DIETARY APPROACHES TO STOP HYPERTENSION 2 (DASH2)

PROTOCOL

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DASH2 Protocol

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1. Overview

The Dietary Approaches to Stop Hypertension 2 (DASH2) study is a multicenter, randomized clinical trial designed to compare the effects of three levels of sodium intake and two dietary patterns on blood pressure among persons with higher than optimal blood pressure or with Stage 1 hypertension (up through 95 mm Hg diastolic). The two dietary patterns are based on the previous Dietary Approaches to Stop Hypertension (DASH) trial. They are: a "control" diet that is typical of what many Americans eat, and a "combination" diet. Compared to the control diet, the combination diet is rich in fruits, vegetables, and low-fat dairy foods, and low in saturated fat and total fat. It is also low in cholesterol, high in dietary fiber, potassium, calcium and magnesium, and moderately high in protein. The diets meet the participant's energy requirements for maintaining stable weight. Alcoholic and caffeinated beverages are limited and monitored. Participants are randomly assigned to one of these two dietary patterns using a parallel group design, and are fed each of three sodium levels using a randomized crossover design. The three sodium levels are: "higher" (150 mmol), reflecting current US consumption; "intermediate" (100 mmol) reflecting the upper limit of current US recommendations for sodium; and "lower" (50 mmol), reflecting potentially more optimal sodium levels. Collaborating on this trial are four field centers, a coordinating center, a central food analysis laboratory, and the National Heart, Lung, and Blood Institute.

Study participants are 400 adults, aged 22 and older, and with systolic blood pressure between 120 and 159 mm Hg and diastolic blood pressure between 80 and 95 mm Hg inclusive. After attending a series of three screening visits to determine eligibility and collect baseline data, participants begin a two-week run-in feeding period using the control diet at the higher sodium level. This is followed within three days by three 30-day intervention feeding periods, one at each of the three sodium levels in random order. Randomization to combination or control diet occurs during the second week of run-in feeding.

The study provides participants with all of their food during the run-in and three feeding periods, although participants resume their normal diets for up to five days between the feeding periods. During the run-in and intervention feeding periods participants are required to attend the clinic for at least one meal per day, five days per week, and to take home food to eat for their other meals. Weight is monitored and individual energy intake adjusted so that participants neither gain nor lose weight during the study. Clinics deliver the interventions in four successive cohorts, with approximately 25 randomized participants per cohort, over a two-year period.

The primary outcome variable is systolic blood pressure. The study also compares as secondary outcomes the impact of the diets on diastolic blood pressure and on 24-hour systolic blood pressure as determined by ambulatory blood pressure monitoring (ABPM).

2. Aims and Objectives

The overall objective of the trial is to study the effects of sodium intake within two dietary patterns that have been shown by the previous DASH trial to have markedly contrasting effects on blood pressure. Participants are randomized to a control or combination dietary pattern, and within each dietary pattern sodium intake is varied using a randomized crossover design. The study also assesses whether the influences on blood pressure of the two dietary patterns vary according to race, sex, baseline blood pressure, age, and other personal characteristics.

While we are aware that salt (i.e., sodium chloride) accounts for the bulk of dietary sodium found in processed foods (1) throughout this document we refer to sodium without specifying the associated anion. We do recognize, however, that several studies have suggested that the blood pressure effects of sodium are dependent on its associated anion (2,3).

The primary outcome variables are systolic blood pressures (SBP) measured at the end of each intervention feeding period. These are contrasted for the three sodium levels using traditional analytic models for crossover designs. Secondary outcomes are diastolic blood pressures (DBP) measured at the end of each intervention feeding period as well as 24-hour systolic blood pressures as measured by 24-hour ambulatory blood pressure monitoring.

Primary Specific Aims

Specific Aim #1: Determine the effect on blood pressure of three levels of dietary sodium fed in (a) **a control dietary pattern**, typical of what many Americans eat, and (b) **a combination dietary pattern** that, by contrast, is rich in fruits, vegetables, and low-fat dairy foods, and reduced in saturated fat, total fat, and cholesterol.

The sodium levels range from "**higher**" (equivalent to 150 mmol of sodium at a caloric intake of 2100 Kcal, and thus typical of current US consumption), to "**intermediate**" (100 mmol of sodium, reflecting the upper limit of current US recommendations), to "**lower**" (50 mmol of sodium, reflecting potentially optimal levels).

For the control dietary pattern, the distribution of potassium, magnesium, and calcium content centers on the 25th percentile of intake of the US population, while the macronutrient profile and fiber content generally reflect current US consumption. For the combination dietary pattern, the distribution of potassium, magnesium, and calcium content centers on the 75th to 85th percentiles of US population intake. The macronutrient distribution of the combination dietary pattern is reduced in saturated fat, total fat, and cholesterol, and is moderately high in protein. The distribution of monounsaturated and polyunsaturated fats is the same as that of the control dietary pattern, 13% and 8%, respectively, for both diets.

Although the effects of sodium intake within each dietary pattern are initially assessed by comparing the higher- vs. lower-sodium diets, interest is centered on the higher- vs. intermediate-level and the intermediate- vs. lower-level contrasts, both of which have clinical and public health relevance. These effects are formally analyzed both overall and separately by gender, race (African American vs. Other), and hypertensive status (hypertensive vs. non-hypertensive).

Rationale: No dose-response trial of dietary sodium, with intakes in a range relevant to clinical practice and public health, has ever been conducted in a population with higher than optimal blood pressure or stage 1 hypertension. We address this question both in a diet that is broadly representative of what many Americans currently eat, and in a combination diet rich in fruits, vegetables, and low-fat dairy foods and reduced in saturated fat, total fat, and cholesterol that

has already been shown in DASH to substantially reduce blood pressure at a level of sodium consumption of approximately 130 mmol / day. The effect of dietary sodium in this context has never been reported. The potentially additive effects of lower sodium intake along with the combination dietary pattern could have major clinical and public health implications in the prevention and control of hypertension.

Modest effects on blood pressure have been documented when sodium intake is reduced from an average level of 150 mmol to the proposed intermediate level of 100 mmol. Few or no controlled clinical trials have investigated the effect of a further reduction in sodium intake to 50 mmol in persons with stage 1 hypertension or higher than optimal blood pressure. Previous trials have found substantial reduction in blood pressure with very low sodium intakes (e.g., <25 mmol), but such low levels are impractical for clinical or public health use.

We hypothesize that blood pressure will be lowered more when sodium intake is reduced from 100 to 50 mmol than from 150 to 100 mmol.

The trial is powered to analyze the gender, race, and hypertensive subgroups separately. This decision reflects evidence in the literature suggesting differential salt sensitivity in African Americans vs. non-African Americans, in males vs. females, and in hypertensives vs. non-hypertensives.

Specific Aim #2: Determine the effect on blood pressure of the combination dietary pattern, relative to the control dietary pattern, at each of the three levels of sodium intake.

Rationale: In the DASH trial, sodium intake was kept constant across the three dietary patterns but varied among participants within a range of 100-174 mmol depending on total energy intake. This aim addresses the important public health question of whether a reduced fat, high fruit, vegetable, and low-fat dairy diet is effective in lowering blood pressure at levels of sodium consumption that are at or below current public health guidelines for maximal intakes. The blood pressures measured at the end of each feeding period are compared between the control and combination diets for each level of sodium intake.

We hypothesize that the combination dietary pattern will significantly reduce blood pressure relative to the control diet at each level of sodium intake.

Secondary Specific Aims

Specific Aim #3: Assess whether the effects of sodium intake on blood pressure are similar for the two dietary patterns. Specifically, assess whether the BP-effect of going from the higher to the lower levels of sodium intake differs for participants in the control and combination dietary patterns.

Rationale: The issue of whether the BP-effects of sodium reduction and the combination dietary pattern are additive is of important public health interest. Additive or synergistic (i.e., positive interaction) effects would argue for a public health campaign promoting both interventions. Similarly, it would be important to know whether an interaction effect is not additive (negative interaction). Although specific aim #2 addresses the issue of interactions indirectly, this aim provides a direct test of the additivity of these effects, and is a two-sided hypothesis.

Specific Aim #4: Determine whether the BP-effect associated with reducing sodium from the higher- to intermediate-level of intake differs from the BP-effect associated with reducing

sodium from the intermediate- to lower-level of intake. This is equivalent to testing whether the BP-effects of sodium are linear across the three sodium levels. This question is addressed both overall and separately for each dietary pattern.

Rationale: Much of the research on the relationship between sodium intake and blood pressure occurs in the upper ranges (i.e., > 50 mmol) of intake. Although some evidence exists for a greater than expected lowering of blood pressure at very low levels of sodium intake, an analysis of INTERSALT data reported that no model fit the data better than a linear model. This specific aim will help fill this knowledge gap.

We hypothesize that the relationship between sodium and blood pressure is not linear, with greater than linear responsiveness at lower levels of sodium. We will test the above four specific aims using RZ-SBP as the primary outcome variable and RZ-DBP and 24-hour SBP as secondary outcome variables.

Other Objectives

We also address the following additional objectives.

1. To assess whether the observed effects of both the DASH combination dietary pattern and sodium intake on blood pressure vary according to baseline characteristics such as race, sex, baseline blood pressure, body mass index, waist circumference, education, and diet prior to entry into the study.
2. To determine the extent to which age influences blood pressure response to the DASH combination diet and to sodium reduction. Because salt sensitivity increases with age, we would expect age to be an effect modifier for the sodium intervention. Although age had no apparent effect on response to the DASH combination diet, an effect may be present when both the DASH combination diet and sodium reduction are combined.
3. To determine the extent to which renin profile influences blood pressure response to the DASH combination diet and sodium reduction.
4. To assess the effects of the DASH combination dietary pattern and sodium intake (*Specific Aims 1-4*, above) on selected measures of ambulatory blood pressure, including 24-hour DBP, waking SBP and DBP, and sleeping SBP and DBP. In addition, as exploratory analyses, we will assess these ambulatory pressure indices in the analyses outlined in *Other Objectives* 1-3, above.
5. To assess the effect of the DASH combination dietary pattern and sodium intake on dipper/non-dipper status (a measure of nocturnal blood pressure decline).
6. To estimate the magnitude of change in plasma low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), total cholesterol, and triglycerides produced by the DASH combination diet, compared with the control dietary pattern, and to determine whether dietary sodium affects blood lipid levels.
7. To collect biological storage samples including plasma, serum, urine, and buffy coat for future analysis.

3. Background and Rationale

Introduction

High blood pressure, defined as systolic blood pressure ≥140 mm Hg, or diastolic blood pressure ≥90 mm Hg, or taking antihypertensive medication, affects almost 50 million people in the United States and places them at high risk for cardiovascular diseases (4). In addition, the risk of cardiovascular disease increases progressively throughout the entire range of blood pressure levels. For this reason, elevated blood pressure affects the health of a large segment of the population who are not defined as hypertensive (5). Lowering the blood pressure of the general public through dietary means thus holds great promise as a strategy to help prevent this highly prevalent public health problem (6). National guidelines for the primary prevention of hypertension recommend reduction of dietary salt, excess body weight and alcohol intake, and possibly increased potassium intake (4,6). The DASH trial has now demonstrated that a dietary pattern that is high in fruits, vegetables, and low-fat dairy foods, but reduced in saturated fat, total fat, and cholesterol, substantially lowers blood pressure (7).

The DASH2 trial extends research on sodium and the DASH dietary patterns to address several issues of relevance to clinical practice and public health. Sodium reduction, coupled with the successful DASH combination diet, has great promise to lower blood pressure to an extent hitherto not demonstrated for any nonpharmacological treatment. This reduction may be comparable to, or in excess of, that produced by single-agent drug therapy.

Sodium Intake And Blood Pressure

Sodium intake across populations worldwide correlates with blood pressure levels and with the upward slope in blood pressure with age (8,9). Within populations as well, sodium intake in individuals correlates directly with blood pressure levels (8,10). Randomized clinical trials confirm that the relationship between dietary sodium and blood pressure is causal, as shown in meta-analyses (11,12) and other reviews (13, 13a). For these reasons, national guidelines for the prevention and treatment of hypertension recommend that daily sodium intake be lowered to ≤100 mmol (6). Despite much research, however, several critical issues remain unanswered.

The Dose-Response Effect

The relationship between sodium intake and blood pressure may not be linear. In INTERSALT, four remote populations that had an average sodium intake ≤60 mmol all had low average blood pressure that did not rise with age (8). These four underdeveloped societies had blood pressures that were lower than that predicted from the relationship between sodium and blood pressure in the 48 populations with sodium intakes >100 mmol. In clinical studies, reducing sodium intake to very low levels (e.g., <25 mmol) has an exaggerated effect on blood pressure compared with reductions of equal magnitude but within a higher range (14-18). In the early 1950's, studies of the Kempner rice diet in moderate-to-severe hypertension found that a sodium intake <25 mmol was required for large, clinically relevant antihypertensive effects (14,15). Modest changes in blood pressure would not have been detected in the small samples of these studies. Results of later studies have also suggested greater than linear responsiveness of blood pressure to very low levels of dietary sodium, e.g., 9 mmol. Blood pressure decreased more when sodium was reduced from 109 to 9 mmol than from 240 to 109 mmol per day (17, 18). However, the findings regarding linearity of the response are not definitive because the sequence of sodium levels was not randomized and there was no formal statistical testing of the blood pressure differences. Cutler et al. (12) analyzed the results of 31 trials that met rigorous quality criteria and that studied a range of sodium intake (28 to 273 mmol) that was within the 95% limits of distribution of the US population. The results showed that a decrease in sodium intake corresponding to 100 mmol lowers blood pressure by 6.0/2.7 mm Hg in hypertensives, and by 2.3/1.4 mm Hg in non-hypertensives. Law et al. (11) found double this effect in a meta-analysis of 70 sodium reduction trials, many of which tested very low sodium intakes. Cutler et al. (12) pointed out that establishing a dose-response relationship

by meta-analysis is not as desirable as by a single, well-designed trial, since outlying trials in meta-analysis disproportionately affect regression results. The results of the Trials of Hypertension Prevention Phase I (TOHP-I) also provide circumstantial evidence for a proportionately greater response in a lower sodium range, since women had lower sodium excretions at baseline and on treatment than did men and also showed a greater reduction in blood pressure (19). However, these results could also be explained by greater responsiveness in women compared to men regardless of the sodium range. MacGregor et al. (20) reported the only well-controlled dose-response trial that assessed the effect of sodium within a clinically relevant range (50, 100, and 200 mmol). Twenty moderately hypertensive patients with mean baseline blood pressure of 162/107 mm Hg consumed a 50 mmol sodium diet supplemented by either sodium or placebo tablets. Compared to the 200 mmol sodium diet, blood pressure decreased by 8/3 mm Hg with 100 mmol and by an additional 10/6 mm Hg with 50 mmol. This apparent doubling of the dose-response in the 100 to 50 mmol range compared to the 200 to 100 mmol range suggests a log-linear rather than a linear relationship. There are no similar dose-response studies in persons with stage 1 hypertension or with normal blood pressure. There are also no dose-response studies of sodium provided as part of food, the customary mode of ingestion, rather than in tablet form. Current guidelines recommend lowering sodium to 100 mmol (6). The possibility of substantial additional blood pressure lowering by reducing sodium intake to 50 mmol appears promising and requires careful testing.

Effects of Dietary Sodium in African Americans, Women, and in Non-hypertensive Individuals
African Americans have a higher rate of hypertension and suffer disproportionately from its deleterious effects compared to other racial groups in the US. Sensitivity to dietary salt is thought to be a contributing factor (16, 21-25). Much still needs to be learned, however, about dietary salt in African Americans. Paradoxically, the greater sensitivity of African Americans to dietary salt has at times hampered acquisition of knowledge on this issue. For example, the otherwise useful analyses of epidemiological studies of sodium and blood pressure by Law and colleagues (9) excluded African American populations because the authors considered that it was established that African Americans differ in salt sensitivity and should not be included with other racial groups (9,10). Virtually no information exists from well-controlled trials on dose-response in African Americans, particularly within the clinically relevant range to be studied in DASH2. DASH2 will quantify the dose-response effect of sodium on blood pressure in African Americans as well as in non-African Americans.

Little information is available on blood pressure responses to sodium in women compared to men. INTERSALT data (26) and a meta-analysis of population data (27) suggest that lower sodium diets lower blood pressure more in women than in men. In TOHP-1, women had greater blood pressure lowering than men, although the lower range of sodium intake in women could have been responsible (19). Other clinical trial data are lacking on the effects of sodium on blood pressure in women.

