The Effects of a Hypocaloric Diet Enriched in Oleic Acid Using Almonds Versus Complex Carbohydrates on Metabolic and Anthropometric Parameters During Weight Reduction

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THE EFFECTS OF A HYPOCALORIC DIET ENRICHED IN OLEIC ACID USING ALMONDS VERSUS COMPLEX CARBOHYDRATES ON METABOLIC AND ANTHROPOMETRIC PARAMETERS DURING WEIGHT REDUCTION

by

Michelle Ann Wien

A Dissertation in Partial Fulfillment of the Requirements for the Degree of Doctor of Public Health in Nutrition

June 2002
Each person whose signature appears below certifies that this dissertation, in
his/her opinion, is adequate in scope and quality as a dissertation for the degree Doctor
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ABSTRACT OF THE DISSERTATION

The Effects of a Hypocaloric Diet Enriched in Oleic Acid Using Almonds Versus Complex Carbohydrates on Metabolic and Anthropometric Parameters During Weight Reduction

by

Michelle Ann Wien

Doctor of Public Health in Nutrition

Loma Linda University, Loma Linda, California, 2002

Joan Sabaté, Chairman

The purposes of this study were to determine the effect of almonds rich in oleic acid as an alternative to complex carbohydrates on metabolic and anthropometric parameters during weight reduction and to evaluate self-reported satiety and satisfaction. A convenience sample of 57 adults was recruited from the Diabetes and Cardiovascular Risk Reduction Program at City of Hope National Medical Center. Subjects were randomized to an almond-enriched hypocaloric diet or to a complex carbohydrate-enriched hypocaloric diet for twenty-four weeks. Mixed model analysis showed that the use of almonds more favorably influenced weight loss (p < 0.0001), body mass index (p = 0.0001), waist circumference (p = 0.0255) and fat mass (p = 0.0205) compared to complex carbohydrates. Additionally, blood ketone levels were
statistically significantly higher in the almond group (p = 0.0230) than in the complex carbohydrate group. No significant difference was found in the total cholesterol: HDL ratio between the groups, however both groups had statistically improved ratios over time with resulting decreases in cardiovascular risk. The glucose (p = 0.0006), insulin (p< 0.0001), insulin:glucose ratio (p = 0.0008), HgbA1c (p<0.0001), systolic blood pressure (p = 0.0506) and diastolic blood pressure (p = 0.0371) improved in both groups over time. Results on self-reported levels of satiety and satisfaction were unequivocal, thus reflecting the ability of both almonds and complex carbohydrates to maintain satiety and satisfaction in the context of medically supervised weight reduction. Individuals participating in medically supervised weight reduction programs could be offered almonds and perhaps other high-oleic nuts within their prescribed weight loss diet. The potential for using almonds as a source of oleic acid to improve the metabolic and anthropometric parameters of the obese adult undergoing weight reduction is discussed.
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LIST OF ACRONYMS

apoA-1  apolipoprotein A-I
apoB  apolipoprotein B
apoE  apolipoprotein E
AHA  American Heart Association
BIA  bioelectrical impedance analysis
BMC  bone mineral content
BMD  bone mineral density
CAD  coronary artery disease
CHD  coronary heart disease
CHF  congestive heart failure
CHO  carbohydrate
COH  City of Hope
COPD  chronic obstructive pulmonary disease
CVA  cerebrovascular accident
D & CVRRP  Diabetes and Cardiovascular Risk Reduction Program
DIT  diet-induced thermogenesis
DJD  degenerative joint disease
EDI  Eating Disorders Inventory
FSL  food selection list
GLUT-4  glucose transporter-4
HCLF  high-carbohydrate low fat
HDL  high-density lipoprotein
HDL-C  high-density lipoprotein cholesterol
HDL-TG  high-density lipoprotein triglyceride
HTN  hypertension
HMR  Health Management Resources
IBW  ideal body weight
IGR  insulin to glucose ratio
LBM  lean body mass
LCAT  lecithin:cholesterol acyltransferase
LCHF  low-carbohydrate high-fat
LDL  low-density lipoprotein
LDL-C  low-density lipoprotein cholesterol
LDL-TG  low-density lipoprotein triglyceride
LP(A)  lipoprotein A
Mg  magnesium
mRNA  messenger RNA
MUFA  monounsaturated fatty acid
NO  nitric oxide
NPY  neuropeptide Y
P/S  polyunsaturated to saturated fat ratio
PRO  protein
PSMF  protein sparing modified fast
PUFA  polyunsaturated fatty acid
SFA  saturated fatty acid
TC  total cholesterol
TNF-α  tumor necrosis factor-alpha
VAS  visual analog scale
VLDL-C  very-low-density lipoprotein cholesterol
VLDL-TG  very-low-density lipoprotein triglyceride
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CHAPTER 1

