Omni-Heart Protocol

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1. SUMMARY

While there is widespread consensus that the optimal diet to reduce cardiovascular risk should be low in saturated fat **and cholesterol**, the type of macronutrient that should replace saturated fat (carbohydrate, protein or unsaturated fat) is uncertain. The Omni-Heart trial evaluates the effects of these 3 macronutrients on established coronary risk factors and a selected group of emerging risk factors. The core design is a randomized, three period cross-over feeding study that compares the effects on blood pressure and plasma lipids of a carbohydrate-rich diet (CARB) to a diet rich in protein, predominantly plant-based protein, and another diet rich in unsaturated fat (UNSAT), predominantly monounsaturated fat. The CARB diet has been shown to reduce blood pressure and LDL-cholesterol substantially, and is currently recommended by policy makers. During a 1 week run-in, all participants are fed samples of the 3 study diets (CARB, PROTEIN and UNSAT). Using a three period cross-over design, participants are then randomly assigned to the CARB, PROTEIN OR UNSAT diet. Each feeding period lasts 6 weeks; a washout period of at least 2 weeks separates each feeding period. Throughout feeding (run-in and the 3 intervention periods), participants are fed sufficient calories to maintain their weight. Trial participants (n=160, ~ 50% female, ~ 50% African-American, **~30% hypertensive**) are 30 years of age or older, with a blood pressure that is above optimal but less than stage 2 hypertension (systolic blood pressure of 120-159 mmHg or diastolic blood pressure of 80-99 mmHg). Primary outcomes variables are blood pressure and plasma lipid risk factors (LDL-C, HDL-C and triglycerides). Other outcomes are **total cholesterol,** apolipoproteins VLDL-apoB, VLDL-apoCIII, apolipoprotein B, non-HDL cholesterol, and lipoprotein(a).

2. SPECIFIC AIMS

Cardiovascular disease (CVD), including coronary heart disease (CHD) and stroke, remains the leading cause of death in the western world. In economically developing countries, CVD is the second highest cause of death with a rising trajectory. High BP and dyslipidemia are the major, diet-related risk factors for CVD. There is much divergence of opinion among experts as to what diet or diets are ideal for improving these risk factors (Connor and Connor, 1997; Katan, Grundy and Willett, 1997). Specifically, although there is widespread agreement that saturated fat **and cholesterol** intake increases LDL cholesterol and contributes to coronary artery disease (ATP III, 2001), there is less agreement about the nutrient that is an optimal replacement for saturated fat. Cogent arguments are being made for replacing saturated fat with complex carbohydrate, protein or unsaturated fat, but there are also arguments against each of these choices. The basis for the divergent opinions is the lack of evidence from definitive studies that test these diets in a single trial and that assess the impact of these diets on the major, diet-related CVD risk factors [blood pressure and the plasma lipids, LDL-cholesterol (LDL-C), HDL-cholesterol (HDL-C), and triglycerides].

In the Omni-Heart trial, we compare the effects of a carbohydrate-rich diet (CARB), based on the DASH diet, to a diet rich in protein, predominantly plant-based protein, and another diet rich in unsaturated fat (UNSAT), predominantly monounsaturated fat. The core design is a three period crossover study with 6 week periods. Participants are fed each of three iso-caloric diets, presented in random order. Study participants (n=160, ~50% female, ~50% African-American, **~30% hypertensive**) are 30 years of age or older, with a blood pressure that is above optimal but less than stage 2 hypertension (systolic blood pressure of 120-159 mmHg or diastolic blood pressure of 80-99 mmHg). In the whole cohort of enrolled participants, we will address the following specific aims:

2.1 Primary Specific Aims

Specific Aim 1 (Blood Pressure): test **whether the PROTEIN and UNSAT diets reduce** blood pressure (BP) **in comparison to CARB and whether PROTEIN and UNSAT differ in their effects on BP.** Systolic BP is the primary BP outcome and diastolic BP a secondary outcome.

Hypothesis: **compared to CARB,** the PROTEIN and UNSAT diets will reduce systolic and diastolic BP. **Compared to UNSAT, PROTEIN will reduce systolic and diastolic BP.**

Specific Aim 2 (Traditional Lipid Risk Factors): test the effects of each diet on plasma LDL-cholesterol, HDL-cholesterol and triglycerides. LDL-cholesterol is the primary lipid outcome; HDL-cholesterol and triglycerides are secondary lipid outcomes.

Hypothesis: compared to the CARB diet, the PROTEIN diet will reduce plasma LDL-cholesterol, will have no effect on HDL-cholesterol, and will reduce triglycerides. Compared to the CARB diet, the UNSAT diet will have no effect on LDL-cholesterol, will raise HDL-cholesterol, and will reduce triglycerides.

2.2 Other Specific Aims

Specific Aim 3 (Glucose/Insulin): assess the effects of each diet on fasting levels of glucose and insulin, and the insulin resistance index, as measured by Homeostasis Model Assessment (HOMA; Mathews 1985).

Specific Aim 4 (Other Lipid and Lipoprotein Risk Factors): assess the effects of each diet on total cholesterol, on the plasma concentrations of VLDL and LDL particle types, VLDL with apoCIII, VLDL without apoCIII, LDL with apoCIII, LDL without apoCIII; on total plasma apoB, on total VLDL and LDL apoB and cholesterol, on non-HDL cholesterol, , and on Lp(a).

Specific Aim 5 (Cardiovascular Risk): assess the overall impact of each diet on cardiovascular risk, **by applying cardiovascular risk prediction equations derived from prospective observational studies to trial results.**

In subsidiary analyses, the specific aims will be addressed in subgroups, defined baseline level of the outcome variable (e.g. for BP, in hypertensives and non-hypertensives), by gender (women, men), race-ethnicity (African-Americans, non-African-Americans), age (those above the median age of study participants, those below the median age of study participants), menopausal status (pre-menopausal women, post-menopausal women), and weight status (non-overweight, overweight, and obese individuals by BMI).

3. BACKGROUND AND SIGNIFICANCE

3.1 Blood Pressure

3.1.1 Impact of low fat, high carbohydrate diets on blood pressure:

Worldwide, there are many populations that eat low- or **reduced-**fat, carbohydrate-rich diets and that have low BP levels compared to western countries (reviewed in Sacks, 1974). In the US, vegetarians who ate a low-fat diet had BP levels that were similar to those in non-industrialized populations with low BP (ibid). Still, small-scale trials that reduced total dietary fat intake generally did not find reductions in BP (Morris & Sacks 1994), although one well-controlled trial documented significant reductions in BP (Puska et al, 1983).

The largest well-controlled trial of a **reduced-fat** diet and BP was DASH (Dietary Approaches to Stop Hypertension, Appel et al 1997). This trial studied a high-carbohydrate diet that included fruits, vegetables, whole grains, nuts, low-fat dairy products, fish, and poultry with reduced amounts of red meat and sugar-containing foods and beverages. In the setting of stable weight and sodium intake, the DASH diet substantially lowered BP in the overall study population, as well as in major subgroups, including normotensives and hypertensives, Blacks and Whites, and men and women (Appel 1997; Svetkey 1999). In hypertensives, the dietary effects were similar to those expected from initial drug therapy. However, the DASH study tested an overall dietary pattern that differed in numerous aspects from the other two diets, the 'control' diet and the 'fruits and vegetables' diet. The DASH study was not designed to study the effects of individual foods or nutrients. Hence, the results from DASH cannot be interpreted as establishing a BP effect of replacing saturated fat with carbohydrate. For instance, the higher protein, **potassium, magnesium, or fiber** content of the DASH diet might have contributed to the BP effects of the diet.

3.1.2 Impact of high protein intake on blood pressure:

Observational Studies: An extensive, and generally consistent, body of evidence from observational studies have documented significant associations of high protein intake with reduced BP. These data **has** been reviewed by Obarzanek (1996) and by He (1999). In the report by Obarzanek, protein intake was inversely associated with BP in 11 populations, the opposite relationship was found in only 1 study, and no relationship was found in another (Longitudinal analyses of CARDIA, Liu 1992). Populations in which there were inverse associations include 1120 Japanese rural farmers (Yamori 1981; Kihara 1984), 6496 Japanese-American men in Hawaii (Reed 1985), 2325 men and women in the INTERSALT 32-country study (Dyer 1992), 11,342 men in the MRFIT screenees (Stamler 1996), 1922 British men and women (Elliott 1992), an ecological study of 2672 men and women in 10 population groups in China (Zhou 1989), 705 men and women in 2 groups in China (Zhou 1994), and the CARDIA population of 3809 US children (cross-sectional analyses, Liu 1992).

Analyses from 2 contemporary US populations, the Nurses Health Study (NHS) in women and the Health Professionals Followup Study (HPFS) in men, extend the above findings and highlight the importance of our trial (A Ascherio, unpublished analyses). In both studies, significant inverse associations of protein intake and BP were present. Of note is the finding, evident in both studies, that those who ate a high protein diet also had a high intake of fruits and vegetables, whole grains, fish and poultry rather than red meat, and low intake of desserts, i.e. a diet similar is several respects to the CARB diet. This degree of collinearity impeded the ability of multiple regression analysis to distinguish between protein and the other components of the CARB diet. Such findings reinforce the need for a clinical trial to determine whether, and by how much, dietary protein lowers BP.

Whether the source of protein (animal versus non-animal) has a differential impact on BP has been uncertain. Animal protein was inversely associated with BP in some studies (Yamori 1981; Kihara 1984; Dyer 1992; Zhou 1989; Zhou 1994; **Stamler 2002**) and with vegetable protein in others (Liu 1992;A. Ascherio unpublished analyses from NHS and HPFS). Data from the INTERMAP study (International Study of Macronutrients and Blood Pressure) provide the most persuasive evidence that increased consumption of vegetable protein is associated with lower BP. This cross-sectional study enrolled **4,860** men and women, ages 40-59, from 4 countries (Japan, China, United Kingdom and the United States). Measurements were notable for meticulous collection of dietary data (four standardized 24hr dietary recalls, two 24hr urine collections) and blood pressure (eight measurements across four visits). Vegetable protein (% kcal) was significantly and inversely associated with both systolic and diastolic BP after controlling for other dietary determinants of BP (sodium, potassium, and alcohol) and for other factors (age, sex, education, height, pulse, cigarettes/day, use of BP medication). In corresponding models, animal protein was not associated with

systolic or diastolic BP. In view of these data, protein from vegetable sources are emphasized in the PROTEIN arm of this trial.

