DIETARY APPROACHES TO STOP HYPERTENSION (DASH)

PROTOCOL

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DASH Protocol

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Dietary Approaches to Stop Hypertension (DASH)

Protocol

1. Overview

DASH is a four-center, randomized clinical trial designed to compare the effects of three dietary patterns on blood pressure among persons with high normal diastolic blood pressure or with Stage 1 (mild) hypertension. Collaborating on this trial are four field centers, a Coordinating Center, and the National Heart, Lung, and Blood Institute.

Participants are 22 or more years of age. After eligibility screening, all participants begin a three-week run-in diet, the "control"diet, that is approximately equivalent to the typical American diet in nutrient content. Those with adequate compliance are then randomized to one of three dietary patterns: 1) the control diet; 2) a diet (the "combination" diet) that is reduced in total fat content, high in fruits, vegetables, and dairy products, and moderately increased in protein; or 3) an intermediate diet (the "fruit & vegetable" diet) that is high in fruits and vegetables, but otherwise similar to the control dietary pattern. Participants continue on their assigned intervention diet for eight weeks.

The study provides participants with all of their food during the eleven-week feeding period. Participants are required to attend the clinics for at least one meal per day, five days a week, and take home food to eat for their other meals. Clinics will deliver the interventions in five-to-six successive waves, with approximately 23 to 29 participants per wave, over a period of two years.

The primary outcome is the change in diastolic blood pressure after eight-weeks of intervention feeding. The study will also compare the impact of the diets on systolic blood pressure and on mean waking, sleeping, and 24-hour blood pressure as determined by ambulatory monitoring.

2. Objectives

This study compares the effects of three dietary patterns on blood pressure in a population of adults with high normal diastolic blood pressure or with Stage 1 (mild) hypertension. The study also will: assess whether the influences of the dietary patterns on blood pressure vary according to race, sex, and other personal characteristics; compare the diets' effects on blood lipid levels; and determine whether ambulatory blood pressure monitoring (ABPM) provides a more suitable endpoint for blood pressure trials than do standard blood pressure measurements.

Primary Objectives

The primary objective is to test whether the DASH dietary patterns have different effects on diastolic blood pressure (DBP). DBP was selected over systolic blood pressure (SBP) as the primary endpoint in order to provide historical comparability with previous blood pressure studies, and also because persons with isolated systolic hypertension may respond differently than those with essential hypertension.

We will test the null hypothesis of no treatment effect versus the following three alternative hypotheses.

1. The change in DBP differs between the combination and control dietary patterns.

2. The change in DBP differs between the fruit & vegetable and control dietary patterns.

3. The change in DBP differs between the combination and fruit & vegetable dietary patterns.

We hypothesize that both the combination and fruit & vegetable diets will significantly reduce DBP relative to the control diet, and that the combination diet will reduce DBP more than the fruit & vegetable diet. The dietary patterns are defined briefly below. A more detailed description is provided in sections 8 and 9.

For the **"control"** dietary pattern, the distribution of potassium, magnesium, and calcium content centers on the 25th percentile of intake of the US population. The macronutrient profile and fiber content generally reflect current US consumption.

The **"fruit, vegetable, dairy, and reduced fat,"** or **"combination,"** dietary pattern is high in minerals and has a favorable macronutrient profile. The diet is high in fruits, vegetables, and dairy products, resulting in a high intake of potassium, magnesium, calcium, and fiber. The macronutrient distribution is reduced in total fat, low in saturated fat and cholesterol, has a high polyunsaturated to saturated fat (P/S) ratio, and is moderately high in protein. The distribution of monounsaturated and polyunsaturated fats matches that of the control dietary pattern.

The **"fruit & vegetable"** dietary pattern is generally similar to the control pattern, but is high in fruits and vegetables, resulting in a high potassium, magnesium, and fiber content.

Secondary Objectives

The secondary objectives are to determine the effects of the dietary patterns on SBP and on both DBP and SBP as measured by ambulatory blood pressure monitoring (ABPM). To address these objectives we will test the following hypotheses.

1. The change in SBP will vary across the three dietary pattern groups as hypothesized in 1-3 above.

2. The change in mean waking, sleeping, and 24-hour diastolic blood pressure will vary across the three dietary pattern groups as hypothesized in 1-3 above.

3. The change in mean waking, sleeping, and 24-hour systolic blood pressure will vary across the three dietary pattern groups as hypothesized in 1-3 above.

In each case we hypothesize that blood pressure reduction will be greatest for the combination diet and least for the control diet.

Other Objectives

We will also address the following additional objectives.

1. To assess whether the observed relationship between dietary patterns and blood pressure change varies according to baseline characteristics such as race, sex, age, blood pressure, weight, body fat composition and distribution, diet prior to entry into the study, insulin sensitivity, and the levels of renin, ionized calcium, vitamin D, and PTH in the blood.

2. To assess the extent to which the observed relationship between dietary patterns and blood pressure change is explained by the change in the levels of renin, ionized calcium, vitamin D, and PTH in the blood.

3. To estimate the magnitude of LDL-C and TC reduction produced by the combination diet compared with the control dietary pattern.

4. To compare the variance of change in mean waking, sleeping, and 24-hour ambulatory blood pressure (ABP) with the variance of change in blood pressure as measured using a random zero sphygmomanometer.

5. To compare the variances of mean 24-hour and mean waking ABP.

6. To compare the effects of the DASH dietary patterns on selected other ABPM measures, including peak ABP, mean waking-sleeping ABP, and diurnal patterns of ABP.

7. To assess the relationship between change in mean waking, sleeping, and 24-hour ABP and change in blood pressure as measured by a random zero sphygmomanometer.

8. To assess the relationship between blood pressure response and body fat composition as measured by dual x-ray absorptometry (DEXA).

3. Background and Rationale

Background

High blood pressure (SBP $\geq$ 140 mm Hg, DBP $\geq$ 90 mm Hg, or use of antihypertensive medications) affects almost 50 million people in the United States (1). The prevalence of high blood pressure is 30% among black Americans and 19% among white Americans (1a). In addition, a large segment of the population have blood pressures that, while within "normal" limits, are still associated with an increased risk of mortality (2). The more precise identification of eating patterns that reduce blood pressure may lead to improved dietary guidelines for the general public that help to prevent blood pressure- related cardiovascular morbidity and mortality.

The strong relationships between diet and blood pressure are well recognized (3). Striking differences exist in blood pressures among various populations (4). It has been reported that nonindustrialized nations have generally lower levels of blood pressure and less steep increases in blood pressure with age than do industrialized societies (3). This suggests that environmental factors, including diet, are related to blood pressure. Weight and consumption of alcohol and salt are directly associated with blood pressure (1,5), and strong evidence exists that potassium intake is inversely related to blood pressure (12). Vegetarian diets are associated with lower blood pressure. Even in industrialized countries, vegetarians have lower average blood pressure levels than in comparable nonvegetarian populations (6-8). Furthermore, trials testing the effects of vegetarian diets on nonvegetarians (9,10) consistently show that, when vegetable products replace animal products in the diet, blood pressure is reduced both among normotensive (9, 11) and mildly hypertensive participants (10).

These studies of vegetarian diets have led to two primary nutritional hypotheses concerning blood pressure independent of caloric balance, salt, and alcohol: (1) minerals, fiber, or other dietary components in vegetable products lower blood pressure, and (2) macronutrients such as amount or type of dietary fat or protein influence blood pressure.

Minerals and Fiber

Many studies, primarily observational, have shown significant associations between blood pressure and minerals. These associations have been strongest for potassium (12,13), but also exist for calcium (14) and magnesium (15-17). In addition, two large cohort studies in women and men found all three of these minerals were inversely related to blood pressure, to change in blood pressure over time, or to the risk of developing hypertension (17,18). In multiple regression analysis, magnesium intake was related to blood pressure, whereas potassium and calcium generally were not. Intakes of fiber and various vegetable products were also inversely associated with blood pressure (17,18).

Individual trials of cation and fiber supplementation, however, have often produced inconsistent results for potassium (19,20), calcium (14,21), magnesium (22-24), and fiber (25,26). These trials may have lacked statistical power to detect small changes in blood pressure. Meta-analysis of the effects of potassium intake on blood pressure (27) found a significant reduction of SBP and DBP in hypertensive patients, although the evidence was less convincing in the larger and better designed trials that employed random assignment. A meta-analysis of calcium (28) showed a small blood pressure-lowering effect for SBP but not for DBP. Results from randomized trials of magnesium (29) vary widely and show no clear overall blood pressure-

lowering effects. The large, well-designed Trials of Hypertension Prevention clinical trial (30), which had power to detect a reduction in DBP of 1.5 mm Hg, investigated the effect of either potassium, calcium, or magnesium supplements in normotensive adults and found no consistent significant reductions in blood pressure.

