

DETAILED PROTOCOL
CARBOHYDRATE: AMOUNT AND TYPE AFFECTING RISK FOR CVD AND DIABETES
(OMNI-CARB)

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Version 1.12

Version 1.12, Protocol edits made on 6/12/2008:

Page 13, Section 4.f. Intervention Periods: Altered “During the last 7 days of each intervention period, all outcome measurements are obtained as described subsequently” to “During the last two weeks of each intervention period, all outcome measurements are obtained as described subsequently.”

Page 14, Section 4.g. Measurement of outcome variables: Altered, “In summary, the outcome measurements, BP, lipids, and OGTT, and symptoms questionnaires are measured at baseline, and in the final fifth week of each of the four diet periods” to “In summary, the outcome measurements BP and symptoms questionnaires are measured at baseline and in the final fifth week of each of the four diet periods, while lipids and OGTT are measured at baseline and in the final ten days of each of the four diet periods.”

Page 15, Table 3 (Schedule of measurements): Added the following footnote to the OGTT, “The OGTT collection time window extends through the last ten days of each Intervention period.”

Version 1.11, Protocol edits made on 4/8/2008:

Page 15, Table 3 (Schedule of measurements): Satiety questionnaire was removed from Run-In and Week 4, and added to Week 3.

Page 15, Table 3 (Schedule of measurements): Altered Blood Pressure Intervention measurements to “9 readings collected total, at least 2 readings taken in the last week.”

Page 17, Section 4.g.iv. Measurement of other variables: Satiety-related symptoms was changed from collection during Run-In and the fourth week to collection during the third week of each feeding period.

Page 17, Section 4.g.iv. Measurement of other variables: Weight will be measured using a calibrated Tanita BWB 800 digital scale, changed from using a balance beam scale.

Version 1.1, Protocol edits made on 10/22/2007:

Page 6, Secondary Aims: Secondary Aim #5, BMI <25 was removed.

Page 11, Inclusion and Exclusion criteria table: Symptomatic ischemic heart disease, e.g. angina pectoris was removed from the Medications Exclusions section.

Page 11, Inclusion and Exclusion criteria table: coumadin (warfarin), was added to the list of exclusionary drugs under the Medications Exclusions section.

Page 11, Inclusion and Exclusion criteria table: Wording was changed in the ‘Other exclusions’ sections to say: Weight loss or gain of $\geq 5\%$ or more during prior 2 months.

Page 12, Participant eligibility visits (4d). Measurement of weight was added to the SV2 visit.

Page 13, Intervention Periods section (4f). Collection of a symptoms questionnaire was removed from the first week of each feeding period.

Page 13, Intervention Periods section (4f). The timing of the collection of a satiety questionnaire was changed from week 3 to week 4.

Page 14, Table 3 (Schedule of measurements): Weight was added to the SV2 measurements.

Page 14, Table 3 (Schedule of measurements): Symptoms questionnaire was removed from the first week of each feeding period.

Page 14, Table 3 (Schedule of measurements): 24 Hr urine collection was expanded to weeks 4 and 5 of each feeding period.

Page 14, Table 3 (Schedule of measurements): Diet Acceptability questionnaire was moved from week 4 to week 5 of each feeding period.

Page 14, Table 3 (Schedule of measurements): Satiety questionnaire was added to the Run-In measurements.

Page 15, Section 4.g.iii. Insulin sensitivity, glycemic response, fructosamine: The following sentence was removed: The arm is kept in a heated container to produce arterialized venous blood.

Page 16, Section 4.g.iii. Insulin sensitivity, glycemic response, fructosamine: For the postprandial responses, the 7:45 collection sample was removed.

Page 16, Section 4.g.iv. Measurement of other variables: Measurement of weight was added to the SV2 visit.

Page 16, Section 4.g.iv. Measurement of other variables: The 24 Hour urine collection time was expanded to the last 2 weeks of each intervention period.

Page 16, Section 4.g.iv. Measurement of other variables: Symptoms questionnaire. Sentence was changed to state: Symptom Questionnaire is administered once during screening, once during run-in, and once during each feeding period.

1. SUMMARY

The optimal diet to prevent cardiovascular disease (CVD) and diabetes is uncertain. Reducing saturated fat and transunsaturated fat lowers CVD risk. However, within the context of low saturated and transunsaturated fat, diets can vary widely in other energy-providing nutrients, particularly carbohydrate but also protein. Some authorities advocate reducing carbohydrate and replacing it with fat and protein. It is hypothesized that such dietary changes will improve blood lipids and insulin resistance. It has also been argued that slowly absorbed carbohydrates, i.e. those with a low glycemic index, improve blood lipids and insulin resistance. These points are much debated. The objective of this trial is to establish definitively the biological effects of dietary carbohydrate level and type on risk of CVD as well as on insulin resistance.

This study is a controlled dietary trial that examines the effects of reducing carbohydrate from a high level (58% kcal) to a lower level (40% kcal) and of lowering glycemic index from a higher level (>65 on the glucose scale) to a lower level (<45) on the major established diet-related CVD risk factors as well as insulin sensitivity, strongly linked to type 2 diabetes. Trial participants (n=160, approximately 50% women, 50% African-American) are overweight or obese with elevated blood pressure, and are at high risk for CVD and diabetes. The core design is a randomized controlled feeding study. Four diets are being tested for 5 weeks each: high or low carbohydrate, each with high or low glycemic index. The participants are maintained in the weight-stable state. The primary endpoints are systolic blood pressure; plasma LDL cholesterol, HDL cholesterol and triglycerides; and insulin sensitivity determined directly by frequently sampled oral glucose tolerance test (OGTT). Secondary endpoints include diastolic BP; apolipoproteins B, CIII, and A-I, and atherogenic VLDL and LDL particle types that contain apolipoprotein CIII; first and second phase insulin response and glucose effectiveness by OGTT; fructosamine; and postprandial responses of glucose, insulin, lipids and hormones. The primary diet contrast is high carbohydrate, high glycemic index vs low carbohydrate, low glycemic index. Other contrasts aim to disentangle level of carbohydrate and glycemic index; i.e. the effects of high vs low glycemic index at each carbohydrate level, and the effects of high vs low carbohydrate level at each glycemic index. Effects of each diet on overall cardiovascular risk is estimated by standard risk equations.

2. SPECIFIC AIMS

While there is widespread consensus that diet to reduce CVD risk should be low in saturated and transunsaturated fat, the optimal level and type of carbohydrate is a major, unresolved research question with substantial public health and policy implications. An expanding body of evidence from epidemiologic studies and small trials suggests that the type and amount of carbohydrate influence CVD risk, yet such evidence was insufficient to influence dietary recommendations (USDA, 2005; IOM-NAS 2002). Ideally, the results of large scale clinical trials with clinical CVD events would be used to establish dietary guidelines. However, there are insurmountable obstacles to the conduct of such trials, including huge sample size (likely tens of thousands of individuals); long duration of follow-up; difficulties in maintaining an experimental contrast; and extremely high cost, likely hundreds of millions of dollars. The next best approach is to test the effects of different diets on established risk factors for CVD.

The DASH diet, a low fat, high carbohydrate diet, is effective for lowering BP (Appel 1997) and LDL cholesterol (Obarzanek 2001), and is considered the benchmark in national guidelines for prevention of CVD (USDA, Dietary Guidelines for Americans 2005). Still, the DASH diet has other effects, which suggest that modifications to the DASH diet can further reduce CVD risk. Specifically, the DASH diet reduced HDL cholesterol, and had no effect on triglycerides (Obarzanek 2001). It also unclear whether the DASH diet with its high dietary carbohydrate content is best for glycemic control. The OmniHeart trial studied the effect of replacing some of the carbohydrate content of a DASH-type diet with either protein or unsaturated fat. The results showed that BP, LDL cholesterol, triglycerides, and HDL cholesterol levels can be further improved by lowering carbohydrate levels from 58% to 48%.

Deliberations on the amount of carbohydrate are intricately woven with considerations on the type of carbohydrate. It has been argued that slowly absorbed carbohydrates, i.e. those with a low glycemic index, improve BP, blood lipids and insulin resistance (Jenkins 2002; Ludwig 2002). In essence, the purported problems identified with carbohydrate (e.g. increased triglycerides, reduced HDL, increased glycemia) could be related more to the type than amount of carbohydrate.

In this setting, we conduct a controlled feeding trial that examines the effects, separate and combined, of reducing carbohydrate from a high level (58% kcal) to a low level (40% kcal) and of lowering the glycemic index from a high level (glycemic index >65 on the glucose scale) to a low level (glycemic index <45) on the major established diet-related CVD risk factors, as well as insulin sensitivity.

Primary Specific Aim

To determine the combined effects of reducing carbohydrate from a high level (58% kcal) to a low level (40% kcal) and of lowering the glycemic index from a high level (glycemic index >65) to a low level (glycemic index <45) on systolic BP, LDL cholesterol, HDL cholesterol, triglycerides, and insulin sensitivity (as determined directly by frequently sampled oral glucose tolerance test (Bergman-Cobelli minimal model)). We hypothesize that the low carbohydrate, low glycemic index diet has favorable effects on each of these risk factors.