Clinical Trials: Prior to the publication of Obarzanek's review (1996) only small, underpowered trials had tested the impact of protein on BP. Five were randomized controlled trials, all with ≤25 patients per diet group. Only 1 investigated whether increasing dietary protein affects BP; no effect of soy protein was found in 13 strict vegetarians with lower than average baseline BP (Sacks 1984). In this study, effects on BP of 4 - 5 mmHg would not have been detected. The other studies investigated the effect of substituting animal and vegetable proteins without changing the total amount (Brussaard, 1981; Sacks,1988; Prescott 1987; Kestin 1989), and these also found no effect of protein type on BP

In contrast to the small underpowered trials, noted above, recent trials have been somewhat larger and more appropriately designed. Through literature searches and queries of opinion leaders, we identified 6 trials (Burke, 2001; He, 2000; Teede, 1999; Crouse, 1999; Washburn, 1999; Williams, 1999), several published in just abstract form. Each trial tested the impact of a soy-based intervention on BP. Of these trials, 5 documented a significant effect of increased protein intake on systolic and/or diastolic BP. The trials of Burke and He are especially relevant, because their primary focus was BP and their BP entry criteria were similar to those used in this protocol. Burke's trial is also noteworthy because it is a controlled feeding study. Each trial reported significant reductions in both systolic and diastolic BP, reductions that our trial should detect given our minimum detectable effect sizes..

To summarize, observational epidemiological studies strongly support the hypothesis that raising protein intake lowers BP. However, this hypothesis has not been adequately tested in a clinical trial of sufficient size and rigor. Obarzanek, Velletri and Cutler conclude in their review, "Because of insufficient data and limitations in previous investigations, better controlled and adequately powered human studies are needed to assess the effect of dietary protein on blood pressure." It is conceivable, then, that replacing saturated fat with protein rather than carbohydrate could enhance the BP lowering of a **reduced**-fat diet like DASH.

3.1.3 Impact of unsaturated fat intake on blood pressure:

In parts of Greece where traditional diets were eaten, the incidence of hypertension was half that of western Europe and the US (Keys 1980). Moreover, among the communities in the Seven Countries Study, including rual Japan, the incidence of CVD was lowest in Crete, and it was low in several of the rural Mediterranean areas. A small-scale clinical trial found that a Mediterranean diet reduced BP in Italians (**Strazzullo** 1986). Although many nutrients differ between the diets of Mediterranean countries and western Europe, a higher intake of monounsaturated fats is one of most prominent differences, and could contribute to lower BP.

The specfic effects of monounsaturated fatty acids have been neglected among the many studies of dietary fats (Morris 1994). Replacement of carbohydrate with monounsaturated fat significantly reduced BP in a small trial of normotensive individuals with non-insulin dependent diabetes (Rasmussen, 1993). More recently, a double-blind study in non-diabetic medication-treated hypertensives found that replacing saturated fat with olive oil significantly lowered BP and reduced the need for antihypertensive medications, whereas sunflower oil, rich in linoleic acid, did not affect BP (Ferrara 2000). Since a diet rich in monounsaturated fat is being advocated by some experts as ideal for reducing cardiovascular risk, it is important to know the effects of this type of diet on BP. To this end, the Omni-Heart trial tests whether an increased intake of unsaturated fat, predominantly monounsaturated fat, **reduces BP** in comparison to a **reduced-**fat, carbohydrate-**rich** diet.

3.2 Plasma Lipids and Lipoproteins

3.2.1 Effects of dietary fat by gender and race:

There is broad consensus that reducing dietary saturated fat **and cholesterol** improves LDL-cholesterol concentration (ATP III, 2001; Mensink 1992). Replacing saturated fat with monounsaturated or polyunsaturated fat lowers LDL slightly more than using carbohydrate (Mensink 1992). The effects of dietary fats in women and in non-white populations are less well-known. In the meta-analysis of Mensink and Katan (1992), there was a trend for the LDL of women to respond to diet less than men. The DASH trial recently documented that the reduced-fat, carbohydrate-rich DASH diet had significantly less effect on LDL in women compared to men (Obarzanek 2001). These findings provide a strong rationale for studying the effects of substituting dietary saturated fat with carbohydrate, unsaturated fat, and protein in women, and to enroll enough women for sufficient statistical power in separate analyses. In African-Americans, there is little information on the effects of diet on plasma lipids. The DASH trial is one of few trials with sufficient power to detect differences between African-Americans and non-African-Americans. In this trial, the DASH diet had similar effects on lipids in African-Americans and non-African-Americans. As in the DASH and DASH-Sodium trials, Omni-Heart has been designed to provide adequate power in subgroups defined by race.

3.2.2 Effects of dietary fat by initial lipid level:

Dietary effects on plasma lipids may be influenced by baseline levels. It has been long assumed that cholesterol-lowering effects of diet are proportional to the initial level, based on studies of Keys (1957). However, evidence from experimental studies is sparse. In DASH, the initial level did not affect the extent of LDL reduction from the **reduced**-fat, carbohydrate**-rich** diet. This unexpected finding needs to be confirmed and extended to the effects of unsaturated fats and protein. As regards protein, Anderson et al (1995) reported in a meta-analysis that soy protein reduced cholesterol in hypercholesterolemic patients but only minimally in those with average cholesterol concentrations. This result is not fully convincing because the studies in hypercholesterolemic patients were not as well controlled as those in normocholesterolemics.

It has been only recently established that the triglyceride-lowering effects of the statin drugs, even when expressed as a percentage change, are directly proportional to the baseline triglyceride level (Stein 1998). It is considered a truism in the lipid field that the hypertriglyceridemic effect of dietary carbohydrate is increased in persons with higher triglyceride levels, although this has not been rigorously established in a controlled trial. Triglycerides are gaining increased importance as a risk factor in view of meta-analyses that demonstrate an independent prognostic effect (Hokanson and Austin, 1996). It is noteworthy that **OmniHeart** has sufficient power to compare effects in persons with triglyceride levels above vs below 150 mg/dl, a level recommended by NCEP-ATP-III as a cutpoint for defining a normal triglyceride.

Finally, the influence of baseline HDL needs to be investigated. Recently Azstalos (2000) reported that HDL reduction from a low-fat diet only occurs in patients with above-average baseline levels, a finding also observed in DASH (Obarzanek 2001). The influence of baseline lipid concentrations on the effects of dietary fat, carbohydrate and protein could have immense clinical implications and lead to dietary guidelines more tailored to the lipid phenotype of patients.

3.2.3 Effects of dietary protein on lipid levels:

The effects of amount of dietary protein on plasma lipids are uncertain. Most research on the effects of dietary protein on plasma lipids focused on soy protein. Soy protein lowers LDL-cholesterol in humans (Anderson 1995), although it is unclear to what extent the effect is due to protein or to non-protein phytoestrogens, that is isoflavones, that are present in variable amounts in certain preparations of soy protein; this specific topic is currently being studied by several research groups. More practical and relevant to human nutrition, in general, are the effects of a mix of common dietary proteins on LDL and the other plasma lipids. In a series of small feeding studies by Wolfe (1991, 1992, 1999), replacement of carbohydrate with protein from a variety of sources had favorable effects on lipids in normocholesterolemic, moderate hypercholesterolemic, and familial hypercholesterolemic individuals. In these trials, the diets that provided ~25% kcal from protein significantly lowered total cholesterol, LDL cholesterol and triglycerides; rises in HDL were non-significant. In summary, increasing the amount of dietary protein may favorably affect both LDL cholesterol and triglycerides. However, there is a clear need for additional trials.

3.2.4 HDL as a Cardiovascular Risk Factor

HDL is a well-established cardiovascular risk factor; high levels are associated with reduced risk (Gordon 1989). Many lines of evidence support a causal relationship between HDL and coronary atherosclerosis. These include evidence that (1) HDL removes cholesterol from macrophages foam cells (Bernard 1990), (2) HDL inhibits oxidative damage to LDL (Parthasarathy 1990), (3) HDL (apoAI) overexpression in mice inhibits diet-induced atherosclerosis (Rubin 1991), (4) HDL delivers cholesterol to the liver and steroidogenic cells by binding to the receptor SRB-1 (Williams 1999), and knockout of the SRB-1 gene promotes atherosclerosis (Rigotti 1997), and (5) clinical trials found that increases in HDL in response to lipid drugs are correlated with reduction in atherosclerosis and clinical coronary events (Gordon 1989). Dietary carbohydrate lowers HDL concentrations by reducing apoAI production (Brinton 1990; Velez-Carrasco W 1999). There are 2 opinions about the clinical significance of this fact. Some experts are concerned about the reduction in concentration and production of an anti-atherogenic lipoprotein, and would prefer a dietary option such as monounsaturated fat that does not reduce HDL (Katan et al 1997). Others note that it is not proven that dietary carbohydrate causes an atherogenic change in HDL metabolism that inhibits cholesterol removal, and some suggest that the deleterious reductions in HDL are less important than the favorable reductions in LDL (Connor 1997). There is currently no test that can distinguish between an atherogenic reduction and an innocuous reduction in HDL. The emergence of such a test will depend on advances, perhaps occurring during the period of this research project. For this reason, we plan to interpret the overall dietary effects on cardiovascular risk with and without inclusion of changes in HDL. As information develops about HDL metabolism, additional phenotypic and genotypic measurements related to HDL can be added.

3.2.5 Triglycerides as a Cardiovascular Risk Factor

After years of controversy, the concentration of triglycerides is now established as an independent risk factor for CHD (Hokanson 1996; Gotto 1998; Stampfer 1996; Jeppeson 1998). In univariate analysis, triglyceride concentration consistently predicts incident coronary disease. However, when HDL and triglycerides are included together in multivariate analyses, triglyceride often becomes non-significant. Hokanson and Austin (1996) performed a meta-analysis of observational studies and demonstrated that adjustment for HDL did not remove the predictive value of triglycerides. Individual large-scale cohort studies subsequently found that triglycerides independently predicted CHD (Stampfer 1996; Jeppson 1998). However, as an independent lipid risk factor, triglyceride concentration is weaker than LDL- or HDL-cholesterol. For example, in men, a 30% change in plasma lipid concentration corresponds to a change in coronary risk of 7% for triglycerides (Hokanson1996) versus 30% for LDL-cholesterol or HDL-cholesterol (Holme 1995; Gordon 1989). Triglyceride in plasma is carried in lipoprotein particles; about 2/3 of the plasma total triglycerides is in VLDL, and the rest in LDL and HDL.