INTRODUCTION

A. Statement of the Problem

Obesity is a chronic disease that is prevalent among adults in the United States (US). The new obesity guidelines established by the National Institutes of Health (NIH) led to the astounding report that 54.9% of the US adult population is either overweight or obese (NIH, 1998). It is well recognized that obesity contributes significantly to both increased individual morbidity and mortality, as well as to increased national health care costs. The NIH estimates that obesity-related costs are approaching $100 billion annually in the US (NIH, 1998).

Obesity is a strong independent risk factor for coronary heart disease. A graded relationship appears to exist between obesity and elevated levels of atherogenic total cholesterol and low density lipoprotein (LDL) cholesterol (Stone et al., 1996). Mild to moderate overweight status is associated with a substantial increased risk of type 2 diabetes mellitus, hypertension (HTN) and coronary heart disease (CHD). Obesity-related modifiable risk factors of cardiovascular disease include metabolic syndrome-related abnormalities such as hyperinsulinemia, insulin resistance, HTN, dyslipidemia and diminished physical activity.

Obese patients are typically advised to reduce their weight by to improve their cardiovascular risk profile. Most weight loss methods are hopelessly ineffective and costly. Surgical interventions for weight loss are associated with significant mortality, hence are only advocated for the morbidly obese. Appetite suppressant medications have a long history of risk, addiction and potential harm. However, medically
supervised weight reduction utilizing hypocaloric diets can be effective in facilitating weight loss concurrent to ameliorating other cardiovascular risk factors, i.e., dyslipidemia and HTN, in obese adults.

Some studies have shown that weight reduction produces beneficial changes to lipoprotein levels (Carmena, Ascaro, Tebar & Soriano, 1984; Wood et al., 1988; Sedgewick, Thomas, Davies & Baghurst, 1990; Cordero-MacIntyre et al., 2000), however others have found a deterioration in lipoprotein levels (Weinsier et al, 1992; Phinney, Tan, Waggoner, Tezanos-Pinto & Davis, 1991). Inconsistent findings across studies may be due to changes in variables that have an impact on lipoprotein concentrations, i.e., energy restriction (Weinsier et al., 1992), changes in macronutrient composition (Mattson & Grundy, 1985; Mensink & Katan, 1989; Mensink & Katan 1992), and exercise (Wood et al., 1988; Katan, 1990).

An effective weight reduction program must integrate comprehensive medical, nutritional, exercise and behavior modification components to meet the challenges of weight reduction and weight maintenance. The Diabetes and Cardiovascular Risk Program (D & CVRRP) at the City of Hope (COH) National Medical Center in Duarte, California, provides a comprehensive approach aimed at reducing obesity-associated health risks through safe and consistent weight reduction among obese adults. However, the conventional hypocaloric dietary intervention advocated to induce weight loss for patients enrolled in the D & CVRRP can result in other effects that may be deleterious to their health, i.e., a lowering of HDL-C due to the high-carbohydrate low-fat (HCLF) macronutrient composition.
Dietary fatty acid composition is known to influence plasma concentrations of total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C) and triglycerides (Grundy & Denke, 1990) under isocaloric conditions. Some investigators have found that no changes or even a slight increase in HDL-C occurs when MUFA-enriched isocaloric diets are tested (Mattson & Grundy, 1985; Mensink & Katan, 1989; Berry et al., 1991; Baggio et al., 1988). Additionally, long-term compliance with a low fat diet is difficult to maintain due to lower levels of satiety and palatability. Thus, further research is necessary to determine the optimal macronutrient and micronutrient composition for the obese adult undergoing medically supervised weight reduction.