Macronutrients

Cross-sectional and prospective observational studies have generally not demonstrated a significant relationship between dietary fat and blood pressure (31). Trials testing the effects of saturated, monounsaturated and polyunsaturated (linoleic acid) fatty acids, and carbohydrates on blood pressure have been reviewed (31). In most trials, substituting carbohydrates or unsaturated fats for animal fat did not lower blood pressure. Most of the trials were small and did not have the power to detect small changes in blood pressure (less than 3-5 mm Hg). Results of studies of normotensives had point estimates that were very close to zero. Results from studies of hypertensives, when taken together, indicated a small reduction in blood pressure. No study attempted to test an overall dietary fat pattern that included low total fat, low saturated fat, high polyunsaturated to saturated fat ratio (P/S), and low cholesterol.

Epidemiologic studies of blood pressure and stroke in Japanese populations (32,33), the international INTERSALT study (34), several observational epidemiologic studies in the United States [CARDIA(35), the MRFIT (36a), Western Electric (36b), Nurses Health Study (NHS), and Health Professionals Follow-up Study (HPFS) (personal communications)], and the British Diet and Nutritional Survey (37), found significant inverse relationships between dietary protein (or urinary nitrogen as a surrogate for dietary protein intake) and blood pressure. In INTERSALT the significant inverse relationship to both SBP and DBP of 24-hour urinary total nitrogen and urea nitrogen is independent of age, sex, BMI, alcohol intake, and 24-hour excretion of sodium, potassium, magnesium, and calcium. In men randomized into MRFIT, dietary protein (% kcal) was significantly related to DBP, after controlling for age, race, education, BMI, alcohol intake, and dietary sodium, potassium, calcium, saturated fat, polyunsaturated fat, cholesterol, and caffeine. Further analyses in NHS and HPFS demonstrate a complex association of protein intake with many other nutrients. In the NHS and HPFS cohorts, persons whose intake of protein is high also have high intake of calcium, magnesium, fiber, fish, chicken, and low-fat dairy products, but low intake of meat, saturated fat, and trans-unsaturated fat. However, in NHS and HPFS, dietary protein was not significantly associated with blood pressure after adjustment for magnesium and fiber. No well-controlled trial has been conducted in the general population to test whether increasing dietary protein lowers blood pressure.

Rationale for Studying Dietary Patterns

The studies of diet and blood pressure suggest that dietary constituents other than calories, alcohol and salt influence blood pressure, and that dietary interventions may lower blood pressure. The contrast in results between the generally positive observational studies and the relatively inconsistent intervention studies may be due to differences in study design. The blood pressure-lowering effects of single nutrients may be too small to detect in modest-sized clinical trials, but when consumed in combination (as in observational studies), their cumulative effect may be sufficiently large to be detectable. In addition, for reasons not yet entirely apparent, supplements may not affect blood pressure to the same extent as do nutrients naturally occurring in foods, and it is also possible that nutrients not generally measured are responsible for the blood pressure effects of vegetarian diets. Because of these possibilities, the DASH trial was designed to test the blood pressure-lowering effect of dietary patterns and not individual nutrients or nutritional supplements. A test of dietary patterns would parallel more closely the

observational studies, where dietary intakes consist of nutrients that naturally occur together. Each dietary pattern would have a distinct nutrient profile, sufficiently different from the others, to allow testing of the hypotheses.

Because the magnitude of the effect is likely to be low, and because it is essential that the combination of nutrients packaged into the dietary patterns is clearly distinct in each experimental arm, the DASH trial will provide all the food for the participants to consume. This will permit accurate assessment of the nutrient profiles of each dietary pattern and will maximize compliance to the diets.

DASH Dietary Patterns

Because the effects of weight, alcohol, and salt on blood pressure have been intensively studied, the DASH trial directs its attention on dietary patterns that target minerals, macronutrients, and fiber. The investigators will conduct a trial of three dietary patterns. The "control" dietary pattern is relatively low in minerals and has a macronutrient profile and fiber content reflecting current U.S. consumption. The total fat, saturated fat, and protein content is, as a percent of energy, 37, 16, and 15, respectively. The P/S ratio is 0.5. The potassium, calcium, and magnesium content centers around the 25th percentile of intake from the NHANES II data (38).

The "fruit, vegetable, dairy and reduced fat" dietary pattern offers the greatest likelihood of lowering blood pressure. It is high in fruits, vegetables, and dairy products and has a "favorable" macronutrient profile (that is, reduced in total fat, low in saturated fat and cholesterol, high P/S ratio, and moderately high in protein). The total fat, saturated fat, and protein content is, as a percent of energy, 27, 6, and 18, respectively. The P/S ratio is 1.3. The potassium, magnesium, and calcium content centers around the 75th percentile of NHANES II data (38). Dietary fiber is also high. This diet involves a decrease in fat consumption and an increase in consumption of fruits, vegetables, and dairy products compared to the control diet.

The "fruit & vegetable" dietary pattern increases consumption of fruits and vegetables relative to the control dietery pattern. Its macronutrient profile and calcium content resemble the control dietary pattern, while potassium, magnesium, and dietary fiber are at levels similar to the combination dietary pattern. This diet, which is high in fruits and vegetables, was chosen as a pattern to test because the evidence is strongest, based on observational and trial data of vegetarian and similar diets, that this pattern alone may be effective in lowering blood pressure.

Each dietary pattern will be prepared at four calorie levels (1600, 2100, 2600, and 3100 kcal/day). Dietary cholesterol is at currently recommended levels of 300 mg per day for the control and the fruit & vegetable dietary patterns. For the combination pattern, cholesterol is 150 mg per day as part of a reduced fat macronutrient profile.

To avoid its potential confounding effects, sodium levels are held constant across the diets and range from 2.5 to 4.0 g, depending on the calorie level. In addition, 0.4 g of sodium is provided for discretionary use. These sodium levels are based on estimates of sodium intake from the TOHP I study, and thus represent recent estimates of typical American intake. Although these sodium levels are higher than optimal recommendations, lowering them to more optimal levels might obscure the effects of the interventions on blood pressure. In particular, sodium may interact with both calcium and potassium in such a way that their effects are most pronounced at higher sodium levels (39).

Milk and juices are provided as part of the diets. Additional discretionary beverages (including alcohol) are permitted. Daily allowances for these beverages are set so as to limit the amount of discretionary caffeine and micronutrients from these sources. In addition, heavy drinkers are excluded from the study.

Hypotheses to be tested

Blood Pressure

We hypothesize that both the fruit & vegetable and the combination dietary patterns will lower blood pressure in comparison to the control dietary pattern. Because the two dietary patterns contain several dietary components that reportedly lower blood pressure, demonstration of a significant reduction in blood pressure cannot identify specific component(s) that are responsible. Depending on the observed results of the study, however, some general hypotheses can be made. For example, if both the fruit & vegetable and the combination patterns lower blood pressure equally, it would suggest that the high potassium, magnesium, and fiber intake associated with high consumption of fruits and vegetables is the effective blood pressure-lowering component of both treatment arms. If the fruit & vegetable pattern fails to lower blood pressure while the combination pattern does, we would hypothesize that the high calcium intake associated with dairy foods or the macronutrient distribution of the combination diet is the active component. Finally, if the fruit & vegetable pattern has an intermediate blood pressure-lowering effect, ranging between the control and combination patterns, we would hypothesize that the total mineral and macronutrient composition of the combination diet is necessary for its full blood pressure-lowering effect. Ultimately, however, we will not be able to ascribe with any certainty specific blood pressure lowering effects to any specific nutrient(s). It may be that some unmeasured nutrient that correlates with the target nutrients is key, or it may be that a complex pattern of nutrients occurring together in the diet produces the effect.

ABPM

Several researchers have advocated use of ABPM technology in clinical trials (40-42). Purported benefits include enhanced precision leading to reduced sample size and/or increased study power, elimination of observer bias, and identification of “placebo-responders.” The latter refers to persons with high standard blood pressure but low ambulatory blood pressure; a few small studies suggest that such persons experience no blood pressure reduction from pharmacologic therapy, a phenomenon which alternatively can be explained by regression to the mean (43-45).

A reasonable (but inconclusive) database suggests that ABPM, compared with standard blood pressure measurements, should reduce the variance of blood pressure change (40, 46-48). Unfortunately, none of these studies has directly compared ABPM with rigorously measured standard blood pressures as typically recorded in clinical trials, i.e., multiple measurements across multiple visits obtained by trained, certified observers.

However, three small studies of non-pharmacologic therapy indicated the net effect size measured by ABPM tended to be smaller than that detected by standard measurements (49-51).

Currently, empiric evidence supporting the use of ABPM in trials of nonpharmacological therapy is still unavailable. Without a study of this type, clinical researchers will continue to debate the use and role of ABPM in clinical trials of blood pressure-lowering therapy and will continue making critical decisions in the absence of fundamental data.