VLDL are a diverse group of lipoprotein particles that vary in triglyceride, cholesterol, and apolipoprotein content, and in their metabolism (Alaupovic 1996; Hodis 1999). Apo CIII is a major determinant of the metabolism of VLDL in plasma, and, in animal models, it accelerates atherosclerosis (Ebara 1997). It has been proposed that the apolipoprotein composition of lipoproteins is more closely linked to CHD than the conventional lipoprotein measurements of lipid content and density (Alaupovic 1996; Hodis 1999). In case-control studies, apoCIII concentrations in VLDL and LDL were higher in patients with coronary disease compared to controls (Chivot 1990, Luc 1996) and apoCIII was correlated with worsening coronary stenosis on angiography (Blankenhorn 1990; Hodis 1994; Alaupovic 1997). Apo CIII in VLDL/LDL was a significant independent predictor of recurrent coronary events in the CARE trial, and accounted for the risk associated with increased plasma triglycerides (Sacks, 2000). In this trial, triglyceride concentration was an independent risk factor in univariate analysis. However, in multivariate analysis, the relative risk of a recurrent event for the fifth vs the first quintile of apoCIII was 2.7, whereas the relative risk for triglycerides was reduced from 1.7 to 1.1. Finally, the strongest TG-related risk factor in the CARE trial was VLDL-apoB, the concentration of VLDL particles in plasma. This is reasonable, since entire VLDL particles enter the arterial intima. These findings were extended to determine specific VLDL or LDL particle types that are predictors of MI. The patients in the CARE trial who were diabetic at baseline and who experienced a recurrent CHD event (N=121) were matched to those who did not have a recurrent event. Plasma was separated by anti-apoCIII immunoaffinity chromatography and ultracentrifugation into VLDL with apoCIII, VLDL without apoCIII, LDL with apoCIII, and LDL without apoCIII. The LDL fraction contained "IDL". LDL with apoCIII was the strongest predictor of events, RR=5.6, P<0.001, for 4th vs 1st quartiles, in univariate as well as multivariate models that included the standard lipid risk factors. LDL without apoCIII, comprising most of the LDL particles, and VLDL without

apoCIII, were borderline, RR=2.0, P=0.07-0.09. VLDL with apoCIII, a very large triglyceride-rich particle was not a predictor (RR=0.5) perhaps because particles this large have difficulty passing through the vascular endothelium. These apoB lipoprotein types will be measured in the study to provide increased understanding of whether the dietary effects on triglycerides are associated with atherogenic or innocuous apoB lipoproteins.

3.2.6 Lipoprotein(a) as a Cardiovascular Risk Factor

Lipoprotein(a) is an LDL-like lipoprotein that has apolipoprotein(a) attached to apoB. The apo(a) adds a thrombogenic factor to the atherogenic LDL. The vast majority of retrospective case-control studies and most of the prospective studies have found that Lp(a) is an independent predictor of coronary heart disease. It was unexpected that in a recent well-controlled multi-center study, carbohydrate *increased* Lp(a) compared to saturated fat (Ginsberg 1998). In another well-controlled study (Clevidence 1997), monounsaturated fat increased Lp(a) compared to saturated fat. These results need re-evaluation in a careful dietary protocol.

3.3 Fasting glucose and insulin

The effects of high carbohydrate, **reduced**-fat diets and high monounsaturated fat diets on fasting and postprandial glucose and insulin concentrations, and on insulin sensitivity have been controversial. Some studies found that high carbohydrate, low fat diets compared to higher saturated fat diets did not affect insulin sensitivity in normal participants (Borkman 1991) or in patients with type 2 diabetes (Garg 1992). Other studies found worsening of post-prandial hyperglycemia or hyperinsulinemia in diabetic patients (Parillo 1996) or in hypertensive patients (Parillo 1988) after replacement of dietary fat with carbohydrate. **The divergent findings on glycemic or insulinemic responses might have resulted from the type (or amount) of fat, carbohydrate, and fiber. In some instances, extremes of dietary intake were tested.** When high carbohydrate diets (60% carb) were compared with high monounsaturated fat diets (50% fat; 33% mono) in patients with type 2 diabetes mellitus, the high monounsaturated fat diet significantly lowered glucose and insulin and had favorable effects on triglycerides (-25%), LDL (-35%) and HDL cholesterol (13%) (Garg 1988). These results were supported by Parillo (1992).

Protein intake may also influence insulin sensitivity. A hypocaloric, high protein diet (45% protein) has been shown to improve insulin sensitivity in comparison to an otherwise similar diet high in carbohydrate (Piatti 1994). Similar results were reported by Baba et al (1999). In contrast, there is scant evidence on the effects of increased protein intake in the eucaloric state.

Jenkins et al have advanced the concept of “lente” carbohydrates that are digested slowly and that produce less glycemic response than other carbohydrates. These carbohydrate-rich foods include whole cereal grains, legumes, nuts, and fruits, i.e. core aspects of the DASH diet. The glycemic index of foods has been used to quantitate this effect. Thus high carbohydrate diets that have low-glycemic index foods produce less postprandial glucose and insulin responses, and have been hypothesized to improve insulin sensitivity. More recently, the beneficial effects of a high carbohydrate diet (55%) that is enriched in dietary fiber (50 g/day) were demonstrated in patients with type 2 diabetes mellitus. In comparison to a high carbohydrate diet with a standard American Diabetes Association diet (with lower fiber content [25 g/day]), the high fiber diet significantly lowered 24 hour glucose (-10%) and insulin concentrations (-12%) (Chandalia 2000).

An ancillary study to the DASH trial tested the effect on fasting and post-challenge glucose and insulin of the DASH diet, a high-carbohydrate, high fiber diet that is reduced in total and saturated fat, and that replaces most of the fat with complex carbohydrate. A subset of DASH participants (N=80) received glucose tolerance tests (75 g glucose challenge) at baseline (at the end of the 3 week run-in period on the high-fat control diet) and after 8 weeks on either the control diet or the DASH diet. The DASH diet did not significantly affect fasting or post-challenge glucose or insulin levels. Thus, considering these and previous findings, it seems likely that increasing carbohydrate intake does not necessarily worsen markers of insulin resistance, if the carbohydrate is mainly complex and/or fiber is increased. The issue still remains whether such a high-carbohydrate diet differs in effects from a high-monounsaturated fat diet or high-protein diet. In addition to fasting levels of glucose and insulin, we will assess the effects of the diets on the HOMA insulin resistance index, which correlates highly (R=0.8-0.9) with insulin resistance determined by euglycemic clamp (Mathews 1985; Emoto 1999).

3.4 Overall effects of diet on coronary disease risk

Observational epidemiology has provided a substantial body of evidence that links levels of the risk factors, BP and plasma lipids, with disease incidence. These relationships have been confirmed by randomized clinical trials with a variety of agents, including antihypertensive medications, and lipid treatment with diet, and several classes of drugs, including bile acid sequestering resins, statins, nicotinic acid, and fibrates. We consider it important to evaluate the overall effects of the dietary changes in risk factors to give clinical and public health applicability of the findings, and will do so using established risk equations. Available risk equations have been derived from the Framingham Heart Study, NHANES I and II, **MRFIT, Pooling Project, and ARIC**. Presently, several health organizations in the US (e.g. AHA and NHLBI) and Europe are developing prediction models to estimate cardiovascular disease risk in diverse populations. Because the relationships between risk factors and CVD risk may differ according to gender and race, exemplified by the increased coefficients found for HDL and triglycerides in women (Hokanson 1997; Gordon 1989), risk predictions will be done separately in these groups.

4. DESIGN

4.1 Study Population/Eligibility Criteria

Trial participants are 160 community-dwelling persons (~80 in Baltimore and ~80 in Boston), ages 30 and older, with a blood pressure that is above optimal but less than stage 2 hypertension (systolic blood pressure of 120-159 mmHg or diastolic blood pressure of 80-99 mmHg). This BP range includes Stage 1 hypertensives, isolated Stage 1 systolic hypertension, as well as non-hypertensive individuals whose BP is higher than optimal BP. The Working Group Report on the Primary Prevention of Hypertension (1993) designates non-hypertensive individuals with higher than optimal BP as a group at high risk for hypertension, justifying special attempts to lower BP. For most individuals with Stage 1 hypertension, JNC-VI recommends non-drug therapy as initial treatment. Table 1 displays the inclusion and exclusion criteria of the trial.