Because the greatest public health impact of dietary intervention in lowering blood pressure for risk reduction occurs in the nonhypertensive segment of the population and because clinicians have particular interest in effects on blood pressure in persons with frank hypertension, the effects of sodium reduction and the DASH combination diet will be assessed according to hypertension status. The analyses will be conducted to address the mail DASH2 specific aims. These results will provide important information to help guide public health recommendations as well as treatment guidelines for individuals with high blood pressure.

Need For Controlled Feeding Studies Of Dietary Sodium
Controlled feeding: The controlled feeding approach successfully carried out in DASH provides a powerful means to assess the true biological effects of dietary sodium on blood pressure.

Sodium reduction in the clinical setting necessitates changes in the overall dietary pattern, since most of the salt that is ingested comes from food rather than the salt shaker (28). Small but significant changes in the intake of various nutrients have been reported in dietary sodium trials. In TOHP-I, for example, total fat and iron intake changed during sodium reduction (19). Statistical modeling in TOHP-I showed that blood pressure lowering was greater than predicted from the Na-blood pressure dose-response relation (19). This was interpreted to suggest that additional, but unidentified, factors in the low-sodium diet affected blood pressure. In the Dietary Intervention to Stop Hypertension (DISH) trial, increases in fiber, ascorbic acid, folic acid, iron, zinc, and other vitamins occurred as a result of the low-sodium intervention (29). In a Finnish trial, intake of several vitamins and minerals increased (30). Most dietary sodium trials that used foods rather than salt tablets did not control or even measure nutrient intake. In view of the considerable effects of dietary patterns on blood pressure found in DASH, it is highly desirable to control background nutrient intake while sodium intake is altered. Only this approach can address the first specific aim of this study, which is to determine the true effects of dietary sodium, excluding issues of compliance and potential effect modifiers.

Public health applicability: Adherence is a major obstacle not only for investigating dietary sodium reduction, but also for implementing clinical and public health recommendations. Since most dietary sodium comes from prepared foods rather than from adding salt during cooking or at the table, the success of sodium reduction in community-based clinical trials, e.g., TOHP I & II, is impeded by the lack of enough prepared foods that are low in sodium. Without such foods being available, studies fail to achieve goals for sodium reduction. However, food companies are reluctant to modify the sodium content of their products. Therefore, we consider that the controlled feeding approach is useful to break this "futile cycle". If sodium intake of ≈50 mmol substantially lowers BP, priorities for population guidelines and product development for controlling blood pressure could be better synchronized.

Potential Interactions Between Sodium and Other Nutrients in Effects on Blood Pressure
Few clinical trials have studied combinations of dietary sodium and nutritional modifications other than weight loss. Two studies suggest an interaction between sodium reduction and potassium supplementation. In one, the blood pressure lowering effect of potassium supplementation was attenuated at lower sodium intakes (31). Conversely, the hypertensive effect of sodium loading by intravenous administration was blunted when the patients were potassium replete rather than depleted (32). Calcium administration also may blunt the hypertensive effects of sodium loading (33). This interaction of sodium reduction with potassium supplementation and possibly also with calcium supplementation could apply to the DASH combination diet, which is high in both potassium and calcium.

It is more likely that the BP-lowering effects of dietary sodium reduction and the DASH combination diet are at least additive. If this is true, the potential for diet to lower blood pressure may be equivalent to or greater than that of low-dose diuretic therapy. Law et al. (9) found that the slope of the regression line of blood pressure on sodium intake was similar for "developed" and "undeveloped" communities throughout the adult age range. We consider this as evidence for the independence of the effects of sodium and other dietary components on blood pressure, since the diets in "undeveloped" and "developed" communities are quite different. The diets of non-industrialized populations usually have important components of the DASH combination diet. Finally, the blood pressures of four remote communities with very low sodium intakes were lower than expected based on the regression of blood pressure on sodium in developed communities having higher sodium intakes (8). This suggests additive effects for sodium and other aspects of the diet or lifestyle. Whatever the relationship is between sodium reduction and the DASH combination diet, the information will be important for public health recommendations. The potential for additive effects from lower sodium intake coupled with the

DASH combination dietary pattern could have major clinical and public health implications in the prevention and control of hypertension.

The DASH Combination Dietary Pattern and Blood Pressure

The DASH combination dietary pattern was developed from studies relating blood pressure to dietary differences among populations, from observational epidemiology, and from clinical trials of dietary patterns and individual nutrients. It is rich in fruits, vegetables, and low-fat foods, and low in saturated fat and total fat. It is also low in cholesterol, high in dietary fiber, potassium, calcium, and magnesium, and moderately high in protein. The diets meet the participants' energy requirements for maintaining stable weight. Alcoholic and caffeinated beverages are limited and monitored. It was originally tested with a salt content slightly below average American consumption. This section provides the rationale for this dietary pattern, summarizes its observed impact on blood pressure in the DASH trial, and discusses the implications of those findings for DASH2.

Epidemiology

Striking differences exist in the blood pressures of populations worldwide (34,8). In isolated non-industrialized societies, blood pressure levels are generally low and rise minimally with age compared to industrialized societies (34,35). Many such populations eat a diet predominantly composed of vegetable products (35). In industrialized countries, vegetarians have lower average blood pressure levels than do comparable nonvegetarian populations (36-38). Some of the lowest average blood pressures found in any society were in strict vegetarians in Massachusetts who consumed a low-fat diet virtually devoid of animal products of any kind (35,38). The difference in blood pressure between these vegetarians and two nearby nonvegetarian populations was 6-8 mm Hg systolic and 11-14 mm Hg diastolic after adjustment for differences in age, sex, and body weight. Lacto-ovo-vegetarians, whose diet was high in fat from dairy products, vegetable products, and eggs, had blood pressures intermediate between strict vegetarian and nonvegetarian groups (36). No intervention studies replicating the dietary contrast between strict vegetarians and nonvegetarians have been reported, and other variables besides the vegetarian dietary pattern could have contributed to the low blood pressure. Dietary trials of lacto-ovo-vegetarian diets found BP-lowering effects of 4-7 mm Hg systolic and 2-3 mm Hg diastolic (39,40).

Two large US cohort studies, one of women and the other of men, found that intakes of various vegetable products or fruits were inversely associated with blood pressure or with the change in blood pressure over time (41,42). In these populations, a dietary pattern that favored vegetable products and fruits, vegetable oil rather than animal fat, fish and chicken over red meat, and low-fat over full-fat diary products was associated with lower blood pressure (Ascherio et al., unpublished findings, 1996). Hypotheses regarding nutrients and blood pressure that are consistent with these findings can be divided into two broad categories: (1) minerals, fiber, or other dietary components in plant foods lower blood pressure, and (2) the amount, type, or combination of macronutrients, specifically fat and protein, influences blood pressure. In addition, nutrients found in dairy products may also reduce blood pressure.

Studies of Specific Nutrients

Minerals and fiber: Many observational epidemiologic studies have demonstrated significant associations between blood pressure and minerals such as potassium (8,43), calcium (44,45), and magnesium (46,47). In addition, two large cohort studies, one of women and the other of men, found that intakes of all three minerals, as well as of fiber, were inversely related to blood pressure, to change in blood pressure over time, or to the risk of developing hypertension (41,42). Meta-analyses that considered trials of potassium supplementation (48,49)

documented a significant blood pressure lowering effect that was stronger in hypertensive than in non-hypertensive participants. Meta-analyses of calcium supplementation (44,50,51) showed a small antihypertensive effect for systolic blood pressure but not for diastolic blood pressure. Trials of magnesium supplementation (47,52-54), taken together, show no overall blood pressure-lowering effect. The large, well-designed Trials of Hypertension Prevention (55), which had sufficient power to detect a reduction in diastolic blood pressure of 1.5 mm Hg, found borderline effects for potassium supplements, but none for calcium or magnesium supplements, in non-hypertensive adults. For fiber, only small-scale trials are available, and they do not show significant effects on blood pressure (56,57). These fiber studies have not been powered to detect blood pressure effects of $\leq$ 3 mm Hg, however. The contrast between the generally negative results from clinical trials of single nutrients and the positive results from observational studies provided some of the scientific underpinning for the original DASH study.

Fat: A review of cross-sectional and prospective observational studies on dietary fat and blood pressure has reported that generally no significant relationships between dietary fat or cholesterol and blood pressure have been found (58). More recently, analyses of MRFIT data indicated independent direct associations of saturated fat and dietary cholesterol and inverse associations of polyunsatursated-to-saturated fat ratio with blood pressure (63, 64). Unpublished data from the Western Electric cohort also suggested a direct relationship of dietary cholesterol at baseline and ten-year change in blood pressure (58a). Trials testing the effects of changes in intake of fatty acids (including saturated, monounsaturated and polyunsaturated [linoleic acid]) and carbohydrates on blood pressure have also been reviewed (58). With one exception (30), trials that substituted carbohydrates or unsaturated fats for animal fat did not lower blood pressure. Although most of the trials were small and did not have the power to detect changes in blood pressure less than 3-5 mm Hg, their point estimates of the effects were very close to zero. However, no well-controlled study having sufficient statistical power to detect small but clinically important blood pressure changes (e.g., 2 mm Hg) has tested an overall dietary fat pattern that was reduced in total and saturated fat, had a high polyunsaturated to saturated fat (P/S) ratio, and was low in cholesterol.

Protein: Epidemiologic studies of stroke in Japanese populations (59,60) indicate that a high dietary protein intake is protective, and a recent review of protein and blood pressure suggests that protein is inversely related to blood pressure (61). Significant inverse relationships between dietary protein (or urinary nitrogen as a surrogate for dietary protein intake) and blood pressure have been found in two large studies, INTERSALT (62) and the Multiple Risk Factor Intervention Study (63,64), in several preliminary reports of observational studies (65-68), and in unpublished analyses of other cohort studies (Nurses' Health Study and Health Professionals Follow-up Study [personal communication, A Ascherio, 1996]). In addition, these studies have found a complex association of protein intake with many other nutrients that could have blood-pressure-lowering properties.

Rationale for Studying Dietary Patterns

The evidence that multiple dietary factors, either characterized by foods or specific nutrients, affect blood pressure contributed strongly to the rationale for the DASH trial to specify dietary patterns, rather than specific nutrients, as the type of intervention (69). Several theoretical reasons were considered. First, the blood pressure-lowering effects of single nutrients may be too small to detect in small-scale clinical trials. Second, when several nutrients, such as certain minerals and fiber, are consumed together, as in observational studies and trials of vegetarian diets, their additive effects may be sufficiently large to be detectable. Third, interactions could exist among nutrients to amplify the effect of combinations. Fourth, untested or unknown nutrients in plant foods may lower blood pressure. Fifth, nutrient supplements may not affect blood pressure to the same extent as do the same nutrients occurring naturally in foods.

Finally, a test of dietary patterns would parallel more closely the observational studies, where dietary intakes consist of nutrients that occur together in common foods. Because of these possibilities, the DASH trial tested the BP-lowering effect of dietary patterns as opposed to individual nutrients or nutritional supplements.

The DASH Trial
Based on these considerations, the DASH trial compared the effects on blood pressure of two dietary patterns. DASH enrolled 459 adults, age 22 years and older, with systolic blood pressures less than 160 mm Hg and diastolic blood pressures of 80 to 95 mm Hg. For three weeks participants were fed a control diet similar to what many Americans generally eat. The participants were then randomized to receive for eight weeks the control diet, a diet rich in fruits and vegetables, or a combination diet rich in fruits, vegetables, and low-fat dairy foods and reduced in saturated fat, total fat, and cholesterol. Sodium intake and body weight were maintained at constant levels. Salt levels were slightly below average American consumption, and alcoholic and caffeinated beverage intakes were limited. Compared to the control diet, the combination diet reduced systolic and diastolic blood pressure by 5.5 and 3.0 mm Hg and the fruit and vegetables diet reduced systolic and diastolic blood pressure by 2.8 and 1.1 mg Hg (7). These results suggest that nutrients such as potassium, magnesium, and fiber, which are associated with a high intake of fruits and vegetables, are effective in lowering blood pressure, and that additional blood pressure lowering is produced by other components of the combination diet, such as low-fat dairy foods, that reduce saturated fat, total fat, and cholesterol and increase calcium and protein (7).

The results of DASH also suggest that a study of sodium reduction with the DASH combination diet has the potential, through additivity, to cause major beneficial blood pressure effects. For example, if the blood pressure lowering effects of the DASH combination diet and of sodium reduction are additive, we would expect a total effect of as much as -10/-6 mm Hg in a controlled dietary experiment. Fifty percent adherence in a community setting could produce an effect that is considered clinically important (e.g., -5/-3 mm Hg) and that is greater than those typically seen in well-controlled, long-term weight-loss studies. These hypothetical effects are clinically important, and the need to test them is essential.

The DASH2 Trial Primary and Secondary Outcome Variables

Unlike DASH, where the primary outcome was diastolic blood pressure and systolic blood pressure was secondary, the primary outcome in DASH2 is systolic blood pressure, with diastolic and 24-hour SBP secondary. Although systolic and diastolic blood pressure are highly correlated with each other, systolic blood pressure was chosen as the primary outcome because large epidemiologic studies have found that systolic blood pressure is a better predictor than diastolic for cardiovascular disease (70,71).

Rationale for Other Objectives

Baseline Characteristics
Because of evidence that the blood pressure response to sodium intake is greater in African Americans than in non-African Americans (21-25) and greater in women than in men (19,26,27), we designed DASH2 to test Specific Aim #1 separately by race and sex. Because of the public health importance of the potential for risk reduction in nonhypertensives as well as hypertensives, the blood pressure response to reduction in sodium intake will be studied separately in the hypertensives and non-hypertensive participants in DASH2. Secondary analyses of this and the other specific aims for subgroups defined by sex, race, baseline blood

pressure, age, education, body mass index, waist circumference, and diet prior to entry into the study will enhance the generalizability of the results. For example, DASH found that the blood pressure lowering effects of the combination diet were present in a broad array of subgroups and were significantly greater in hypertensive participants than in non-hypertensive participants (7). The effect of age on blood pressure response to diet will be examined as it has been reported that older people have greater blood pressure responsiveness to salt intake than younger people (13a). Waist circumference is used as an indicator of central adiposity (72,73). Growing evidence indicates that centrally located adiposity is associated with carbohydrate metabolism abnormalities, hyperlipidemia, and hypertension (74,75). In DASH2, we expect centrally located fat to be associated with higher blood pressure for any given level of body fat, and increased central fat may enhance the blood pressure lowering effects of the intervention diets (75).

Ambulatory Blood Pressure Monitoring (ABPM)
Similar to DASH, mean waking, sleeping, and 24-hour blood pressures as measured by 24-hour ambulatory blood pressure monitoring (ABPM) will be measured to determine the effects of our dietary interventions on diurnal patterns of blood pressure in DASH2. ABPM has been used extensively in trials of antihypertensive medications, but not in trials of non-pharmacologic measures. No information exists on how variation in salt intake affects blood pressure during waking and during sleep, or on major aspects of the diurnal cycle of blood pressure change. In the DASH study, the fruits and vegetables diet and the combination diet reduced mean 24-hour, waking, and sleeping blood pressure. Also, we found that the blood pressure effect size of the DASH diets measured by ABPM correlated well with the blood pressure effect size measured by random zero sphygmomanometer. The additional information provided by ABPM particularly in relation to sodium intake, will be equally valuable in DASH2.

ABPM provides unique information pertaining to the pattern of blood pressure over a 24 hour period. Such information may have prognostic relevance. Typically, blood pressure is higher during awake hours and then falls during sleep. In cross-sectional analyses, those persons with less of a reduction during sleep (so called “non-dippers”) tend to have higher left ventricular mass. ABPM may also be more highly associated than clinic measurements with cardiovascular disease events, although at present the literature is inconsistent on this issue. In DASH2, we plan to examine the impact of the DASH diet and sodium levels on the difference between waking and sleeping blood pressures and on other selected areas of the ABPM profile, including peak and minimum levels of ambulatory blood pressure.