We hypothesize that the variance of replicate waking, sleeping, and 24-hour mean ambulatory DBP and SBP and the variance of pre-to-post changes in mean ambulatory DBP and SBP will be less than those obtained through standard RZ measurements. We further hypothesize that the intervention effect sizes for SBP and DBP will not differ for the two measurement techniques.

Renin and Insulin Resistance

Categorizing participants by renin status and insulin resistance is also important. Resnick et al. (52-55) have reported abnormalities in calcium and magnesium homeostasis in hypertensives divided into low, normal, and high renin subgroups. Participants with low renin levels had the greatest blood pressure-lowering effect with calcium supplementation, while high-renin participants responded well to magnesium supplements. In addition, hyperinsulinemia and insulin resistance occur in both hypertensives and normotensives predisposed to developing hypertension (56-58). Insulin resistance is associated with salt-sensitivity and with cellular magnesium depletion in both white and black participants (59). Thus, renin status and insulin resistance have both been linked to alterations in the regulation of calcium and magnesium.

We therefore believe that both renin status and insulin resistance may affect response to the experimental diets. Because increasing renin levels appear to have opposite influences on the blood pressure lowering effects of calcium and magnesium, it is unclear how renin levels might modify the relative effects of the three dietary patterns. Because of the improvement in insulin sensitivity seen with magnesium supplementation in animal studies, we hypothesize that the blood pressure lowering effects of the combination and fruit & vegetable diets will increase with increasing insulin resistance.

In addition, we hypothesize that the combination dietary pattern will normalize renin status and calcium-regulating hormone levels because that diet provides an optimal intake of calcium and magnesium and is hypothesized to lower blood pressure. This effect should be most pronounced in low-renin subjects. The diet, as it lowers blood pressure, should raise normal plasma renin activity (PRA) by comparison to the control pattern and should decrease plasma 1, 25 dihydroxy vitamin D and PTH levels.

Plasma renin activity varies inversely with sodium intake. Although sodium intake is constant across the three diets, individual intake will vary from 2.5-4.0 g/day depending on the amount of discretionary sodium used, and even more widely as a consequence of noncompliance and the switch from the run-in to the assigned intervention diet. Therefore, we also anticipate salt-induced variations in PRA in addition to the variation resulting from changes in divalent cation intake and blood pressure status.

Body Fat Composition and Distribution

Growing evidence indicates that body fatness, particularly central location of body fat, is associated with carbohydrate metabolism abnormalities, hyperlipidemia, and hypertension (60). Central obesity has also been associated with increased mortality and increased incidence of

myocardial infarction, coronary heart disease, hypertension, stroke, and noninsulin dependent diabetes mellitus (60). We expect that centrally located fat will be associated with higher blood pressure for any given level of body fat, and that increased central fat may reduce the blood pressure lowering effects of the intervention diets.

Accurate assessments of body fat composition made in the context of a controlled dietary intervention on blood pressure may prove valuable in clarifying the link between diet and blood pressure. In this case, evaluations of central or abdominal obesity become of particular interest. Of current evaluation techniques, dual x-ray absorptiometry (DEXA) is the accepted standard for gauging these body fat composition traits. Two centers participating in the DASH study have access to DEXA machines and will include body fat composition in the clinical assessment package. In addition, all clinical centers will collect estimates of body fat composition from skinfold thicknesses measured at the triceps and subscapular sites.

Multi-Center Feeding Study

A unique feature of this blood pressure trial is that it is a feeding study. Dietary blood pressure trials have typically used nutritional counseling as a means of intervention. A wide range of adherence is typically observed, where perhaps one-third of the intervention group achieves the stated dietary goals. Low adherence is related to the difficulty encountered in achieving behavioral changes that require educational and behavioral efforts to promote lifestyle modifications. The provision of foods to the participants will maximize compliance because the participants do not have to learn how to make changes in their diet. However, the participants do have to make some lifestyle changes and make a substantial time commitment to accommodate the requirements of the study.

A successful multi-center feeding study requires that all the detailed quality control procedures necessary for the conduct of a multi-center clinical trial, including a standardized protocol and centralized training, must apply to the menus and food service as well. The same menus must be offered in all four field centers and thus the menu items must be acceptable for a wide geographic area covering a diverse population. Special steps must be taken to minimize nutrient variability between centers arising from food procurement, storage, preparation, cooking, and meal delivery.

4. Study Design

DASH is a four-center, randomized clinical trial designed to compare the effects of three dietary patterns on blood pressure. The three dietary patterns include: a “control” diet; a “combination” diet that is high in fruits, vegetables, and dairy products and has a reduced fat content; and a diet that is high in fruits and vegetables, but otherwise similar to the control diet. Study participants are adults, aged 22 and older, with high normal diastolic blood pressure (defined as 80-89 mm Hg DBP) or with Stage 1 (mild) hypertension (defined as 90-95 mm Hg DBP). In addition, study participants must have SBP < 160 mm Hg.

DASH participants are required to attend a series of three screening visits to determine eligibility and to collect baseline data. Individuals with uncomplicated hypertension who are currently taking antihypertensive medications need to go through a period of supervised medication withdrawal before beginning the formal screening process. The screening visits are followed by a three-week run-in feeding period using the control diet and an eight-week

intervention feeding period. Randomization occurs during the beginning of the third week of run-in feeding, and participants are not told their dietary assignment.

The study provides participants with all of their food during the eleven-week feeding period. 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. Clinics will deliver the interventions in five-to-six successive waves, with approximately 23 to 29 participants per wave, over a period of two years.

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 PI, be repeated once and the participant retained if the second value falls within eligible limits. Exceptions to this rule are diabetes and hyperlipidemia. Repeat testing for these conditions requires fasting blood samples, and criteria are described below. 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, or on medications, that would affect micronutrient metabolism and those with potentially serious chronic health conditions.

6. Recruitment and Screening

Study Population

The study sample will consist of approximately 456 healthy, free-living adult men and women, age 22 years and older, who have a DBP of 80-95 mmHg and a SBP < 160 mmHg. Given the disproportionate burden of hypertension and its complications in minority populations, two-thirds of DASH participants will be from a minority background. To reach this goal, the Pennington Biomedical Research Center will recruit a cohort that is 100% African-American and the other three sites will achieve at least 55% minority (though not necessarily African-American) participation.

In the event that a site exceeds its overall recruitment objective of 114 randomized participants, the minority target will pertain only to the initial 114 participants. Thus in absolute numbers the minority targets are 114 African-American participants for Pennington and 63 minority participants for each of the other sites.

Recruitment

Each DASH clinical center will recruit its participants in five-to-six 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 worksite screenings, 4) mass media (e.g., radio and television advertisements and public service announcements; posters), and 5) referrals from local physicians.

Table 1.

Inclusion Criteria

- SBP<160 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 11 weeks
- 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 local laboratory's gender-specific normal range (unless PI has reason to believe this is not due to nutritional deficiency)
- History of CVD event within past 6 months (stroke, CHD)
- Currently taking antihypertensive drugs and having a history of previous stroke, MI, heart failure, CABG, hospitalization for unstable angina, coronary angioplasty, or peripheral arterial disease
- Inflammatory bowel disease, colostomy, malabsorption, or any prior GI resections other than localized colonic resections
- Hepatitis (transaminase > 1.5 times the local laboratory's upper range of normal)
- ER visit or hospital stay for asthma or COPD in last six months, or other evidence of recent instability in asthma or COPD
- Renal insufficiency (GFR≤60ml/min as estimated using Cockroft-Gault formula)
- 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 >1+
- 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 >35 Kg/m^2
- DASH staff or household member of DASH staff person

Medications:

- lithium
- insulin
- oral corticosteroids
- unstable doses of psychotropics or phenothiazines
- cholestyramine
- colestipol
- oral breathing medications
- dilantin
- antacids containing magnesium or calcium, unless they can be discontinued

—digitalis
—blood pressure drugs and not willing/able to withdraw

Other Exclusionary Criteria:
—Total cholesterol >260 mg/dL. Repeat testing may include fasting cholesterol or lipid profile. For fasting cholesterol, exclude if >260 mg/dL. For lipid profile, exclude if LDL $\geq$ 160 mg/dL or if LDL = 130-159 mg/dL and participant has two or more CHD risk factors present (see Appendix 1, Fig. 2)
—Poor compliance during run-in diet
—Consumption of more than 14 alcoholic drinks per week
—Investigator discretion for safety or compliance reasons
—Unwilling or unable to modify current diet
—Current use of food supplements that cannot be stopped
—Planning to leave the area prior to the anticipated end of the intervention period
—Pregnant/planning pregnancy prior to the anticipated end of intervention
—Breast feeding
—Significant food allergies or preferences that would interfere with diet adherence.

Each center has a recruitment coordinator who oversees recruitment efforts and serves on the DASH 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

In order to be randomized, participants must complete a series of screening visits, a run-in period, and, if on antihypertensive medications, a period of medication withdrawal. 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 DASH dietary requirements and to verify the caloric level needed to maintain weight.