Table 1: Inclusion and Exclusion Criteria of the Trial

Inclusion Criteria

- Baseline SBP 120-159 mmHg or DBP 80-99 mmHg (mean over three screening visits) [note: stage 2 hypertension (SBP $\geq$ 160 or DBP $\geq$ 100 mmHg) based on the mean over three screening visits will be excluded, as well as a mean systolic BP > 170 or diastolic BP > 105 at any one visit]
- age 30 or older
- willing to eat at least one on-site meal/day, five days/week, and willing to eat study diets and nothing else for the 19 weeks of controlled feeding

Medication Exclusions

- Use of antihypertensive drugs (any in two months prior to SV1)
- Chronic use of medications that raise or lower blood pressure
- Use of a lipid lowering agent (any in 3 weeks prior to SV1)
- Unstable dose of hormone replacement therapy, psychotropic medications and thyroid hormone replacement therapy (defined as a change in dose within two months of the SV1 visit)
- Use of lithium, insulin, oral hypoglycemic agent, oral corticosteroid, anti-psychotic drugs, weight loss medications, oral breathing medication, nitrate, or digitalis

Medical History Exclusions

- Active or prior cardiovascular disease (stroke, MI, PTCA, CABG, congestive heart failure, symptomatic ischemic heart disease (angina), or ASCVD-related therapeutic procedure)
- Cancer diagnosis in past two years (however, persons with non-melanoma skin cancer, localized breast cancer, or localized prostate cancer can enroll if they did not require systemic chemotherapy)
- Inflammatory bowel disease, colostomy, malabsorption, or major GI resection
- Renal insufficiency as determined by a serum creatinine > 1.2 mg/dL for women or > 1.5 mg/dL for men. These participants can enroll if their estimated GFR is $\geq$ 60 ml/min by either the Cockcroft-Gault equation or the simplified MDRD equation.
- Emergency room visit or hospital stay for asthma or COPD in last six months
- Any serious illness not otherwise specified that would interfere with participation

Laboratory Exclusions*

- Fasting LDL cholesterol > 220mg/dL, triglycerides > 750 mg/dl, fasting blood sugar > 125 mg/dl
- Urine dipstick protein > 1+
- Serum transaminase > 2 times the upper range of normal, or a clinical diagnosis of hepatitis

Other Exclusions

- Consumption of more than 14 alcoholic drinks per week, or consumption of 6 or more drinks on an occasion, one or more occasions per week
- Significant food allergies, preferences, intolerances, or dietary requirements that would interfere with diet adherence
- Weight over 350 pounds
- Weight loss or gain of 10 pounds or more during prior 2 months
- Planning to leave the area prior to the anticipated end of participation
- Pregnant, breast feeding, or planning pregnancy prior to the end of participation
- Requirement for use of thigh cuff (arm circumference > 41 cm) or inability to obtain accurate blood pressure measurements
- Current participation in another clinical trial with an intervention that affects blood pressure or lipids
- Investigator discretion (e.g. for concerns over safety, adherence, or follow-up or for inappropriate behavior) reasons
- Vitamin, fish-oil, weight-loss, soy, mineral, or herbal supplements that cannot be stopped prior to run-in

* For any laboratory-based exclusion, one repeat laboratory test is permitted if the initial value would have excluded the individual

4.2 Recruitment Strategies

Both clinical centers focus on mass mailing of brochures as their primary recruitment strategy. Yields from mass mailing are typically on the order of 2 to 5 randomizations per 10k mailed brochures. Despite this apparently low yield, mass mailings are easy to implement and can predictably reach a large audience. Mass mailings have the added advantage of targeted recruitment, i.e. focused effort on one group because most lists can be sorted on gender or race-ethnicity or on a particular zip code. The primary sources of mailing lists are commercial vendors and local governments (for lists of registered voters or drivers). Other strategies may include ValPac coupon distribution, advertisements or articles in local newspapers, flyers, and mass screenings.

4.2.1 Recruitment of minorities and women

Because of the disproportionate burden of hypertension and its complications in African-Americans and because of the BP responsiveness to dietary interventions in African-Americans, 50% is the trial recruitment goal for African-Americans. In subgroup analyses, African-Americans will be compared to non-African-Americans (whites and non-African-American minorities). We anticipate that over 50% of trial participants will be women and that approximately 30-40% will have Stage 1 hypertension. Using recruitment procedures similar to DASH and DASH-Sodium, these goals are readily achievable without targeted recruitment efforts. However, both centers are fully capable of implementing targeted recruitment efforts if there is a shortfall in recruitment of an important subgroup.

4.3 Contact Pattern

4.3.1 Screening

Participant eligibility for the trial is determined in a series of three formal screening visits, each of which includes questionnaires and clinical measurements. Data collected in the screening visits also provide baseline levels used to describe participants and to classify individuals for subgroup analyses. For example, hypertension status will be based upon the average of all BP obtained during screening prior to run-in. Data collection instruments and procedures are adapted from those used in the DASH and DASH-Sodium trials. Table 2 displays blood pressure eligibility criteria by screening visit.

Pre-Screen Contact: The pre-screening evaluation is a quick, inexpensive means to identify potential candidates as definitely ineligible or potentially eligible. This contact is conducted over the phone or in-person. The pre-screening form includes brief questions on major eligibility criteria and may also include a single, exclusionary BP measurement. Eligible and interested individuals from the pre-screen contact are scheduled for the first formal screening visit.

Screening Visit 1 (SV1): Informed consent for screening and run-in are obtained. SV1 identifies major exclusionary criteria in a more comprehensive fashion. This visit includes three BP measurements, measurement of height and weight, and review of dietary habits and preferences (used to assess a participant's ability and willingness to consume all diets and to adhere to the feeding protocol). Eligibility for the second screening visit is determined at the end of SV1.

*Screening Visit 2 (SV2:**at least 7 days after SV1**)*: SV2 includes BP measurement; eligibility is based on the average of the three BPs from SV1 and the three BPs from SV2 (six in all). At either SV2 or SV3, a fasting blood specimen is drawn for eligibility (local laboratory measurement of lipids and transaminases and storage of specimens for possible central analyses and storage) and a dipstick urinalysis for proteinuria. Also, the Food Frequency Questionnaire is completed at SV2.

*Screening Visit 3 (SV3:**at least 7 days after SV2**)*: SV3 includes three BP measurements; eligibility is based on the average of the nine BP measurements taken at SV1, SV2, and SV3. Other data collection procedures include measuring weight, completing a symptoms questionnaire, and processing the 24-hour urine specimen (analyzed centrally for sodium, potassium, phosphorus, urea nitrogen, and creatinine). Participants complete a detailed study foods checklist and meet with a study dietitian to confirm their willingness to comply with the controlled feeding protocol. The medical eligibility questionnaire is re-administered if it was last completed 60 days prior to the anticipated start of run-in.

Table 2: Blood pressure eligibility criteria by screening visit		
Visit	Blood Pressure	Eligibility Range (mmHg)
SV1[a]	Systolic BP	118 – 170
	Diastolic BP	78 – 104
SV2[b]	Systolic BP	119 – 165
	Diastolic BP	79 – 102
SV3[c]	Systolic BP	120 – 159
	Diastolic BP	80 -99

[a]Participants must meet EITHER the systolic BP criteria OR the diastolic BP criteria based on the cumulative average of BP across screening visits to date. At any one visit, persons with a systolic BP > 170 OR a diastolic BP > 105 will be excluded.
[b]Based on average of SV1 and SV2 blood pressures.
[c]Based on average of SV1, SV2 and SV3 blood pressures.

4.3.2 Run-in and Randomization

*Run-In Period (RI: **no more than 180 days after SV1**)*: The 6-7 day run-in phase has two main objectives: 1) to introduce participants to the feeding protocol and 2) to identify and exclude individuals who cannot adhere to the feeding regimen. During run-in, participants are provided all of their food, snacks and most beverages. On weekdays, they eat their major meal on-site, either lunch or dinner, and receive the remainder of their meals to be eaten off-site. For weekend meals, they are provided all of their food on the preceding Thursday or Friday. For each day of controlled feeding, participants complete a daily diary which asks about study food not eaten, non-study food eaten, the number of caffeinated beverages consumed, and the number of alcohol beverages consumed.

The initial calorie level at the start of run-in is estimated using sex, height, weight and physical activity level. During run-in, participants are provided each diet for two days. A three day meal cycle will be used (e.g. CARB, PROTEIN, UNSAT) so that the two days on each diet will be non-consecutive. Each weekday, weight is measured. To ensure that weight remains stable, calorie intake is adjusted by providing another calorie level for the same diet (1600, 2100, 2600, 3100, or 3600 kcal/day) or by increasing or decreasing the number of 100 kcal unit foods.

Additionally, recent physical activity, a symptoms questionnaire, medical, and social history (e.g. family history of hypertension and cardiovascular disease, socioeconomic variables) are collected. BP is measured on one day. Participants may be excluded during run-in for non-adherence to the protocol. Individuals are also excluded during run-in (and intervention), if their BP exceeds certain pre-specified safety levels (see Safety Monitoring section). Participants meet with a study dietitian to review progress and assess their continued interest in the trial. At a subsequent case conference, a team that includes the clinical center dietitian, study coordinator and principal investigator confirms suitability for randomization

Randomization (RZ: 0 – 7 days after end of run-in): Upon successful completion of run-in, eligible and interested participants are asked to sign an informed consent statement that covers the main portion of the trial (randomization and the subsequent 3 feeding periods). Participants are randomized to one of six sequences of the three diets (CARB , UNSAT, PROTEIN). Randomization is stratified by clinic. Participants are not told the sequence of diets to which they have been assigned. Except for staff involved in meal preparation, data collection personnel are blinded to diet sequence. As in the DASH and DASH-Sodium trials, each clinic operation is organized, in terms of space and personnel, to accomplish blinding of data collectors.

4.3.3 Intervention Periods

Each of three feeding periods lasts 6 weeks, during which time participants are provided all of their food, snacks and most beverages. A washout period of at least 2 weeks separates each period; the washout period allows ad libitum food intake. Participants are fed sufficient calories to maintain their weight. During the initial four weeks of each period, BP is measured once each week. During the last 10 days of each intervention period, BP is measured on 5 days; 2 days of these 5 measurement days occur during the last 5 days of the intervention period. The requirement for 5 measurements during the last 10 days (2 measurements during the last 5 days) of each period ensures that participants will have been exposed to the diet for the full 6 weeks of feeding.

During the last 10 days, one 24 hour collection of urine is also obtained. A fasting blood specimen is drawn during the 4^{th} and 6^{th} week of each intervention period. For each day of controlled feeding, participants complete a daily diary. In the first and last week of each intervention period, participants also complete a symptoms questionnaire. During the last week of the last feeding period, participants complete an anonymous adherence questionnaire.

Table 3 displays major measurements and data collection activities by visit. The following sections describe our approach to measuring all outcomes variables and other selected measurements.