Secondary Aims

1. To determine whether and how much the effects found in the Primary Aim are explained by lowering the glycemic index from a high level to a low level. We hypothesize that a low glycemic index improves risk factor levels even when carbohydrate amount is held constant. We further hypothesize that the effect of glycemic index is more pronounced when total carbohydrate intake is high rather than low.
2. To determine whether and how much the effects found in the Primary Aim are explained by lowering carbohydrate from the high level to the low level. We hypothesize that reducing carbohydrate amount improves risk factors even when glycemic index is held constant. We further hypothesize that the effect of reducing carbohydrate amount is more pronounced when the glycemic index is high than low.
3. To determine the effects of the diets on other outcomes (diastolic BP; apolipoproteins B, CIII, and A-I, and atherogenic VLDL and LDL particle types that contain apolipoprotein CIII; first and second phase insulin response and glucose effectiveness by OGTT, and fructosamine). We hypothesize that the response of these outcomes will generally follow those of the primary outcomes.
4. To determine the effects on overall CHD risk using established risk equations (Framingham and PROCAM). We hypothesize that the low carbohydrate low glycemic index diet will reduce risk the most compared to estimated baseline risk.

5. To examine the effects of the diets in pre-specified subgroups, defined by gender, race-ethnicity (blacks and non-blacks), age (above and below median), metabolic syndrome, baseline level of each outcome variable (above and below clinical thresholds, e.g. hypertensive and pre-hypertensive), and weight status based on standard BMI categories (25-29.9, ≥ 30). Determine if dietary effects significantly differ among subgroups (interaction analysis).
6. To estimate changes from baseline for each of the 4 diets.
7. To examine the effects of the diets on postprandial glucose, insulin, lipids and hormone levels.

3. BACKGROUND AND SIGNIFICANCE

3.a. Diets to Reduce CVD and Diabetes

Cardiovascular disease (CVD), including coronary heart disease 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 modifiable CVD risk factors. It has become disturbingly clear that as certain risk factors for CVD like BP and LDL cholesterol are decreasing (Gregg 2005), diabetes, hypertriglyceridemia, and the metabolic syndrome have been increasing (Gregg 2005; Eckel 2005; Alexander 2003; Ford 2004) to threaten the gains in BP and LDL cholesterol. As drug therapy is becoming ever more multivalent, challenging, and expensive, and targeted simultaneously to BP, lipids and glucose, it is all the more important to understand the full biological potential of diet to treat these risk factors.

The modifiable risk factors for myocardial infarction and stroke that are related to diet are BP and the plasma lipids, LDL-cholesterol (LDL-C), HDL-cholesterol (HDL-C), and triglycerides. In addition, insulin resistance increases CVD risk directly through its effects on vascular cells and the atherosclerotic process and indirectly through its effects on lipids. Insulin resistance is also closely associated with hypertension and is a precursor to type 2 diabetes, considered to be a “coronary disease risk equivalent”. Finally, hyperglycemia is a risk factor for microvascular disease in the retina, kidney, and elsewhere. The ideal diet should be effective in preventing CVD and diabetes, by improving each of these risk factors.

There is still much divergence of opinion among experts as to what diet or diets are ideal for improving lipid risk factors, and preventing type 2 diabetes. Specifically, although there is wide agreement that saturated and transunsaturated fat intake increases LDL cholesterol and decreases HDL cholesterol, and contributes to coronary artery disease (Krauss et al, AHA Guidelines 2000; American Diabetes Association, 2003), there is disagreement about the nutrient that is an optimal replacement for saturated and transunsaturated 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. There is also much discussion among experts on the role of quality of the carbohydrate containing foods, high or low glycemic index, as regards insulin sensitivity and glycemic control (Sheard, 2004; Reaven, 1988; Ludwig, 2002; Jenkins, 2002; Eckel, 2004).

The basis for the divergent opinions is the lack of evidence from definitive studies that assess the impact of diets on each major risk factor. The result is confusion among clinicians and the public. We believe that the recently published OmniHeart study (Appel 2005) has made progress in extending the science for evidence-based nutritional guidelines, by determining the benefits of a modest reduction in dietary carbohydrate.

The following sections discuss what is known about the effects of carbohydrate on BP, blood lipids, and blood glucose control and insulin resistance.

3.b. Carbohydrate Type and Amount: Overview and controversies

All dietary carbohydrates, from starch to table sugar, share a basic biologic property: they can be digested or converted into glucose. Digestion rate and, therefore, blood glucose response has been commonly thought to be determined by saccharide chain length, giving rise to the terms “complex carbohydrate” and “simple sugar.” This view, which has its origins in the beginning of the last century (Allen 1920), receives at least tacit support from current nutritional recommendations to increase consumption of starchy foods but decrease consumption of sugar.

Over the past 25 years, however, the relevance of chain length to carbohydrate digestion rate has been questioned. Wahlqvist et al (1978) demonstrated similar changes in blood glucose, insulin and fatty acid concentrations after consumption of glucose as a monosaccharide, disaccharide, oligosaccharide or polysaccharide (starch). Bantle et al (1983) found no differences in blood glucose responses to meals with

25% sucrose compared to meals containing a similar amount of energy from either potato or wheat starch. Nevertheless, the physiologic effects of carbohydrates may vary substantially, as demonstrated by marked differences in glycemic and insulinemic responses to ingestion of isoenergetic amounts of white bread versus pasta (Granfeldt 1991). For this reason, Jenkins et al (1981) proposed the glycemic index as a system for classifying carbohydrate-containing foods according to glycemic response.

Glycemic index is a property of a carbohydrate-containing food that quantifies the rise of blood glucose occurring after a standardized amount of the food is consumed. It is defined as the incremental area under the blood glucose curve following consumption of 50 g carbohydrate from a test food divided by the area under the curve following consumption of 50 g carbohydrate from a control food, either white bread or glucose (Wolever 1991). Foods that are rapidly digested and absorbed or metabolically transformed into glucose have a high glycemic index (Ebbeling 2001; Foster-Powell 2002). In general, most refined starchy foods eaten in the U.S., white bread, prepared breakfast cereals, rice, and potato products, have a high glycemic index; unprocessed grains, pasta, nonstarchy vegetables, fruit, nuts and legumes tend to have a low glycemic index. Regular consumption of high-glycemic index meals, compared to isoenergetic and nutrient-controlled low glycemic index meals, increases 24-hr blood glucose and insulin levels, C-peptide excretion and glycosylated hemoglobin concentrations in normal or diabetic subjects (Jenkins 1987; Miller 1994).

The glycemic index is considered by the World Health Organization (FAO, WHO 1997) and several other professional health agencies to be a useful method for the classification of *carbohydrate type*, as it compares foods with equal amounts of available carbohydrate. Small-scale clinical trials have shown that a low glycemic index diet favorably affects BP, blood lipids, postprandial glucose and insulin concentrations, and insulin sensitivity. Nonetheless, this evidence, reviewed subsequently, has been insufficient to guide public policy. The following issues have been raised by colleagues regarding the glycemic index (e.g. Coulston & Reaven 1997; Pi-Sunyer 2002; Sheard 2004; Eckel 2004):

- Skepticism that a glycemic index value for a food, based on the glucose response to a single meal, is a meaningful indicator of the postprandial glucose response to a complete meal or to the long-term glycemic effect of an overall diet.
- Skepticism that an increase in blood glucose produced by a high glycemic index food, could as part of a complete diet reduce insulin sensitivity.
- Lack of appropriately designed trials of glycemic index on CVD risk factors and insulin resistance. Trials to date have been small, typically $n < 15$, sometimes lacking appropriate controls, sometimes not in high risk patients, and very brief, often < 2 weeks; and results have been mixed. Hence, there is uncertainty whether the transient glucose elevations in response to high glycemic index foods has long term effects on risk factors and health.

We now turn to a review of evidence on dietary carbohydrate and the risk factors we propose to study.

3.c. Carbohydrate amount and type: Effects on BP

Worldwide, there are many populations that eat carbohydrate-rich low-fat diets 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, observational studies tend to be inconsistent. For instance, Stamler (1996) documented a direct association between starch intake and BP in observational analyses of the MRFIT trial, while other studies documented either no association of carbohydrate with BP (Reed 1985) or an inverse association (Stamler 2002). In early trials, increasing carbohydrate by reducing total fat generally did not reduce BP (Morris & Sacks 1994).

The largest well-controlled trial of a high carbohydrate low-fat diet and BP was DASH (Dietary Approaches to Stop Hypertension, Appel et al 1997, see Appendix). The diet, termed the DASH diet, emphasizes fruits, vegetables, and low-fat dairy products; included whole grains, nuts, fish, and poultry; and was reduced in red meat and sugar-containing foods and beverages. The DASH diet substantially lowered BP (while body weight and sodium intake were held constant) in the overall study population, as well as in major subgroups, including normotensives and hypertensives, blacks and whites, and men and women (Svetkey 1999). In hypertensives, the dietary effects were similar to those expected from initial drug therapy. However, the DASH study was not designed to determine effects of individual foods or nutrients. The DASH diet differed in numerous dimensions from the 'control' diet, similar to a common US

dietary pattern. Hence, the results from DASH cannot be interpreted as establishing a BP effect of simply replacing saturated fat with carbohydrate.

Evidence has been developing that increased intake of either unsaturated fat or protein lower BP. Still, it is difficult to tease apart a BP lowering effect of fat or protein from a BP raising effect of carbohydrate, because carbohydrate is the macronutrient that is typically reduced when fat or protein are increased. Observational evidence suggests an inverse association of unsaturated fat with BP. In parts of Greece where traditional diets were eaten, the incidence of hypertension was half that of western Europe and the US (Keys 1980). A small-scale clinical trial found that the Mediterranean diet reduced BP in Italians (Strazzullo 1986). This was followed by another trial that confirmed a BP lowering effect (Esposito, 2004). 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. Rasmussen et al (1993) found that a high monounsaturated fat diet reduced BP in type 2 diabetic patients.