B. Purpose of the Study

The overall purpose of this study was to evaluate the effects of a complex carbohydrate (CHO)-enriched or almond-enriched hypocaloric diet on the plasma lipoprotein levels of obese adults during medically supervised weight reduction. The primary study objective was to assess the effect of a HCLF hypocaloric diet enriched in complex carbohydrate (CHO) versus a LCHF hypocaloric diet enriched with monounsaturated fatty acids (MUFA) using almonds in obese patients enrolled in the 24-week D & CVRRP on the following: total cholesterol (TC); high density lipoprotein (HDL); triglycerides (TG); high density lipoprotein subfractions (HDL-C and HDL-TG); low density lipoprotein subfractions (LDL-C and LDL-TG); very low density lipoprotein subfractions (VLDL-C and VLDL-TG); and, LDL-C/HDL-C ratio. If there is a significant difference in plasma lipoprotein levels found between the complex-CHO enriched and the almond-enriched (MUFA) hypocaloric diets, additional studies will be
warranted using a high-oleic oil versus almonds to separate the effects of almonds from those of a high fat or high MUFA diet. In the future, it would also be appropriate to evaluate the plasma levels of lipoprotein A [Lp(a)], apolipoprotein A-I (apoA-I), apolipoprotein B (apoB) and apolipoprotein E (apoE), however analysis of these parameters is beyond the scope of this dissertation. Kiortsis et al. (2001) found a 7.5% loss in body weight produced a 17.6% reduction in Lp(a) after 6 weeks of dietary intervention in obese subjects with high pre-treatment Lp(a). The effect of the diets on change in total body weight loss, body mass index (BMI), body composition, girth measurements, glucose, insulin, HgbA1c, blood pressure and blood ketone levels in study participants will also be evaluated in this dissertation. A secondary objective was to evaluate self-reported levels of satiety and satisfaction among the study participants during the 24-week study.

1. Importance to Nutrition

This study is important to the field of nutrition in light of the aforementioned prevalence of obesity in the US population. The results of this study could provide an alternative and novel approach within the field of medically supervised weight reduction and weight management of obese adults.

C. Research Questions

Question #1: What are the effects of a HCLF hypocaloric diet enriched in complex CHO versus a LCHF almond-enriched (MUFA) hypocaloric diet on the plasma lipoprotein levels in obese adults enrolled in the medically supervised D & CVRRP?
Question #2: Does the use of a LCHF almond-enriched (MUFA) hypocaloric diet confer a beneficial effect on self-reported levels of satiety and satisfaction in obese adults as compared to a HCLF hypocaloric diet enriched in complex CHO?

Question #3: Can the inclusion of almonds within a hypocaloric dietary regimen be successfully used during weight loss and be sustained for a 24-week medically supervised weight reduction program?

Question #4: Does the use of a LCHF almond-enriched (MUFA) hypocaloric diet confer a beneficial effect on blood pressure in obese adults as compared to HCLF hypocaloric diets enriched in complex CHO?

Question #5: What is the relationship between total body weight loss, BMI, body composition, and girth measurements in obese adults receiving a HCLF hypocaloric diet enriched in complex CHO versus a LCHF almond-enriched (MUFA) hypocaloric diet?

Questions #6: Does the use of a LCHF almond-enriched (MUFA) hypocaloric diet confer a beneficial effect on plasma glucose, insulin, HgbA1c and blood ketone levels as compared to HCLF hypocaloric diets enriched in complex CHO?
CHAPTER 2
REVIEW OF LITERATURE

A. Obesity and the Risk of Coronary Heart Disease

Epidemiological data suggest that obesity is associated with an increased risk of coronary heart disease and that weight reduction may be beneficial to the obese population to reduce mortality (Ashley & Kannel, 1974). A 10% reduction in relative weight for men in the Framingham Study (Castelli et al., 1986) resulted in a decrease in serum cholesterol of 0.292 mmol/L, which translates into a predicted 20% decrease in the incidence of coronary artery disease.