The sequence and timing of data collection during the screening visits was designed to maximize efficiency in excluding ineligible subjects. Information obtained out-of-sequence may, however, still be used to exclude ineligible participants. For instance, if a participant mentions during the pre-screening visit that he has a medical condition that makes him ineligible, he should be excluded from further participation even though the medical eligibility questionnaire 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 population being recruited. The PSV is intended as a fast, efficient way to eliminate ineligible participants and to identify participants who must undergo medication withdrawal prior to completing the screening process. The visit includes questionnaire data for exclusion and a single, optional, exclusionary blood pressure measurement. Individuals who complete the PSV are either excluded from further participation, referred to the drug evaluation visit (DEV)

pathway for possible medication withdrawal, or scheduled for SV1. If more than 90 days elapse between the PSV and SV1, the PSV must be repeated as part of SV1.

For individuals not currently taking antihypertensive medications, no eligibility limits are established for the PSV blood pressure measurement, although it is recommended that individuals with DBP value less than 76 mm Hg be excluded. Those who meet the PSV eligibility criteria are scheduled for SV1, which may occur immediately.

Participants who <u>are</u> taking antihypertensive medications are excluded from participation if their PSV DBP $\geq$ 90 mm Hg or if their SBP $\geq$ 150 mm Hg. Those who meet the blood pressure and other PSV eligibility requirements and who indicate a willingness to withdraw from medications for the duration of the study are scheduled for the initial DEV visit (DEV1). If no PSV blood pressure measurement is available, then it must be taken at the start of DEV1 and the participant excluded if this blood pressure is not within the above limits.

Any participant who is on antihypertensive medications at the PSV but who is not identified as such until a later visit is started on DEV1 at the time his antihypertensive medication use is determined. After completion of the DEV process the participant then re-enters the screening process at SV1. Further, the preceding blood pressure exclusion criteria applies to any blood pressure recorded while a participant is still on antihypertensive medications .

Beginning with the fifth feeding wave, all participants who are eligible to participate after completing the PSV visit, and who subsequently decline to participate, are asked to complete a refusal survey indicating their reasons for dropping out. This holds, no matter when the dropout occurs.

Medication Withdrawal (DEV Visits)

Individuals meeting PSV eligibility criteria but taking antihypertensive medications must complete an initial drug evaluation visit (DEV1). The purpose of the DEV1, which may coincide with the PSV, is to verify that the participant does not have any other medical condition that might put him/her at adverse risk should blood pressure medication be withdrawn. In particular, individuals taking antihypertensive medications and having a history of stroke, heart failure, peripheral arterial disease, MI, CABG, hospitalization for unstable angina, or coronary angioplasty cannot participate in DASH. Further, persons with a history of severe hypertension, those on blood pressure medication for a reason other than hypertension, and those taking more than two medications for blood pressure control should not be withdrawn from antihypertensive medications. (For assessing polytherapy, multiple diuretics should be considered as a single medication.)

Clinical judgment should be used to minimize withdrawal of medications from persons with a high likelihood that blood pressure will rise above 159 mm Hg systolic or 95 mm Hg diastolic during the course of withdrawal. In no case shall participants taking three or more blood pressure medications be allowed to enter the study. Prior to drug withdrawal, all persons on drug therapy must complete the medical eligibility questionnaire (otherwise distributed at SV1) and a special form designed to elicit symptoms of occult coronary artery disease and other conditions that would contraindicate drug withdrawal.

While a face-to-face consultation is not required to withdraw a participant from medication, a study clinician (MD or equivalent, PA, NP, or RN) needs to review each case prior to withdrawal of medication. In addition, the clinical center must obtain written informed consent from the participant and must document permission (either written or oral) from the participant's physician in order to withdraw medications. If this information is available at the time of DEV1 and the necessary clinical review has occurred, medication withdrawal may begin at this visit. Otherwise medication withdrawal must be initiated, either by phone or face-to-face, at some later time. No maximum time limit is set between DEV1 and the initiation of medication withdrawal.

Medication withdrawal must be supervised by a qualified clinician who shall determine the schedule of withdrawal, including tapering if indicated. DEV visits must be spaced no further than 21 days apart, and at least 21 days must elapse from completion of medication withdrawal until entry into SV1. No maximum number of DEV visits is specified. At each DEV visit blood pressure is measured and a symptom checklist completed for the purpose of safety monitoring. These measurements do not constitute study data.

All DEV blood pressures are taken as the mean of two measurements, and participants excluded if either SBP $\geq$ 160 mm Hg or DBP $\geq$ 96 mm Hg. If, at any DEV visit occurring 21 or more days post-withdrawal, SBP < 160 mm Hg and DBP is in the range 78-95 mm Hg, the participant immediately begins the SV1 visit using these blood pressure values as the SV1 blood pressures.

Prior to 21 days post-withdrawal, during which time the BPs are being collected only for safety monitoring and do not constitute study data, the BP technician need not be DASH certified and the BP device may be any appropriately maintained sphygmomanometer. All other aspects of the DASH blood pressure protocol must be followed. In particular, the subject must rest quietly for five minutes prior to the first measurement, and the second measurement should be taken 30 seconds after the first. All DEV blood pressures taken 21 or more days post-withdrawal

must be recorded using a random zero sphygmomanometer and performed by a DASH certified technician.

The DEV exclusion limits make the SV1 blood pressure eligibility range more restrictive for DEV participants than for participants not withdrawing from medications. After SV1, all study participants have the same blood pressure eligibility ranges. The various blood pressure eligibility ranges are summarized in Table 3. Participants excluded from screening at any time based on blood pressure may re-enter the screening process at a later date, but only as part of recruitment for a separate feeding wave.

In addition to the eligibility ranges stated in Table 3, safety limits have been established as described in section 13 (Safety Monitoring). Prior to randomization, individuals whose blood pressure levels exceed these limits at any given visit are not only excluded from further participation in the study, but they also are referred for medical counseling. The only exception to this rule is for the PSV blood pressure measurement. As this is a single, non-RZ measurement, no escape limits are established. Instead, each clinical center should decide on their own limits and should refer to a clinician anyone who is excluded based on a high PSV blood pressure.

Table 3. DASH Blood Pressure Eligibility Criteria

Visit		No BP Medication	BP Medication
PSV	DBP	≥76mm Hg[1]	<90 mm Hg
	SBP	--- ---	<150
DEV	DBP	--- ---	≤95
	SBP	--- ---	<160
SV1	DBP	78-100	78-95
	SBP	<170	<160
SV2[2]	DBP	79-98	79-98
	SBP	<165	<165
SV3[3]	DBP	80-95	80-95
	SBP	<160	<160

[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 0-90 days of the PSV. If more than 90 days has elapsed the PSV must be repeated. The visit should last about 20 minutes and include blood pressure, a review of general dietary information, and instructions for completing the medical eligibility questionnaire at home. The blood pressure eligibility cutpoints are listed in Table 3 and are based on the average of the two SV1 blood pressure measurements.

All DASH participants must provide written, informed consent for screening visits, DEV visits (if applicable), run-in and intervention. The number and timing of these consents will be determined by the local IRBs 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; measurement of height and weight; review of the medical eligibility questionnaire; 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. 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 and skinfold measurements; a follow-up review of questions from the medical eligibility form (if needed); a review of DASH 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, Mg, Ca, urea nitrogen, and creatinine; and completion of the physical activity questionnaire.

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 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 prior to randomization. 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 (61) to estimate resting energy expenditure and an activity factor, derived from the physical activity questionnaire, that is used to obtain an estimate of total energy requirements.

Run-in feeding must begin within 120 days after the completion of SV1, and all laboratory eligibility criteria must be met prior to the start of run-in. 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 logistical reasons the clinics will conduct the feeding in five-to-six successive waves, with 23 to 29 randomized participants per wave, over a period of two years. In order to allow for dropouts and exclusions during the run-in phase, the run-in cohorts will vary in size from approximately 24 per site (six feeding waves) to approximately 29 per site (five feeding waves). Run-in feedings will be scheduled to start on the same day for all

participants in a given feeding wave in a given clinic. However participants may be allowed to enter the run-in feeding up to two days late if the clinic 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 subjects finish run-in feeding on the same day.

The total duration of run-in feeding is three weeks, although participants are randomized during the beginning of the third week. This lag between randomization and the start of intervention feeding is required in order for the clinics to assemble and prepare the necessary foods for the start of intervention feeding. Table 2 summarizes the information collected during the run-in.