An alternative explanation that applies to some of the studies is that reducing carbohydrate rather than increasing fat, per se, is responsible for lowering BP. Pertinent to this idea, Psaltopoulou et al (2004) analyzed the diets of Greeks, and designed an index representing a traditional Greco-Mediterranean diet. This index was strongly associated with lower BP. They then analyzed the individual components of the traditional diet index and found that olive oil, vegetables, and fruits but not bread, i.e. carbohydrate, had a favorable association with BP. Bread had an adverse coefficient, and olive oil was the most favorable. This suggested that the traditional Greek diet could be improved, as regards BP control, if it were reduced in content of carbohydrate. Finally, an extensive, and generally consistent, body of evidence from observational studies have documented significant associations of higher protein intake with reduced BP (Obarzanek 1996; He 1999, Liu K 1992; Yamori 1981; Kihara 1984; Dyer 1992; Zhou 1989; Zhou 1994).

There is a paucity of direct experimental evidence that glycemic index affects BP. Acute or chronic consumption of high glycemic index carbohydrate, typically sucrose and high-fructose corn syrup, increases BP in several animal models (Hwang 1987, Martinez 1994, Preuss 1992, Zein 1990, Gondal 1996, Zhang 1999). Potential mechanisms for a direct BP effect include increased catecholamine production or release, and promotion of sodium retention. A few trials have also tested the effects of acute sugar consumption on BP in humans. Rebello (1993) assessed the BP response after ingestion of solutions of five simple sugars using a Latin square design, among 20 healthy normotensive men. Both glucose and sucrose ingestion were associated with significant increases in systolic BP at 1 hour (+9 to 10 mm Hg). In a cross-over study of 24 men and women classified as "carbohydrate sensitive" on the basis of exaggerated insulin response to a sucrose load, diastolic BP was significantly increased after 6 weeks of a diet containing 33% of calories as sucrose (Israel 1983). In contrast, 50g of glucose led to reduced BP in a trial of 10 elderly individuals (Visvanathan 2004). Finally, there is a large body of evidence from mechanistic studies that connects insulin resistance to high BP (e.g. Reaven 1988; Ferrannini 1987; DeFronzo 1975; Kuroda 1999; Egan 2003).

3.d. Carbohydrate: Blood Lipids and Lipoproteins

LDL-cholesterol is the paramount lipid risk factor used in guidelines to prevent and treat CVD. The effects of dietary macronutrients on plasma lipids are defined much better than the effects on BP. There is broad consensus that reducing dietary saturated and transunsaturated fat improves LDL-cholesterol concentration (Krauss RM 2000; Mensink 1992). The DASH trial documented that the low-fat, high-carbohydrate DASH diet, replacing about 10% of energy of saturated fat with carbohydrate, significantly reduced LDL cholesterol (Obarzanek 2001), consistent with the literature. The OmniHeart trial, described subsequently, found that a reduction in carbohydrate content of the DASH diet by increasing protein significantly lowered LDL cholesterol by 3% whereas an increase in unsaturated fat (mainly monounsaturated) resulted in a nonsignificant 1.4% reduction (Appel 2005). This beneficial effect on LDL cholesterol of a mix of dietary proteins (approximately one-third meat, one-third dairy, and one-third plant) replacing carbohydrate confirms earlier work in small groups of subjects (Wolfe 1992,1999). Eight interventional studies have examined the effects of glycemic index on LDL cholesterol under macronutrient controlled conditions (reviewed in Ludwig 2002). The low glycemic index diets lowered LDL cholesterol levels in seven of them by 5-10% (Jenkins 1985 ; Jenkins 1987a,b; Jenkins 1988 ; Brand 1991 ; Wolever 1992 ; Jarvi 1999), and in one study there was a 2% increase (Luscombe 1999).

HDL is a well-established CVD risk factor; high levels are associated with reduced risk (Gordon, 1989). Much evidence supports a causal relationship between low levels of HDL and atherosclerosis. Clinical trials

found that increases in HDL in response to lipid drugs are correlated with reduction in atherosclerosis and clinical coronary events (reviewed in Gordon 1989; Sacks 2002a). Direct intravenous infusion of HDL-like lipoprotein particles reverses atherosclerosis in rabbits (Badimon 1990) and humans (Nissen 2003). Of concern are consistent findings that dietary carbohydrate lowers HDL concentrations (Mensink 1992). The mechanism is reduced production of apo A-I by the liver (Brinton 1990; Velez-Carrasco 1999). Some experts are concerned about the reduction in production of an anti-atherogenic lipoprotein, and would prefer a dietary option such as monounsaturated fat that does not reduce HDL (e.g. Katan 1997). Recent experiments in transgenic mice demonstrated that increased human apoA-I production by the liver directly causes cholesterol removal from macrophage-foam cells and excretion into the bile in vivo (Zhang 2003). The DASH diet lowered HDL, as expected from its high carbohydrate content. In OmniHeart, protein reduced HDL cholesterol by 3% when it replaced carbohydrate, and by 5% when it replaced unsaturated fat. Thus it appears that unsaturated fat has the best HDL raising effect among the macronutrients.

There is limited information on whether the glycemic index of carbohydrate affects HDL concentrations. Three observational studies found that glycemic index was associated with reduced HDL levels (Liu S 2001; Ford 2001; Frost 1999). Twelve interventional studies have reported the effects of a low glycemic index on HDL cholesterol under macronutrient controlled conditions. No particular pattern emerged. HDL cholesterol increased in 6 of them (Jenkins 1988; Brand 1991; Fontvieille 1992; Luscombe 1999; Jarvi 1999; Giacco 2000) and decreased in 6 (Jenkins 1985; Jenkins 1987a,b; Collier 1988; Fontvieille 1988; Wolever 1992). The mean changes among the studies ranged widely from +16% to -19%, making it difficult to ascertain whether there is a true effect or no effect. Another problem in interpreting these results is that the studies were often very small in sample size and short in duration, e.g. 1-2 weeks.

The concentration of triglycerides is now established as an independent risk factor for CHD (Hokanson 1996; Gotto 1998; Stampfer 1996; Jeppeson 1998, Sacks 2002b, Hopkins 2005). 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, as shown by subsequent individual large-scale studies (Stampfer 1996; Jeppeson 1998; Sacks 2002b, Hopkins 2005). Dietary carbohydrate increases plasma triglyceride levels (Mensink 1992) by increasing hepatic triglyceride production and VLDL secretion (Parks and Hellerstein 2000). Thirteen interventional studies have examined the effects of dietary glycemic index on plasma triglycerides under macronutrient controlled conditions (Jenkins 1988; Brand 1991; Fontvieille 1992; Luscombe 1999; Jarvi 1999; Giacco 2000; Jenkins 1985; Jenkins 1987a,b; Collier 1988; Fontvieille 1988; Wolever 1992a,b). The low glycemic index diets reduced triglycerides in 10 of the studies; mean changes ranged from -20% to +6%. Interestingly, the DASH diet did not raise triglyceride levels despite its high carbohydrate content, supporting the likelihood that a lower glycemic index diet may protect against the triglyceride-raising effect of dietary carbohydrate (Obarzanek 2001).

3.e. The OmniHeart Study.

We conducted a trial, OmniHeart that manipulated the macronutrient composition of the DASH diet by modestly lowering carbohydrate and raising either unsaturated fat or protein. The OmniHeart trial asked the question whether the low-fat, high-carbohydrate content of DASH is optimal, or whether replacement of some of the carbohydrate with unsaturated fat (primarily mono) or protein (primarily from plant sources) would improve BP and lipids. In OmniHeart, three diets were studied in 164 adults in a crossover protocol that provided all meals for three 6-week periods: (1) CARB - high carbohydrate, similar to the DASH diet, (2) PROT – rich in protein, about half from plant sources, and (3) UNSAT – rich in unsaturated fat, primarily mono. In brief, each of the three diets tested in OmniHeart was healthy and consistent with nutrient intakes recommended by the Institute of Medicine. Each had beneficial effects on blood pressure, low density lipoprotein cholesterol, and estimated coronary heart disease risk, relative to baseline. Systolic BP was significantly higher during the CARB diet compared to either PROT or UNSAT in the total group and in the hypertensive subgroup. LDL cholesterol was higher during CARB compared to PROT by 3.3 mg/dl (2.8%, $p=0.01$) and compared to UNSAT by 1.5 mg/dl ($p=0.22$). Mean triglycerides was significantly higher during CARB by 15.7 mg/dl (15.5%, $P<0.01$) compared to PROT and by 9.6 mg/dl (9.5%, $P=0.02$) compared to UNSAT. Among participants with a triglyceride level > 150 mg/dl, triglycerides were 26.9 mg/dl ($p=0.04$) lower during PROT than during CARB. This pattern of CARB having unfavorable changes compared to PROT and UNSAT was not seen in HDL cholesterol. Changes in HDL cholesterol were small; compared to the concentration on CARB, HDL cholesterol decreased by 1.3 mg/dl on PROT ($P=0.02$) and increased by 1.1 mg/dl on UNSAT ($P<=0.03$). In summary, OmniHeart showed that the DASH diet can be improved by modestly lowering carbohydrate from 58% to 48%, i.e. by 10% kcal which is two-thirds of the contrast in the proposed

study. We note that glycemic index for all 3 diets was similar and moderate, approximately 70 on the white bread scale (the white bread scale yields GI values that are 20 points higher than the glucose scale referred to in this protocol). Thus, the question remained unanswered whether a lower glycemic index could improve the effects of a high carbohydrate diet, and narrow or eliminate the difference in dietary effects on risk factors.