Obese individuals tend to have an unfavorable lipoprotein pattern, i.e., elevated TC, LDL-C, VLDL-C, TG, apoB, and Lp(a) whereas their levels of HDL-C, apoA-I and apoE tend to be reduced (Leenan et al., 1993). The dangerous combination of an elevated TC and reduced HDL-C produces an increased risk for the development of coronary heart disease (Gordon and Rifkind, 1989). Hence, obese adults, especially when they are hypercholesterolemic, are typically advised to reduce their weight by a magnitude of at least 10 to 15% in order to improve their lipid profile.

Medically supervised weight reduction programs can be quite effective in ameliorating cardiovascular risk in the obese adult. However, the standard dietary interventions advocated for obese adults to induce weight loss can result in other effects that may be deleterious to their health. The weight loss regimen commonly recommended by the nutrition community is a HCLF hypocaloric diet, which may produce a deleterious decrease in HDL-C levels (Grundy, 1986). Thus the information compiled in this literature review addressing the substitution of dietary complex CHO
with a source of MUFA under hypocaloric conditions could provide additional options for the weight reduction and weight management of obese adults.

B. Development of Atherosclerosis

The relationship between serum cholesterol and the development of atherosclerotic lesions has been established through the joint effort of animal research, epidemiological studies and clinical trials. There is a wealth of circumstantial evidence from animal studies suggesting that HDL-C is causally involved in atherosclerosis and that reducing plasma HDL-C concentrations increases the risk of cardiovascular disease (Katan, 1997). However, the landmark epidemiological Framingham Study revealed the relationship between low HDL-C levels and an increased prevalence of coronary heart disease (CHD), and, high HDL-C levels conferring protection from CHD.

Epidemiologic findings among the Mediterranean countries deserve comment. The Seven Countries Study (Keys, 1970) showed that CHD incidence in middle-aged men from the Mediterranean area of Southern Europe was lower than expected from their cholesterol concentration, perhaps due to differences in nutrient intake. The traditional Mediterranean diet features a low saturated fatty acid (SFA) content in the background of a high total fat intake (40% to 45% of total energy) due to a high consumption of olive oil, which is rich in MUFA, mainly oleic acid (C 18:1, n-9). The low incidence of CHD observed in these populations may be related to the beneficial effects of diets rich in MUFA on lipoprotein levels.

Keys, Anderson and Grande (1957) initiated pioneer work in the field of dietary effects on serum lipids. In their classic experiment, men were studied in a mental institution consuming isocaloric diets differing in fat content over a range of 9 to 44%
of calories from fat for two to nine weeks duration (usually four weeks). Sixteen isocaloric diets were studied and yielded 41 sets of comparisons for analysis, which produced a prediction equation for the change in serum cholesterol resulting from an adjustment of dietary fatty acid intake. The prediction equation provided the basis for planning safe and effective nutritional interventions to improve the health of the US population.

C. Serum Lipoproteins

The ideal blood lipid profile for reducing the risk of CHD is one that reflects a low TC, low LDL-C and high HDL-C. According to the Third Report of the National Cholesterol Education Program Expert Panel (NIH, 2001), the desirable level of TC is less than 200 mg/dl, LDL-C less than 160 mg/dl and HDL-C greater than 40 mg/dl.

1. High Density Lipoprotein Cholesterol

Becker et al. (1983) compared the effects on plasma lipids of three isocaloric cholesterol-free formula diets containing 40% total fat, in which the predominant fatty acids were SFAs, MUFAs or polyunsaturated fatty acids (PUFAs). These investigators found similar mean HDL-C levels on all three dietary interventions after three, four-week periods using a randomized crossover design.

