provided and/or eat other foods, you will be asked to leave the study.

Study risks:

The diets to be used in these studies are eaten by significant numbers of typical Americans. Because you were recruited on the basis of abnormalities in fat and/or glucose metabolism, the average American diet is not optimal for you. However, we do not believe that 7 weeks on the average American diet will significantly affect your health. The other two diets that we will feed you are within the guidelines for diets to be used in people who have abnormalities in fat and/or glucose metabolism.

You may be overweight at the start of the study. We must maintain your weight throughout the study and we will weigh you twice weekly. You will not be allowed to lose weight during the study. However, because it may be beneficial to your health to lose weight, we will offer you weight loss counseling at the conclusion of the study. You may choose to join a weight loss program elsewhere at this time, rather than joining our diet study.

The risks involved with blood drawing include some local pain and bruising from venipuncture: we will use well trained and experienced phlebotomists. Blood sampling can also cause light-headedness and dizziness; we will watch for this and if it occurs we can alleviated symptoms by having the subject lay flat with feet raised. The total blood taken on any day (including the days when 3 blood samples are obtained) is small and we do not expect any problems to arise.

Alternatives:

As noted above, you have several alternatives to entering our study. You might wish to see your physician for treatment of your elevated level of triglycerides or reduced level of HDL cholesterol. You might choose to see your physician if we have found that your blood pressure is greater than 140/90 mm Hg. You might wish to join some weight loss program rather than enter our study (during which your weight will be steady).

Compensation:

All foods, meals, snacks, fluids will be provided at no cost to participants. In some cases, subjects will be reimbursed for travel/parking fees.

Confidentiality:

Patient files and results will all be coded. All data will be locked in a file cabinet by the Principal Investigator.

Participation is voluntary:

Your participation in this study is completely voluntary. You can refuse to participate or withdraw from the study at any time, and such a decision will not affect your medical care, employment, or student status at Columbia Presbyterian Medical Center now or in the future. As noted above, the investigators may ask you to leave the study if you cannot comply completely with the diets.

Questions:

If you have any questions, please ask. In the future, should you have any questions you can reach Dr. Berglund or Dr. Ginsberg at 305-3741 and he wil do his best to answer them. If you have any questions on your rights as a research subject you can call the Institutional Review Board (212-305-5883) for information.

Consent to Participate in the Study

I have discussed this study with Dr. Ginsberg (or his designated associate) to my satisfaction. I understand that my participation is voluntary and that I can withdraw from the study at any time without prejudice. I have read the above and agree to enter this research study. Signing this form does not waive any of my legal rights.

I have been informed that if I believe that I have sustained injury as a result of participating in a research study, I may contact the Principal Investigators, Henry N. Ginsberg at 305-3741, or the office of the Institutional Review Board, at 305-5883, so that I can review the matter and identify the medical resources which may be available to me.

I understand that:

a) The Presbyterian Hospital will furnish the emergency medical care determined to be necessary by the medical staff of the hospital;

b) I will be responsible for the cost of such care, either personally or through my medical insurance or other form of medical coverage;

c) No monetary compensation for wages lost as a result of injury will be paid to me by the Columbia Presbyterian Medical Center.

d) I will receive a copy of this signed consent form.

Signed_____

Date_____

Print Name_____

Witnessed_____ Date_____

The Institutional Review Board of the Columbia Presbyterian Medical Center has approved the recruitment of subjects for this study.

CONSENT FORM FOR CHOLESTEROL SCREENING

The effects of dietary fats plasma lipid metabolism and hemostasis.

Principal Investigator: Henry N. Ginsberg, M.D.
Wahida Karmally M.S., R.D.

1. I have been recruited as a possible participant, as a normal volunteer, in a study of the effects of dietary fats on blood lipids (cholesterol and fats) and thrombotic factors (things that make the blood clot). As a first step toward participating, I have been asked to have a blood sample that will be taken by either finger stick or venipuncture, so that my plasma cholesterol will be measured. Nor more than one teaspoon of blood will be taken if the venipuncture is needed; only a drop of blood will be needed if the doctors can use the finger stick method. I will also be asked questions about my personal medical and dietary history.

2. I understand that if my plasma cholesterol concentration is between the 25th and 85th percentile for my age and sex in the United States, and if nothing in my medical or personnal history exclude me from the study, I will be asked if I still wish to participate in the study. If I state that I am still interested in participating in such studies, I will return, after a 12 hour overnight fast and have a second blood sample taken for repeat determination of blood cholesterol levels. This blood sample will be a venipuncture and about two tablespoons of blood will be taken. The investigators will ask me further questions about my health habits and my personal medical history. If I am not interested in participating further in these studies, or if my cholesterol level is below the 25th or above the 85th percentile, or if my medical or personnal history preclude my participation, I will be given the results of the present test and released.

3. I understand that the possible risks associated with these studies include local pain during the finger stick (a pinprick at the top of my finger) or venipuncture (a needle into a vein in my forearm). This discomfort should be minimal. In addition, I may feel dizzy and weak during or after blood sampling, but this will be watched for and is easily treated by raising the legs and lowering the head. The loss of a drop of blood from the finger stick or of 10 ml of blood from venipuncture should cause no ill effects and my body should replace that amount during the next few days.

4. I understand that there will be no direct benefit to me from this study other than learning my blood cholesterol level. If my cholesterol level is elevated (above the 85th percentile), I may wish to consult a physician concerning this matter.