3.f. Carbohydrate: glucose response and insulin sensitivity

The effects of high carbohydrate, low 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 reduce insulin sensitivity in normal subjects (Borkman 1991) or in patients with type 2 diabetes (Garg 1992). Other studies found that high carbohydrate compared to high fat diets worsened postprandial hyperglycemia or hyperinsulinemia in diabetic patients (Parillo 1996) or in hypertensive patients (Parillo 1988). 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 (Garg 1988). These results were confirmed by Parillo (1992).

The effects of glycemic index on insulin resistance have been studied in 5 brief trials, each with < 30 participants. Frost et al (1998) conducted a randomized controlled trial with 28 women and found greater insulin sensitivity as determined by insulin tolerance test after 3 weeks on a low- compared to a high-glycemic index diet. Piatti et al (1993) examined changes in M/I (insulin sensitivity index) determined by hyperglycemic clamp among obese subjects during energy restricted diets, a predominately grain-based diet (thus relatively high in glycemic index, n = 11) versus a predominately fruit-based diet (thus relatively lower in glycemic index, n = 7). After the 3-week dietary intervention, insulin sensitivity decreased on the starch-based diet, but increased on the fruit-based diet. Rizkalla et al (2004) compared low- and high-glycemic index diets in 12 men with type 2 diabetes in a 4-week cross-over study. Insulin sensitivity, measured directly as glucose disposal during a hyperglycemic euglycemic clamp, was significantly greater following the low- versus high-glycemic index diet (7 ± 1.3 vs. 4.8 ± 0.9 mg glucose/kg/min, $p < 0.001$). In contrast, Jarvi et al (1999) compared high and low glycemic index diets in 20 type 2 diabetic patients, mean age 66 years and with BMI ≤ 27 , in a cross-over study of 24 days length. Although 12-hour blood glucose and insulin concentrations were significantly lower during the low glycemic index diet, insulin sensitivity determined by euglycemic hyperinsulinemic clamp was not significantly different. Kiens and Richter (1996) found no difference in glucose disposal during physiological insulin concentrations in 7 lean, fit young men fed low- or high-glycemic index diets for one month. However, findings from this last study in young men with optimal insulin sensitivity may not be applicable to obese insulin resistant individuals who are at greatest risk of type 2 diabetes.

In summary, a large body of evidence is suggestive but not conclusive that a high carbohydrate intake and a high glycemic index each adversely affect risk factors for CVD and reduce insulin sensitivity. It is not clear whether amount or type (glycemic index) of the carbohydrate is paramount, and in particular whether the effects of high carbohydrate are mostly explainable by a high glycemic index. The present study aims to disentangle the effects of carbohydrate amount and glycemic index to determine optimal macronutrient contents to reduce CVD and insulin resistance.

4. DESIGN

4.a. Design Summary

The core design is a feeding study that uses the same methodology as the DASH and OmniHeart trials. Inclusion/exclusion criteria were chosen to enroll a population at high risk for CVD and diabetes. Specifically, trial participants (n=160, ~50% women, ~50% African-American) have systolic BP of 120-159 mmHg and diastolic BP of <100 mmHg (not on anti-hypertensive medication). Four diets are being tested for 5 weeks each: high or low carbohydrate, each with high or low glycemic index, using a 4-period cross-over design. A washout period of at least 2 weeks separates each period. Participants are fed sufficient calories to maintain their weight. The total number of feeding weeks in the study (21 weeks) is only slightly longer than that of OmniHeart (19 weeks) and is therefore feasible. The primary endpoints are systolic BP; plasma LDL cholesterol, HDL cholesterol, and triglycerides; and insulin sensitivity determined directly. Secondary endpoints include diastolic BP; apolipoproteins and atherogenic lipoprotein particle types; first and second phase insulin response and glucose effectiveness by OGTT; fructosamine; and postprandial responses of glucose, insulin, lipids and hormones. The primary dietary comparison is between high carbohydrate - high glycemic index diet (CG) and the low carbohydrate - low glycemic index diet (cg). Secondary aims are to

determine whether level of carbohydrate or glycemic index or both are components of the response. Effects will be determined on overall CHD risk, as estimated by the Framingham and PROCAM risk equations. Dissemination of recommendations on superiority of a diet or diets will depend on overall risk being improved. The objective of this type of “feeding study” is to establish definitely the biological effects of dietary carbohydrate level and type on risk of CVD and diabetes.

4.b. Study population/Eligibility Criteria

This trial will recruit 160 generally healthy participants (80 at Brigham and Woman’s Hospital and 80 at Johns Hopkins). Participants will be men and women, ages 30 years or older, who have an average systolic BP of 120-159 mmHg and diastolic BP of 70-99 mmHg (not on anti-hypertensive medication). The table displays inclusion and exclusion criteria of the trial.

Table: Inclusion and Exclusion Criteria of the Trial

Inclusion Criteria

- Baseline SBP 120-159 mmHg and DBP <100 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
- Overweight or obese, as defined by a Body Mass Index (BMI) \geq 25 kg/m²
- Willing to eat at least one on-site meal/day, five days/week, and willing to eat study diets and nothing else during controlled feeding periods (run-in and intervention)
- Willingness to complete measurement procedures, including five OGTTs.

Medication Exclusions

- Regular use of medications during the past 2 months that raise or lower BP
- Use of a lipid lowering agent (any in 3 weeks prior to first screening visit)
- Unstable dose of hormone replacement therapy, thyroid hormone replacement therapy and psychotropic medications known to cause weight gain or affect the outcome variables (unstable defined as a change in dose within two months of the SV1 visit)
- Use of insulin, oral hypoglycemic agent, lithium, coumadin (warfarin), oral corticosteroid, anti-psychotic drugs, weight loss medications, nitrate, or digitalis.

Medical History Exclusions

- Active or prior CVD (stroke, MI, PTCA, CABG, congestive heart failure, symptomatic ischemic heart disease (angina), or ASCVD-related therapeutic procedure).
- Diabetes mellitus
- Cancer diagnosis or treatment 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)
- Active inflammatory bowel disease, malabsorption, or major GI resection
- Renal insufficiency as determined by a serum creatinine > 1.2 mg/dL for women or > 1.4 mg/dL for men. These participants can enroll if their estimated GFR is \geq 40 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 glucose >125 mg/dl
- Serum transaminase > 2 times the upper range of normal, or a clinical diagnosis of hepatitis

Other Exclusions

- Body weight over 420 pounds
- 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 loss or gain of $\geq 5\%$ 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
- Current participation in another clinical trial that manipulates diet or that will affect the outcome of this study (this criterion may be waived at the site PI's judgment).
- Investigator judgment (e.g. for concerns over safety, adherence, or follow-up or for inappropriate behavior)
- Vitamin, fish-oil, weight-loss, soy, mineral, or herbal supplements that cannot be stopped (supplement use is discouraged, but this criterion may be waived at site PI's judgment and if participant remains on a constant dosage throughout the study).
- Unable to measure baseline blood pressure (due to arm circumference > 50 cm or atrial fibrillation) or obtain baseline OGTT.
- Unable to maintain a stable weight during Feeding Period 1, which is defined as a loss or gain of $> 3\%$ of their initial weight at Week 4.

4.c. Recruitment

The primary recruiting tool is mass mailing of brochures, flyers and coupons (Appel 1999). Yields from mass mailing are typically on the order of 2 to 5 randomizations per 10k mailed brochures. The primary sources of mailing lists are commercial vendors and local governments (for lists of registered voters or drivers). It is noteworthy that (1) each center has ready access to lists from commercial vendors and local governments and (2) each center has identified a commercial mail distributor that can generate mailing labels from computer files and then affix the labels and postage to the brochures. In the end, we anticipate that there will be 60-70 randomizations per center from mass mailings. At both centers, secondary strategies include distribution of flyers, print ads, word-of-mouth, posters in buses and subways, and ValPack coupons. The anticipated number of randomizations from each secondary strategy is 5 to 15.

Because of the disproportionate burden of hypertension, diabetes, metabolic syndrome and its complications in blacks, we set 50% as the trial recruitment goal for blacks. In subgroup analyses, blacks will be compared to other race/ethnicities, mainly non-Hispanic whites. We anticipate that $\sim 50\%$ of trial participants will be women and that approximately 20-30% will have Stage 1 hypertension. Targeted recruitment is used if there is a shortfall in recruitment of an important subgroup. Children will not be included, as has been our policy in DASH, DASH-Sodium, and OmniHeart, because of the strict requirements of the feeding protocol and, in the proposed study, the intensity of measurements which include OGTT and postprandial meal studies. The trial also requires maintenance of stable weight over an extended period of time. This requirement is inappropriate for children who would be expected to grow.

4.d. Participant eligibility visits

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.

Pre-Screen Contact: 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 the exclusionary criteria. 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).

Screening Visit 2 (SV2:at least 7 days after SV1): SV2 includes 3 BP measurements, weight, a Food Frequency Questionnaire, and waist circumference.