Information on medical and social history issues relevant to the trial (e.g., family history of hypertension, history of significant weight changes, socioeconomic status) is collected during the run-in period and included as part of the study database. Weight is recorded at each clinic visit, blood pressure is assessed during four of the final 13 days of run-in feeding, and a fasting blood is drawn during the last eight days of run-in feeding. This latter is sent to a central lab for analysis of fasting blood lipid levels, renin, ionized calcium, vitamin D, and PTH. A 24-hour urine sample is collected at some time during the final week of run-in and sent to a central lab for analysis of Na, K, Mg, Ca, urea nitrogen, and creatinine, which becomes part of the official study database. All participants complete medication and side effects surveys during the final week of the run-in period. Concurrent with the fasting blood draw, a subset of participants also complete a two-hour oral glucose tolerance test. A different subset of participants have their body composition assessed via DEXA measurements at some time during run-in. Beginning with the second feeding cohort at each site, all participants wear an ABPM device for 24-hours. This occurs one time during the final 13 days of run-in feeding.

In addition to the exclusionary criteria applied during the screening visits, participants may be excluded during the first two weeks of the run-in 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 and first three run-in weights defines the participant's target weight and is used as the baseline against which to measure weight change during run-in feeding. The overall caloric content of the participant's meals should be 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, clinics 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 noncompliant behavior during the final week of run-in feeding, but after randomization, are retained in the study. However, randomized participants who drop out of the study before starting intervention feeding are replaced provided that they have no knowledge of any sort concerning their randomization assignment.

Randomization

Randomization occurs during the beginning of the third week of run-in feeding (some time during days 15-17 of run-in feeding). Following randomization, participants remain on the run-in

diet until intervention feeding begins. Randomization will be stratified by clinic and, within each clinic, structured to assure comparable treatment group sizes over time.

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 14 (Quality Control and Data Management).

For participants, staff shall refer to the diets by color. The red diet is the control diet, the yellow diet is the fruit & vegetable diet, and the green diet is the combination dietary pattern.

8. Intervention

The eight-week intervention feeding period begins exactly 21 days after the scheduled start of run-in feeding. During this period participants continue to receive all of their food from the clinic and to eat on-site at least one meal per day, five days per week. As with the run-in feeding, the on-site meal should, if possible, be a lunch or dinner meal.

During this period weight is recorded at each clinic visit and blood pressure is assessed weekly. For the first six weeks the blood pressure assessment consists of a single day's set of two measurements. Five daily sets of two blood pressure measurements are recorded over the final 13 days of intervention feeding, and at least two of these sets must be taken during the final week. A 24-hour ABPM reading is also recorded during these final 13 days for all subjects except those in the first feeding cohort. Additional intervention measurements include: a 24-hour urine (during final 13 days); fasting blood lipid levels, renin, ionized calcium, Vitamin D, PTH (last 8 days); formal medication and side effects monitoring (weeks 4 and 8); and a repeat of the physical activity questionnaire (week 8). A sample from the 24-hour urine specimen is sent to a central laboratory for assessment of Na, K, Mg, Ca, urea nitrogen, and creatinine for group analyses. All randomized participants, including those who do not complete feeding, are also asked about their reasons for participating in DASH. This should be done during week 8.

The average of all weight measurements recorded during the final 13 days of run-in feeding 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 the participant's meals should be adjusted as needed in order to assure that the participant's weight remains stable during intervention feeding.

Participants will complete their required study measurements at various times during intervention week 8. Once these measurements are completed, participants may be excused from the remaining intervention meals. Note, however, that several measurements (including at least two sets of blood pressure readings) are required to be taken during intervention week 8.

At the conclusion of each feeding wave, study participants receive a summary of their study blood pressures and dietary counseling for heart disease prevention. Participants who were withdrawn from antihypertensive medications are also advised that they should consult their physicians about resuming their medications. At the conclusion of the study, all participants are unblinded to their treatment status and receive a summary of the study results. All participants will also be counseled on cardiovascular risk factors and given the opportunity to ask questions if desired at the end of the study.

9. Dietary Patterns and Menus

Dietary Patterns

Three dietary patterns will be compared for their efficacy in lowering blood pressure. The **control** dietary pattern approximates the nutrient profile of the average American diet and serves as the reference diet against which the performance of the other two diets is evaluated. The **combination** dietary pattern contains higher amounts of the target nutrients -- potassium, calcium, magnesium, fiber, and protein -- but lower amounts of total fat, saturated fat, and cholesterol relative to the control diet. The **fruit & vegetable** dietary pattern contains a nutrient profile between the control dietary pattern and the combination dietary pattern. It will be high in magnesium, potassium, and fiber, but will contain the same amount of fat, cholesterol, calcium, and protein as the control diet.

Food consumption survey data from the USDA's Continuing Survey of Food Intakes of Individuals (CSFII) (62,63), the National Health And Nutrition Examination Survey series I and II (NHANES I and II) (64,65) and the National Health Interview Survey (NHIS) (66) were used to set nutrient levels for the three experimental diets. This was done by first determining the 10th and the 90th percentile of energy intake from the combined survey data and using this as the reference range of energy within which nutrients could vary. Next, four energy levels (1600, 2100, 2600, and 3100 Kcal) likely to be consumed by 75-80 percent of the study population were selected for purposes of menu development. Finally, the 10th, 50th, and 90th percentiles of calcium, magnesium, potassium, and dietary fiber were used to set the limits of variation in the contol and the combination diets, such that a given nutrient could vary with energy from the 10-50th percentile (centering around the 25th percentile) in the control diet and from the 50-90th percentile (centering around the 75th percentile) in the combination diet. To illustrate, the 10th, 50th and 90th percentiles of potassium intake based on the combined survey data were determined to be about 1100, 3500, and 6700 mg/day. Indexing these levels to the four working energy levels yields a range of 1440-2290 mg/day for the control diet, while the range for the combination and fruit & vegetable diets is 4140-5740 mg/day.

This approach indexes nutrient intake against energy consumption, while ensuring that levels of key nutrients do not overlap among the dietary patterns. Because the information used to design the diets comes from actual population intakes, the diets have application potential should they prove efficacious in lowering blood pressure.

Fat intake levels for the control and fruit & vegetable diets (37 percent calories from fat and P/S ratio of 0.5) were set by determining the prevailing intake levels based on the CSFII, NHIS, and NHANES data. These figures are also consistent with unpublished findings from the Coronary Artery Risk Development in Young Adults (CARDIA) study.

Dietary intake data from the 1992-1993 survey of this cohort, 50% of whom are African Americans, was examined to assess typical intake of total fat. The median percent of energy from total fat was 39.2% for black males and 37.7% for black females. The respective median P/S ratios were 0.5 and 0.6. The amount of fat in the combination dietary pattern (27 percent of energy; P/S ratio of 1.3) is similar to step II AHA recommendations (67). Protein levels were set at 18 percent of energy for the combination diet since preliminary data from a number of epidemiological studies suggest that higher protein intake is associated with lower blood pressures.

The carbohydrate content of the control diet is derived from complex carbohydrates in cereals, fruits, and vegetables, and from simple sugars in candies and cakes. The increase of carbohydrates in the combination diet, which results as a consequence of the decrease in total fat levels, is accomplished primarily through the inclusion of nutrient-dense complex carbohydrates. Few simple sugars are present in the combination diet since they represent "empty calories."

In addition to the target nutrients, sodium levels are held constant across all three dietary patterns, but vary between 2500-4000 mg/day for the 1600-3100 Kcal energy range. These levels were based in part on baseline urinary sodium excretion data from phase I of the TOHP study (30), which was estimated to be 151 mmoles (3476 mg) at the 2000 Kcal level. Each day participants also are provided a (sodium and potassium free) seasoning packet and two packets of discretionary sodium (200 mg of sodium per packet). Participants are encouraged to use the seasoning packet in place of the discretionary salt. The nutrient profiles of the experimental diets are shown in Table 4.

Table 4. Nutrient Profile Targets for DASH Diets--Calorie levels: 1600, 2100, 2600, and 3100

Macronutrient Composition of Diets[1]:	**Control**	**Fruit & Vegetable**	**Combination**
Fat, %	37	37	27
Sat, %	16	16	6
Mono, %	13	13	13
Poly, %	8	8	8
P/S ratio	0.5	0.5	1.3
CHO, %	48	48	55
Pro, %	15	15	18
Range Among Calorie Levels:			
K, mg	1440-2290	4140-5740	4140-5740
Mg, mg	140-210	430-630	430-630
Ca, mg	380-580	380-580	1040-1640
Fiber, g	7-12	26-41	26-41
Chol, mg	300	300	150

[1] (% of Kcal)

Variation for nutrients:

± 10% of target for magnesium and fiber
± 5% for all other targeted micronutrients
± 5% for cholesterol
± 1 percentage point for macronutrients

The clinical centers provide participants with milk and juice to drink. Participants are also allowed three servings per day of coffee, tea, or a diet soft drink. 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 alcoholic beverages that are relatively low in the micronutrients of interest. Unused daily beverage allowances cannot be carried over to subsequent days.