Table 3: Schedule of activities by visit

		Screening Visits			Run In	Each of 3 Intervention (INT) Periods					
	PSV	SV1	SV2	SV3		INT Wk 1	INT Wk 2	INT Wk 3	INT Wk 4	INT Wk 5	INT Wk 6
Informed consent		T			T						
Blood pressure	opt	T	T	T	T	once per week ---------- >				5 x in last 10 days; at least once in wk 5; 2 x in last 5 days	
Health questionnaire		T			T						
General dietary information questionnaire		T									
Weight		T		T	each weekday of feeding ---------- >						
Height		T									
Urine dipstick			T								
24 hour urine collection*			T							T	
Food Frequency Questionnaire			T								
Fasting Blood**			T						T		T
Symptoms questionnaire				T							T
Feeding activities					daily ---------- >						
Randomization					T						
Patient history questionnaire					T						
Brief physical activity questionnaire					T						T
Medication questionnaire					T						T
Diet Acceptability q'aire								T			
Satiety questionnaire								T			

* Sodium, potassium, phosphorus, urea nitrogen, creatinine

** Total cholesterol, HDL-C, triglycerides, LDL-C, glucose, insulin, and storage specimen at each phlebotomy; VLDL-apoB, VLDL-apoCIII, total plasma apolipoprotein B, and lipoprotein(a) at week 6 of each period; whole blood for subsequent DNA extraction (just once).

4.4 Measurement of outcome variables

4.4.1 Blood Pressure (BP)

Blood pressure is determined by the OMRON 907 device which records BP using an oscillometric technique. **The OMRON 907 device does not provide a thigh cuff. For persons who require a thigh cuff, the SpaceLabs 90207 device will be used.** The decision to use automated devices reflects concerns about the continued availability of mercury, which could be banned from one or both of the clinical centers during the course of the trial. The OMRON device has been shown to have sufficient accuracy during a validation study (White, 2001). **The OMRON 907 device** is currently used in the NHLBI-sponsored ACCORD study. **In the DASH trial (Moore, 1999), BP change as measured by the Spacelabs 90207 ambulatory monitor, was similar to BP change measured by random-zero device in the DASH trial (Moore 1999). Likewise, in the DASH-Sodium trial (Appel 2000), BP change as measured by the Spacelabs ambulatory monitor and the random-zero device were similar.** We considered aneroid devices but decided against use of this type of device because BP measurements can not be obtained in a blinded fashion.

Blood pressure will be obtained by trained and certified data collectors according to a standard protocol, adapted from that used in the DASH-Sodium trial. Three measurements **(each separated by 30 seconds)** are obtained at each visit on the right arm of participants after they rest quietly in the seated position for at least 5 minutes. A cuff of appropriate size is identified at the initial visit and used thereafter at all subsequent visits. Heart rate is also recorded by the OMRON device.

4.4.2 Lipids, glucose and insulin

Traditional lipid fractions, glucose and insulin are measured at baseline and in weeks 4 and 6 of the 3 dietary periods from blood collected after an overnight (8-12 hour) fast and then stored at -70C. Apolipoproteins and Lp(a) will be measured in week 6; depending on sufficient resources, these variables will also be measured at baseline and/or in week 4. At the CLCS laboratory directed by Dr. Cole, plasma total cholesterol and glycerol-blanked triglycerides are measured using enzymatic kits from Miles-Technicon and Roche on the Hitachi 917 analyzer. HDL-C is measured as above in the supernatant of plasma after the precipitation of apo B-containing lipoproteins with dextran sulfate (50,000 MW, Genzyme). Glucose is measured using the enzymatic hexokinase kit from Roche on the Hitachi 917. Insulin is measured using Microparticle Enzyme Immunoassay technology on the Abbott IMx analyzer. Consistency of measurement is evaluated by an in-house Quality Assurance program based on the standard Westgard rules for analytical run acceptance. Accuracy of measurement is verified by participation in the CDC Lipid Standardization Program, the CDC Cholesterol Reference Method Laboratory Network, the Pacific Biometrics, Inc., ALERT Program and the College of American Pathologists external proficiency program. Accuracy of cholesterol, HDL cholesterol and triglyceride measurements are also evaluated by a comparison of routine methods to reference methods or designated comparison methods in-house using patient specimens.

From the fasting glucose and insulin levels, the HOMA insulin resistance is calculated (fasting plasma insulin concentration in uU/ml x fasting plasma glucose concentration in mmol/l ÷ 22.5). Quantification of insulin resistance using this model has been validated using a euglycemic hyperinsulinemic clamp, a hyperglycemic clamp, monocyte and erythrocyte insulin receptor status; the HOMA index also correlates well with the degree of obesity (Matthews 1985).

4.4.3 VLDL and LDL subfractions

VLDL and LDL subfractions are measured in Dr. Sacks's laboratory at Harvard School of Public Health. Plasma, 1 cc, stored at -70C, is thawed, and applied to Sepharose to which anti-apoCII is coupled. The unretained fraction that does not have apoCIII is collected, and the retained fraction that has apoCIII is eluted with sodium thiocyanate, 3M, and desalted. The unretained and retained fractions is ultracentrifugated to isolate VLDL ($d<1.006$ g/ml) and LDL ($1.006 <d<1.063$ g/ml). Note that this LDL is the standard LDL density that contains IDL. This procedure isolated 4 VLDL and LDL types: VLDL without apoCIII, VLDL with apoCIII, LDL without apoCIII, and LDL with apoCIII. Apolipoprotein B is measured in each fraction, and is the principal measurement of interest (each VLDL and LDL particle has 1 molecule of apoB). In addition, cholesterol and triglyceride are measured in each type.

These VLDL and LDL measurements are combined to provide direct measurements of total VLDL cholesterol, VLDL apoB, LDL cholesterol, LDL apoB, and total apoB.

4.5 Measurement of other variables

Weight is measured by trained, certified staff using a calibrated balance beam scale. Weight will be recorded during screening at the SV1 visit (to determine eligibility), at the SV3 visit (to estimate calorie requirements) and every weekday during feeding periods (to adjust calorie intake in order to maintain weight).

Height (collected once at the SV1 visit) is measured by trained staff using a stadiometer.

Waist Circumference is measured at SV2 or SV3 by trained, certified staff using an anthropometric measuring tape, at a horizontal plane that is one cm above the navel.

24 Hour Urine Collections is obtained once during screening and once during the last week of each intervention period. To ensure proper and complete collections, we provide verbal and written instructions prior to each collection, initiate the collections in the clinic on a weekday, and obtain repeat collections if an initial collection is deemed unsatisfactory (collection period < 22 or > 26 hours; more than one missed void; total volume < 500 cc; or collection during menstruation). Aliquots are stored at -70E and then shipped to the Central laboratory, upon completion of feeding. At the laboratory of Dr. Thomas Cole, urines are analyzed for sodium, potassium, phosphorus, urea nitrogen, and creatinine. Aliquots are stored for possible analyses, e.g. magnesium, calcium, and markers of bone mineral metabolism.

The Willett Food Frequency Questionnaire is administered once during screening by certified staff as a means to describe the usual diet of participants. This questionnaire is checked locally and then sent to the Channing Laboratory where it is optically scanned and analyzed by computer.

Symptom Questionnaire is administered once during screening and twice during each feeding period. This self-administered checklist, used in both the DASH and DASH-Sodium trials, collects information on symptoms including gastrointestinal problems (diarrhea/loose stools, constipation, bloating/uncomfortably full, and nausea/upset stomach). Each symptom is classified by severity (mild, moderate and severe). The checklist had sufficient sensitivity to detect differences between diets in GI symptoms and headaches in the DASH and DASH-Sodium trials (Appel, 1997; Sacks, 2001).

Storage specimens of plasma and serum from each fasting blood collection are obtained. In addition, aliquots from each 24 hr urine collection are also stored. Finally, whole blood is obtained and stored for subsequent extraction of DNA. Candidate assays that might be performed include those related to inflammation (e.g. hsCRP, IL-6), oxidative damage (eg Antibody to OxLDL), kidney function (e.g. proteinuria and albuminuria), diet composition (e.g. urinary excretion of isoflavones), and bone mineral metabolism (e.g. osteocalcin, serum C-terminal telopeptide of type I collagen (CTX), serum PTH, urinary calcium and cyclic AMP). Also, the VLDL and LDL subfractions at week 4 may be measured on stored specimens, if there is a diet effect at week 6 (see section 4.4.3).

4.6 Description of Diets

Table 4 displays the nutrient targets of the three intervention diets at the 2100 kcal level. After randomization, participants are fed three diets, each of which is low in saturated fat **and cholesterol**. One diet, termed CARB, is based on the DASH diet; the CARB diet emphasizes carbohydrates (58% of kcal). Another diet, termed PROTEIN, emphasizes protein (25% of kcal), predominantly plant-based protein, while the third diet (UNSAT) emphasizes unsaturated fat (21% of kcal), predominantly monounsaturated fat. The three diets have a similar micronutrient profile and provide a similar amount of sodium, 2,300 mg per day in the 2,100 kcal diet. This level corresponds to the upper limit of current US recommendations for preventing and treating hypertension.

For each diet, five calorie levels are prepared (1600, 2100, 2600, 3100 and 3600 kcal). The macronutrient profile is identical at each calorie level. Micronutrient and fiber levels are identical to those of the DASH diet and are also identical across diets at the same calorie level (e.g. 5,700 mg of potassium in the 3,100 kcal level of each diet). The micronutrient levels are based on a Linear Index Model that indexes micronutrient levels to energy levels (Lin, 1999). This model is based on actual population consumption data and thus provides a realistic range of micronutrient intakes at lower and higher calorie levels, rather than a fixed ratio applied to all calorie levels (e.g. 4,700 mg of K per 2,100 kcal, or 2,238 mg of K per 1,000 kcal).

The CARB diet is a high carbohydrate diet (58% of kcal) similar to the DASH diet (55% of kcal from carbohydrate) that effectively lowered BP and reduced LDL-C without a change in triglycerides despite its relatively high carbohydrate content. This type of diet is currently recommended for the prevention and

treatment of hypertension (JNC VI, 1997). The DASH diet emphasizes fruits, vegetables and low-fat dairy products; includes whole grains, poultry, fish, and nuts; and is reduced in fats, red meats, sweets, and sugar-containing beverages (Karanja, 1999). To establish a contrast in protein between the CARB and PROTEIN diets, we reduced the protein content of the DASH diet from 18% kcal to 15% kcal and increased the carbohydrate from 55 to 58% kcal. Otherwise, the CARB diet is similar to the DASH diet. Note that 15% kcal from PROTEIN is close to national estimates of average protein intake.