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 collected include weight, a symptoms questionnaire, and 24-hour urine specimen for sodium, potassium, urea nitrogen, and creatinine. Participants meet with a study staff member to confirm their willingness to adhere to the protocol. Baseline levels of fasting lipids, glucose and creatinine will be drawn at this time. The fasting

laboratory studies and urine collection may be done at the SV2 visit, depending on logistic considerations. Subsequently, an OGTT will be scheduled prior to run-in.

4.e. Run-in and Randomization

Run-In Period (no more than 180 days after SV1): The 8-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 all calorie-containing 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. Each weekday, weight is measured. Assessment of satiety-related symptoms will be obtained. To ensure that weight remains stable, calorie intake is adjusted by providing another calorie level for the same diet (1500, 2000, 2500, 3000 and 3500 kcal/day) or by increasing or decreasing the number of 100 kcal unit foods.

Additionally, a symptoms questionnaire, medical, and social history (e.g. family history of hypertension and CVD, 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 Section 5. 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 investigator confirms suitability for randomization

Randomization 0 – 7 days after end of run-in): Upon successful completion of run-in, eligible participants are asked to sign an informed consent form that covers the main portion of the trial. Each participant is randomized to one of 8 sequences of the 4 intervention diets:

CG, Cg, cG, cg
 cg, cG, Cg, CG

Randomization is stratified by clinic. Participants are not told their assigned sequence of diets.

4. f. Intervention Periods

Each of 4 feeding periods lasts 5 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, allowing ad libitum food intake. Participants are fed sufficient calories to maintain their weight. For each day of controlled feeding, participants complete a daily diary to document self-reported dietary adherence. During the initial three weeks of each period, BP is measured once each week. During the last two weeks of each intervention period, all outcome measurements are obtained as described subsequently. Thus, all participants will have been exposed to the diet for at least a full 4 weeks of feeding before outcome measurements are obtained. In the last week of each intervention period, participants complete a symptoms questionnaire. During week three, subjects complete a pre and post meal satiety questionnaire for 5 days. A diet acceptability questionnaire will be administered the last week of each of the four dietary interventions.

The duration of the intervention feeding periods (5 weeks) reflects both scientific and practical considerations. In OmniHeart, we measured BP and lipids at 4 and 6 weeks and found no difference. Other major dietary factors that affect BP (sodium, potassium, and dietary patterns) have each reduced BP within 4 weeks and typically by 2 weeks (DASH, DASH-Sodium, OmniHeart; Brancati 1996). Previous studies found that dietary changes on lipids reach steady state within 4 weeks (Keys 1965; O'Hanesian 1996). Several dietary studies of carbohydrate and the glycemic index found significant effects on insulin sensitivity in 2 to 4 weeks (Frost 1998; Jarvi 1999; Piatti 1993; Kiens 1996; Rizkalla 2004). Thus, we consider 5 weeks sufficient.

A break between feeding periods also reflects both scientific and practical considerations. The 14 day break ensures that outcome variables measured at the end of feeding periods are separated by at least 7 weeks. A carryover effect from the prior diet, even if present, is likely to be minimal at that point. Participants also appreciate the break from controlled feeding, thereby promoting goodwill, adherence and retention.

Closeout: At the closeout visit within one month after the end of the last intervention period, participants receive nutrition counseling on prevention of CVD. Upon presentation of trial results at a national scientific meeting or publication, participants receive a summary of trial findings and a summary of their own results with interpretation. A group meeting is held at this time for the participants to discuss the study and their results with the PI and nutrition director, followed by a celebratory event.

4.g. Measurement of outcome variables

In summary, the outcome measurements BP and symptoms questionnaires are measured at baseline and in the final fifth week of each of the four diet periods, while lipids and OGTT are measured at baseline and in the final ten days of each of the four diet periods. In a sub-sample of 25%, a 12-hour postprandial study will be conducted to determine responses of blood glucose, insulin, lipids and blood pressure to each diet. This postprandial study will be conducted in the final 2 weeks of each diet period, and not at baseline. Data collection personnel are blinded to diet sequence. See Table 3 for a schedule of measurements.

	PSV	Screening Visits			Run In (RI)	Each of 4 Intervention (INT) Periods				
		SV1	SV2	SV3	RI	INT Wk 1	INT Wk 2	INT Wk 3	INT Wk 4	INT Wk 5
Informed consent		✓			✓					
Blood pressure	opt	✓	✓	✓	✓	9 readings collected total, at least 2 readings taken in the last week.				
Health questionnaire		✓			✓					
General dietary information questionnaire		✓								
Weight		✓	✓	✓	each weekday of feeding ----- >					
Height		✓								
Waist circumference			✓							
24 hour urine collection ¹			✓						✓	
Food Frequency Questionnaire			✓							
Fasting Blood ²			✓							✓
Symptoms questionnaire				✓	✓					✓
OGTT ³				✓						✓ ⁵
Postprandial responses (12 hours) ⁴									✓	
Feeding activities					daily ----- >					
Randomization					✓					
Patient history questionnaire					✓					
Brief physical activity q'aire					✓					✓
Medication questionnaire					✓					✓
Diet Acceptability q'aire										✓
Satiety questionnaire							✓			

- 1 Sodium, potassium, phosphorus, urea nitrogen, creatinine, microalbumin
- 2 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), fuctosamine at week 5 of each period; whole blood for subsequent DNA extraction (just once).
- 3 Insulin and c peptide
- 4 25% sub-sample, insulin and c peptide
- 5 The OGTT collection time window extends through the last ten days of each Intervention period.

4.g.i. Blood Pressure (BP)

BP is determined by the OMRON 907 device which records BP using an oscillometric technique. The OMRON device has been validated (White 2001). BP is obtained by trained and certified data collectors according to a standard protocol, adapted from that used in the Omni-Heart 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.g.ii . Lipids

The lipid outcome variables are measured at baseline and in week 5 of the 4 dietary periods from blood collected after an overnight (8-12 hour) fast and then stored at -70C. Lipids are measured in Dr. Sacks's laboratory at Harvard School of Public Health, standardized by the Centers for Disease Control Lipid Standardization Program. Plasma and lipoprotein cholesterol and triglycerides are measured using enzymatic kits on ELISA plates using automated dilution and kinetic reading. 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). LDL-C is determined directly by ultracentrifugation as described subsequently.

VLDL and LDL particles are measured from plasma, 1 cc. Plasma is ultracentrifugated to prepare VLDL, and LDL (1.006 <d<1.063 g/ml). In VLDL and LDL, cholesterol, triglyceride and apolipoprotein B are measured directly. To measure atherogenic VLDL and LDL particles with apolipoprotein CIII, whole plasma is applied to Sepharose to which anti-apoCIII 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 are ultracentrifugated to isolate VLDL (d<1.006 g/ml) and LDL (1.006 <d<1.063 g/ml). This procedure isolates 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. In addition, cholesterol, triglyceride, apoCIII and apoE are measured in each type.

4.g.iii. Insulin sensitivity, glycemic response, fructosamine

Insulin sensitivity and pancreatic beta cell function will be measured directly by the frequently sampled oral glucose tolerance test (OGTT), using the 2-hour 7-sample minimal model technique (Dalla Man 2005). We consider it essential to use a direct measure of insulin sensitivity and beta cell function to determine whether glycemic index or level of carbohydrate has true health implications. The frequently sampled OGTT was developed by Bergman, Cobelli and colleagues (Caumo 2000; Breda 2001), following their success with the frequently sampled intravenous glucose tolerance test analyzed by the minimal model (Steil 1993; Bergman 1981). The IVGTT was developed for use in intervention and population studies to replace the "gold standard" method of insulin sensitivity, the euglycemic hyperinsulinemic clamp (Bergman 1981). In addition, the IVGTT has an advantage over clamp methods since it can measure beta cell function in addition to insulin sensitivity. Gradually, the glucose tolerance methods have been simplified to facilitate their use in large intervention studies. First, the original 33-sample IVGTT was reduced to 12 samples taken during 3 hours while maintaining accuracy and low intra-individual variation (Steil 1993,1994; Duysinx 1994). Oral glucose administration was developed next not only for convenience but as a more physiological condition than intravenous glucose (Caumo 2000; Breda 2001; Cretti 2001). Oral glucose stimulates incretins, gut hormones that affect beta cell sensitivity to glucose and insulin secretion (Campioni 2007). Initially, the OGTT was performed during 5 hours using 22 samples (Breda 2001). The technique was validated extensively against the IVGTT, clamp techniques, and isotopic tracer techniques, and in a wide range of nondiabetic people including those with obesity and insulin resistance (reviewed in Cobelli 2007). It has not been validated sufficiently in patients with type 2 diabetes and they will not be enrolled in this trial. Later, it was shown that the quality of the information is preserved using a 7-sample 2-hour protocol at 0,10,20,30,60,90, and 120 minutes (Dalla Man 2005; Cobelli 2007). The key element in the sampling schedule is the frequent early sampling which resolves beta cell function. The minimal model OGTT has been used in recently published clinical studies (Nair KS et al, N Engl J Med 2006;355:1647; Petersen KF et al PNAS 2006;103:18273). The OGTT is performed after an overnight fast. An intravenous catheter is placed in an arm for blood sampling. Blood samples are analyzed for glucose, insulin and C-peptide. The data are modeled using published equations in the SAAM-II software program (Simulation, Analysis and Modeling) to compute the parameters of insulin response and action (Dalla Man 2002).