Menus and Feeding Cycles

Seven-day cycle menus were prepared using the four working energy levels; 1600, 2100, 2600, and 3100 Kcal. Since participants usually do not visit clinic sites during weekends, two days of prepared meals are distributed to participants for the weekends.

Because some participants consume energy levels intermediate between those used to formulate the menus, a series of 2-4 unit foods (food modules) containing the target nutrient profile of the primary diets were designed for the purpose of adjusting energy intake.

10. Food Handling and Distribution

Purchasing and Acquisition

DASH 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 control nutrient variability, some food items are purchased from one source and then shipped to each clinical center.

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 more than 10g. 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.

Prior to feeding, the nutrient composition of the menus is verified by chemical analysis of aliquots to ensure that the designed menus achieve the target nutrient values predicted by the nutrient database (see Section 11).

Feeding Logistics

Daily food production lists include participant, day, menu cycle, meal, 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. 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. Nonstudy 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 will accommodate individual food preferences through the use of comparable substitutes. Such modified diets must remain within the limits of the protocol. All Centers provide at least one on-site meal, five days a week, with weekend meals packed for eating at home.

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 & run-in log 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 feedings and summarized into a run-in log for purposes of analysis. The daily diary and run-in logs are intended to be used as monitoring tools, 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** and do not have a good reason for having done so. Participants having good reasons may be counseled or excluded at local discretion.

All participants missing a **third meal** during the first two weeks of run-in are excluded from further participation, regardless of reason. 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 & nonstudy foods Clinics use the daily diary to estimate the number of study foods that are not consumed and the number of nonstudy foods that are consumed. Participants for whom these numbers add up to 10 or more during the second week of run-in feeding are considered noncompliers and are considered for exclusion during the case conference.

case conference At the end of the second week 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 a participant's 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 & intervention log 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 into an intervention log 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 eight weeks of intervention feeding provides a measure of overall compliance.

24-hour urine Each participant provides 24-hour urines during the third week of run-in feeding and the final 13 days of intervention feeding. Aliquots from these two specimens are sent to a central laboratory for analysis of Na, K, Mg, Ca, urea nitrogen, and creatinine for group analyses.

Persons who withdraw from the feeding program after randomization are asked to re-enter their assigned feeding wave 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 Measure

The primary outcome measure for the study is the change in DBP from baseline to the end-of-study. These terms are defined below.

Change in DBP—within-participant change in DBP from baseline to end-of-study.

Baseline DBP—the average of all mean daily DBP measurements taken during screening (SV1-SV3) and run-in. All available run-in blood pressure measurements shall be used in computing the baseline DBP. The decision to include the screening visit blood pressure measurements as part of the baseline DBP was based on simulation results that suggested (i) a negligible regression to the mean effect from including them, and (ii) an estimated reduction in the standard deviation of DBP change of 0.4 mm Hg.

End-of-Study DBP - the average of the five mean daily DBP measurements recorded during the final 13 days of intervention feeding. In the event that five sets of final blood pressure measurements are not available, the end-of-study DBP shall be computed as the average of all mean daily DBP readings (minimum of two) recorded during the final 13 days of intervention feeding. For participants who need to start on antihypertensive medications during the feeding period, every effort will be made to collect five sets of two daily blood pressures prior to the onset of therapy, and these measurements will be used to calculate the end-of-study blood pressure. However, participants will not be asked to delay the start of clinically indicated medications. For all other participants who terminate their participation prior to the completion of the intervention feeding, end-of-study blood pressure will be calculated using the two most recent sets of blood pressure measurements recorded while on the intervention diet. In all cases, the end-of-study DBP shall be computed as the average of the mean daily DBPs.

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) are still considered to be in the trial for analysis purposes. For participants who start intervention feeding but have no intervention blood pressure measurements, the end-of-the study DBP is defined to be the average of the mean SV1, SV2, and SV3 DBPs. The rationale for this rule is that participants who drop out of the study are likely to revert to their usual diet, and the screening blood pressures provide our best estimate of what their blood pressure is on their usual diet.

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, they are retained in the study and included in the final analysis.

With the exception of pre-screening and previously defined DEV 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) (68). 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 and drinking caffeinated beverages are not allowed 30 minutes prior to any reading.

Secondary Outcome Measures

Change in SBP from baseline until end-of-study serves as the secondary study outcome. The terms are defined analogously to those for DBP.

DASH participants are asked to wear an ABPM device toward the end of the run-in feeding period and toward the end of the intervention feeding period. The DASH ABPM measurement protocol is adapted from that advocated by the British Hypertension Society. We 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 returning 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 acceptable. If fewer than 14 acceptable readings are available during this timespan, the subject is asked to repeat the monitoring.

Measurements of primary interest to be derived from the ABPM data include change in mean waking, sleeping, and 24-hour DBP and SBP from baseline until end-of-study. Secondary ABPM outcomes include change in peak SBP and DBP, variation in ABPM blood pressure measurements, and change in the diurnal pattern of ABP measurements.

Funding for the ABPM substudy was obtained after start-up of the main study, and as a result collection of ABPM measurements began with the second feeding cohort at each site. More complete details concerning the measurement and analysis of the ABPM data are contained in a separate DASH ABPM Protocol, which is appended to this document.

Other Variables

Fasting lipids are collected at the end of the run-in and intervention feeding periods for the purpose of assessing any effects of the diets on lipids, particularly LDL. Blood is collected after a 12 hour fast and direct measurement made of HDL and triglyceride levels. LDL and VLDL levels are calculated. A minimum fast of eight hours is required for this measurement.

The Block Food Frequency questionnaire (69) is used to assess usual nutrient intake, and the Stanford 7-day Physical Activity Recall Interview (70) is used to assess physical activity levels and to help calculate caloric requirements.

Three 24-hour urine collections are completed: between SV2 and SV3; during week 3 of run-in; and during the final 13 days of intervention feeding. All three specimens are analyzed for Na, K, Mg, Ca, urea nitrogen, and creatinine by the central laboratory for purposes of group analyses.

Plasma renin activity is measured in samples drawn after an overnight fast and after at least 90 minutes in the upright position during run-in week 3 and intervention week 8. Baseline renin level is treated as a continuous variable and analyzed as an effect modifier in our analysis of

the blood pressure effect for each diet comparison. Ionized calcium, PTH, and vitamin D levels will also be analyzed during run-in week 3 and intervention week 8.

Change in renin levels from baseline to end of intervention will be treated as a continuous variable and used to determine the relative impact of the intervention diets on renin status. Change in renin will also be related to changes in other calcium regulating hormones (Vitamin D and PTH) and to changes in ionized calcium.

All randomized participants at some of the clinical centers receive a standard DEXA determination during run-in. Variables to be used in analysis are total percent fat, total lean body mass, percent of abdominal fat, and ratio of abdominal to peripheral fat for each individual.

For participants at another subset of clinical centers, insulin sensitivity/resistance will be measured by calculating the area-under-the-curve (AUC) for plasma insulin levels drawn at 0, 60, 90, 120 minutes after a 75 gram oral glucose load. The insulin AUC will be treated as a continuous variable and analyzed as an effect modifier in our analysis of the blood pressure effect for each diet comparison.

A sample of each participant's urine and serum will be frozen for future analyses. In addition, a sample of buffy coat (white blood cells) will be frozen in case study results suggest that genetic analyses would be helpful in understanding the relationship between blood pressure and diet.

13. Safety Monitoring

Blood Pressure Escape Levels

For reasons of safety, the following blood pressure escape levels have been established to assure that appropriate evaluation and therapy are offered when clinically indicated. Individuals who meet either escape blood pressure level prior to randomization are excluded from further participation in the study. The only exception to this rule is for the PSV blood pressure measurement. As this is a single, non-RZ measurement, no escape limits are established. Instead, each clinical center should decide on their own limits and should refer to a clinician anyone who is excluded based on a high PSV blood pressure.

Individuals whose blood pressure meets escape levels at any point during the post-randomization phase of the study are referred to a physician to determine if medications are needed. If the participant does not have a personal physician, qualified blinded personnel at the clinical center may make the recommendation for treatment. The clinical center should seek to obtain a set of up to five end-of-study blood pressure measurements and, until such time as blood pressure medication is initiated, the participant may continue in the trial.

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.

Escape level #2: The mean blood pressure for each of two successive sets of two random zero blood pressure readings, spaced no more than one week apart, exceeds either a SBP of 170 mm Hg or a DBP of 105

mm Hg. The second blood pressure must be scheduled for 1-7 days following the initial reading.

During the screening visits (SC1, SV2, SV3), individuals are excluded after the first such visit. The reference to two successive sets of measurements pertains only to the run-in and intervention phases of 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 DASH. If clinical problems arise from DASH participation, the problem may be dealt with at the clinical center or through referral as is most appropriate.