5. I understand that this is not a study of therapy, so no alternatives are available.

6. I understand that all records and results will be kept in the confidential files of the Principal Investigator.

7. I understand that I may contact Dr. Ginsberg at (212) 305-3741 if a problem arises related to this research test. I may also contact the Institutional Review Board at (212) 305-5883.

8. I understand that participation in these studies is voluntary. I am free to ask any questions related to this study and can expect an appropriate reply. I may withdraw from or terminate this procedure at any time. Such action will not interfere with any treatment I am receiving

or was to have received in association with this study or with my student or employee status at the CPMC.

9. I have been told that I will not receive monetary compensation for travel, any time lost from employment, or inconvenience.

10. Dr. Ginsberg (or his designated associate) has answered all of my questions concerning this research. I understand that any further questions will be readily answered. If I have any future questions about this study, I may contact the Principal Investigator, Dr. Henry Ginsberg, at (212) 305-3741. If I have any questions about my rights as a research participant, I may contact the Institutional Review Board at (212) 305-5883.

11. I have been informed that if I believe that I have sustained injury as a result of participating in a research study, I may contact the Principal Investigator, Dr. Ginsberg, at (212) 305-3741 or the Office of the Institutional Review Board, at (212) 305-5883, so that I can review the matter and identify the medical resources which may be available to me.

I understand that:

a) The Presbyterian Hospital will furnish whatever emergency care is determined to be necessary by the medical staff of this hospital;

b) I will be responsible for the cost of such care, either personally or through my medical insurance or other form of medical coverage;

c) No monetary compensation for wages lost as a result of injury will be paid to me by the Columbia-Presbyterian Medical Center.

Signed:_____ Date:_____

Print Name:_____

Investigator:_____
Date:_____

The Institutional Review Board of the Columbia-Presbyterian Medical Center has approved the recruitment of subjects for this study.

The subject will receive a copy of this signed consent form.

Signed:_____ Date:_____

DELTA STUDY

Columbia University

Participant Agreement

I understand that if I agree to participate in Dietary Effects of Lipoproteins and Thrombogenic Activity (DELTA), I will be expected to do the following:

1. Come to Bard Hall (B1)-60 Haven Avenue for my lunch and dinner Monday through Friday. The study will last 7 1/2 months, and I will eat 3 different diets lasting 7 weeks each during that time. Between Diet periods 1 and 2, I will have a 7 week break where I can eat any food and during which I do not need to come to the study facility. Between Diet periods 2 and 3, I will have 4 weeks where I can eat any food and during which I do not need to come to the study facility.

2. Pick up prepackaged containers of breakfast, and a snack for daily weekday consumption as well as prepackaged meals for my consumption during weekends.

3. Avoid all foods other than those provided by Bard Hall and eat all foods provided.

4. Weigh in twice weekly (Monday and Thursday - before dinner) at Bard Hall to allow adjustment of calories to maintain my current body weight.

5. Avoid use of alcoholic beverages during each 8 week diet period.

6. Allow blood samples to be drawn during each diet period.

7. I will be asked to collect a 24 hour urine sample during each diet period.

My signature indicates that I have read and that I understand the above description of my responsibilities in the DELTA study.

_____

Prospective Participant

APPENDIX B

COMMUNICATIONS

Communications

Design characteristics of single-center versus multicenter trials differ in the fact that single-center trials are easier to design and operationalize because all study personnel are located in the same institution. For single-center trials, it is not necessary to maintain communications and decision-making structures for execution of the trial. In addition, the physical proximity of study personnel may make it possible for them to work more efficiently and to achieve a higher degree of uniformity in the procedures they perform than might be expected in a multicenter trial. The main weaknesses of a single-center trial are the sample size and resource limitations. One center and a few investigators will find it difficult to recruit and follow the numbers of patients needed (Meinert, Curtis L, CLINICAL TRIALS: Design, Conduct and Analysis, Oxford University Press, New York/Oxford 1986).

The communications network of a multicenter trial relies on frequent and timely communiques during the design, implementation and conduct of a research trial. This makes it imperative that the decision-making structures, the Steering Committee and all the subcommittees, provide the facility to communicate in such a fashion that all timeframes and deadlines are met with some reasonable degree of timeliness. The vast differences in individual's daily work schedules and traveling schedules makes the burden of interactive communications a serious one.

Purpose

To provide a structure to facilitate timely, efficient, and economical transmission of information between the DELTA Coordinating Center, central laboratories, field centers, and NHLBI program office.

Communications from the Coordinating Center

The Coordinating Center is responsible for ensuring that the DELTA protocols are properly implemented and carried out and for protecting the integrity and security of the data. This responsibility is carried out through assisting in the development and distribution of protocols, procedure manuals, forms, and data entry systems. The Coordinating Center maintains a continuing quality assurance program through ongoing monitoring of data, site visits, inspection of forms completion, and assay of the experimental diets. Any problems encountered in this process will be reported to the appropriate principal investigator, sub-committee Chairperson, NHLBI project scientist, or Steering Committee.

Communications from Field Centers

Field Centers and central laboratories have a responsibility for on site monitoring of the protocol and procedures. It is important that any ambiguities in the written procedures be brought to the immediate attention of the Coordinating Center. Likewise, deviations from protocol or problems encountered in carrying out the protocol should also be brought to the immediate attention of the Coordinating Center.

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