The primary outcome from the OGTT is S_i , insulin sensitivity. In addition, first (dynamic) and second (static) phase insulin response, measuring beta cell response to glucose, and glucose effectiveness,

representing insulin-independent glucose disposal, are secondary outcomes. Finally, specimens from the OGTT are stored at -80C for future analysis.

The postprandial responses of glucose, insulin, C-peptide, blood lipids and hormone levels to each diet are determined in a 25% random subsample. Participants in this substudy are given breakfast, lunch, and supper at 8AM, noon, and 5PM, and have blood sampling from an indwelling intravenous catheter during 12 hours. We use the protocol of Gannon et al (Am J Clin Nutr 2003;78:734). Blood samples are taken at 7:30, 8:00, 8:30, 9:30, 10:30, 11:30, 12:30, 13:30, 14:30, 15:30, 16:30, 17:30, 18:30, 19:30. The recent ADA statement commented on the need to document how strongly daily glucose responses are affected by carbohydrate content and glycemic index of the daily diet (Sheard 2004). We will also determine how correlated the glycemic responses are to the changes in insulin sensitivity among individuals, i.e. treat 14-hour glycemic response as an explanatory variable.

Fructosamine is determined as a secondary outcome. Fructosamine concentration, like glycosylated hemoglobin, is an integrated measure of blood glucose concentrations. However, as a glycosylated protein, fructosamine reaches steady state within 2 weeks of change in plasma glucose. The American Diabetes Association statement (2003) commented on the lack of information linking glycemic index to longer term blood glucose concentrations measured by fructosamine or hemoglobin A1C.

Glucose, insulin, C-peptide, creatinine, and fructosamine will be measured in the core laboratory of the GCRC at BWH.

4.g.iv. Measurement of other variables

Weight is measured by trained, certified staff using a calibrated Tanita BWB 800 digital scale. Weight will be recorded during screening at the SV1 visit (to determine eligibility), at the SV2 and SV3 visits (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 by trained, certified staff using an anthropometric measuring tape, at a horizontal plane that is one cm above the navel.

24 Hour urine collections are obtained once during screening and once during the last 2 weeks of each intervention period. The purpose of the urine collections is to monitor sodium and potassium intake which can affect BP, and to measure urea nitrogen which is a biomarker for dietary protein. These and creatinine are measured at the BWH GCRC core laboratory. Aliquots are also stored for future analyses.

A food frequency questionnaire (NCI) is administered once during screening as a means to describe the usual diet of participants during the previous 3 months. The participants enter their diet information on-site using the NCI web-based system. NCI software then analyzes the data which are downloaded into the study database by the coordinating center.

Symptom Questionnaire is administered once during screening, once during run-in, and once during each feeding period. This self-administered checklist 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).

Satiety-related symptoms are obtained during the third week of each feeding period.

Specimens of blood from each fasting blood collection, the OGTT, and the postprandial study, and urine from each 24 hour collection will be stored for additional analyses. Samples of whole blood for DNA for later gene-diet interaction studies will also be stored. A full set of samples will be stored at HSPH and at Johns Hopkins. Both facilities have alarm and back-up procedures in case of freezer malfunction or power outages. Candidate assays that might be performed include those related to metabolomics, proteomics, inflammation (e.g. hsCRP, IL-6), oxidative damage (e.g. 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). We store samples of whole blood for DNA for later gene-diet interaction studies, as well as plasma for proteomics and metabolomics. Inflammatory and other disease markers will be considered.

4.g.v. Description of Diets**Table: Macronutrient targets of study diets**

Diet*	Carb Level	GI Level	GI	Carb (%)	Prot (%)	Fat (%)	% Sat	% Mono	% Poly
CG**	High Carb	High GI	> 65	58	15	27	6	13	8
Cg	High Carb	Low GI	< 45	58	15	27	6	13	8
cG	Low Carb	High GI	> 65	40	23	37	6	20	11
cg	Low Carb	Low GI	< 45	40	23	37	6	20	11

*Key: C=high carbohydrate, c=low carbohydrate, G=high glycemic index, g=low glycemic index; **Reference diet: High carbohydrate, high glycemic index (CG)

The table displays the macronutrient targets of the four study diets. Two diets have a high carbohydrate composition (58% Carb), one with a high glycemic index (GI > 65) and the other with a low glycemic index (GI < 45). Another two diets have a low carbohydrate composition (40% carb) also with either a high or a low glycemic index composition. The carbohydrate content of the low carbohydrate diet is set at 40% kcal to ensure a large contrast in carbohydrate intake (18% kcal=58% kcal – 40% kcal) between the high and low carbohydrate diets; both protein and total fat are increased in comparison to the high carbohydrate diets again because both nutrients had favorable effects in OmniHeart. In NHANES-III, the 25th and 75th percentiles of carbohydrate intake (%kcal) are 44% and 59%, respectively (data analyses by WenYen Juan, Nutritionist, USDA-CNPP). The contrast in glycemic index (GI < 45 vs. GI >65) is substantial, spanning the first to fifth quintiles (Michaud 2005), and similar to contrasts in other studies.

Complementary high and low glycemic index foods are used to achieve the target contrast between high and low glycemic index diets (e.g. instant potatoes vs. pasta al dente; steel cut oats vs. cornflakes; white bread vs. whole kernel bread). Thus, high and low glycemic index meals are constructed around similar types of foods that have different glycemic indexes. The macronutrient distribution of each meal (breakfast, lunch, dinner, and snack) is similar for each diet type to better achieve the desired distribution of carbohydrate and glycemic control throughout the day.

Two major principles guide menu development. First, to enhance the public health relevance of the trial, we decided that the diets should be constructed with naturally occurring foods and should not be artificial. Hence, we avoid supplements and unusual foods. Second, within the constraints imposed by typical foods, the diets should provide equivalent amounts of other nutrients that might affect trial outcomes. The levels reflect prevailing guidelines. For example, the trial diets are similar in saturated fat intake (6% kcal), sodium intake (2300 mg/d), cholesterol (150 mg/day), potassium (4,700 mg/d), magnesium (500 mg/day), and calcium (1,200 mg/d). Fiber intakes are similar in the four diets to disentangle the influences of glycemic index and fiber, two frequently confounded variables. At the 2000 kcal level, dietary fiber is ~25 gm/d in all four diets.

Seven menu cycles are used for each intervention diet at 5 calorie levels (1600, 2000, 2500, 3000, 3500 kcals). Food Processor® SQL, V 9.9 (ESHA Research, Salem, OR, 2006) is used for menu calculation. Unit foods are used to meet caloric needs between the menu calorie levels and correspond to the nutrient distribution in the 4 intervention diets. Recipes are taste tested for participant acceptance. The glycemic index values of individual foods are calculated using published tables (Foster-Powell 2002) and other published data.

4.g.vi. Food Production and Distribution

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

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 specified alcoholic 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 is provided for consumption off-site. On any given feeding visit, participants complete and review a daily food diary, and are weighed. A meal monitor (dietitian or diet technician) is present during all on-site feeding visits for the purpose of facilitating compliance, collecting daily data (weights, daily diaries, etc) , tray checks, and distributing take-home meals.

Prior to study commencement and prior to run-in, a group participant orientation is held to introduce all team members, welcome participants, review study requirements and time frames, and to further explain the feeding details of the study.

Once run-in feeding starts, participants experience the actual demands of the study. Efforts to promote adherence include palatable and interesting menus convenient to their lifestyles; maintaining easy access to staff; providing daily, supportive contacts; and providing a variety of incentives (raffle tickets, movie coupons, newsletters) that promote good rapport. Acceptance of the controlled feeding protocol is increased by allowing participants to consume up to 3 caffeinated and 2 alcoholic beverages per day, as well as an unlimited amount of water and artificially sweetened soft drinks. It is noteworthy that in the DASH trial, < 50% of participants consumed any alcohol, and daily intake was rare.

Following run-in, a case conference is held with the study dietitian, study coordinator and principal investigator to review the progress of all participants and assess their continued interest and overall commitment to the trial. After randomization, every effort is made to promote adherence.

4.g.vii. Adherence Assessment

Adherence assessment includes both self-reported and objective measures. The subjective measures are used to determine suitability for randomization and subsequently to counsel participants and promote adherence during the trial. Self-reported measures are obtained from information provided on a daily diary and from subjective judgment of clinic personnel. Each day, an overall compliance score (0=compliant; 1,2, or 3 for various degrees of non-compliance) is calculated based on staff observation and information from the daily diary. The objective measures, available at the end of the study, are used to document the overall success of our procedures. Objective measures include urinary urea nitrogen (reflective of protein intake); urinary sodium and potassium intake (reflective of dietary intake), meal attendance and on-site meal consumption; and body weight.