Morbid Event

Participants who suffer a morbid event with a lasting effect on blood pressure (myocardial infarction, stroke) are considered terminated as of the date of the morbid event. Their end-of-study blood pressure is calculated as the average of the two most recent mean daily blood pressure measurements taken during the intervention feeding. These participants 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 own 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.

14. 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 Validation and Monitoring

Menu Validation

Eight weekday and four weekend menus from each of the three diets at each of the four calorie levels (144 menus total) were calculated by the Diet Committee. Validation of these

menus takes place during the planning phase of the trial through food compositing and chemical analysis of nutrients. Each clinical center prepares selected weekday and weekend menus from the 2100 and 3100 calorie diets in a predetermined manner such that validation occurs on two sets of the same menus at both the 2100 and 3100 calorie levels, with each menu set prepared by two different clinical centers. Thus a total of 144 menus are prepared for compositing and assay. The menu sets are shipped frozen to Hazelton Laboratories for compositing. Frozen composited samples are then shipped to the Food Analysis Coordinating Center (FALCC) at Virginia Polytechnical Institute for nutrient analysis.

For the menu validation, nutrients analyzed include: calcium, magnesium, potassium, sodium, iron, total fat, protein (via nitrogen), moisture, and ash. Carbohydrates and total calories are available as calculated variables. 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 aliquots from each of the diet-menu-calorie composites for fatty acids (total SFA, total MFA, 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.

The unit foods for each of the three DASH diets are validated on a food by food basis. Up to four unit foods are used per diet. Each unit food-diet combination is prepared at two field centers and shipped frozen to the FALCC where they are composited and assayed. The nutrient assays for the unit foods are done in the same manner as described for the menu validation.

Diet Monitoring

Because nutrient levels may vary over time, both as a result of variations in foods available and as a result of local preparation practices, the menus are monitored sequentially during the intervention phase of the trial. Each center prepares one-to-two menu cycles (seven days of menus) per diet during each cohort. These seven-day menu sets are shipped frozen to the FALCC for compositing and assaying.

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 variables. FALCC also assays each diet-clinical center combination once during the study for fatty acids (total SFA, total MFA, total PUFA), cholesterol, and dietary fiber.

Staff Training and Certification

DASH staff are trained and certified in three main areas: clinical evaluations, data collection and management, and food preparation and handling. In addition, a detailed set of 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, ABPM, height, weight, skinfolds, physical activity, food frequency questionnaire, and (where applicable) insulin resistance and body composition as measured by DEXA. Staff are

also trained in procedures for drawing and processing blood specimens and for processing 24-hour urine specimens.

DASH uses the same blood pressure training materials as the TOHP. 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. Recertification for all technicians occurs biannually and is done locally. Recertification of trainers is done annually through a central, trial-wide process. Each DASH site must maintain on staff at least two certified, practicing blood pressure technicians. Each technician must be observed by another certified technician doing a mock RZ BP at least once every other month. The Coordinating Center monitors certification training, recertification, and quality control.

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. At the end of 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 recertified biannually.

Data Collection and Management

DASH employs a distributed data entry system in which data may be entered directly into laptop computers and transferred to a central file server at the end of each day. A complete paper-based system is available for backup. Paper backup forms are entered into the laptop computers locally at a later time. In addition, self-administered forms not used to determine eligibility are sent to the Coordinating Center for data entry. Finally, shipments of laboratory specimens and food samples need to be regularly logged.

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 uploading and downloading laptop data to the file server and 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 in May 1994. 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

Clinical 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 about four times a week 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.

Quality Control

Direct and distributed data entry via laptop computers enhances quality control through range, logic, and missing data checks performed at the time of data entry. The data management system also provides the capability to perform cross-form edits. Data inconsistencies occurring across forms are summarized in edit reports that must be resolved by local clinic staff. These audits are rerun bimonthly 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 and the Data and Safety Monitoring Board, as well as to individual clinical centers.

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 group they have been assigned, they will obviously know what they are eating.

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. 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 if the participants' physicians demand to see the data for reasons of medical management. This option is not disclosed to participants in advance. 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 intervention, participants may be given an average of all blood pressures taken. 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 feeding group until all intervention cohorts have been completed.

15. Sample Size

The study design is a three-arm randomized trial with a three-week run-in period of controlled feeding followed by an eight-week intervention feeding period. The primary trial endpoint is the change in DBP from the end of the run-in period (DBP1) to the end of the intervention feeding period (DBP2).

In order to derive a model for the variance of blood pressure change, we first assume the following variance component model for absolute blood pressure.

$$BP = \mu + \gamma + \beta + \varepsilon, \qquad (1)$$

where

μ is a fixed constant describing the true mean blood pressure in the population,

$\gamma \sim N(0, \sigma_p^2)$ is the true individual deviation about this population mean,

$\beta \sim N(0, \sigma_d^2)$ reflects day-to-day variation,

and

$\varepsilon \sim N(0, \sigma_e^2)$ reflects within-day variation.

If we assume that both DBP1 and DBP2 are computed as the mean of k daily measurements over n days, then the variance of each of these measures is given by

$$V(x) = \sigma^2 = \sigma_p^2 + \sigma_d^2/n + \sigma_e^2/(nk). \qquad (2)$$

The variance of the difference, Δ=DBP2-DBP1, may then be expressed as

$$Var(\Delta) = 2\sigma^2(1-\rho), \qquad (3)$$

where ρ is the observed tracking correlation for blood pressure measurements recorded over, in our case, 2 months. As Satterfield et al (71) point out, ρ will vary with both k and n. Following Rosner's computational method, we calculate that ρ varies from 0.84 for one measurement on each of three days to 0.89 for three measurements on each of seven days.

Using data from the Hypertension Detection and Follow-Up Program (72), we obtain the following estimates for the various variance components. These are the same estimates as used in the design of phase I of TOHP.

$\sigma_p^2 = 109.11$

$\sigma_d^2 = 26.76$

$\sigma_e^2 = 7.42$

Plugging these values into equations (2) and (3) yield the following estimates for the estimated standard deviation of Δ.

Table 5. Estimated Standard Deviation Of Blood Pressure Change When Each Blood Pressure Is Based On The Mean Of k Daily Measurements Recorded Over n Days.

n=	3	4	5	6	7	8
k=1	6.27	5.80	5.49	5.27	5.12	4.99
k=2	6.06	5.61	5.35	5.15	5.00	4.98
k=3	5.99	5.56	5.30	5.12	4.99	4.98

These suggest standard deviations in the range of 5-6 mm Hg and, for the design being proposed, a SD between 5.35 and 5.00 mm Hg. These figures compare favorably to unpublished estimates derived from the TOHP data (n=k=3) and are, if anything, conservative when compared to the TOHP data.

The preceding calculations do not, however, account for the population selection effects that will result from the blood pressure screening criteria. The true participant-to-participant variation in blood pressure among randomized participants should be much less than 109.11 mm Hg^2. To address this we conducted a simulation study following the above model and using 84.1 mm Hg as the overall mean DBP for the population. This latter figure was also derived from the HDFP data (72) The simulation used identical diastolic blood pressure cutpoints as are being proposed for this study and followed successive individuals until they either were rejected or successfully passed the three screening exams. A total of 4000 participants were accrued through SV3 and thus used to generate the official blood pressures. These results suggest a standard deviation of 3.1 mmHg, which is well below the estimates in Table 5.

Table 6 shows the sample size required per treatment group in order to detect a given difference Δ assuming a standard deviation of the change ranging from 4 mm Hg to 5.25 mm Hg. Separate estimates are presented for powers ranging from 80% to 90%. All assume a type I error rate of 5% and adjust for two multiple comparisons (see Data Analysis). The sample size estimates were derived using the standard formula:

$$n = 2*(Z_{\alpha/4} + Z_{\beta})^2*Var(\Delta)/\Delta^2,$$

where $Z_{\alpha/4}$ and Z_{β} are standard normal percentiles corresponding to significance level $\alpha/4$ and power $1-\beta$.

For purposes of this study, the Steering Committee assumed a standard deviation of 5.0 mm Hg. This is in line with the TOHP estimates and yet still substantially higher than the simulation estimates. It thus represents a realistic, yet conservative, basis for developing sample size. The Steering Committee also chose to power the study to have an 85% power to detect a mean difference in DBP change of 2.0 mm Hg.