Plasma Lipids
It is anticipated that the combination diet will significantly lower blood lipids, thus serving as a measure of dietary compliance and also providing evidence for other cardiovascular benefits of the diet. In addition, because of reports suggesting that very low sodium diets raise total and LDL-C and triglycerides (76), it will be important to determine whether sodium intakes at the upper limit of current recommendations (100 mmol) or at potentially optimal (50 mmol) levels have putatively adverse effects on plasma lipids, with either the control or the combination diet, or both.

Plasma Renin
Hypertensive individuals display a broader range of plasma renin levels than do non-hypertensive individuals (77). Laragh (78) found that hypertensives with low renin levels (about 20% of essential hypertensives) responded especially well to “volume” treatment, i.e., diuretics, while those with high renin levels responded to beta-blockers, which reduce renin levels. These findings formed the basis for the volume-vasoconstrictor theory of hypertension pathophysiology (78). Of particular relevance to DASH2, low renin levels are reportedly more

common in African Americans and older persons, also populations with greater salt sensitivity. Thus, the renin status of DASH2 participants may be a confounder for the blood pressure response to salt intake. In addition, Resnick et al. (79) reported that low renin individuals have a greater antihypertensive response to calcium supplementation, suggesting that the response to the DASH combination diet itself may be affected by renin status. Based on these considerations, we will categorize renin status in DASH2 by measuring plasma renin activity at the end of the 50 mmol sodium diet period. We have chosen this level of sodium because renin levels will be maximally stimulated, providing the clearest definition of low renin participants (80,81).

Biological Storage Samples
Numerous endogenous vasoactive substances and candidate genes related to blood pressure or cardiovascular risk may influence blood pressure response to the DASH combination diet and to sodium reduction. To facilitate future investigation of biological variables (e.g., plasma renin and angiotensin) and candidate genes (e.g., angiotensinogen), we will store plasma, serum, and 24-hour urine specimens taken at baseline (screening) and at the end of each feeding period, and buffy coat specimens taken once during intervention.

4. Study Design

DASH2 is a multicenter, randomized clinical trial designed to compare the effects of three levels of sodium intake and two dietary patterns on blood pressure among persons with higher than optimal blood pressure or with Stage 1 hypertension (up through 95 mm Hg diastolic). The two dietary patterns are a control diet that is typical of what many Americans eat and a combination diet. Participants are randomly assigned to one of these two dietary patterns using a parallel group design, and are fed each of three sodium levels using a randomized crossover design. Collaborating on this trial are four clinical centers, a Coordinating Center, a central food analysis laboratory, and the National Heart, Lung, and Blood Institute.

Study participants are 400 adults, aged 22 and older, with systolic blood pressure between 120 and 159 mm Hg and diastolic blood pressure between 80 and 95mm Hg inclusive. Individuals who are currently taking antihypertensive medications are not eligible to participate. After attending a series of three screening visits to determine eligibility and collect baseline data, participants begin a two-week run-in feeding period using the control diet at the higher sodium level (Figure 1). This is followed within three days by three 30-day intervention feeding periods, one at each of the three sodium levels in random order. Randomization to combination or control diet occurs during the second week of run-in feeding.

The study provides participants with all of their food during the run-in and intervention feeding periods, although participants resume their normal diets for up to five days between each of the feeding periods. During the controlled feeding periods, participants are required to attend the clinic for at least one meal per day, five days per week, and to take home food to eat for their other meals. Weight is monitored and individual energy intake adjusted so that participants neither gain nor lose weight during the study. Clinics deliver the interventions in four successive cohorts, with approximately 25 randomized participants per cohort, over a two-year period.

The primary outcome variable for each of the trial's specific aims is systolic blood pressure. The study also compares the impact of the diets on diastolic blood pressure and on 24-hour, waking, and sleeping ambulatory pressure.

5. Eligibility

Table 1 presents the eligibility criteria for the study. Any initially abnormal laboratory values that would result in exclusion may, at the discretion of the local Principal Investigator (PI), be repeated once and the participant retained if the second value falls within eligible limits. Exceptions to this rule are for renal insufficiency, elevated blood sugar, and hyperlipidemia and are noted in the table. Repeat testing for these conditions requires an alternative measure to that used for the initial assessment (Table 1). All laboratory assessments for eligibility are performed locally and eligibility is based on local normal ranges. Eligibility criteria were selected to exclude individuals with conditions, special dietary requirements, or taking medications, that would affect blood pressure or micronutrient metabolism and individuals with potentially serious chronic health conditions.

Table 1. DASH2 Eligibility Criteria

Inclusion Criteria

- SBP 120-159 mm Hg and DBP 80-95 mm Hg based on mean values over three screening visits
- Age ≥ 22 years
- Willing to eat at least one on-site meal/day, five days/week, and willing to eat study diets and nothing else for the 15 weeks of controlled feeding
- Willing to provide informed consent

Exclusion Criteria

Medical Conditions:

- Any serious illness not otherwise specified that would interfere with participation
- Currently on cancer chemotherapy or with evidence of active malignancy or radiation therapy within past six months
- Hematocrit at least 5 percentage points below the local laboratory's gender specific normal range (unless PI has reason to believe this is not due to nutritional deficiency)
- History of CVD event (MI, CABG, angioplasty, symptomatic ischemic heart disease, or stroke)
- Clinical diagnosis of congestive heart failure
- Inflammatory bowel disease, colostomy, malabsorption, or any prior GI resections other than localized colonic resections
- serum transaminase > 2 times the local laboratory's upper range of normal, or a clinical diagnosis of hepatitis as determined locally
- Emergency room visit or hospital stay for asthma or COPD in last six months, or other evidence of recent instability in asthma or COPD
- Renal insufficiency as determined by a serum creatinine > 1.2 mg/dL for women or > 1.5 mg/dL for men. Participants rejected by this criteria can be retained if the glomerular filtration rate, as estimated by the Cockrault-Gault formula, is ≥ 60 ml/min
- Hypo- or hypercalcemia (serum Ca >0.3 mg/dL above or below local laboratory normal range)
- Hypo- or hyperkalemia (serum K >0.2 mg/dL above or below local laboratory normal range)
- Urine dipstick protein ≥ 2+
- Random glucose ≥180 mg/dL or positive urine dipstick for glucose; repeat testing may include fasting blood sugar (FBS) or HgbA1C. For FBS, exclude if ≥140 mg/dL. For HgbA1C, exclude if ≥8 (or local lab equivalent to an average blood sugar ≥200 mg/dL)
- Body mass index > 40 Kg/m^2
- DASH2 staff or household member of DASH2 staff

Medications:

—use of blood pressure lowering drugs within the last three months
—lithium
—insulin
—oral corticosteroids
—unstable doses of psychotropics or phenothiazines
—cholestyramine
—colestipol
—unstable doses of statins or other lipid lowering agents not already excluded
—oral breathing medications
—dilantin
—antacids containing magnesium or calcium, unless they can be discontinued
—digitalis
—weight reducing medications
— OTC medications or other consumer products providing 3 or more mmol of sodium per serving, unless they can be discontinued

Other Exclusion Criteria:

— Given the trial's CVD exclusions, hyperlipidemia carries less risk than it did in DASH. Exclude if total cholesterol >260 mg/dL. Repeat testing based on LDL-C (determined either directly from a nonfasting blood sample or computed from a fasting blood sample). If this would require pharmacotherapy according to NCEP(82), then exclude. The NCEP guidelines to initiate pharmacotherapy for LDL cholesterol are:
  - $\geq$ 220 mg/dL for young adults (men under 35 and premenopausal women) without 2+ CVD risk factors,
  - $\geq$190 mg/dL for older individuals without 2+ CVD risk factors, and
  - $\geq$ 160 mg/dL for individuals with 2+ CVD risk factors.

—Consumption of more than 14 alcoholic drinks per week
—Investigator discretion for safety or compliance reasons
—Inability to provide reliable blood pressure measurements
—Current use of vitamin or mineral supplements or salt substitutes that cannot be stopped
— Use of chewing tobacco, snuff, or other smokeless tobacco products
—Planning to leave the area prior to the anticipated end of the intervention period
—Pregnant, planning a pregnancy prior to the end of intervention, or breast feeding
—Significant food allergies, preferences, or dietary requirements that would interfere with diet adherence

6. Recruitment and Screening

Study Sample

The study sample consists of approximately 400 healthy, free-living adult men and women, age 22 years and older, who have a SBP of 120-159 mm Hg and a DBP of 80-95 mm Hg . Given the disproportionate burden of hypertension and its complications among African Americans, one-half of DASH2 participants are of African American background and dietary sodium effects will be examined separately by race. We also include equal numbers of men and women to study the dietary sodium effects separately for each gender. Hypertensives and non-hypertensives are also examined separately because of the public health and medical care implications associated with each subgroup. Approximately 30 percent hypertensives and 70 percent nonhypertensives will be recruited. The unequal split between hypertensives and non-hypertensives reflects the strong evidence in the literature that the effects of salt reduction are more pronounced in hypertensives than in non-hypertensives (9, 11, 12), and hence provides reasonable power to study effects in both hypertensive and non-hypertensive subgroups.

Recruitment

Each DASH2 clinical center recruits its participants in four separate feeding cohorts. Specific recruitment approaches include 1) targeted mailings to specific groups (e.g., employees of local industries, previous screenees), 2) mass mailings (e.g., vis-à-vis inserts in coupon packs and brochures to registered voters or licensed drivers), 3) community and work-site screenings, 4) and mass media advertising (e.g., radio and television advertisements and public service announcements).

Recruitment efforts at each site are broad-based. Although previous DASH participants are not excluded from participation in DASH2, recruitment is not focused on these individuals and the number who do enroll in DASH2 is monitored.

Each center has a recruitment coordinator who oversees recruitment efforts and serves on the DASH2 Recruitment Subcommittee. The recruitment coordinator is the primary liaison with the Coordinating Center for issues related to recruitment. The Coordinating Center monitors recruitment activities and facilitates recruitment efforts by providing regular recruitment reports and organizing conference calls. It also facilitates brochure development and prepares other recruitment materials for common use at the clinical sites.

Screening

Participants must complete a prescreening evaluation, three formal screening visits, and a run-in feeding period in order to be randomized (Figure 1). Each screening visit includes questions and procedures designed to determine eligibility for the trial. The run-in period is designed to identify and exclude those individuals not likely to comply with the DASH2 dietary requirements and to verify the caloric level needed to maintain weight. Table 2 summarizes the content of each screening visit as well as the procedures done during run-in and intervention.

The sequence and timing of data collection during the screening visits is designed to maximize efficiency in excluding ineligible participants. Information obtained out-of-sequence may, however, still be used to exclude ineligible participants. For instance, if a screenee mentions during the pre-screening visit a medical condition that is exclusionary, the screenee should be

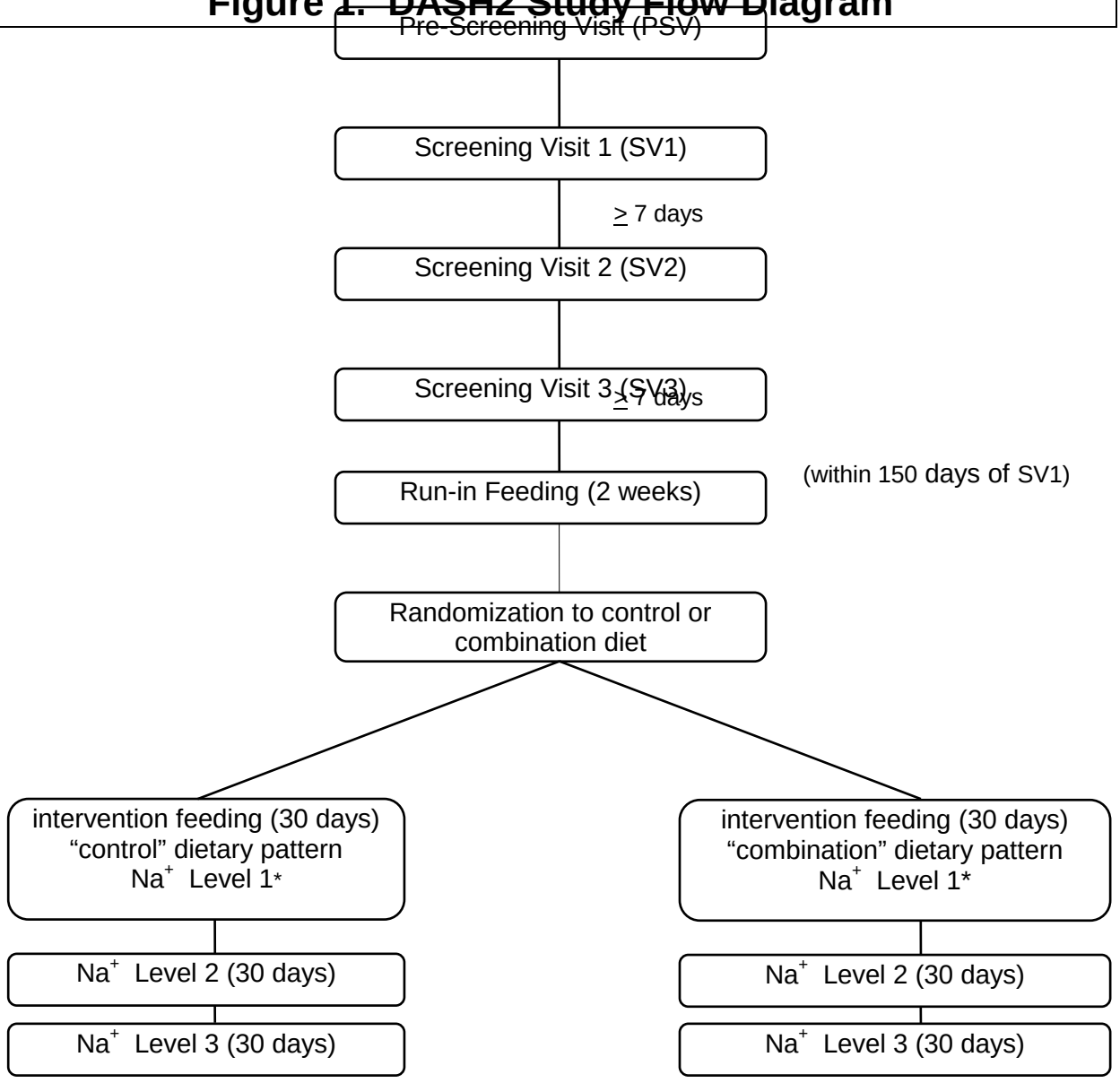
excluded from further participation even though medical eligibility is normally not reviewed until a later visit.

Pre-Screening Visit (PSV)
The PSV may take place at the clinical center (e.g., coincident with the initial screening visit), via telephone, or at a location in the community convenient to the people being recruited. The PSV is intended as a fast, efficient way to eliminate ineligible participants prior to the formal screening visits. The visit includes questionnaire data for exclusion and a single, optional blood pressure measurement for exclusion. Individuals who complete the PSV are either excluded from further participation or scheduled for SV1. If more than 120 days elapse between the PSV and SV1, the PSV must be repeated as part of SV1. As with all screening visits, screening may be stopped as soon as the participant is determined to be ineligible.

No eligibility limits are established for the PSV blood pressure measurement, although it is recommended that individuals with a SBP value less than 116 mm Hg or a DBP value less than 76 mm Hg be excluded. Those who meet the PSV eligibility criteria are scheduled for SV1, which may occur immediately.

Other than for basic demographic information (e.g., gender and race), data collected at PSV are not considered study data and are not incorporated into the study database. The demographic data are entered only for participants who are eligible to continue on to SV1.