4.g.viii. Sample size calculations

A sample size of 160 participants provides sufficient power to investigate the dietary effects on BP, lipids, and insulin sensitivity, as well as effects in subgroups comprising approximately 50% of the population, i.e. women, men, African-Americans, non-African-Americans, age > or <45 years, and metabolic syndrome (present, absent, defined by the NCEP ATP-III criterion). The following table provides SD and minimal detectable differences (MDDs) for full cohort analysis (N=160) and for half-sample subgroup analyses. For the primary analyses, the significance level is 0.01 (reduced from 0.05 because of Bonferoni adjustment for 5 outcomes)

Outcome variable	Estimated SD	N=160 MDD at $\alpha=0.01$	N=80 MDD at $\alpha=0.01$
SBP (mmHg)	6.1	1.7	2.4
HDL (mg/dl)	7.4	2.0	2.9
LDL (mg/dl)	17.3	4.7	6.7
Triglycerides (mg/dl)	49.0	13.4	19.1
Insulin SI(/min/pmol/l)	.22 x 10 ⁻⁴ l	.06 x 10 ⁻⁴	.09 x 10 ⁻⁴

SDs for BP and lipids are within-individual taken directly from OmniHeart. SD for SI is taken from Steil (1994), using the reduced 12-sample in the minimal model. The minimal detectable differences for BP and lipids are similar to those found in OmniHeart, small but meaningful to the public health. The MDD for SI represents about a 7% change from a baseline value of 0.8 as reported in Steil (1994). Judging from our experience in the DASH and OmniHeart trials, we expect minimal drop-outs and loss-to-follow-up. However, as a sensitivity test, we determined that even for a loss-to-follow-up rate as much as 20%, the MDD values increase only by

13% (e.g., MDD for SBP increases from 1.7 to 1.9 mmHg for the full cohort analysis).

For the VLDL and LDL particle 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 2002a). Since variability would be expected to be greater in free-living patients over 1 year than in the subjects in the present trial who will be eating a controlled diet for 5 weeks, the minimum detectable differences derived from these SDs, while quite small, are likely to be greater than those actually detectable in the proposed study.

4.g.ix. Analysis plan

Overview. This trial is a randomized, four-period crossover study that tests the effects of 4 diets on a selected set of outcomes. The four diets are jointly described by level of carbohydrate (C for high and c for low carbohydrate) and by level of glycemic index (G for high and g for low glycemic index), so that the four diets can be denoted CG, Cg, cG, and cg. Each participant receives all four diets. Each diet is eaten for five consecutive weeks. The order in which participants eat these diets is random. A washout period of at least 2 weeks separates each consecutive set of feeding periods. The five primary outcomes of the trial are the established CVD risk factors (systolic BP (SBP), LDL-cholesterol (LDL), HDL-cholesterol (HDL), and triglycerides (TG)), as well as insulin sensitivity (SI). The primary diet contrast is diet CG versus diet cg (**Primary Specific Aim**). Secondary diet contrasts focus on the effects of G versus g, separately by level carbohydrate (**Secondary Aim 1**: CG versus Cg, and cG versus cg) and the effects of C versus c, separately by level of glycemic index (**Secondary Aim 2**: CG versus cG, and Cg versus cg). The trial will assess the effects of these diet contrasts on other protocol-specified outcomes, namely, diastolic BP; apolipoproteins B, CIII, and A-I, and atherogenic VLDL and LDL particle types that contain apolipoprotein CIII; first and second phase insulin response and glucose effectiveness by OGTT, and fructosamine (**Secondary Aim 3**) and in pre-specified subgroups (**Secondary Aim 5**). In addition to these analyses which evaluate each outcome separately, we will estimate the impact of the four diets on overall CHD risk by using established risk prediction equations (**Secondary Aim 4**).

Analysis plan. This crossover trial permits assessment of within-person effects of all dietary contrasts of interest using all 160 enrollees for each overall statistical test. Any subgroup test can be performed using all the participants who possess the demographic conditions defining the subgroup.

As in OmniHeart, primary analyses will be conducted on a per protocol (PP) basis, excluding information on study dropouts whenever incompleteness interferes with a given comparison. A per protocol analysis is appropriate for this study, as it was in OmniHeart, when the primary inferences relate to biological mechanisms. Primary analyses will be supplemented by an intention-to-treat analysis. To perform ITT analysis with subjects who are lost-to-follow-up prior to outcome measure determination, multiple imputation (Rubin 1987) methods will be used to conduct valid analysis under the assumption that missing data arising from study dropout are missing at random. Full investigation of missingness patterns, with attention to association of dropout with current diet assignment, baseline conditions, and outcome changes, will be conducted. These secondary analyses 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 DASH and OmniHeart trials, such incompleteness was very rare.

For the **Primary Aim**, diets CG and cg will be compared using within-person differences in outcome levels obtained on those diets. The distribution of differences is expected to be well-approximated by the Gaussian model, so that the one-sample, paired t-test of $H_0: \text{mean diet difference equals } 0$ is expected to be an efficient approach to conducting inference on diet impacts. Decision making for the primary inferences will employ a significance level of 0.01; see the section below on multiple comparisons. For each outcome, estimates of the mean difference will be accompanied by 99% confidence intervals. A parallel approach will be used for **Secondary Outcomes 1, 2, 3 and 5** except that the significant level will be 0.05 and 95% confidence intervals will be reported.

Extended data analysis will be performed for all primary inferences, to assess sensitivities and stabilities of simple statistical procedures employed. For example, regression modeling that evaluates the conditional mean change in outcome response given a difference in end-of-period weights between two diets can be used to assess the impact of weight change, if any. Such a procedure provides an estimate of the conditional mean change in outcome given zero weight change.

The group of study outcomes can be viewed as a high-dimensional vector of repeated measures on individual participants, with diet assignment, measured body weights, demographic, behavioral, and other measured variables viewable as time-dependent covariates. This provides a very general framework for the extended data analysis mentioned above. Subvectors of the entire outcome vector may be appropriately analyzed using the (potentially partly nonlinear) mixed effects model:

$$Y_{ijk} = X_{ijk} \beta + f(W_{ijk}; \gamma, \delta) + e_{ijk}$$

where i indexes individuals, j indexes diet and k indexes repeated measures on outcome Y and potentially time-dependent covariate vectors X (of dimension $p \times 1$) and W (of dimension $(q + r) \times 1$). The model component $f(w; \gamma, \delta)$ is some parametric function of w with q -dimensional parameter vector γ (to be estimated as a fixed effect and r -dimensional parameter vector δ (to be estimated as a mean-zero Gaussian random effect. The error term e_{ijk} is, conditional on the values of the random effects parameters, Gaussian with mean zero. This generic model specializes to a number of models of interest. For example, if the diet assignments CG and cg are being contrasted, Y_{ijk} is taken to be the mean end-of-period difference in SBP, f is stipulated to be identically zero and X_{ijk} is an indicator function of diet CG, then the ratio of the estimate of γ to its standard error is equivalent to the one-sample paired t statistic based on within-subject end of period differences. To investigate dynamics of BP change with increasing duration of diet exposure, we put repeated BP measure k as Y_{ijk} , (as opposed to a change in BP), and it may be desirable to take f to be a quadratic, or parametrically asymptotic, or simply a smooth nonparametric function of time elapsed on diet. Between- and within-subject variability in repeated outcome measures can be assessed by incorporating random effects (δ) and evaluating components of variance due to random effects and residual error. We will address these extended modeling activities using the NLME module of R (Pinheiro and Bates, 2000).

It will also be of interest to understand the relationship between certain outcome variables, e.g. between changes in insulin sensitivity and BP in conjunction with changes in diet. For a conditional or predictive characterization (e.g., insulin sensitivity changes used to predict SBP changes) we can employ the aforementioned model, with data on insulin sensitivity appropriately introduced into the X and W matrices. A complication in this connection is that insulin sensitivity is measured with error, and accurate inference on the resulting model (indeed any model in which the components on the right-hand side of the regression equation include a stochastic component) depends on correction for measurement error. Dr. Rosner has an extensive record of contribution to methodology for inference in the presence of measurement error in nutritional epidemiology (Rosner 1990). Drs. Carey and Rosner (2001) have provided methods for unconditional inference on covariation of multiple longitudinal outcomes (e.g., joint behavior of SBP and DBP over time), and this approach will also be of interest in extended modeling of the data in this trial.

Multiple comparisons. We have adopted a nominal significance level of 0.01 for each of the outcomes to be evaluated in the primary analysis. This constitutes a Bonferroni-type penalty for the multiplicity of outcomes. This has been adopted as a conservative and easy-to-explain approach to multiple comparison adjustment. For the secondary analyses, which will be numerous, as catalogued in the secondary specific aims, multiple comparison adjustments are not adopted. Full descriptive statistics will be provided, with estimates and standard errors and unadjusted 95% confidence intervals for all estimated effects of interest. A crucial feature of our approach to study interpretation is the requirement of biological coherence of significant findings. Estimates that do not fit biologically with the bulk of our findings, even those that are large relative to estimated standard errors, will be reported with explicit warnings about coherence and the risk of chance errors. Unfortunately, because many of the proposed analyses are at the frontier of scientific knowledge, coherence constraints are no panacea. Unexpected significant findings will be reported as requiring independent confirmation. Findings that do not arise from prespecified comparisons noted in the study protocol will be explicitly identified as such.

Missing data. Incomplete responses are an important source of uncertainty in nutrition science. The primary solution is improved retention. We will use real-time data analysis to identify patterns of non-response or dropout and will use findings to direct retention efforts. We will strive to obtain full outcome measures on individuals who explicitly decline to continue diet participation. We will analyze weight and biomarker trajectories to assess possible relationships between dropout tendency and study outcomes. If there are strong indications that missingness occurs "at random" (i.e., that tendency to drop out appears to be independent of the responses that would have been observed had participation continued), then likelihood-

based inferences such as those derivable from the mixed effects model described above, are valid. Multiple imputation will also be performed as confirmation. If, however, there are indications that non-ignorable missingness processes are present (i.e., tendency to drop out is associated with study outcome values), model-based sensitivity analyses will be presented to foster clear interpretation of study results (Daniels and Hogan, 2000).