The PROTEIN diet provides 25% kcal from protein. While the protein sources are varied and include meat, dairy and plant sources, most of the increase in protein from the CARB diet (with 15% kcal from protein) to the PROTEIN diet (with 25% kcal from protein) comes from plants. The PROTEIN diet includes some soy products. However, soy products are not emphasized because of the potential for confounding from isoflavones that are present in variable amounts in certain preparations. Unfortunately, current nutritional databases are insufficient to estimate the soy content of foods. Hence, there are no specific quantifiable guidelines for the soy content of this diet or the other study diets.

The UNSAT diet is rich in unsaturated fat, predominantly monounsaturated fat. Accordingly, the distribution of monounsaturated, saturated, and polyunsaturated fats differs from the CARB and PROTEIN diets. This diet uses nuts, seeds, and oils such as olive, canola, and safflower oil to meet its target fat distributions. Specially formulated fat products are not used.

Table 4: Nutrient target levels of the study diets, 2100 kcal level

	CARB	PROTEIN	UNSAT
Carbohydrate (% kcal)	58	48	48
Fat (% kcal)	27	27	37
Saturated	6	6	6
Monounsaturated	13	13	21
Polyunsaturated	8	8	10
Protein (% kcal)	15	25	15
Meat	5.5	9.0	5.5
Dairy	4.0	4.0	4.0
Plant	5.5	12.0	5.5
Cholesterol (mg/day)	150	150	150
Fiber (g/day)	$\geq$ 30	$\geq$ 30	$\geq$ 30
Sodium (mg/day)	2300	2300	2300
Potassium (mg/day)	4700	4700	4700
Magnesium (mg/day)	500	500	500
Calcium (mg/day)	1200	1200	1200

4.7 Menu development, evaluation, and monitoring

During the planning phase, a seven day menu cycle is developed for each diet (CARB, UNSAT, PROTEIN). In brief, the planning process involves preparation of 8 – 9 full day menus for each diet at each of 5 calorie levels (1600, 2100, 2600, 3100 and 3600); each full day menu is designed to meet the nutrient targets as displayed in Table 4. The difference between the nutrient target and the corresponding estimate from the Food Processor® software (version 7.9) is assessed. For each of the 3 seven day menu cycles, acceptable variation is a difference of ± 1% kcal for total fat, saturated fat, monounsaturated fat, polyunsaturated fat, carbohydrate, total protein, and plant protein. Acceptable variation (the difference as a percent of the target) is ± 5% for sodium, potassium, and calcium; ± 10% for magnesium; and ± 50 mg/**day** for cholesterol. In addition to the full day menus, diet-specific unit foods are developed to meet caloric needs between the calorie levels. The nutrient distribution of the unit foods corresponds to nutrient targets of the diet.

After menus are developed, recipes are taste tested for acceptance. Also, chemical (nutrient) analyses of composited menus are performed. Cost and feasibility considerations preclude chemical analyses of the 270 full day menus (9 candidate menus for each of 3 diets, at 5 calorie levels, at 2 sites). For this reason, composite analyses of just the 2100 kcal level are performed; this level was the most commonly used calorie level in the DASH and DASH-Sodium trials. Chemical analyses will include measurement of protein, carbohydrate, total fat, saturated fat, polyunsaturated fat, monounsaturated fat, sodium, potassium, magnesium, calcium and fiber. The final selection of menus is based on taste test results and chemical analyses of composited menus, as well as food production and cost considerations.

During the trial, menu monitoring is also performed. Once during each year of feeding, a full week of menus from each diet at the 2100 kcal level will be collected for composite analysis. In addition, analyses of urinary electrolyte excretion are also performed.

4.8 Food Production and Distribution

Once menus are formulated, all foods are identified and selected to promote consistency at both feeding sites. Specific national brands are selected, as well as purchasing specifications for meats and produce. Detailed food preparation procedures and standardized recipes are developed to ensure that participants receive the same diets at the two feeding centers. Food production is also conducted according to respective state or county public health guidelines and JCAHO regulations. Quality control procedures developed in the DASH trials are used to monitor food procurement, preparation, and distribution. Research kitchens are monitored for safety, sanitation and equipment accuracy.

The process of preparing foods is a labor intensive process. In this process, food service workers weigh, portion, and package individual food items for the three diets, according to the calorie levels of the participants being fed. The cooks prepare all cooked items (e.g. casseroles, meats); in the process, they measure raw product, cook the items following standard hygienic procedures, and store the individualized portions. Some portion-controlled packaged food items are used for production efficiency. A diet technician assembles the trays for on-site meals and the bags and coolers for distribution of off-site meals, by diet and calorie level. A second person independently rechecks the assembled foods to confirm the accuracy of food delivery.

The feeding protocol is identical to that used in the DASH and DASH-Sodium trials. All study food is provided to participants who are instructed to eat all their food and to consume no additional food other than approved selected beverages. They are allowed to consume up to 2 alcoholic beverages per day and 3 non-caloric caffeinated beverages per day. Participants are required to eat one meal (the main meal of the day) at the feeding center Monday through Friday. The remaining food for the day and weekend food are provided for consumption off-site. On any given feeding visit, participants are expected to complete a daily food diary and be weighed. The daily diary asks about study foods not eaten, non-study foods eaten, and beverages consumed over the past day.

A dietitian case manager meets participants on a daily basis, receives and reviews their progress, elicits general feedback, tracks weight, and adjusts calorie level, if needed. The participant then proceeds to the dining area to eat the on-site meal. A meal monitor checks the completed tray and provides the take-home meals. On each day of feeding, participants complete a daily diary, which elicits information about food and beverage consumption over the preceding day, including non-alcohol beverages consumed, alcohol beverages

consumed, unit foods eaten, study foods not eaten, non-study foods eaten, and vitamin/supplement consumed.

4.9 Promotion of Adherence

Efforts to promote adherence begin at the earliest stages of the study. During screening and orientation, participants are repeatedly provided with information about key features of the study. At the third screening visit, they are provided a detailed list of foods provided in the diets. Individuals must be willing to eat each of these foods; otherwise, they are excluded. Key contacts with dietary staff include a group orientation session during screening and an in-person evaluation by a dietitian. The intent of these efforts is to identify and exclude, prior to run-in and certainly prior to randomization, participants who are unwilling or unable to comply with the feeding protocol.

Once run-in feeding starts, participants get a chance to experience the actual demands of the study. At this time, efforts to promote adherence center on making the foods palatable and convenient to their lifestyles; maintaining easy access to staff; providing daily, supportive contacts; and providing a variety of items and incentives (raffle tickets, movie coupons) that promote good rapport. Acceptance of the controlled feeding protocol is increased by allowing participants to consume up to 3 non-caloric caffeinated beverages and 2 alcoholic beverages per day, as well as an unlimited amount of water and artificially sweetened soft drinks.

During a case conference at the end of run-in, the study dietitian, the study coordinator and principal investigator review all participants to assess their progress, continued interest and overall commitment to the trial. After randomization, every effort is made to promote adherence. In many instances, these efforts are tailored to the specific needs of the participant (e.g. meals delivered to home or work).

4.10 Sample size

Table 5 displays the minimum detectable, between-diet differences for primary, secondary and other outcome variables in the full cohort (n=160) and in subgroups (n=80 and 70) at powers of 80% and 90% (2-sided alpha, p=0.05). The sample size of the trial (n=160) was selected because it provided adequate power to detect between-diet differences in our primary outcome variables that have public health significance, both overall and in subgroups. Specifically, the minimum detectable effect size for our primary outcomes, systolic BP and LDL-C, are < 3 mmHg and < 10 mg/dl, respectively, even in subgroups that comprise 40 % (**n=64**) of participants. For instance, Stamler (1989) has estimated that a population wide 3 mmHg reduction in systolic BP will lower coronary heart disease mortality by 5% and stroke mortality by 8%. Furthermore, the BP effect sizes are plausible based on extrapolations from the INTERSALT observational study (Stamler, 1996), i.e. a difference in total protein intake of 40 gm per day (a conservative estimate of the contrast in protein between CARB and PROTEIN) is associated with a 3.2 mmHg reduction in systolic BP and 2.6 mmHg reduction in diastolic BP. Likewise, the small trial by Ferrara (2000), which documented a 7 mmHg reduction in systolic BP and 6 mmHg reduction in diastolic BP from replacing saturated fat with olive oil, suggests that our trial is sufficiently powered to detect more modest BP reductions from UNSAT in our population with lower BP. **If non-hypertensives comprise 70% of study participants, the minimum detectable between-diet difference in SBP and DBP at 80% power is 1.7 and 1.3 mmHg for non-hypertensives and 2.6 and 2.0 mmHg for hypertensives. Corresponding estimates at 90% power are 2.0 and 1.5 mmHg for non-hypertensives and 3.1 and 2.3 mmHg for hypertensives.**

For lipids, the minimum detectable effect sizes represent approximately 3% to 6% changes from expected mean concentrations (e.g. 140 mg/dl for LDL-C, 50 mg/dl for HDL-C, and 150 mg/dl for TG). For VLDL-apoB, the differences represent a 13% change from the mean (e.g. 12 mg/dl) for the full cohort and 20% for the n=80 subgroup. For VLDL-CIII, the differences represent a 10% change from the mean (e.g. 5 mg/dl) for the full cohort and a15% change for the n=80 subgroup. For fasting glucose, the differences represent 3 to 6% changes from typical mean values, e.g. 80 mg/dl. For fasting insulin and the insulin resistance index (HOMA), the differences represent changes of 20% and 8% from expected mean values of 5 uU/ml for insulin and 1.2 for HOMA (Mathews, 1985).

For the primary and secondary outcome variables, the standard deviation (SD) of change from beginning to end of the intervention periods in control subjects on the DASH trial were used to compute sample size requirements. These SDs were 6.4 mmHg for systolic BP, 4.8 mmHg for diastolic BP, 23 mg/dl for LDL-C, 5.4 mg/dl for HDL-C, 35 mg/dl for triglycerides, 7.6 mg/dl for fasting glucose, and 4.0 uU/ml for fasting insulin. The SDs for BP are based on means from clusters of 5 days of measurements. Because the eligibility criteria of the Omni-Heart and DASH trials are similar and because our recruitment strategies are likewise similar, these SDs are the most applicable. Still, the BP measurement technique of this trial (oscillometric) differs from that of the previous DASH trials (auscultatory); however, even if the actual SDs are slightly higher than projected, projected power should remain high, especially given the anticipated effect sizes (discussed below).