Figure 1. DASH2 Study Flow Diagram



* sodium levels assigned in random

Table 2. DASH2 Activity Sequence												
	PSV	**SV 1**	**SV 2**	**SV 3**	**1 week prior to RI**	**RI Week 1**	**RI Week 2**	**INT Week 1 ***	**INT Week 2 ***	**INT Week 3 ***	**INT Week 4 ***	**Close-out**
Event												
PSV Eligibility Questionnaire	X											
Standard Blood Pressure	X											
RZ Blood Pressure		X	X	X		X	X	X	X	X	X	
24-hour ABPM											X	
Eligibility Questionnaire		<==X==>										
General Dietary Info. Questionnaire		X										
Weight		<==X==>		X		X	X	X	X	X	X	
Height		<==X==>										
Urine Dipstick Protein/Glucose			X									
Collect Non-fasting Blood Spec.			X									
Distrib. 24-hr Urine Supplies			X								X	
Instructions on Food Freq. Ques.			X									
Review Food Freq. Ques.				X								
Physical Activity Recall				X								
Brief Physical Activity Question.				X							X	
Review Eligibility Labs				X								
Review Study Food Checklist				X								
Review DASH Menus				X								
Process 24-hour Urine Specimen				X							X	
Collect Fasting Blood Specimen				X							X	

Table 2. DASH2 Activity Sequence - Part 2												
	PSV	**SV 1**	**SV 2**	**SV 3**	**1 week prior to RI**	**RI Week 1**	**RI Week 2**	**INT Week 1 ***	**INT Week 2 ***	**INT Week 3 ***	**INT Week 4 ***	**Close-out**
Event												
Symptoms Questionnaire				X			X				X	
Eligibility Review					X							
Run-in Feeding Activities						X	X					
Patient History Questionnaire						X						
Waist circumference						X						
Medication Questionnaire							X				X	
Salt Acceptability							X	X			X	
Randomization							X					
Intervention Feeding Activities								X	X	X	X	
Compliance Monitoring						X	X	X	X	X	X	
Anonymous Compliance Ques											X	
Closeout, Post Study Survey												X
PSV=Prescreening Visit	***SV=Screening Visit***				***RI=Run-in***			***INT=Intervention***				

* 3 separate 30-day intervention feeding periods

Table 3. DASH2 Blood Pressure Eligibility Criteria

PSV	SBP	≥76mm Hg[1]
	DBP	≥116 mm Hg[1]
SV1	SBP	118-170
	DBP	78-100
SV2[2]	SBP	119-165
	DBP	79-98
SV3[3]	SBP	120-159
	DBP	80-95

[1] this is an optional limit
[2] average of SV1 and SV2 measurements
[3] average of SV1, SV2, and SV3 measurements

Screening Visit 1 (SV1)
As with the PSV, SV1 is intended as a brief visit to identify major exclusionary criteria at minimal expense. SV1 must occur within 120 days of the PSV. If more than 120 days has elapsed, the PSV must be repeated. At a minimum, the visit should include blood pressure and a review of general dietary information concerning the trial. Both the general eligibility review and body mass eligibility may be assessed at this visit. If the general eligibility review is not completed at this visit, the participant should receive instructions for completing the eligibility questionnaire at home so that it can be reviewed at SV2. The blood pressure eligibility cutpoints are listed in Table 3 and are based on the average of the two SV1 blood pressure measurements.

All DASH2 participants must provide written, informed consent for screening visits, run-in and intervention. The number and timing of these consents is determined by the local Institutional Review Boards and is not included in this description of the screening visits.

Screening Visit 2 (SV2)
SV2 must occur at least seven days after SV1. The visit includes: blood pressure assessment; a nonfasting blood sample for eligibility (cholesterol, creatinine, blood sugar, transaminase, Ca, K); urine dipstick for protein and glucose; instructions for completing the food frequency questionnaire; and instructions and supplies for a 24-hour urine collection. If not already done as part of the SV1 visit, the SV2 visit must also include measurement of height and weight (for determination of body mass eligibility) and a review of the eligibility questionnaire. The SV2 blood pressure eligibility is determined by averaging the two blood pressures from SV1 and the two blood pressures from SV2 (four in all). The blood pressure eligibility cutpoints for SV2 are listed in Table 3.

Screening Visit 3 (SV3)
SV3 must occur at least seven days after SV2. The visit includes: blood pressure measurement; weight; a follow-up review of questions from the eligibility questionnaire (if needed); a review of DASH2 menus and specific food items; a review of the food frequency questionnaire; processing of the 24-hour urine specimen, which is analyzed centrally for Na, K, Ca, phosphorus, urea nitrogen, and creatinine; a fasting blood draw that is analyzed centrally for lipids; and completion of physical activity and GI symptom questionnaires. The 24-hour urine and fasting blood specimens are also processed for long-term storage for future analyses. The blood is separated into serum, plasma, and buffy coat.

Laboratory results from the SV2 blood draw should be reviewed prior to SV3 in order to determine if additional blood work (either fasting or nonfasting) is needed. It is not necessary to collect repeat eligibility bloods at SV3; such blood draws may occur either before or after SV3. Participants may not begin run-in feeding, however, until they have met all laboratory eligibility requirements.

SV3 blood pressure eligibility is based on the average of the six blood pressure measurements taken at SV1, SV2, and SV3. Table 3 lists the blood pressure eligibility cutpoints for SV3.

7. Run-In and Randomization

Run-In

All participants who are eligible based on the three screening visits undergo a run-in period on the control diet (at the higher sodium level) prior to randomization. The higher sodium level was chosen because it is most reflective of current intake and because, if a participant is going to reach a blood pressure escape level during the study, it is most likely to occur at this level of sodium intake.

The run-in phase has two main objectives: 1) to identify and exclude individuals who will not comply with the eating requirements of the trial; and 2) to determine the appropriate caloric level for each participant that is needed to maintain weight. Initial caloric requirements for the start of run-in feeding are calculated using the WHO equation (83) to estimate resting energy expenditure and an activity factor, derived from the Stanford 7-day physical activity questionnaire(84), previously used in DASH, that is used to obtain an estimate of total energy requirements.

Run-in feeding must begin within 150 days after the completion of SV1; no minimum length is required between SV3 and the start of run-in, however. In addition, all laboratory eligibility criteria must be met prior to the start of run-in, and medical eligibility and medication use must be re-affirmed within one week prior to the start of run-in feeding.

During the run-in period, participants receive all of their food from the clinic and are required to attend the clinic for at least one meal per day, five days per week, for lunch or dinner, if possible. For logistical reasons, the clinical centers conduct feeding in four successive cohorts, with roughly 25 randomized participants per cohort over a period of two years. In order to allow for dropouts and exclusions during the run-in phase, clinical centers attempt to recruit 30 participants for the start of each feeding cohort. Run-in feeding is scheduled to start on the same day for all participants in a given feeding cohort at a given clinical center. This date may vary across centers, however. Also, participants may be allowed to enter the run-in feeding up to two days late if the clinical center determines this is due to exceptional circumstances not likely to affect future compliance. In this latter case, the length of run-in feeding for those participants is shortened so that all participants finish run-in feeding on the same day.

The total duration of run-in feeding can be 12-14 days, so long as it is the same for all participants in a specific feeding cohort at any given clinical center. Table 2 summarizes the information collected during this time. Information on medical and social history issues relevant to the trial (e.g., family history of hypertension, level of education, and socioeconomic status) is collected during the run-in period in the Patient History Questionnaire and is included as part of the study database. Weight is recorded at each clinic visit and blood pressure (sets of two) is

assessed once each week. Waist circumference is measured, and the participants are surveyed regarding symptoms and medication use.

In addition to the exclusion criteria applied during the screening visits, participants may be excluded prior to randomization for unusually large weight swings or for noncompliance with the protocol. All participants whose weight changes by five percent or more between SV3 and the first day of run-in are excluded from the trial at that point. The average of the SV3 weight and the weights measured on the first two full days of run-in feeding the participant's *target* weight and is used as the reference against which to assess weight change during run-in feeding. The overall caloric content of the participant's meals is adjusted as needed in order to assure that the participant's weight remains stable.

Participants may also be excluded prior to randomization for missed meals, poor clinic attendance, and over- or under-consumption of food. Finally, clinical centers subjectively evaluate each participant's overall compliance and attitude just prior to randomization and may exclude participants on the basis of this assessment as well. A more detailed discussion of compliance assessment is provided in section 11 (Dietary Compliance).

Participants who exhibit non-compliant behavior during run-in feeding, but after randomization, are retained in the study. However, randomized participants who drop out of the study before starting any of the intervention feeding intervals are replaced provided that they have no knowledge concerning their randomization assignment.

Randomization

Randomization occurs during the second week and prior to the end of run-in feeding. The timing of randomization may vary from cohort to cohort, provided that it occurs at least nine days after the start of run-in feeding and at least three working days prior to the start of intervention feeding. The former criterion permits adequate assessment of dietary compliance, while the latter assures that the kitchen staff have adequate time to assemble and prepare the foods that are needed for the start of intervention feeding (they need to know each participant's dietary assignment). Within a feeding cohort at any given site, however, all participants are randomized at the same time.

Following randomization, participants remain on the run-in diet until the run-in period ends. Participants are not told to which group they have been assigned and, except for staff involved in meal preparation, clinic personnel are also blinded to intervention assignment. Blinding is discussed further in section 16 (Quality Control and Data Management).

Randomization is stratified by clinic and, within each clinic, structured to assure comparable treatment group sizes over time with respect to both dietary pattern assignment and the sequence in which the sodium levels are administered. Although randomization is not stratified by gender, race, or baseline hypertensive status, the distribution of these factors across the treatment groups is monitored to assure that a balanced distribution is achieved.

8. Intervention

The total three-month intervention period is divided into three separate one month (30 day) feeding periods, each at a different level of sodium intake. For each participant, the underlying dietary pattern, control or combination, is the same for all three 30-day intervention feeding periods.

Feeding Breaks

The initial intervention feeding period begins from 0-3 days after the scheduled end of run-in feeding. This could therefore be anywhere from 12-17 days from the start of run-in feeding. Subsequent intervention feeding periods may be separated by breaks of up to 5 days in duration. These breaks are not mandatory, and their duration may vary from site-to-site, and from cohort-to-cohort. Within a given cohort at a given site, however, all participants follow the same feeding schedule. During the breaks between feeding periods, participants are not provided any food and are allowed to return to their original diets. No specific guidelines are provided to participants for what they should eat during these breaks in feeding.

The breaks between feeding periods are intended to serve several purposes. First, it is hoped that they will boost overall adherence to the feeding regimen and retention in the trial by breaking the overall feeding period (total of 102-104 days) into a series of smaller feeding intervals with which the participants can better comply. Second, the breaks provide the clinical centers with some flexibility in scheduling that should minimize the need to feed participants over major holidays, when compliance is most at risk. And finally, the breaks should help minimize the fatigue factor for clinical center staff and should also allow them to restock food supplies in a less rushed atmosphere.

The breaks between feeding periods are not intended as “washout” periods. In a drug trial, such intervals truly can, in many cases, permit washout of the experimental agent from a participant’s system, thus minimizing the likelihood of carryover effects. The potential for carryover effects is unavoidable in this trial, however, since the experimental agent is one’s diet and participants must eat something during these intervals. If anything, using breaks between the three intervention feeding periods is likely to increase the variability of response somewhat, since the foods consumed during these breaks can and will vary arbitrarily from person to person.

Ultimately the real issue is not washout, but how long the intervention feeding periods need to be for whatever carryover effects are present to become negligible. The experience from the DASH trial (7) suggests that the impact of the dietary patterns on blood pressure (at least in the context of a controlled feeding trial and at sodium levels comparable to those used in the DASH2 higher sodium diets) is achieved very quickly (within two weeks), so that even if carryover is an issue, its effect is likely to be minimal for blood pressure measurements taken during the final 9 days of intervention feeding.

A four-week duration was chosen for the sodium periods. This is justified by several individual trials and by meta-analyses. Two trials conducted by MacGregor et al, 1982 and 1989 (20, 85) provide the best evidence from individual trials that the effects on the blood pressure of changes in sodium intake are fully established by 4 weeks. In the first trial (85), reduction in sodium intake from 191 to 83 mmol reduced blood pressure by 13/10 mm Hg after 2 weeks, and 13/7 at 4 weeks. The addition of 75 mmol sodium tablets raised blood pressure by 5/5 at 2 weeks, and 8/5 at 4 weeks. The average effects at each time point were identical. In the second trial (20), reduction in sodium intake for 4 weeks produced the same blood pressure lowering as did 1 year of treatment. Chalmers et al (86) found that decreases in blood pressure were stable within 1 mm Hg during 2-6 weeks after reducing sodium intake. The same trial found that raising sodium intake increased blood pressure slightly more at 2 weeks than it did at 4 or 6 weeks. Meta-analyses by Cutler et al (12) and by Midgley et al (12a) both found that a longer duration of treatment was not associated with greater reduction in blood pressure. In the Cutler analysis, similar blood pressure effects were found in crossover trials of 1 or 2 months’ duration. These effects, moreover, were similar to those in parallel group trials

which were longer in average duration. In contrast, the meta-analyses of Law et al (11) found that durations of 2-4 weeks produced less blood pressure lowering than those at 5-8 weeks. However, the effects were not adjusted for hypertension status of the study participants, which varied for each time interval. The direction of the bias is toward less effect in the shorter trials. The mean effect on systolic blood pressure observed in the eight 4-week trials was 4.8 mm Hg. This effect size is consistent with the overall effects found in hypertensives and greater than those found in non-hypertensives in the other meta-analyses. This is also compatible with the effect sizes used in the power calculations for DASH2. In conclusion, the preponderance of evidence indicates that the effects on blood pressure of reduced sodium intake are established rapidly and that a 4-week duration is sufficient to establish a new steady state having a magnitude of reduction that is planned for in DASH2.

Procedures

During each intervention feeding period participants receive all of their food from the clinic and eat at least one on-site meal per day, five days per week. As with the run-in feeding, the on-site meal should, if possible, be lunch or dinner.

Weight is recorded at each clinic visit during the intervention feeding periods. Blood pressure is assessed weekly during the first three weeks of each intervention feeding period. Five blood pressure measurements are recorded during the final nine days of each intervention feeding period, and at least two of these must occur during the final four days of feeding. All blood pressure assessments consist of a set of two random zero measurements. A 24-hour ABPM reading is also recorded during the final nine days of each intervention feeding period. Additional intervention measurements taken during the last nine days of intervention feeding include: a 24-hour urine (for analysis of Na, K, Ca, phosphorus, urea nitrogen, and creatinine for group analyses) and a fasting blood collection for measurement of lipid levels. Fasting blood samples are also obtained for renin determination, but only the samples obtained during the lower sodium level feeding period are actually analyzed. (The other samples are collected to preserve blinding among clinic staff.) Samples of urine, serum, and plasma, will be frozen and stored for future analysis. Medication use and symptom monitoring, an anonymous compliance survey, and a brief assessment of physical activity occur during the final seven days of each intervention feeding period.

All randomized participants complete an evaluation at the end of the third intervention feeding period summarizing their overall experience with the trial.

The average of all weight measurements recorded during run-in feeding plus the weight taken at SV3 defines the participant's *baseline* weight and is used as the baseline against which to measure weight change during intervention feeding. The overall caloric content of each participant's meals is adjusted as needed in order to assure that weight remains stable during intervention feeding.

Participants may discontinue feeding during a given feeding period provided that all of their measurements are complete and that the resulting break does not exceed the limits set above (i.e., two days following run-in and five days otherwise).

At the conclusion of the third intervention feeding period, study participants receive a summary of their study blood pressures (average of all blood pressure measurements) and counseling for heart disease prevention. Individuals with persistently elevated blood pressure levels during feeding are referred to their physicians for evaluation. If the participant does not have a personal physician, qualified personnel at the clinical center may make the recommendation for

treatment. If this clinician is associated with the study, he/she must remain blinded to the participant's intervention assignment.

At the conclusion of the trial, all participants are unblinded to their treatment status, receive their individual blood pressure results and a summary of the study results, and are given the opportunity to ask questions.

9. Dietary Patterns and Menus

Dietary Patterns

DASH2 compares the effect on blood pressure of two dietary patterns and three levels of sodium intake. The control dietary pattern is typical of what many Americans eat. The combination dietary pattern is rich in fruits, vegetables, and low-fat dairy foods, and reduced in saturated fat, total fat, and cholesterol and is fully described on page 1. Compared to the control dietary pattern, the combination dietary pattern is designed to contain higher amounts of potassium, magnesium, calcium, fiber, and protein, and lower amounts of saturated fat, total fat, and cholesterol.

Nutrient Goals

The nutrient targets for the control and combination diets are shown in Table 4 for all nutrients except sodium.