Carryover effects. Carryover effects are not expected, given the interval between outcome ascertainment in consecutive periods (at least 7 weeks) and the time course of dietary effects on BP, plasma lipids, and insulin sensitivity. Evaluation of carryover in the three-period OMNI Heart study employed several approaches to detection of differential carryover. First, outcome (SBP) sums were obtained across treatment sequence pairs. For example, the mean of sums of outcomes obtained for individuals given sequence AB consecutively was compared to the mean of sums obtained for individuals given sequence BA, where A (B) denotes one of PROT, CARB, UNSAT. The minimum p-value over all such pairwise contrasts was 0.54. A further global test was performed using sums obtained over the six sequences (PCU, PUC, UCP, UPC, CUP, CPU); the F test on five degrees of freedom had $p=0.80$. We conclude on both biological and empirical grounds that this design is not at risk for differential carryover effects, but similar methods of analytic assessment of carryover will be conducted to confirm this.

Risk prediction equations. The impact of the diets on overall cardiovascular risk (**Specific Aim 4**) will be assessed using prediction equations derived from prospective observational studies. Because of several methodological issues [i.e., equations without triglycerides, LDL cholesterol, and insulin sensitivity]; concerns about generalizability (e.g. most equations derived from populations of European Americans)], estimated CHD risk is a secondary outcome. 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) and PROCAM (Assmann, 2002), we will assess the overall effects of the diets on CHD 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. Optimally, risk prediction should include the impact of all 5 of our primary outcomes.

5. Organizational structure

The research team includes 2 field centers (one at Johns Hopkins and another at BWH), a data management and statistical unit at BWH, and a core laboratory at BWH and HSPH. Drs. Sacks and Appel co-chair the trial. Dr. Appel directs the field center at Hopkins. Dr. Sacks directs the field center at BWH. Dr. Carey and Rosner direct the data management and statistical unit. A Steering Committee is the primary decision-making body. There are three standing subcommittees (Diet, Design and Measurements, and Recruitment). Ad hoc working groups will be assembled as needed. An External Advisory Committee (DSMB) will be established.

6. Time-line

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 4 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, as was done in the OmniHeart trial. 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 21 weeks (1 week of run-in plus four 5 week periods) with at least 2 weeks separating each period. Recruitment for the initial cohort commences during the 4th quarter of the first study year, in anticipation of initial feeding in the 1st quarter of the second study year. Recruitment and feeding continues in study years 2 – 4. Laboratory studies are performed in batch, after participants complete a full cycle of feeding. 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 year 5, main results are prepared for publication and presentation.

7. Human Subjects Research - Protection of Human Subjects (See Section 4.b. for type of subject and inclusion and exclusion criteria; Section 4.c. for recruitment methods.)

7.a. Risks To The Subjects, and Protection from risks

Data collection procedures include interviews, self-administered questionnaires, collection of blood and urine specimens, and anthropometric measurements (weight and height). Data are collected by professional staff who are trained to perform these procedures according to detailed study protocols. The data collected in this project are for research purposes; however, copies of clinically relevant laboratory studies will be provided to participants at the end of the study. All records are kept in locked file cabinets, and participant data are identified only by number. Data are used only in aggregate, and no identifying characteristics of individuals will be published or presented. Each local clinical center maintains a file with the names and contact information of their participants by ID number. This information is not sent to the coordinating unit.

This study should not involve any major risk to screenees and trial participants. Substantial effort has been made to identify and minimize potential risks. For instance, those persons who require pharmacologic therapy for elevated BP, dyslipidemia or diabetes are excluded. Specifically, we exclude persons with systolic BP ≥ 160 mmHg and/or diastolic BP ≥ 100 mmHg). Such persons are informed of the elevated BP level, and advised to consult with their physician. Likewise, medication-treated diabetics, persons with prior or active CVD, and persons with renal disease are excluded.

The diet interventions pose minimal risk. Participants may experience some bloating and other minor gastrointestinal discomforts related to the high fruit, dairy and fiber content of the intervention diets. It has been our experience that these problems resolve soon after changes in diet. For those persons with lactose intolerance, we provide lactaid. 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.

Each of the four intervention diets, provided after randomization, is reduced in saturated fat and cholesterol, and should therefore reduce LDL cholesterol. Furthermore, because each diet will provide the micronutrient profile and fiber content of the DASH diet and will provide just 100 mmol/day of sodium, each intervention diet should reduce BP. Safety procedures include regular monitoring of BP. Two escape levels of BP are applied in this study:

- I. 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.
- II. 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.

Other potential risks of the study result from the blood drawings and intravenous line insertion. 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 are obtained by experienced personnel using small gauge needles. The 7-sample 2-hour OGTT procedure will be performed by trained, certified staff.

7.b. Potential Benefits

Individuals often enjoy participating in studies that relate diet to health. Laboratory testing and the questionnaires may lead to the early diagnosis of disease, if present. Participants will be provided with a copy of their BP and clinically relevant laboratory studies after the study is complete. At the end of the study, participants will receive a nutrition counseling session.

7.c. Importance of the Knowledge To Be Gained

The importance of the trial is the potential to determine the diet or diets that have optimal potential to reduce risk of CVD and diabetes. This is directly applicable for clinical counseling and treatment by physicians, nurses, and dietitians; and for public health efforts.

7.d. Data and Safety Monitoring Plan

This study is a 2 center clinical trial with minimal risk to participants. Still, an External Monitoring Committee (DSMB) (to include experts in biostatistics, CVD prevention, nutrition studies, and clinical trials) will be appointed by the Steering Committee to review the protocol prior to field work and to monitor trial progress and safety. Prevailing NIH, NHLBI and IRB policies will dictate the specific roles of this Committee. During fieldwork, the DSMB will convene every 6 months, either in person or by phone. Adverse events will be reviewed initially by the principal investigator and then reported to local IRBs, the NHLBI project office and the DSMB according to prevailing policies of these review bodies. This plan should ensure the safety of participants.

8. Data management, Quality assurance, Quality control

8.a. Data management

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 sent weekly to the data monitor. Corresponding forms from the DASH and OmniHeart trials serve as prototypes. The data monitor checks the forms for completeness and appropriateness. The data monitor sends reports of missing or inappropriate entries to the project coordinators every week, for clarification and resolution. The data monitor provides 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 cohort, 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 variability.

The data forms are double entered by a data entry service (Trade Quotes, Boston) which the investigators have used in many previous projects. 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 BWH GCRC Core laboratory and from Dr. Sacks's lipoprotein laboratory; if appropriate, replicate assays are performed.

A detailed Manual of Operations, annual training meetings for staff, and site visits each year minimizes the potential for error. The Measurements Subcommittee monitors the performance of each site and recommends corrective procedures in case deficiencies are noted.

Randomization assignments to one of 8 sequences of the 4 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 to ensure a balance of sequences at each site in each cohort. Randomly varying block sizes are used to prevent predictability of sequence assignment; note that all patients receive all diets and that only the sequence varies, so there is no plausible scenario in which study personnel would prefer a given allocation for a given patient.

Blinding: Due to the nature of the intervention, kitchen staff needs to have knowledge of participant diet assignment. The assignment is communicated to the kitchen personnel in confidence. All BP observers and lab personnel are blinded to diet sequence assignment, and participants are blinded to post-randomization BPs until the end of the trial. Until the end of the trial, all investigators, staff and participants are masked to all trial outcome data, with the exception of the trial statisticians, the data manager, and the External Monitoring Committee (DSMB). However, a mean of all readings, across diets, is provided to each participant at the end of his/her participation.

8.b. Quality assurance and Quality control

Quality Assurance (QA) pertains to activities that promote collection of high quality data, while Quality Control (QC) pertains to activities that detect emerging issues. The trial places considerable effort on QA and QC activities.

Our approach to QA is as follows:

- Have a well-documented protocol and a manual of operations,
- Have master trainers at each site,
- Train and certify all primary data collectors, with special emphasis on procedures related to trial outcomes,
- Recertify data collectors on an annual basis (semi-annual for blood pressure)
- Establish proficiency requirements before initial certification of technicians,

- Establish requirements for a minimum number of procedures to maintain certification,
- Routinely observe technicians,
- Routinely calibrate equipment, and
- Maintain logs of certified staff and calibrated equipment.

To identify problems with sufficient time to institute appropriate corrective actions and to quantify the quality of data collected during the trial, we perform the following QC activities:

- monitor counts of completed visits and key data collection items,
- monitor distributions of trial outcomes, overall, by center and by technician
- assess reproducibility of laboratory studies
- record lag time in data entry,
- review types and distribution of data entry errors.
- review diet adherence measures (e.g. weight, number of feeding days by diet and period)
- obtain nutrient analyses of composited menus periodically throughout the trial.

9. Dissemination/Public Access Data Set

This study has the potential of providing results that are immediately applicable to public health. Dissemination projects of results and menus used will occur in the latter part of the final year (Year 5) and, if feasible, during a no-cost extension period of a sixth year. We will propose workshops and symposia at major clinical meetings for physicians, dietitians, and health plan managers. We will work with major websites for healthcare professionals to feature the results. We will work with public relations, communications, and education departments at our institutions, NIH, and major health organizations (e.g. American Dietetics Association, American Diabetes Association, American Heart Association, American College of Cardiology) to develop programs that reach the public and health care professionals. We will amplify the model used by us for the DASH and DASH-Sodium studies, now widely known and considered the benchmark diet for cardiovascular health. Specific funding will facilitate the dissemination program. In addition, the trial will prepare a public access data release that complies with prevailing NIH and HIPPA guidelines.

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