For the VLDL measurements, apoB and apo CIII, SDs of change are 6.1mg/dl and 2.0 mg/dl, respectively. These are based on the differences between baseline and 1 year in a random sample of 100 patients in the control group of the CARE trial (Sacks, unpublished). However, variability would be expected to be greater in free-living patients over 1 year than in our trial participants who eat in a controlled setting for 6 weeks. Hence, the minimum detectable differences derived from these SDs, while quite small, are likely to be greater than those actually detectable in this trial. For the insulin resistance HOMA index, the reproducibility data of Mathews (1985) was used, i.e. a CV of 31% (mean 1.2, SD 0.37).

Table 5: Minimum Detectable Differences

Table 5: Minimum Detectable Differences for Primary, Secondary and Other Outcome Variables for the Full Cohort (N=160) and Subgroups (N=80 and 70) at Powers of 80% and 90% (2-sided Alpha, P=0.05)

	Full Cohort (n=160)			Subgroup (n=80/n=70)	
Primary Outcomes	**80 % Power**	**90 % Power**		**80 % Power**	**90 % Power**
SBP (mmHg)	1.4	1.7		2.0/2.2	2.3/2.5
LDL-C (mg/dl)	5.1	5.9		7.3/7.8	8.4/9.0
Secondary Outcomes					
DBP (mmHg)	1.1	1.2		1.5/1.6	1.8/1.9
HDL-C (mg/dl)	1.2	1.4		1.7/1.8	2.0/2.1
TG (mg/dl)	7.9	9.0		11.1/11.9	12.8/13.8
Other Outcomes					
VLDL-apoB (mg/dl)	1.4	1.6		1.9/2.1	2.2/2.4
VLDL-apo CIII (mg/dl)	0.45	0.52		0.63/0.68	0.73/0.79
Glucose (mg/dl)	1.7	2.0		2.4/2.6	2.8/3.0
Insulin (uU/ml)	0.89	1.0		1.3/1.4	1.5/1.6
Insulin resistance index (HOMA)	0.082	0.095		0.12/0.13	0.14/0.15

4.11 Analysis plan

Primary analyses compare the BP and plasma lipid concentrations measured at the end of the CARB diet with those at the end of the PROTEIN and UNSAT diets, within each individual among all participants.

Outcome definitions. For **Specific Aim 1**, the primary outcome is constructed as follows. The SBP measure associated with a given diet is the mean of the five SBP measures obtained in the final two weeks of the intervention period for that diet. The primary outcome for each participant for the PROTEIN diet is the difference between the participant's SBP measure for that diet and the corresponding SBP measure for the CARB diet. The primary outcome for each participant for the UNSAT diet is the difference between the participant's SBP measure for that diet and the corresponding SBP measure for the CARB diet. The secondary outcome for Specific Aim 1 is constructed analogously using DBP instead of SBP measures. For **Specific Aim 2**, the lipid values associated with a given diet are obtained from the week 6 fasting blood sample for that diet. The primary lipid outcome for each participant for the PROTEIN diet is the difference between the participant's LDL-C from that diet and his/her corresponding LDL-C measure from the CARB diet. The primary outcome for each participant for the UNSAT diet is the difference between the participant's LDL-C for that diet and the corresponding value for the CARB diet. Other lipid outcomes in **Specific Aim 2** and all outcomes for **Specific Aims 3 and 4** are derived in like fashion for each of the components identified in the statement of the relevant Specific Aim.

Analysis framework. The inferences of interest concern efficacy of the proposed diets in reducing risk of cardiovascular disease. All pairwise comparisons between diets will be evaluated for all outcomes. Primary analyses will be conducted on a per protocol basis, excluding information on study dropouts whenever incompleteness interferes with a given comparison, i.e., an individual who completes only two diets contributes information to only one comparison. Primary analyses will be supplemented by detailed information on dropouts, and by analysis of sensitivity of inferences to the occurrence of dropouts. These secondary analyses will investigate patterns of missingness, will attempt to discern if missingness is associated with features of the response profile, and will employ multiple imputation from the posterior predictive distribution of the outcome on the opposing arm to obtain a conservative secondary inference. The standard multiple imputation variance (Rubin, 1987) will be used to obtain secondary test statistics and confidence intervals. In the previous DASH trials, such incompleteness was very rare.

Inferential contrasts will be calculated using the simplest appropriate statistical method. For example, with the SBP outcome, the equal-variances paired t-test of equal blood-pressure effects on two diets will likely be satisfactory. Ninety-five (95) percent confidence intervals will be reported for the effects of PROTEIN and UNSAT relative to CARB. The effect of PROTEIN compared to UNSAT will be reported, but this pairwise contrast is not independent of the other 2 primary comparisons. Supplementary detailed modeling of the trajectory of repeatedly observed outcomes and its dependence on covariates will be conducted in the mixed effects models framework (Laird and Ware, 1982).

Multiple comparisons. Statistical significance will be reported without adjustment for multiple comparisons, and all point estimates and p-values will be accompanied by unadjusted 95% confidence intervals for the mean difference. For each analysis, statistical significance will be defined by p (unadjusted) <0.05.

Subgroup analyses. Analyses will be performed separately within subgroups. Pre-specified subgroups of interest are those defined by gender (women, men), race-ethnicity (African-Americans, non-African-Americans), menopausal status (pre-menopausal women, post-menopausal women), age, weight status, and baseline level of the outcome variable. Subgroups analyses defined by other pre-randomization variables may also be conducted, but these analyses will be reported as exploratory.

In general, prevailing national recommendations (e.g. JNC and ATP) guide selection of cut-points for subgroup-defining variables with a continuous distribution. Hypertension is defined by baseline SBP $\geq$ 140 mmHg and/or DBP $\geq$ 90 mmHg, and non-hypertension by baseline SBP < 140 mmHg and DBP < 90 mmHg. Other prospectively defined cutpoints are as follows: LDL cholesterol (130 mg/dl, 160 mg/dl), HDL cholesterol [40 mg/dl (all), 40 mg/dl (men), 50 mg/dl (women)], triglycerides (150 mg/dl), baseline weight [non-overweight (BMI < 25 kg/m^2), overweight (25 kg/m^2 $\leq$ BMI < 30 kg/m^2), obese (BMI $\geq$ 30 kg/m^2)]. The cutpoint for age is the median (above median age, below median age). For continuous subgroup-defining factors such as age, baseline BP, and baseline lipids, more powerful inferences may be achieved using continuous formulations of the predictor in analysis of covariance. Also, in some cases, the dispersion in a factor of interest may require a non-prespecified stratification definition, or introduction of a sophisticated analysis tool such as spline regression. Such analyses will be reported as exploratory.

Kinetic modeling. Kinetic modeling of outcomes are used to assess whether a steady state has been achieved. To this end, we also employ mixed effects models. The 6 week duration of the feeding perio makes it feasible to fit detailed models of outcome trajectories. These outcomes include BPs measured weekly during each intervention period and laboratory measurements measured during weeks 4 and 6 of each period. A standard tool is the nonlinear mixed effects model (Pinheiro 2000). For intensively monitored outcomes such as BP components, it is anticipated that asymptotic regressions, that is, of the form mean Y = a + (b-a)exp(-c*t), where a is final level, b is initial level, and c is a rate constant, can be fit at the individual level. For lipids, glucose and insulin, which are sampled at weeks 4and 6, inference on variance components corresponding to subject-specific slopes will be used to assess achievement of steady-state kinetics by week 6.

Carryover effects. Carryover effects are not expected, given the interval between outcome ascertainment in consecutive periods (at least 8 weeks) and the time course of dietary effects on BP and plasma lipids. Nonetheless, we cannot categorically dismiss the possibility that a small carryover effect could occur. All period

one treatments are free of potential carryover effects; hence, unbiased but inefficient profiles of treatment effects are available using only period one observations. Detection of carryover effects is facilitated by the detailed kinetic modeling described above. If carryover is found for any diet, it will be quantified, and then adjusted estimates of the dietary effects will be provided as secondary analyses. Special designs for the accommodation of carryover effects (Koch 1989; Peace 1990) present drawbacks (e.g., confinement of participants to longer periods with fewer treatments) that are considered undesirable given the low probability of carryover in this setting.

Risk prediction equations. The impact of the diets on overall cardiovascular risk (**Specific Aim 5**) will be assessed using prediction equations derived from prospective observational studies. Because of several methodologic issues [i.e., equations without relevant covariates (e.g., none with triglycerides); concerns about generalizability (e.g. most equations derived from populations of European Americans); uncertainties about the meaning of diet-induced reductions in HDL], these analyses will be considered exploratory. Still, an assessment of the overall impact of the diets on cardiovascular risk has strong conceptual appeal and obvious public health significance. Using available risk equations from the Framingham Heart Study (Wilson, 1998), from NHANES I and II (Liao, 1999), **and from other cohorts (MRFIT, Pooling Project, ARIC),** we will assess the overall effects of the diets on cardiovascular risk in our study population, in relevant subgroups, and, more broadly, to the US population. Presently, several health organizations in the US (e.g. AHA and NHLBI) and Europe are developing prediction models to estimate cardiovascular risk in diverse populations. Once these new equations become available, we will refine our plans and amend the protocol accordingly.

5. ORGANIZATIONAL STRUCTURE

The research team includes 2 field centers (one at Johns Hopkins University and another at Brigham and Women's Hospital, a data management and statistical unit at BWH, and a core laboratory at Washington University. The Steering Committee is the primary decision-making body. There are three standing subcommittees (Diet, Design/Measurements, and Recruitment/Study Coordinators). Ad hoc working groups are assembled as needed. A Data, Safety and Monitoring Committee is appointed to review and approve the protocol and then monitor all aspects of the trial.

The **Steering Committee** (co-chaired by the PIs of the field centers) includes the chair of each subcommittee; the lead dietitian from each center; and the data manager. During field work, the study coordinator from each center joins the Steering Committee. For the vast majority of deliberations, decision-making occurs by consensus. In the event that a consensus cannot be reached, the PIs of the field centers will vote formally. In the event that the PIs cannot reach agreement on a vital matter, the NHLBI project officer is asked to adjudicate. This committee is responsible for the overall design, conduct and analyses of the trial and for presentation and publication of trial results. To this end, it deliberates on all major issues, including protocol amendments.