Table 4: Nutrient Targets for DASH2 Diets (1600 - 3600 Kcal)

Nutrient Component[1]	**Control Diet**	**Combination Diet**
% Energy Protein	15	18
% Energy Carbohydrate	49	56
% Energy Total Fat	36	26
% Energy SFA[2]	15	5
% Energy MUFA[2]	13	13
% Energy PUFA[2]	8	8
P/S Ratio	0.5	1.6
Nutrient Ranges Among Energy Levels		
Cholesterol (mg)	220-477	104-230
Fiber (g)	9-17	26-46
Calcium (mg)	380-647	1040-1840
Magnesium (mg)	138-215	430-697
Potassium (mg)	1440-2573	4140-6273

[1] Acceptable ranges for target nutrients are as follows:
All macronutrients = ± 1%
Magnesium and Fiber = ± 10%
Calcium and potassium = ± 5%

[2]SFA = Saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids

Energy Levels
In DASH four energy levels--1600, 2100, 2600, and 3100--formed the basis for menu development. Since 6% of DASH participants required energy levels above 3600 Kcal, a fifth energy

level, 3600 Kcal, is included for DASH2 menus to expand our ability to meet participant energy needs.

Macronutrients and Fiber
The fat intake goals for the DASH control diet were based on intake levels from national surveys (87-91) and are consistent with unpublished findings from the 1992-93 survey of the Coronary Artery Risk Development in young Adults (CARDIA) study. In CARDIA, 50% of the participants were African American and the median percent of energy from total fat was 39% for African American men and 38% for African American women. The nutrient goals for total fat and saturated fat for the DASH2 control diet (36% and 15% of energy, respectively) reflect actual fat values achieved in DASH based on chemical analyses of the DASH diet composites. The amounts of total fat and saturated fat in the combination dietary pattern (26% and 5%, respectively) also reflect the actual values achieved in DASH and are based on the National Cholesterol Education Program (82) and American Heart Association Step II dietary recommendations (92). The polyunsaturated and monounsaturated fat goals for each diet are 8% and 13%, respectively (Table 4). The cholesterol ranges reflect the empiric dependence of dietary cholesterol on energy intake in DASH and DASH2. For the control diet, the range is centered around 350 mg, and thus tends to be somewhat higher than the median intake of adults (90). For the combination diet, the cholesterol range is centered around 165 mg, or about one-fifth lower than the Step II dietary recommendations for cholesterol (82,92).

As in DASH, protein levels were set at 15% for the control diet, because that reflects current US consumption (90,93), and 18% for the combination diet, because data from several epidemiologic studies suggest that higher protein intake is associated with lower blood pressure (61). The carbohydrate goals are set by difference from total fat and protein. The carbohydrate content of the control diet is derived from complex and simple carbohydrates in cereals, fruits, vegetables, candies, and cakes. The increased carbohydrate in the combination diet is a result of the decrease in total fat content accomplished through the inclusion of nutrient-dense complex carbohydrates. The fiber content of the control diet approximates intake in the general American population (91,93), while fiber in the combination diet is based on 3-fold increase over the control diet values and is somewhat higher than current recommendations (94).

Micronutrients
The DASH2 micronutrient goals are the same as those of DASH and are shown in Table 4. These goals were developed by indexing the micronutrient amounts in proportion to the energy level of the diet. The overall objective is to achieve a 2.5-fold contrast in micronutrients at each energy level between the control and combination diets. A detailed description of how the micronutrient and fiber levels were derived is given elsewhere (95). In general, the 10th, 50th, and 90th percentiles of calcium, magnesium, and potassium intakes averaged from several population surveys (87-91) were used to set the limits of variation in the control and combination diets. Thus, a given nutrient in the control diet could vary with energy from the 10th to 50th percentile, centering around the 25th percentile, and in the combination diet from the 50th to 90th percentile, centering around the 75th to 85th percentile.

Sodium Levels
Each of the two dietary patterns is modified to contain lower, intermediate, or higher amounts of sodium. The higher level (equivalent to 150 mmol of sodium at a caloric intake of 2100 Kcal) reflects current intakes of adults (93) and is consistent with the screening urinary values seen in DASH (160 mmol). The intermediate sodium level of 100 mmol at 2100 Kcal reflects the upper limit of current US recommendations for reducing blood pressure (4), and is consistent with urinary excretion values reported in hypertension prevention trials. The lower sodium level (50

mmol of sodium at 2100 Kcal) has the potential for further reductions in BP than do current US recommendations.

Table 5 shows the range of sodium intakes for the five energy levels that are used in constructing the DASH2 menus. The amount of sodium per 1000 Kcal is approximately 24, 48, and 72 mmol for the lower, intermediate, and higher sodium diets, respectively.

Table 5: Sodium Levels to be Used in DASH2 Diets

Energy level (Kcal)	Sodium levels (mmol/day) Lower	Intermediate	Higher
1600	40	80	120
2100	50	100	150
2600	60	120	180
3100	70	140	210
3600	80	160	240

Acceptability of Low-Sodium Diets

Adherence to low-sodium regimens has been a major obstacle in community-based interventions, in part due to limited availability of lower-sodium products. Because DASH2 will use commonly available foods, it will begin to answer important questions regarding the palatability and tolerance of lower-sodium eating patterns. We plan to assess acceptance of and adherence to these diets using an anonymous survey. This is a questionnaire which, in addition to general questions about the study, contains specific questions regarding the acceptance of, and adherence to, the sodium levels used in DASH2. We also plan to assess salt taste acceptability, and how it might change over time during each of the three feeding periods. Some evidence exists that preference for salt decreases over time when following a reduced salt diet (96,97). It is not clear from the literature as to the time course of these changes, however. Some studies show changes in a couple of weeks (98) and other studies show changes over a period of several months (99). No systematic evaluation of time course has been performed.

Menu Cycles

Seven daily menus are prepared for each of five energy levels: 1600, 2100, 2600, 3100, and 3600 Kcal. During feeding, these menus are rotated so that participants eat each menu once per week. Participants eat one meal per day at the clinical site and pick up the rest of their meals and snacks to take home. Weekend meals are distributed to participants on Fridays.

Because some participants consume energy levels intermediate between the five levels for which menus were developed, unit foods in the form of cookies or muffins containing the target nutrient profile of the primary diets are used to adjust energy intakes. A single unit food contains approximately 100 Kcal. For example, a person requiring 2300 Kcal per day receives the 2100 Kcal menu plus two unit foods each day. The target nutrients in the unit foods are chemically validated prior to feeding.

Menu Development

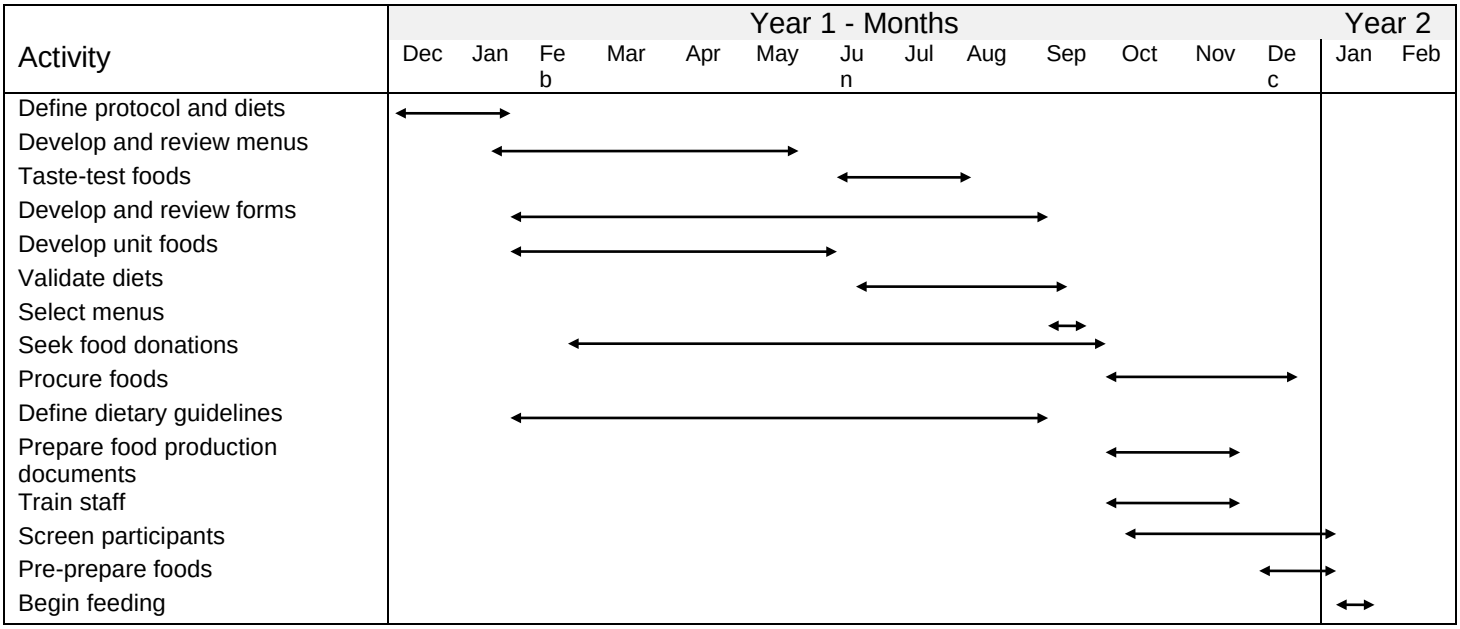
The menu development process consists of three distinct phases. The focus of the first phase is creating menus that approximate target nutrient goals as closely as possible. The diet committee develops eight complete sets of daily menus (i.e., menus for each diet-sodium combination at each of the five energy levels for each of eight days). In all, this represents 240 individual daily menus (eight days times six diet-sodium combinations times five energy levels). The menus are developed using the personal computer version of Moore's Extended Nutrient System (MENu) (Pennington Biomedical Research Center, Baton Rouge, Louisiana).

In the second phase of menu development, specific recipes within the menus undergo taste-testing. In the third phase, the menus are chemically analyzed at the Food Analysis Laboratory Control Center (FALCC) to validate target nutrient levels. Validation is described in section 16.

Following the taste-testing and validation, the menus are evaluated with respect to ease of preparation, taste, conformity to nutrient goals, and cost. The seven menus that perform best across these dimensions are retained to form the seven-day menu cycle for DASH2.

The timeline for developing, validating, and finalizing the diets is shown in Figure 2.

Figure 2. Planning Timeline for the Dietary Component of the DASH2 Study



Strategy For Menu Development And Salt Reduction

Previous DASH menus form the basis for menu development for DASH2. All menus are first developed based on the 100 mmol sodium level (2100 Kcal), and then are altered to decrease or increase the sodium level. Sodium is reduced by using low-sodium baked products, particularly low-sodium breads, avoiding salt-cured foods, and not adding salt to foods. When it is necessary to increase sodium in a menu, kitchen staff add salt during meal preparation, or use the normal sodium version of the baked product or use salt-cured foods. Participants do not receive any discretionary salt with their meals.

Beverages

The clinical centers provide participants with milk and juice to drink. Participants are allowed three servings per day of coffee, tea, or diet soft drinks. Unit servings of these beverages are defined as 1 cup (coffee or tea) and 12 ounces (soft drinks). In addition, participants have a daily allowance of up to two servings of alcohol, where a unit serving is defined as 1.5 ounces of hard liquor, 12 ounces of beer, or five ounces of wine. Participants receive a list of allowed alcoholic beverages that are relatively low in potassium, magnesium, calcium, and sodium. Unused daily beverage allowances cannot be carried over to subsequent days.

10. Food Handling and Distribution

Purchasing and Acquisition

DASH2 uses both centrally provided and locally purchased foods. Centrally provided foods (i.e., donated and reduced cost items) are shipped by the corresponding company to each clinical center or are obtained through a local distributor. Other foods are purchased by each clinical center from local vendors. Each center uses exact specifications during acquisition to assure minimum variation. If necessary to reduce nutrient variability, some food items are purchased from one source and then shipped to each clinical center. To the extent possible, each site purchases the same brand of each particular food item.

When foods arrive at each clinical center they are inspected to ensure proper quality and weight specifications. Any foods not meeting the specifications are returned and replaced with the correct item. Foods are properly stored until ready to use.

Production and Storage

Standardized recipes outlining specific ingredients and gram weights, correct mixing and cooking procedures, timing, and use of equipment are meticulously followed under sanitary procedures. Ingredients are weighed on electronic balances to the nearest 0.1g if they weigh less than 10g and to the nearest 1.0g if they weigh 10g or more. Mixed foods are prepared in batch quantities, individually portioned, weighed, sealed, labeled, and frozen until ready to use. Freezers are maintained at 0^{o}F and refrigerators at 38^{o}F.

Feeding Logistics

Daily food production lists include participant, day, menu cycle, meal, energy level and food items required with portion weights. The food production lists are followed when meal trays are prepared. Foods are placed on labeled individual meal trays until served, or are packaged for take-out or for distribution at a satellite dining center. Take-out foods are packaged with a heat-sensitive label to alert participants if foods have spoiled prior to consumption. At the time of meal pick-up, a staff member reviews the menu with the participant, checking to confirm all foods are provided.

All field centers serve at least one on-site meal a day, five days a week. The meal should, if possible, be lunch or dinner. All other meals are packaged in suitable containers for take-out. Meal trays and carry-out boxes are labeled with the participant's name, ID number, and study. Prepared meal trays and carry-out boxes are handed to each participant as he arrives at the field center. The participants are not told of their dietary treatment assignment, but the menus for each treatment do differ due to the varied nature of the nutrient requirements.

Participants are instructed to consume all of their foods and to record the type and amount of any uneaten foods. Non-study foods are also recorded. Clinic staff regularly review these records to identify potential problems with meal acceptance and to encourage participants to continue on the diets.

11. Dietary Compliance

Promoting Compliance

Each center uses a variety of incentives (e.g., cash and non-cash awards, personal encouragement) to promote compliance with the feeding protocol. In addition, all participants must attend a group orientation session prior to randomization. This session may be conducted as part of a regular screening visit or as a separate visit. The primary purpose of the orientation session is to set expectations of adherence to the experimental routine and diets. It also gives participants a chance to ask questions and to meet the intervention team, as well as other participants.

Where feasible, clinic staff accommodate individual food preferences through the use of comparable substitutes. Such modified diets must remain within the limits of the protocol.

Measures of Compliance

The study uses several measures to assess compliance with the feeding protocol, both prior to randomization for purposes of exclusion, and following randomization for purposes of monitoring and encouraging compliance. These measures, and the corresponding actions they require, are summarized below.

Prior to Randomization

daily diary All participants are expected to maintain a daily diary summarizing study foods and beverages that were not consumed and nonstudy foods that were consumed. Participants also complete questions each day summarizing problems or illnesses they may be having that might interfere with their compliance. This information is reviewed by the intervention staff at each of the daily on-site meals and summarized for purposes of analysis. The daily diary is intended to be used as a monitoring tool, and no compulsory action is taken based on diary information other than as noted below.

missed meals Clinic staff take the following actions in response to missed meals.

For the **first missed meal**, participants are counseled by the clinic staff on the importance of compliance with the study diet.

Participants are excluded from further participation if they miss a **second meal** during the first week of run-in. Exceptions based on extraordinary circumstances may be appealed to the Coordinating Center.

attendance Participants who miss a scheduled clinic meal and do not call in to provide a valid explanation are excluded (this constitutes three missed meals, since they would have failed to pick up their meals for that day). Exceptions based on extraordinary circumstances may be appealed to the Coordinating Center.

Participants who miss a scheduled clinic meal and do call in to explain are treated as having simply missed a single meal and may be kept in the study provided they make arrangements to pick up their other meals.

missed foods & Clinics use the daily diary to estimate the number of study foods that

non-study foods are not consumed and the number of non-study foods that are consumed. Participants for whom these numbers add up to more than 8 during the first week are considered noncompliers and are considered for exclusion during the case conference.

case conference At the end of the compliance assessment phase of run-in and prior to randomization, the clinic staff review each participant's overall compliance history and decide whether the participant is a good candidate for the trial or should be excluded. This conference considers all aspects of participation in the trial to date, including tardiness, attitude, need for special staff effort, and variance from the diet to date.

Post Randomization

daily diary All participants are expected to maintain a daily diary summarizing study foods and beverages that were not consumed and nonstudy foods that were consumed. Participants also complete questions each day summarizing problems or illnesses they may be having that might interfere with their compliance. This is reviewed by the intervention staff at each of the daily feedings and summarized for purposes of analysis.

clinic rating Clinic staff provide a subjective measure of daily compliance for each participant (0/1/2 score) based on the information provided in the daily diary and firsthand observations of foods eaten in the clinic. The sum of these scores over the 30 days of each intervention feeding period provides a measure of overall compliance.

24-hour urine Each participant provides a 24-hour urine during the final 9 days of each intervention feeding period. Aliquots from these specimens are sent to a central laboratory for analysis of Na, K, phosphorus, urea nitrogen, and creatinine for group analyses.