Given these design characteristics, Table 6 suggests a total of 135 participants per treatment group, or 405 overall. Allowing for a ten percent drop out rate following randomization, this suggests that each site will need to randomize 38 participants to each

Table 6. Number Of Participants Required Per Group To Detect Given Difference Δ In Mean Diastolic Blood Pressure Change Assuming Two-Tailed, α = 0.05/2 = .025 Level Test (Bonferroni Adjustment For Two Multiple Comparisons)

(a) power = 80%

	standard deviation of change (in mm Hg)					
Δ (mm Hg)	4.0	4.25	4.5	4.75	5.0	5.25
1.50	136	153	171	191	212	233
1.75	100	113	126	140	156	171
2.00	76	86	97	108	119	131
2.25	61	68	76	85	94	104
2.50	49	55	62	69	76	84

(b) power = 85%

	standard deviation of change (in mm Hg)					
Δ (mm Hg)	4.0	4.25	4.5	4.75	5.0	5.25
1.50	153	173	194	216	239	263
1.75	113	127	142	159	176	194
2.00	86	97	109	122	135	148
2.25	68	77	86	96	106	117
2.50	55	63	70	78	86	95

(c) power = 90%

	standard deviation of change (in mm Hg)					
Δ (mm Hg)	4.0	4.25	4.5	4.75	5.0	5.25
1.50	177	200	224	249	276	304
1.75	130	147	165	183	203	224
2.00	100	113	126	140	156	171
2.25	79	89	100	111	123	136
2.50	64	72	81	90	100	110

treatment arm (or 114 total randomized per site). Each site will actually begin run-in feeding on roughly 142 participants in order to allow for an additional 20% compliance drop-out prior to randomization. Because of the conservative nature of our estimate of σ, it is likely that the actual power of the study to detect a 2.0 mm Hg difference in DBP change will be greater than 85 percent.

Previous controlled feeding trials have lost participants to follow-up because of noncompliance with protocols. The proposed run-in period is intended in part to minimize such losses. Thus we again feel that the figure of ten percent loss to follow-up at each site is conservative.

16. Data Analysis

Analysis of Primary Blood Pressure Change Endpoint

The primary outcome measure for DASH is the change in DBP from baseline to end of intervention. The primary analysis will adjust only for clinic. We will use multiple linear regression analysis to compare the change in DBP between the treatment groups after adjusting for differences in overall blood pressure change among the clinics. Dummy variables will be used to indicate the two intervention diets (fruit & vegetable and combination) and the individual clinics. A secondary analysis will further adjust for the potentially confounding effects of race, sex, age, body mass index, and baseline DBP. Additional dummy variables will be used in this analysis to indicate African-American and "other minority" racial groups.

Because we anticipate that the largest treatment differences will be between the control dietary pattern and the two intervention diets, we will use a hierarchical procedure to compare the treatment groups. First, we will examine the coefficients of the two intervention group indicator variables. These coefficients will have the interpretation of the difference in DBP change between the specific intervention group and the control group. If neither of these coefficients is significant at the α=.05/2 =.025 level (Bonferroni adjustment for two comparisons) the three treatment groups would be declared not significantly different. If either coefficient is significant, then the two intervention diets would be compared at the same significance level, α=.025.

This procedure is similar to the Fisher Least Significant Difference (LSD) procedure, whereby a global F-test is performed and unadjusted pairwise comparisons are made only if the global statistic is significant (73). Our procedure can be viewed the same way, except that our global statistic is the maximum of the t-statistics comparing each intervention group to the control group, and the comparison of the intervention diets is made at significance level α=.025 instead of .05 to make all of the pairwise tests comparable. Our procedure offers strong control of the Type I error rate (74).

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-study blood pressure levels. End-of-study blood pressure values for such participants are discussed in section 8 (Outcome Measures). We will use an intention-to-treat analysis whereby each randomized participant will be included in the analysis according to his randomly-assigned diet group. The only exception to this will be for randomized participants who drop out of the study before beginning intervention feeding and without knowledge of their intervention assignment. These participants will be excluded from all analyses, since their loss should be totally unrelated to randomization assignment.

Fortunately, the run-in period should identify the majority of noncompliers prior to randomization, and the number of participants needing to go on medications during follow-up should also be small. Thus the number of participants with censored data should be minimal.

Analysis of Secondary Outcome Measures

Secondary blood pressure outcomes of interest include change in SBP recorded using the random zero sphygmomanometers and changes in both SBP and DBP as measured using ambulatory blood pressure monitoring. These outcomes will be evaluated in the same manner as described above for change in DBP. For ABPM, blood pressure is measured as the mean waking, sleeping, and 24-hour blood pressure.

Analysis of Other Outcome Measures

In order to determine if any observed treatment effects differ for various subgroups, we will incorporate the appropriate interaction terms into our statistical model and test whether the interaction model significantly improves the fit to the data relative to the basic model. Specific subgroups of interest are male versus female and African American versus non-African American. Additional analyses will test whether baseline renin status and insulin resistance influence treatment differences.

We will also compare the effects of the three dietary patterns on serum lipid levels. Specific outcomes of interest for this analysis are total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides. For each of these outcomes we will compute within-participant differences as for blood pressure and then conduct a multivariate analysis of variance (MANOVA) to simultaneously test whether treatment differences exist for any of the outcomes. If this test is significant, we will conduct separate analyses for each of the four outcomes using an approach analogous to that described for change in DBP.

An additional outcome of interest is whether ABPM provides greater precision for measuring blood pressure change in clinical trials than do standard blood pressure measurement techniques (i.e., use of random zero sphygmomanometers). We will evaluate this hypothesis by using the Morgan-Pitman test statistic for correlated variances (75) to compare the variance of blood pressure change estimates made using ABPM with those made using RZ sphygmomanometers. For ABPM, blood pressure is measured as the mean waking, sleeping, and 24-hour blood pressure. In order to compare effect sizes as measured using the two measurement techniques, we will also regress within participant change in ABPM blood pressure on within participant change in standard blood pressure and test whether the slope of the regression line is significantly different from unity. Finally, we will use this same regression technique to compare standardized effect sizes. Specifically, let (D_i, D'_i) denote blood pressure change measured the standard way and by ABPM, respectively, for the ith participant. Let s and s' denote the estimated standard deviations of the Ds and D′s, respectively. Since these standard deviations will be based on a large number of observations, we can treat these as population standard deviations σ and σ', and compute standardized effect sizes $E_i = D_i/s$ and $E'_i = D'_i/s'$. Comparing these standardized effect sizes will most directly answer the question of primary interest, namely, is ABPM a more sensitive technique than standard blood pressure measurement for assessing blood pressure change in clinical trials? The separate DASH ABPM Protocol lays out a number of additional analyses related to the ABPM data. These are viewed primarily as exploratory data analyses and are not further discussed here.

In addition to the baseline and final blood pressure measurements, numerous intermediate blood pressure measurements will also be available on each individual. In the event that we observe significant mean differences in overall blood pressure change between the treatment groups, these intermediate blood pressure measurements can be used to evaluate the pattern of change over time. Such an analysis would be largely descriptive in nature and would be

used to determine whether the observed declines occurred steadily over time or followed a more curvilinear pattern. For example, one hypothesis is that the bulk of the blood pressure decline would occur fairly soon after the introduction of a new diet and that blood pressure levels would remain fairly stable after that. Random effects models will be used to test for a nonlinear pattern of decline.

17. DASH Timeline

On the DASH timeline, the planning phase extends from August, 1993, through May, 1994. Recruitment begins in June, 1994, and extends through May of 1996. Although the timeline shows seven feeding waves, the clinics plan to deliver one intervention in five-to-six waves of 23-29 participants each. The additional wave provides a backup in the event that our recruitment targets have not been met. All feeding will be completed by September, 1996, with clinic close-out shortly thereafter. Analysis and writing projects will continue through July, 1997.

18. Trial Administration

Participating Sites

Participating institutions in the DASH study include the NHLBI Project Office, the Data 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 (Virginia Polytechnical Institute, Blacksburg, VA) and the Central Laboratory (Oregon Health Sciences University, Portland, OR) are contracted through the Coordinating Center.

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 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 and a vice-chair, and the PI of each participating institution and the NHLBI Project Officer (or their designee) has 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 Committee 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. The 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 director, reviewed the protocol prior to implementation. The PRC provided advice to the Institute regarding the scientific merit of the protocol and made recommendations to improve the protocol and its implementation.

Subsequent to the review of the protocol, a Data and Safety Monitoring Board (DSMB), composed of members of the PRC, was 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, recommends early termination of one or more arms of the trial (1) if the data suggest significant adverse risk to participants in the trial or (2) if the question posed by the trial appears to have been answered. 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 developed. In addition to the PRC or DSMB members, meetings are attended by representatives from the Coordinating Center, the Steering Committee (including the chair and vice chair), and the NHLBI. Only the PRC or DSMB members may vote.

19. Human Subjects

Description of Consent Process

In order to participate in DASH, 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 DASH 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, including copies of the consent forms, are included as part of the Manual of Procedures. Procedures covered by the consent forms must include:

1. Prescreening/SV1
2. SV2/SV3/Run-in
3. Randomization
4. Withdrawal from antihypertensive medication

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 the subject's 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 DASH study are protected by the customary constraints on confidentiality of participant data. Hard copy forms, except those containing contact information, are identified only by a 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 DASH must agree not to disclose any information which 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 which would permit readers to identify any individual participant in the study. When, and if, 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 should be 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.

20. References

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