The **Diet Subcommittee** (chair appointed by the Steering Committee) consists of the core dietitians from each site and the consultant dietitians. This subcommittee is responsible for all aspects related to menu development; food procurement, production, and distribution; hygienic issues; and adherence monitoring.

The **Design and Measurements Subcommittee** (chair appointed by the Steering Committee) consists of the co-investigators from each site and the data manager. This group is responsible for finalizing forms, developing data collection and training procedures, preparing the Manual of Operations, developing specimen collection and shipment procedures, and monitoring quality control at all levels (collection, entry and management of data; follow-up rates, etc).

A **Recruitment/Study Coordinators Subcommittee** (chair appointed by the Steering Committee) consists of recruitment and study coordinators from each center. This subcommittee reviews plans at each center, develops recruitment materials, and monitors progress on a regular basis.

A **Data, Safety and Monitoring Committee** (to include experts in biostatistics, hypertension, cardiovascular disease prevention, feeding studies, and clinical trialist) is appointed by the Steering Committee to review the protocol prior to field work and to monitor trial progress. Prevailing NIH and NHLBI policies will dictate the specific roles of this Committee.

Most of the committees convene by conference calls and at in-person Steering Committee meetings. The frequency of contacts varies during the trial. For instance, during the planning phase, the Steering Committee convenes at least monthly and the diet subcommittee more frequently.

6. TIMELINE

Planning: Initially, the focus of the planning period is finalization of the protocol and menu development. The Manual of Operations, instruments and forms are then prepared, and data entry/management systems developed. Meal cycles are developed for each of 3 diets diet at each of 5 calorie levels. Menus are prepared, analyzed, and taste-tested. Recruitment planning also occurs.

Implementation: In contrast to DASH and DASH-Sodium, in which individuals were enrolled in non-overlapping cohorts, participants in this trial are recruited in smaller waves that overlap. In this fashion, we avoid the wide swings in activities that complicated the conduct of the DASH and DASH-Sodium trials. Feeding for each wave lasts *19* weeks (1 week of run-in plus three 6 week periods) with at least *2 week*s separating each period. Recruitment for the initial cohort commences during the 3rd quarter of the first study year, in anticipation of initial feeding in the 4th quarter. Recruitment and feeding occurs in study years 2 – 4. As participants complete the trial, they receive participant-specific reports with their own data (averaged across study diets).

Analyses/closeout: Clinic closeout occurs in study year 5. During this period, clinical centers complete all data entry, respond to data edits and prepare summary reports of trial data for participants. During study years 4 and 5, laboratory specimens are analyzed; main results are prepared for publication and presentation.

7. SAFETY CONSIDERATIONS

This study does not involve any major risk to participants. Substantial effort has been made to identify and minimize potential risks. For instance, those persons who require pharmacologic therapy for elevated blood pressure or for dyslipidemia are excluded. Specifically, we exclude persons with systolic BP $\geq$ 160 mmHg and/or diastolic BP $\geq$ 100 mmHg. Such persons are informed of the elevated BP level and advised to consult with their physician. Likewise, diabetics, persons with prior or active cardiovascular disease, and persons with renal disease are excluded because the threshold for medication treatment of elevated BP is 130/85 mmHg in these persons (Class C hypertensives).

Each of the three study diets is reduced in saturated fat **and cholesterol,** and should therefore reduce LDL cholesterol. Furthermore, because each diet provides the micronutrient profile and fiber content of the DASH diet and because each diet provides just 100 mmol/day of sodium, each diet should reduce BP. The potential for BP reduction and for improved lipid profiles from the three diets greatly exceeds the negligible risks associated with participation in this trial.

Still, some persons may experience bloating and other minor gastrointestinal discomforts related to the high fruit, dairy and fiber content of the diets. It has been our experience that these problems resolve soon after changes in diet. For those persons with **presumptive** lactose intolerance, we will provide lactase tablets. Our experience suggests that GI discomfort is generally minor and subsides quickly. Participants are monitored for reactions to the diets and, if necessary, the diet can be modified or terminated. To minimize the risk of food-borne illness, kitchen staff follow all prevailing government hygienic regulations. Each of the metabolic kitchens is locally certified to prepare and distribute food.

Other potential risks of the study result from blood drawings. Venipuncture may cause some discomfort and/or bruising at the site of the puncture; and less commonly, the formation of a small blood clot or swelling of the vein and surrounding tissue and/or bleeding from the puncture site. Occasionally, blood drawing can cause someone to become dizzy, lightheaded or nauseated. All blood samples will be obtained by experienced personnel using small gauge needles.

8. SAFETY MONITORING

During the screening and feeding phases of the trial, the investigators collect clinically relevant data. Prior to randomization, BP data and local laboratory data are shared with participants. Provision of such information allows the participant to make an informed decision about whether to participate or to seek medical care (e.g. for stage 1 hypertension or elevated LDL-C). For BP, trained staff provide copies of BP readings. Those individuals with elevated BP (defined by escape BP below) are advised to return for repeat measurement or, if appropriate, seek urgent medical care. Results from local laboratory tests used to define eligibility are reviewed by a clinician-investigator and then provided, typically sent, to participants. For fasting lipids and glucose, an information sheet based on national guidelines (ATP 3) is also provided. Otherwise, locally defined alert values are used to identify clinically relevant thresholds. In these instances, participants are advised to share these results with their personal health care provider. The type of communication channel (phone or letter) depends on the seriousness of the laboratory abnormality.

During the feeding phase, participants have their BP measured at least weekly. As in the DASH and DASH-Sodium trials, two escape levels of BP are applied:

> Escape Level # 1: systolic BP > 180 mmHg or diastolic BP > 110 mmHg. Persons with an escape level #1 BP at any one visit are referred for medical care.
>
> Escape Level # 2: systolic BP > 170 mmHg or diastolic BP > 105 mmHg (and less than escape level #1). Persons with an escape level #2 BP are referred for medical care if a repeat BP obtained within 7 days also exceeds this level.

Serious adverse events, although extremely unlikely, can be reported by participants at any visit. Because of protocol-mandated daily contacts of participants during the feeding phase, ascertainment of adverse events is high and likely complete. Specifically, each weekday during the feeding phase of the study (run-in and intervention), participants complete a daily log and meet with staff. The daily **diaries**, which are reviewed by staff, and the daily in-person encounters provide an opportunity to identify symptoms and intercurrent medical problems which might trigger an adverse event report. In the setting of a potentially serious medical problem, staff contact an investigator-clinician who assesses the severity of the problem, advises the participant on appropriate medical care, and, if needed, reports the problem to the IRB, coordinating unit and NHLBI. The investigator-clinician does not provide medical care, except in the setting of an emergency.

9. DATA MANAGEMENT, QUALITY ASSURANCE AND QUALITY CONTROL

Drs. Carey and Rosner direct the data management and statistical unit, which develops and maintains the trial database; develops and implements randomization procedures; and provides statistical and trial monitoring support throughout the trial.

Data from screening, run-in, and intervention visits (including baseline characteristics, medical history, BP, body weight, symptoms, and adherence assessment) are entered on specific forms at the centers and then **key** entered. Corresponding forms from the DASH and DASH-Sodium trials serve as prototypes. The data monitor sends reports of missing or inappropriate entries to the project coordinators every week, for clarification and resolution. **Data coordinating unit staff** provide reports on the quality and completeness of the data to Drs. Appel and Sacks every month, organized by type of visit, e.g. screening visit 1, run-in, etc, and by specific data form. At the end of each wave, the data manager verifies the completeness of data for each individual. Quality control reports are generated for key aspects of the trial, e.g. BP digit preference and variability.

Key data (exclusion criteria and outcome data) are double entered along with a sample of other data. Range, logic, and missing data checks are performed. After data entry, cross-form edit checks are also performed. Data inconsistencies occurring across forms are resolved with the assistance of clinic staff. These audits are rerun periodically to detect unresolved problems. Standardized edit reports that summarize problems in the database provide an additional method of assuring data quality. Corresponding data checks are performed on data from the Dr. Cole's Core laboratory and from Dr. Sacks' lipoprotein laboratory; if appropriate, replicate assays will be performed.

To minimize the potential for error, we use a detailed Manual of Operations, conduct annual training meetings for staff, and conduct site visits each year. The Measurements Subcommittee monitors the performance of each site and recommends new or corrective procedures in case deficiencies are noted.

Randomization: Randomization assignments to one of six sequences of the 3 diets are generated by the data manager, after confirming, by computer program, that all screening activities have occurred, that the participant meets all eligibility criteria, and that all required baseline data have been collected. Diet sequences are stratified by site with varying block sizes to ensure a balance of sequences at each site.

Blinding: Until the end of the trial, all investigators, staff and participants are masked to trial outcome data, with the exception of the trial statisticians, the data manager, the NHLBI project officer, and the Data, Safety and Monitoring Committee. Due to the nature of the intervention, however, kitchen staff are unmasked to diet assignment. All BP observers are blinded to diet assignment, and participants are blinded to diet-specific, post-randomization BPs until the end of the trial. However, a mean of all readings, across diets, will be provided to the participant **at** the end of feeding.

Confidentiality: Each participant is assigned a unique study identification number. The data management and statistical unit do not have access to the list of participant names and ID numbers and therefore cannot link names to data. Files of study data include the study identification number but not participant names. Any hardcopy of data forms obtained by the coordinating unit (for example, as part of spot-checking for quality control) are maintained in locked file cabinets under the supervision of Dr Carey.

At the clinical centers, data are collected on forms with fields for study identification number but without a field for the participant's name. Still, lists of participant names, identification numbers, and contact information are used during the study to maintain contact with participants and eventually to provide feedback on study and personal results. All hardopies of forms are stored in locked file cabinets. All computer files are password protected. Participant results will only be released to the participant unless he/she provides written approval to release data (e.g. laboratory results to personal provider).

Data will be only be presented and published in aggregate, i.e. no identifying characteristics of participants will be published or presented.

Data Security: All computer files pertaining to the study are maintained on a UNIX system at Channing Laboratory that is subject to daily backup and periodic off-site storage of backed-up data. All computer files are stored in password-controlled accounts on Channing laboratory computers.

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