Persons who withdraw from the feeding program after randomization are asked to re-enter their assigned feeding cohort when and if it seems feasible to make such a request. The underlying goals are to maximize the amount of study foods consumed during the intervention period, minimize the amount of non-study foods consumed, and collect outcome data on as many randomized participants as possible.

12. Outcome Measures

Primary Outcome

The primary outcome for the study is the mean SBP at the end of each intervention feeding period. Baseline SBP will be used as a covariate in some analyses. These terms are defined below.

Mean end-of-intervention SBP— the average of the five mean daily SBP measurements recorded during the final nine days of each intervention feeding period. In the event that five sets of final blood pressure measurements are not available, the end-of-intervention SBP is computed as the average of all mean daily SBP readings (minimum of two) recorded during the

final nine days of intervention feeding. For participants who need to start on antihypertensive medications during the feeding period, every effort is made to collect five sets of two daily blood pressures prior to the onset of therapy, and these measurements are used to calculate the end-of-intervention blood pressure for that sodium level. However, participants are not asked to delay the start of clinically indicated medications. For all other participants who terminate their participation prior to the completion of a specific intervention feeding period, their end-of-intervention blood pressures are calculated using the last blood pressure measurement recorded during that feeding period and all other blood pressure measurements recorded within seven days of that measurement. In all cases, the end-of-intervention SBP is computed as the average of the mean daily SBPs.

Once intervention feeding has begun, participants are considered to be in the trial regardless of their actual compliance to the intervention feeding or measurement schedule. Randomized participants who must discontinue participation due to medical reasons (see section 13, Safety Monitoring) and individuals who elect to drop out of the study prematurely are still considered to be in the trial for analysis purposes. For participants who start intervention feeding but have no blood pressure measurements for a given intervention feeding period, their end-of-intervention SBPs for that feeding period are defined to be the average of their mean SV1, SV2, and SV3 SBPs. The rationale for this rule is that participants who drop out of the study are likely to revert to their usual diets, and their screening blood pressures provide our best estimate of what their blood pressure is on their usual diets.

Baseline SBP—the average of all mean daily SBP measurements taken during screening (SV1-SV3) and run-in (i.e., measurements taken on five days).

Mean daily SBP— the mean daily SBP is defined as the average of the two SBP measurements recorded as part of each blood pressure assessment. In the event that only one blood pressure measurement is available, the mean daily SBP is defined as that measurement.

Participants who drop out of the study after randomization but prior to the start of intervention feeding are excluded from all analyses as if they had never been randomized. Such drop-outs presumably can have no association to intervention assignment, since participants are not told their randomization assignment. However, if such participants have learned of their intervention assignment (e.g., through a DASH2 staffperson), they are retained in the study and included in the final analysis.

Measurement Protocol
With the exception of pre-screening blood pressure measurements, all blood pressures are recorded using random zero sphygmomanometers and following the same procedures as used for the Trials of Hypertension Prevention (TOHP) and DASH (7,100). All blood pressure measurements are taken with participants in a seated position and using the right arm (unless the right arm is missing or unsuitable for use, in which case the left arm may be used). Two measurements are taken at each visit. The first measurement is taken after an initial five-minute period of quiet sitting, and the second is taken 30 seconds after the first. All blood pressure measurements must be taken at least one hour after eating; smoking, drinking caffeinated beverages, and vigorous exercise are not allowed 30 minutes prior to any reading.

Secondary Outcomes

Mean DBP at the end of each intervention feeding period serves as a secondary study outcome. Mean end-of-intervention DBP, baseline DBP, and mean daily DBP are defined analogously to those for SBP.

DASH2 participants also provide a 24-hour ABPM reading during the last nine days of each intervention feeding period. The DASH2 ABPM measurement protocol is adapted from that advocated by the British Hypertension Society and is identical to that used in DASH. Staff obtain both the usual RZ measurements and ABPM readings when the monitor is placed in order to confirm satisfactory readings in the clinic setting. Participants are then asked to wear the device for a full 24-hour period. Upon return to the clinic, the data are downloaded to the computer and checked to confirm that at least 14 acceptable readings were obtained between the hours of 6 a.m. and 12 midnight. If so, the monitoring is deemed acceptable. If fewer than 14 acceptable readings are available during this timespan, the participant is asked to repeat the monitoring.

We will use 24-hour SBP as a secondary outcome analogous to RZ-DBP. Other ambulatory pressure indices, including 24-hour DBP and waking and sleeping pressures, will be used for exploratory analyses. "Waking" and "sleeping" pressures are calculated according to the times of going-to-sleep and awakening indicated by the participants on the day they wear the ambulatory monitor.

Other Outcomes

A standard lipid panel is run on (8-hour) fasting blood samples collected at SV3 and during the last week of each intervention feeding period. Total cholesterol, VLDL-C, HDL-C and triglyceride levels are measured directly, and LDL-C levels are calculated. These data are used to validate differences in dietary consumption between the control and combination diets and also to assess the impact of lower levels of sodium on total and LDL-C cholesterol. All analyses are done centrally, and a minimum fast of eight hours is required for the measurement to be valid.

Plasma renin activity is measured in samples drawn after an overnight fast and at least 90 minutes in the upright position. Samples are collected during the last week of each intervention feeding period, but only the samples corresponding to the lower sodium level diet are analyzed. A minimum fast of eight hours is required for this measurement. Renin level is measured to determine the extent to which renin profile influences blood pressure response to the DASH combination diet and sodium reduction.

The Block Food Frequency questionnaire (91) is completed during screening and used to estimate usual nutrient intake for use in exploratory analyses and to describe usual eating patterns. Physical activity is assessed at the end of each intervention feeding period via a brief self-administered questionnaire in order to document changes that might affect blood pressure. Alcohol intake is assessed through daily food diaries.

Four 24-hour urine collections are completed: during screening and during the final week of each intervention feeding period. All four specimens are analyzed centrally for Na, K, phosphorus, urea nitrogen, and creatinine in order to assess group compliance. Calcium is also assessed in order to examine the influence of sodium intake on calcium excretion.

Samples of each participant's urine, plasma, and serum are frozen for future analyses. In addition, a sample of buffy coat (white blood cells) is frozen for future investigations of genetic influences on blood pressure response to diet.

13. Safety Monitoring

Blood Pressure Escape Levels

The following blood pressure escape levels and protocols have been established to assure that participants are offered appropriate evaluation and therapy when clinically indicated. The actions taken when these escape levels are reached vary somewhat for screening, run-in, and intervention. In all cases, participants may be immediately referred for evaluation if a qualified clinician believes such action is appropriate based on his or her clinical judgment.

In addition to the RZ measurements required below, additional non-RZ measurements may be taken on a more frequent basis to ensure participant safety. All non-RZ blood pressure measurements are recorded in the participant's chart, but are not used for analysis. All RZ measurements become part of the participant's official study BP record.

Screening
Screening criteria for excluding participants from further participation based on elevated blood pressure levels are discussed in section 6. At each screening visit, the exclusion limits are based on all blood pressures averaged up to that point. In the event that the mean SBP

exceeds 180 mm Hg or the mean DBP exceeds 110, the participant is excluded from the study and referred to a physician to determine if medication is needed. If the participant does not have a personal physician, qualified personnel at the clinical center may make the recommendation for treatment.

Run-In Feeding
Two escape levels apply during run-in and intervention feeding. They differ in terms of the frequency with which a follow-up measurement is required. Also, a repeat elevated blood pressure triggers an automatic exclusion during run-in but only a referral during intervention feeding.

Escape level #1: The mean blood pressure recorded at any single visit exceeds either a SBP of 180 mm Hg or a DBP of 110 mm Hg.

Action: Participant may be excluded immediately and referred to a physician for further evaluation. Alternatively a second RZ blood pressure measurement must be obtained within four days and still during run-in. If this second measurement exceeds 170/105 mm Hg, the participant is automatically excluded and referred to a physician for follow-up.

Escape level #2: The mean blood pressure recorded at any single visit exceeds either a SBP of 170 mm Hg or a DBP of 105 mm Hg.

Action: Participant may be excluded immediately and referred to a physician for further evaluation. Alternatively a second RZ blood pressure measurement must be obtained within seven days and still during run-in. If this second measurement again exceeds 170/105 mm Hg, the participant is automatically excluded and referred to a physician for follow-up.

If the participant does not have a personal physician, qualified, blinded personnel at the clinical center may make a recommendation for treatment.

Intervention Feeding

Escape level #1: The mean blood pressure recorded at any single visit exceeds either a SBP of 180 mm Hg or a DBP of 110 mm Hg.

Action: A second RZ blood pressure measurement must be obtained within four days. If this exceeds 170/105 mm Hg, the participant is referred to a physician for follow-up.

Escape level #2: The mean blood pressure recorded at any single visit exceeds either a SBP of 170 mm Hg or a DBP of 105 mm Hg.

Action: A second RZ blood pressure measurement must be obtained within seven days. If this again exceeds 170/105 mm Hg, the participant is referred to a physician for follow-up.

If the participant does not have a personal physician, qualified, blinded personnel at the clinical center who are not affiliated with DASH2 may make a recommendation for treatment.

In the event that a participant is referred to a clinician for evaluation, the clinical center should seek to obtain a set of up to five end-of-intervention blood pressure measurements. Care should be taken, however, that this does not delay or otherwise interfere with appropriate clinical care. If blood pressure medication is not initiated, the participant continues in the trial.

Referral for Non-Blood Pressure Reasons

Abnormalities noted in laboratory or physical assessments that require medical evaluation will result in referral to other medical care sources unless they arise as a direct result of participation in DASH2. If clinical problems arise from DASH2 participation, the problem may be dealt with at the clinical center or through referral as is most appropriate and consistent with the institution's risk management procedures.

Morbid Events Affecting Blood Pressure

Participants who suffer a morbid event with a lasting effect on blood pressure (e.g., myocardial infarction, stroke) are considered terminated as of the date of the morbid event. Similarly participants who are placed on exclusionary medications or special diets by their physicians are also considered terminated as of the date these medications or diets began. In each of the above cases the participant's end-of-intervention blood pressures are calculated as outlined in section 12 and they are excluded from further participation in the study.

Food Safety

Clinic staff are instructed in procedures for handling, preparation, and distribution of foods. These procedures focus on the prevention of contamination of foods and on safe preparation, storage, and consumption practices. Participants are instructed to immediately report symptoms that may arise from food borne illness. Such reports will trigger clinics to investigate whether other participants have experienced similar symptoms, to review their procedures, and to determine if further action is required. In order to avoid food borne illness, participants are provided instructions on food storage and preparation.

Other Adverse Events

Throughout the run-in and intervention feeding periods, participants are questioned daily about possible symptoms from the study foods. Particular attention is paid to symptoms of lactose intolerance and to other gastrointestinal symptoms. Severe or potentially clinically significant symptoms are brought to the attention of a DASH2 clinician. Feeding is terminated if symptoms or signs related to a DASH2 diet are deemed by the participant or DASH2 clinician to be intolerable or dangerous. To the extent possible, staff collect end-of-intervention measurements on such participants, giving first priority to the five end-of-intervention blood pressure measurements.

The clinical centers are expected to formally document the occurrence of any unusual or significant health problems that arise during feeding and require medical intervention. Participants are asked about such events at the end of each feeding period, and their occurrence is documented on forms that are sent to the Coordinating Center.

External Monitoring

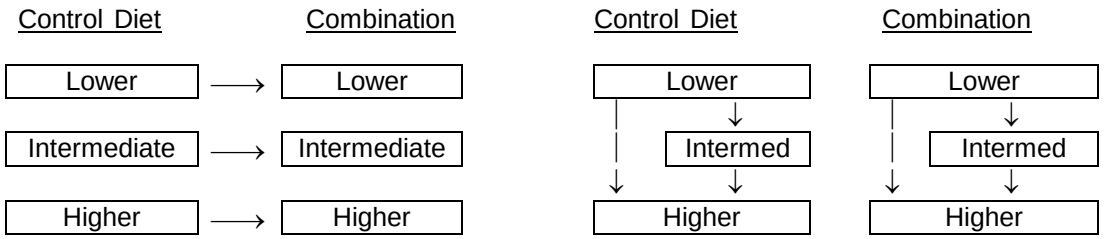
In addition to the above rules for monitoring the safety of individual participants, the external Data and Safety Monitoring Board (DSMB) regularly reviews the trial's progress, including unblinded interim results, and can recommend that the NHLBI terminate the trial early if participants are being subjected to undue risk or if further follow-up would serve no added scientific purpose.

14. Data Analysis

Design

The design is a crossing of two simpler designs, a parallel, two-group comparison of diet (Control vs. Combination), and a complete, three-period crossover of three levels of sodium (Lower, Intermediate, Higher). In effect, each participant is assigned at random to one of the two dietary components, and independently to one of the six possible sequences of sodium treatment. Each participant's blood pressure is measured at baseline, and after each of the three intervention feeding periods (one at each level of sodium).

The first two specific aims of the study are to investigate the effects of one factor within levels of the other. The analysis plan specifies one model for analysis, but parametrized two different ways, each of which addresses one of the specific aims, as indicated in the following diagram. There will be three comparisons of diet effects, within sodium levels (left side), and six comparisons of sodium levels within diet (right side). Using only one model for both analyses should add clarity to the presentation of results.



For each individual, all blood pressure measurements (baseline, and three follow-ups) will be entered into the analysis. For the sodium analyses, results will, in effect, be based on differences between different sodium treatment levels, and so the baseline values will only contribute to the estimates of the variance components. For the diet analyses, however, the baseline values add information and improve the study power.

The fundamental strategy is to fit linear models that include the effects of interest for each specific aim, together with the other factors that are regarded as being relevant to the outcome. Parameter estimates will be determined by the method of maximum likelihood, assuming normally-distributed random terms. The baseline terms are included because they assist in estimating the between-individual component of variance, and models that incorporate this random term are more efficient than those that do not. For some secondary outcomes, such as ambulatory blood pressure, baseline values are not collected, and thus cannot be used in the analysis. While this may result in some power loss, it does not invalidate the basic modeling approach.

An essential feature of the analysis plan is to deal properly with the fact that we must expect multiple observations on the same person to be correlated. There is ample evidence of this

correlation from the original DASH study, where the correlation between two blood pressures from the same person was about 0.90, irrespective of their separation in time . This feature can be dealt with fairly easily, however, by using a mixed-effect regression analysis (also called "repeated measures analysis of variance", or "generalized least squares", or multilevel analysis (101)). Computations that were carried out for the purpose of investigating the consequences of different analysis strategies consistently showed large efficiency gains for this approach. For this reason, the "repeated measures" model is planned for all analyses. This will be implemented by including random effects for individuals in all models. The cohort effects (nested in sites) will also be considered random effects, since it is plausible that participants in the same cohort might give correlated responses.

Corrections for multiple testing, when used, will be based on Holm's method (102). This is done by sorting the p values from smallest to largest, $p_1,\ldots,p_n$, then successively comparing p_i to α /(n-i+1) until the first non-rejection. This method applies in all cases where the more familiar Bonferroni method has been used to bound the overall Type I error probability at α, but it is uniformly more powerful than the latter method.

Aim #1: Blood Pressure Change due to Sodium Levels within Combination and Control Diets

For the first specific aim, the repeated measures model will be parametrized to include:

1. contrast of higher vs. lower sodium within the Control diet;
2. contrast of higher vs. intermediate sodium within the Control diet;
3. contrast of higher vs. lower sodium within the Combination diet;
4. contrast of higher vs. intermediate sodium within the Combination diet;
5. contrast of Combination vs. Control diet
6. site effects
7. cohort effects, nested within sites
8. period effects (for four feeding periods nested within a cohort);
9. person effects

Site effects will be considered fixed, while cohort, period, and person effects will be considered random. The assumptions underlying this approach will be investigated to determine whether the cohort and period should be considered fixed effects.

The primary analysis involves first testing 1 and 3 (higher vs. lower sodium within diet) using equal-tailed, .05-level, two-sided tests with a Holm correction for the two tests. If the first analysis yields any positive results, then 2 and 4 will be tested using the same strategy; specifically, 2 will be tested only if 1 was significant, and 4 will be tested only if 3 was significant. If there is only one test at this second stage, then it will be at .05 level; otherwise, the Holm adjustment will also be used for the two tests at the second stage. For purposes of presentation, the remaining contrast (intermediate vs. lower) will be shown, but its test is implicit in the tests already done.

This analysis will be carried out for SBP as the primary analysis of the trial. Secondary analyses will consist of (1) the same analysis for DBP and 24-hour SPB, (2) SBP, DBP and 24-hour SBP separately for men and for women, (3) SBP, DBP, and 24-hour SBP separately for African Americans and for those not of African American descent, and (4) SBP, DBP, and 24-hour SBP separately for non-hypertensives and for hypertensives. For 24-hour SBP there are

no baseline measurements, and while this may lower the power of the analysis somewhat, the same analytic model and analytic strategy apply.

Multiple testing adjustments will not be performed for the secondary analyses. The reasoning is that the hypothesis tested in the trial is that of the primary analysis (for which a multiple testing protection will be used), but that all subsequent analysis are for the purpose of interpreting and elucidating the primary results. Thus, it is only the primary analysis that involves a decision, for which multiple testing protection is required. Sufficient detail will be reported to allow others to carry out multiple testing adjustments.

A fundamental assumption of the above analysis is that there are no carry-over effects of a sodium level in one period to later periods. The principal advantage of a cross-over design is that when there are no carry-over effects, then the between-individual component of variance (which is large) is eliminated from the estimation of the sodium contrasts. The principal drawback of the design is that if there is carry-over, then there is no standard way to analyze the results. One can formulate a variety of scenarios for how carry-over might have occurred, but none of them can be tested, and so the analysis of sodium effects including some assumption about carry-over becomes rather subjective. It is known that there is no satisfactory statistical solution to this problem (103).

Although there is no good alternative analysis in the presence of carry-over, we will fit a model that allows for exponentially rising and diminishing carry-over effects in order to provide a check on this assumption. Specifically, a BP measured at t time units after the start of feeding will receive a term B(1-exp(-rt)) which rises to an ultimate value of B, and then s time units after a feeding period of duration T the model would include a term B(1-exp(-rT))exp(-rs). This model is suggested by analogous kinetic models, and while it does not provide a complete solution to the carry-over problem, it provides one sensible alternative analysis. If there is evidence of carry-over by this analysis, then this will be reported for the purpose of interpreting and elucidating the primary analysis.

Aim #2: Blood Pressure Change Between Combination And Control Diets within Sodium Levels

The second specific aim pertains to the effect of diet type at the three sodium levels. The model is unchanged, but reparametrized as follows:

1. contrast of Combination vs. Control diet at lower sodium;
2. contrast of Combination vs. Control diet at intermediate sodium;
3. contrast of Combination vs. Control diet at higher sodium;
4. contrast of higher vs. lower sodium
5. contrast of higher vs. intermediate sodium
6. site effects (as before)
7. cohort effects (as before)
8. period effects (as before)
9. person effects (as before)

The analysis will test 1, 2, and 3, using equal-tailed two-sided tests, with a Holm correction for multiple testing. The structure of primary and secondary analyses will be the same as outlined above for specific aim #1.

Aim #3: Potential Interaction between Diet and Sodium

This aim will compare effects 1 vs. 3, and 2 vs. 4, as given in aim #1, together with the corresponding comparison involving intermediate vs. lower sodium level. Again, Holm adjustment will be used to account for the multiple tests, which will all be two-tailed.

Aim #4: Linearity of the Dose-response Relationship Between Blood Pressure and Sodium

The null hypothesis is that the sodium effect, can be represented as a linear function of sodium level, moving from lower to higher. It is assumed that this analysis will need to be carried out separately within each diet, and that there will consequently be a Holm adjustment for four tests.

For all four aims, further secondary analyses will adjust for the potentially confounding effects of race, sex, age, body mass index, education, physical activity, and alcohol intake. Interaction terms are used to determine if any observed treatment effects differ for various subgroups. Additional analyses will test whether baseline renin status influences treatment differences.

Handling of missing data.

Participants not completing the post-randomization follow-up (either due to nonattendance at data collection visits or to protocol escape levels) are likely to represent a nonrandom subset of the overall randomized population. For example, those individuals who are required to initiate antihypertensive medications are likely to be the participants who would have had the highest end-of-intervention blood pressure levels. End-of-intervention blood pressure values for such participants are discussed in section 12 (Outcomes Measures).

Because of the cross-over nature of the study design, some dropouts will not have any BP measurements for one or more sodium levels. As described in section 12, we use the average of these individuals' screening blood pressures as their end-of-intervention blood pressures. The rationale for this rule is that, in essence, these individuals are just very noncompliant with the study diets, and our best estimate of their blood pressure on their usual diets is the average of their screening blood pressures. The only exception to this rule is for those individuals who are randomized but who never start any of the intervention feeding periods. Since loss to follow-up in these individuals cannot be related to treatment assignment, they are treated as if they were never randomized and excluded from all analyses.

Finally, subject to the preceding considerations, analyses are conducted using an intention-to-treat philosophy, whereby each randomized participant is included in each analysis according to his/her randomly assigned diet group.

15. Sample Size / Statistical Power

The proposed sample size of 400 participants (200 in each of the combination and control diet arms) was selected to provide adequate power, both overall and within subgroups, for addressing specific aims #1 and #2. This section summarizes power computations to address all four of its specific aims. We assume two-sided alternative hypotheses for each specific aim, and we also include allowance for multiple testing adjustment, as indicated above.

To compute power, the statistical routine that will actually do the analysis (the generalized estimating equation module of Stata) was employed. Only the design specification (fixed and random effects) is required, since given this, the results depend only on the residual variance estimate. We provide this latter value from the DASH results. This procedure provides standard deviations for each estimate, which is all that is required for power computation.

Using the DASH results to estimate variance components (between = 150 mmHg, within = 40 mm Hg), the following table gives the effect sizes that can be detected with the indicated power, allowing for multiple testing, for each of the specific aims. These computations are based on two-sided .05 (adjusted) level.

Detectable Effects: Systolic BP in mmHg

	Detectable Effect at Power =		
	70%	80%	90%
1. Sodium Effects within Diets (2)	0.8	0.9	1.0
2. Diet Effects within Sodium Levels (3)	1.2	1.3	1.5
3. Interactions (2)	1.1	1.2	1.4
4 Linearity of Sodium Effect (2)	1.4	1.5	1.7

Number of Holm adjustments in parentheses.

The results can be summarized as follows.

Aim #1. A sodium effect of 1.0 mm Hg (difference between two groups) can be detected (power = 90%) between two sodium levels, within a diet.

Aim #2: A diet effect of 1.5 mm Hg (difference between two groups) can be detected (power = 90%) between the two diets within a sodium level.

Aim #3: A diet effect on a sodium effect of 1.4 mm Hg can be detected (power = 90%). The effect here is defined by first computing a sodium effect (such as lower vs. higher) d_1 in the control diet , the same sodium effect d_2 in the combination diet, and then computing $d_2 – d_1$.

Aim #4: A departure from linearity of 1.7 mm Hg/sodium-class can be detected (power = 90%). The effect here is computed as d_1 equal to the intermediate vs. lower sodium effect, d_2 equal to the higher vs. intermediate sodium effect, and then computing d_2-d_1.

As Stamler et al. (5) note, a population-wide reduction of 3mm Hg in SBP could have a great public health impact. This provides a reference value suggesting that the study will have excellent power to detect meaningful effects for Aim #1-#3.

Subgroup Effects on Power

The above figures pertain to the primary analyses for each specific aim. Analyses within subgroups will have lower detectable effect levels, but a single correction can be made in these cases, so long as the variance component estimates still pertain. Let p denote the fraction of data available for analysis (relative to complete data), and then multiply the effect sizes by $1/\sqrt{p}$. To take changes in variance into account, multiply the effect sizes by the ratio of the presumed within-person variance to the overall value of 150+40/5 that was used for the full analysis.

The following table adjusts that given previously, so that it pertains to a subgroup analysis using only half the participants – and is therefore roughly applicable for race-specific or gender-specific analyses.

Detectable Effects: Systolic BP in mmHg
For Half of the Entire Sample

	Detectable Effect at Power =		
	70%	80%	90%
1. Sodium Effects within Diets (2)	1.1	1.2	1.4
2. Diet Effects within Sodium Levels (3)	1.7	1.9	2.1
3. Interactions (2)	1.6	1.7	2.0
4 Linearity of Sodium Effect (2)	1.9	2.1	2.4

The following table repeats the detectable effects, now for the subgroup of hypertensives only (assumed to be 30% of the total sample), and using the with person-specific variance (91+60/5) that was observed among hypertensives in the control group in the DASH study.

Detectable Effects: Systolic BP in mmHg
Hypertensives Only

	Detectable Effect at Power =		
	70%	80%	90%
1. Sodium Effects within Diets (2)	1.8	2.0	2.2
2. Diet Effects within Sodium Levels (3)	2.6	2.9	3.3
3. Interactions (2)	2.5	2.8	3.2
4 Linearity of Sodium Effect (2)	3.0	3.4	3.9

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The final table is for the 70% normotensives, with person-specific variance equal to 71+37/5, again from the DASH control group of normotensives.

Detectable Effects: Systolic BP in mmHg
Normotensives Only

	Detectable Effect at Power =		
	70%	80%	90%
1. Sodium Effects within Diets (2)	0.9	1.0	1.1
2. Diet Effects within Sodium Levels (3)	1.4	1.5	1.7
3. Interactions (2)	1.3	1.4	1.6
4 Linearity of Sodium Effect (2)	1.6	1.7	2.0

16. Quality Control and Data Management

Principles and Philosophy

The objective of quality control efforts is to ensure that project data and activities are standardized, accurate, and timely, thus minimizing variation not associated with treatment effects. To achieve this, staff are trained and certified rigorously and all trial activities are monitored routinely.

Menu Development and Validation

In order to arrive at the trial's 7-day menu plan, the Diet Committee initially develops eight daily menus (breakfast, lunch, and dinner) for each of the six diet-sodium combinations and for each of five calorie levels (1600 Kcal, 2100 Kcal, 2600 Kcal, 3100 Kcal, and 3600 Kcal). Thus 240 separate menus are developed. Validation of these menus takes place during the planning phase of the trial through compositing and chemical analysis for nutrients of interest. Each clinical center prepares selected menus from the 2100 and 3100 calorie diets in a predetermined manner such that each of the six diet-sodium combinations of each menu at both the 2100 and 3100 calorie levels are validated twice, with the replicate menus prepared by two different clinical centers. This represents a total of 192 menus that are prepared for compositing and assay (8 menus * 2 diets * 3 sodium levels * 2 calorie levels * 2 sites). In addition, all eight 1600 Kcal, lower sodium menus for each diet arm are also prepared by two different sites for a total of 32 additional composites. And finally, unit foods for each diet-sodium combination are also prepared at two separate sites and assayed.

Once prepared, the menus are shipped frozen to the Food Analysis Laboratory Control Center (FALCC) at Virginia Polytechnic Institute and State University for compositing and analysis. For the menu validation, nutrients analyzed include: calcium, magnesium, potassium, sodium, iron, total fat, protein (via nitrogen), moisture, and ash. Carbohydrates are calculated as the difference between the total amount of fat, protein, moisture, and ash (minerals), and the total weight of the food. Calories are calculated based on the amount of protein, fat, and carbohydrates, using standard Atwater factors. Menus that do not fall within target nutrient ranges are discarded. Of the remaining acceptable menus, seven are selected for the feeding phases of the trial.

FALCC also assays selected diet-menu-kcal composites for fatty acids (total SFA, total MUFA, total PUFA) and cholesterol. These assays are used for documentation of the nutrient content of menus in the diets, but are not used for menu selection.

Unit food composites (one for each diet-sodium level, i.e., six total) are assayed for calcium, magnesium, potassium, sodium, iron, total fat, protein (via nitrogen), moisture, and ash. For documentation, fatty acids and cholesterol are assayed in the intermediate sodium level unit food composite for each diet (control and combination).

Alcohol and beverage consumption is estimated from the daily diaries. No assays are performed.

Diet Monitoring

Because nutrient levels may vary over time, both as a result of variation in available foods and as a result of local preparation practices, the menus are monitored sequentially during the intervention phase of the trial. Each center prepares one extra menu cycle (i.e., seven daily menus) per diet-sodium level during each cohort. Each of these seven-day cycle menus is shipped frozen to the FALCC, where they are composited as a unit and assayed.

Nutrients analyzed during the diet monitoring phase include: calcium, magnesium, potassium, sodium, iron, total fat, protein (via nitrogen), moisture, and ash. Carbohydrates and total calories are available as calculated values. Unit foods are processed much in the same manner as the primary menus and assayed as distinct composites during monitoring

Staff Training and Certification

DASH2 staff are trained and certified in three main areas: clinical evaluations, data collection and management, and food preparation and handling. In addition, detailed procedures cover the collection and handling of blood and urine specimens.

Clinical Evaluations
Clinic staff from each site are trained to administer and record these measurements: RZ blood pressure, ambulatory blood pressure, height, weight, physical activity, waist circumference, and food frequency questionnaire. Staff are also trained in procedures for drawing and processing blood specimens and for processing 24-hour urine specimens.

DASH2 uses the same RZ blood pressure training materials as the TOHP and DASH studies. This includes centralized training of trainers, who must have at least six months experience taking blood pressures and who are certified to conduct local training of other technicians with similar qualifications. Re-certification for all technicians occurs semi-annually and is done locally. Re-certification of trainers is done annually through a central, trial-wide process. Each DASH2 site must maintain on-staff at least two certified, practicing blood pressure technicians. Each technician must be observed by a certified trainer doing a RZ blood pressure measurement at least once every three months. The Coordinating Center monitors certification training, re-certification, and quality control.

DASH2 uses the same ABP Monitor training as the DASH Study. This includes centralized training of a trainer who is certified to conduct local training of other technicians. Re-certification for all technicians occurs semi-annually and is done locally. Re-certification of the trainer is done annually through a central, trial-wide process. Each DASH2 site must maintain on staff at least 2 certified technicians trained in placement of the ABP Monitor. Each technician must be observed in placement of two ABP Monitors with-in one month prior to the start of each Run-in feeding period. The Coordinating Center monitors certification training and re-certification.

Participants are instructed on the proper methods for obtaining a complete 24-hour urine collection. Urine jugs, and for women a collection “hat,” are dispensed along with written instructions. The instructions specify when to start and when to stop the collection. The initial void is discarded, and all subsequent voids are put into the jug. Participants return the jug to the clinical center upon completion of the collection. Female participants are instructed not to perform the collection during menstrual periods. The study personnel measure and record total volume and freeze duplicate aliquots at -70°C. Twice during each cohort the clinical centers ship all aliquots in dry ice to the central laboratory for analysis.

Appropriate staff from each site are centrally trained as trainers in all other relevant procedures. Following their certification as trainers, these individuals are responsible for training and certification of local clinic staff at their sites. Centralized trainers are recertified annually and local staff are re-certified biannually. In all cases, recertification is scheduled to occur within two months prior to the start of run-in feeding periods.

Data Collection and Management
DASH2 employs a combination of centralized and distributed data entry. The majority of data, including all clinical measurements and eligibility information, are entered into a PC-based application at the clinical centers. In addition, the food frequency questionnaire and potentially some other forms not used to determine eligibility are sent to the Coordinating Center for

centralized data entry. Finally, shipments of laboratory specimens and food samples are regularly logged at the clinical centers.

All staff involved in data collection are trained in the data entry system, including the instructions for administering each of the questionnaires. At least one data coordinator is trained in processing and resolving edit reports.

Data coordinators from each site are introduced to key concepts of the data management system as part of the central training prior to the start of recruitment. Following this, staff from the Coordinating Center visit each site to install the equipment and do in-depth training and certification of the data coordinators. After their certification, the data coordinators conduct additional training of local staff. The Coordinating Center monitors data quality regularly and conducts additional training as needed.

Food Preparation and Handling
Kitchen staff are trained in the preparation and handling of foods to assure that diet composition is uniform across sites; that food is handled, prepared, and distributed safely; and that dietary monitoring composite analyses are collected and shipped appropriately. In addition, clinic nutrition staff are trained in the collection of dietary compliance information and in other aspects of nutritional intervention.

Data Management and Reporting

Data Management System
The official study database is maintained at the Coordinating Center and is updated regularly via telephone access to the file servers located at each clinical site. These data are merged with centrally entered data and with the results of central laboratory analyses. The database is monitored regularly for completeness.

Demographic and prescreen data collected during a participant's initial contact with field site staff are entered to the computer, which then registers the participant and assigns a unique study ID.

Randomization assignments are generated locally using the desktop PC assigned to each clinical center. Prior to randomization, the computer checks the master database to make sure that all screening activities have occurred, that the participant meets all eligibility criteria, and that all required baseline data have been collected. Participants are assigned a diet status (control or intervention) and a sodium sequence from a predetermined allocation table stored on each site's PC. Diet assignments are stratified by site with varying block sizes to ensure a balance of randomization assignments over time. The sequence of sodium levels (higher, intermediate, lower) is also randomly assigned so that all six orderings occur with equal frequency at each site and for each diet arm.

Quality Control
The data management system performs range, logic, and missing data checks on all data at the time of data entry. Cross-form edit checks are also performed locally and for the integrated data maintained at the Coordinating Center. Data inconsistencies occurring across forms are summarized in edit reports that must be resolved by local clinic staff. These audits are rerun periodically to detect unresolved problems. Standardized edit reports that summarize problems in the database provide an additional method of assuring data quality.

Reporting
The Coordinating Center prepares regular reports summarizing the performance characteristics of the study as a whole and of individual clinical centers. These reports are distributed to the members of the Steering Committee, appropriate subcommittees, and to the Data and Safety Monitoring Board.

Blinding

The study is intended to be a double blinded trial. Due to the nature of the intervention, however, kitchen staff need to be unblinded. Further, although participants are not told to which dietary pattern or sodium sequence they have been assigned, they will obviously know what they are eating and may be able to infer their assignments.

All blood pressure measurers are required to be blinded to diet assignment, and participants are blinded to their study blood pressure data from after run-in until the end of their participation in the trial. Participants are alerted, however, if their blood pressure goes above a predetermined escape level (see section 13, Safety Monitoring). Clinical centers are allowed to unblind participants whose physicians demand to see the data for reasons of medical management. This option is not disclosed to participants in advance, however. Clinics will notify the Coordinating Center of any participants who are unblinded to their blood pressure values during the intervention period.

When they have completed the entire intervention, which includes all three feeding periods, participants receive an average of all blood pressure measurements taken during the study. If a participant demands individual blood pressures, the clinical center should arrange for the Coordinating Center to send the information directly to the participant. No participant will be unblinded to his/her dietary assignments until the end of the trial when all cohorts have completed the intervention.

17. DASH2 Timeline

The study will be conducted over a 48-month period.

Months 1-12 (Feb 1, 1997-Jan 31, 1998): The first grant year is devoted primarily to design and development of all aspects of the study. Activities include: planning, developing and validating menus; developing the Protocol and Manual of Operations (MOP); receiving approval of the Protocol by the NHLBI based on recommendations of the Protocol Review Committee; developing, pre-testing, and printing forms; hiring, training, and certifying staff; purchasing hardware and developing programs for the data management system; and procuring food. In addition, clinical sites initiate recruitment during months 7-10.

Months 13-36 (Feb 1, 1998-Jan 31, 2000): Each clinical site feeds four cohorts of approximately 25 randomized participants each over this two-year period. This involves screening visits (three per person), run-in feeding (two weeks), and three 30-day periods of experimental feeding with a break of up to 5 days between feeding periods for each randomized participant. The precise timing of cohorts is determined by each site, and is therefore not necessarily the same across sites. However, all sites conclude the final cohort by Feb 1, 2000.

Months 37-48 (Feb 1, 2000-Jan 31, 2001): The final year of the project is devoted to completion of data entry; cleaning of data files; analysis of data; manuscript preparation, review and submission; presentation of results; and closeout of all activities. Study-wide policies govern access to study data through the Publications and Ancillary Studies Committee (PASC). This

committee reviews and approves all analysis requests, presentations and publications related to DASH2. The PASC policies remain in effect until Jan 31, 2005 or until the PASC is formally dissolved. A complete set of clean data tapes is supplied to all clinical sites and to the Project Office at the end of the grant period. All information that could uniquely identify individual participants (e.g., name) is removed from the data before the data tapes are distributed.

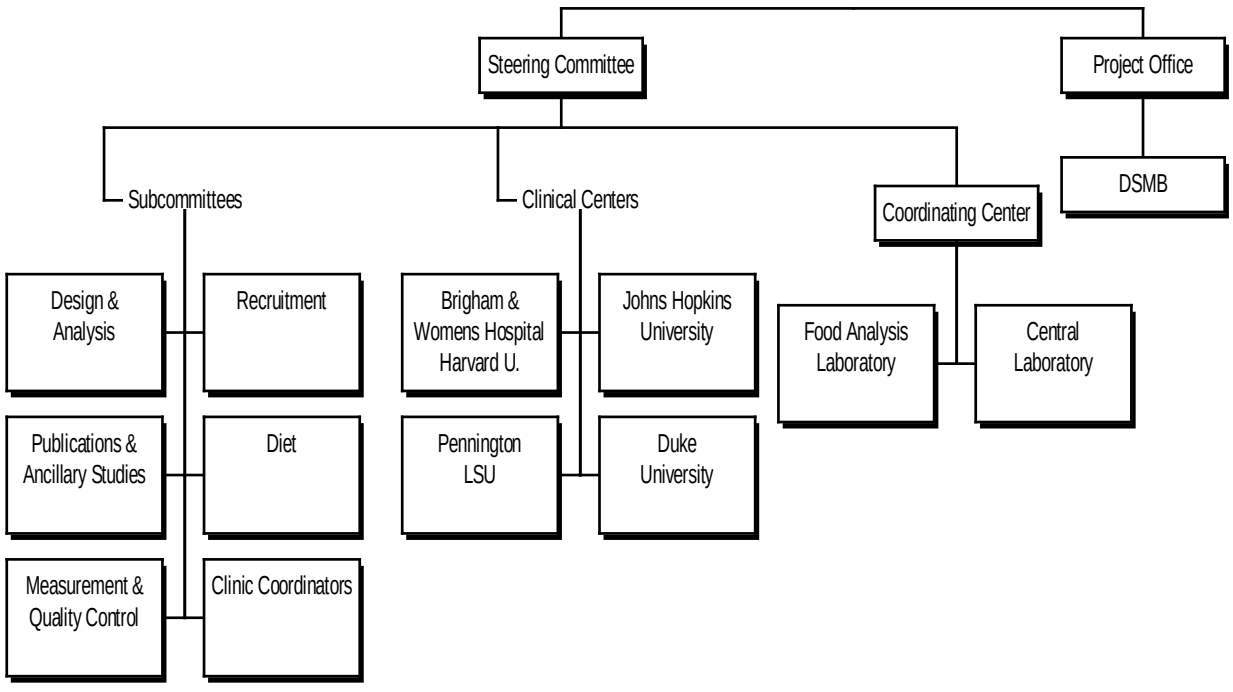
18. Trial Administration

Participating Sites

Participating institutions in the DASH2 study include the NHLBI Project Office, the Coordinating Center (Kaiser Permanente Center for Health Research in Portland, Oregon), and four Clinical Centers: Brigham and Women's Hospital in Boston, Johns Hopkins University in Baltimore, Pennington Biomedical Research Center in Baton Rouge, and Duke University Medical Center in Durham. The Food Analysis Laboratory Control Center (Virginia Polytechnical Institute and State University, Blacksburg, VA) and a central laboratory for analysis of blood and urine specimens are contracted through the Coordinating Center.

These institutions work together, through the mechanism of cooperative agreements, to design and implement a common protocol and to administer the trial. The structure of the trial is shown in figure 3.

Figure 3: DASH2 Organizational Chart



Trial Committees

Steering Committee (SC)
The Steering Committee is the overall decision-making body for the trial and is responsible for developing, reviewing, and approving the study protocol and all policies relating to the conduct of the study. The SC is also responsible for assuring a clear delineation of roles and responsibilities and for clear communications between the participating organizational units. Finally, the

SC tracks overall trial performance, approves (or disapproves) all recommendations of subcommittees, and approves any ancillary studies and access to study data.

The SC meets at least semiannually, with conference calls or additional meetings as needed and with regular information sharing. The Steering Committee is headed by a chair (Frank Sacks) and a vice-chair (Laura Svetkey). The PI of each participating institution and the NHLBI Project Officer (or their designee) have one vote. The chairs of the Diet Subcommittee and the Clinic Coordinators Subcommittee attend Steering Committee meetings as non-voting members.

Design and Analysis Subcommittee
The Design and Analysis Subcommittee recommends to the Steering Committee the basic design and analysis components of the trial. It also considers the trial design during the implementation phase and recommends changes in and additions to the protocol as appropriate.

Measurement and Quality Control Subcommittee
The Measurement and Quality Control Subcommittee recommends to the Steering Committee measures, processes, and procedures for conducting all measurements including those for outcomes, eligibility criteria, compliance, safety monitoring, and quality control. These recommendations include training, certification, and quality control measures and procedures, and other activities directed at assuring that the data are valid and reliable. This subcommittee also reviews quality of data during the conduct of the trial.

Diet Subcommittee
The Diet Subcommittee recommends to the Steering Committee policies, practices, and procedures relating to development, assay, analysis, procurement, preparation, delivery, consumption, and assessment of intake of the various diets. The Diet Subcommittee also recommends appropriate compliance measures for this trial and designs and implements a quality control program to ensure comparability of the diets fed across all clinical sites. The chair of the Diet Subcommittee serves as a non-voting member of the Steering Committee.

Recruitment Subcommittee
The Recruitment Subcommittee facilitates the successful recruitment of study participants and monitors and reports on recruitment progress to the Steering Committee during the trial. The Recruitment Subcommittee also identifies problems in recruitment and develops strategies for dealing with them quickly and effectively.

Publications and Ancillary Studies Subcommittee
The Publications and Ancillary Studies Subcommittee develops and recommends to the Steering Committee policies on publications and presentations and oversees the implementation of these policies. This subcommittee also is responsible for reviewing and recommending ancillary studies to the Steering Committee.

Clinic Coordinators Subcommittee
The Clinical Coordinators from each site meet as a group to discuss study problems and procedures and to communicate about approaches that are successful or unsuccessful. The chair of this group serves as a non-voting member of the Steering Committee.

Protocol Review Committee and Data and Safety Monitoring Board
An independent Protocol Review Committee (PRC), appointed by the NHLBI, reviews the protocol prior to implementation. The PRC provides advice to the Institute regarding the scientific

merit of the protocol and meets once to review the protocol and provide recommendations for approval. A second meeting may be arranged if necessary. The PRC meetings are attended by representatives from the Coordinating Center, the Steering Committee (including the study chair, vice chair, and others as deemed appropriate), and the NHLBI. Only the PRC members may vote.

Subsequent to the review of the protocol, a Data and Safety Monitoring Board (DSMB) is established. The purpose of the DSMB is to serve in an advisory capacity to the Institute in order to monitor, review, and assess the progress of the study. The DSMB has access to unblinded outcome data during the trial and, in order that participants are not exposed to unreasonable or unnecessary research risks, can recommends early termination of one or more arms of the trial if (1) the data suggest significant adverse risk to participants in the trial or (2) further follow-up would serve no added scientific purpose. The DSMB also reviews the timeliness of recruitment and the timeliness and quality of the data, based on data monitoring reports and other materials submitted by the Coordinating Center, and suggests analyses to be included in data monitoring reports.

The DSMB meets at least annually throughout the trial once the protocol has been approved. In addition to the DSMB members, meetings are attended by representatives from the Coordinating Center, the Steering Committee (including the study chair, vice chair, and others as deemed appropriate), and the NHLBI. Only the DSMB members may vote.

19. Human Subjects

Description of Consent Process

To participate in DASH2, participants must provide written, informed consent using procedures reviewed and approved by each clinical center's local IRB. In particular, even though consent to participate in DASH2 must be obtained for all stages of the study, the process and timing of consent may vary by clinic. Descriptions of each clinical center's consent procedures are included as part of the Manual of Procedures, and copies of each clinical center's consent documents are kept at the Coordinating Center. The consent forms must include all procedures done as part of screening, run-in, and feeding.

Elements of Consent

Consent to participate in a research study includes the eight elements listed below. An appropriate consent process must include all elements.

1. Participants must be advised that the study involves research. Staff must explain the purposes of the research, the expected duration of participation, and a description of the procedures to be followed, including identification of experimental procedures.
2. Anticipated benefits of the trial must be explained to the participant.
3. Attendant discomforts and risks "reasonably to be expected" must be described.
4. Appropriate alternative procedures that might be advantageous for the participant must be disclosed.
5. The extent, if any, to which confidentiality of records identifying the participant will be maintained must be described.

6. Prospective participants must be advised of the availability or non-availability of medical treatment or compensation for physical injuries incurred as a result of participation in the study, and, if available, what they consist of, or where further information can be obtained.
7. Persons responsible for the study must explain whom a participant can contact for answer to pertinent questions about the research and his or her rights, and whom to contact in the event of a research-related injury.
8. Participants must be told that participation is voluntary, refusal to participate will involve no penalty or loss of benefits to which the participant is otherwise entitled, and the participant may discontinue participation at any time without penalty or loss of benefits to which he or she is otherwise entitled.

Confidentiality

Participants in the DASH2 study are protected by the customary constraints on confidentiality of participant data. Wherever feasible, study forms identify participants only by an anonymous study ID number. Forms and electronic data are protected by reasonable security procedures including locked rooms and/or file cabinets for paper documents and coded security access for electronic data. Access to identifying information will be limited to study personnel with a need for such access. Personnel involved in DASH2 must agree not to disclose any information that might be protected by confidentiality policies to persons who do not work for the study or who do not have a need to know the information. No published data will include information that would permit readers to identify any individual participant in the study. When the study database is made available to clinical centers and to the Program Office, it will not include actual identities and contact information for participants. Such information is retained only under lock and key at the individual clinical centers and at the Coordinating Center for use in the event the future follow-up of the study participants is necessary.

Data Integrity

Data maintained at the clinical centers are internally backed up each day onto a second hard drive located in the PC. Copies of the master database maintained at the Coordinating Center are backed up daily and archived off-line on a daily, weekly, monthly, and yearly basis.

Risks

The DASH2 study should not involve any major health risk to participants. The most likely physical health risks associated with participation are gastrointestinal upset (e.g., bloating), increased frequency and bulk of stools (resulting from the high fiber content of some diets), and minor discomfort from the venipunctures. These effects are either transient or readily reversible once off the study diets. The DASH experience suggests that GI discomfort, though common, is generally minor and appears to subside over time. Participants are monitored for reactions to the diets and, if necessary, the diet can be terminated (although this was not necessary during the DASH study).

Depending on a participant's baseline diet, some of the dietary patterns and sodium levels under study may lead to slight, transient increases in blood pressure that are not sufficient to significantly raise risk for any morbid consequences. Participant blood pressure is monitored weekly during the feeding periods and potentially "at-risk" individuals, including those taking blood pressure medications, are excluded during screening. In addition, participants are referred for medical evaluation if their blood pressure exceeds pre-established "escape" levels, and are dropped from the study if they subsequently need to start on blood pressure medication.

Additional risks to study participants include: accidental breach of confidentiality; the inconvenience of having to come to the clinic each day to eat a meal and only being allowed to eat assigned study foods; the inconvenience of collecting 24-hour urine specimens; and the inconvenience of wearing an ABPM device.

Benefits

The benefits associated with participation in the study include: free food for 15 weeks, regular blood pressure monitoring, cash reimbursement (amounts vary by center), and free laboratory tests that have a small possibility for early diagnosis of an illness. Participants will also have the satisfaction of participating in a clinical trial with potentially major public health implications.

We anticipate that a majority of participants randomized to the combination dietary pattern will experience a reduction in blood pressure while on this diet. In addition, all participants will receive two months of food that is at or below current US recommendations for sodium intake. Changes in blood pressure associated with the DASH2 diets, however, whether up or down, are unlikely to be of any clinical significance over the relatively short duration of the study.

Gender and Minorities

The DASH2 study will recruit a sample that is 50% African American and 50% female. Recruitment of minorities and women is formally monitored quarterly and reports forwarded by NHLBI. Minorities other than Blacks will also be included, although no targets are set for this category and they will be grouped with White participants for analysis. All of the participating centers have demonstrated in DASH their ability to recruit large numbers of African Americans and women. Further, we have specifically powered the study to have good power to detect sodium effects for race and gender subgroups.

20. References

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