Shepherd et al. (1978) investigated the effects of dietary SFAs and PUFAs on the chemical composition and thermotropic properties of HDL-C in four healthy males during two five-week periods on a metabolic unit. Participants were fed a 40% total fat isocaloric diet containing a P/S ratio of 0.25 and 4.0 with a five-week washout period between study periods. These researchers found that 60% of the PUFA-induced cholesterol lowering resulted from a reduction in the atherogenic LDL-C
fraction, augmented by an 8% decrease in VLDL and 32% decrease in the anti-atherogenic HDL-C fraction. Analysis of the plasma HDL-C composition revealed the percentage of cholesterol was unchanged by dietary manipulation, however PUFA ingestion caused a significant increase in HDL phospholipid, reciprocated by a fall in apoA-I content. Spritz and Mishkel (1969) found similar changes in LDL-C, however not with HDL-C, as a result of PUFA-enriched isocaloric diets, and hypothesized that because unsaturated fatty acids occupy a greater area than SFAs, they increase the space occupied by the lipids into which they are incorporated. Catabolism of apoA-I was not affected by increasing the PUFA level in the diet, however a decreased rate of synthesis of apoA-I was observed. Thus, the weight of the evidence supports the theory that the major mechanistic action of PUFAs is to alter either the production or clearance of the plasma lipoproteins, and not to change the relative proportions of their lipid and apoprotein components.

2. Low Density Lipoprotein Cholesterol

The literature contains conflicting reports concerning the effect of dietary manipulation on LDL-C composition, possibly due to the variability in the length of the intervention periods across studies. For example, Kuusi et al. (1985) noted the LDL-C compositional changes after six weeks of a PUFA-enriched isocaloric dietary intervention involved a decreased cholesterol and phospholipid content, however no change in apoB content. After 12 weeks, a decrease in the apoB content had occurred, and the composition of the LDL-C was that of baseline. Vega et al. (1982) found a decrease of LDL apoB, yet no overall changes in the cholesterol to protein ratio, after feeding males and females an isocaloric high fat (40%) PUFA-
enriched liquid formula diet for four weeks after being fed an isocaloric SFA-enriched liquid formula diet in a metabolic ward setting. Bilheimer (1979) has speculated that transient changes in the LDL-C/apoB ratio occur with changes in dietary fatty acid intake, and may be due to delayed clearance of LDL-C, thus allowing time for greater accumulation of cholesterol esters through the prolonged action of lecithin:cholesterol acyltransferase (LCAT).

D. Influence of Nutrients on Serum Lipoproteins

A well-recognized non-pharmacological treatment approach to prevent or treat non-familial CHD traditionally employs the manipulation of nutrients to fuel favorable apolipoprotein synthesis. The quality and quantities of the macronutrients consumed will influence serum lipoprotein outcomes, thus influence CHD risk within specific populations.

1. Saturated Fatty Acids

The major effect of the inclusion of dietary SFAs on serum lipids is assumed to involve suppression in the activity of LDL-C receptors under isocaloric conditions. As a consequence of a dietary intake high in SFAs, Shepherd et al. (1980) observed a decrease in the fractional catabolic rate for LDL-C and an enhancement in the conversion of VLDL remnants to LDL-C. This group also noted that the hypocholesterolemic properties of PUFAs, when substituted for SFAs under isocaloric conditions appear to be the result of increased activity of LDL-C receptors. Available evidence suggests that the effects of PUFAs may be passive, i.e., the suppression of activity by SFAs is removed and the LDL-C receptors return to their normal activity (Grundy & Denke, 1990). On the basis of current knowledge, a similar passive effect
probably also exists for MUFAs as a substitute for SFAs in the background of an isocaloric diet.

2. Carbohydrates Versus Monounsaturated Fatty Acids

HCLF dietary profiles have been historically recommended for the prevention of CHD. Based on the equations of Keys, Anderson and Grande (1957) and Hegsted (1965), it was accepted for over two decades that dietary MUFAs (i.e., oleic acid) have no effect on serum cholesterol under isocaloric conditions. However, since 1985 there have been investigators who have challenged this conclusion, concurrent with the advent of improved study designs. To investigate the putative hypocholesterolemic effect of dietary MUFAs, the SFAs and PUFAs have to be held constant. In many prior investigations, MUFAs were substituted for either SFAs or PUFAs under isocaloric conditions. However, because both SFAs and PUFAs affect plasma lipid concentrations in opposing directions, the role of MUFAs could not be defined precisely.

Grundy (1986) and Mensink and Katan (1987) observed that in contrast to MUFAs, dietary CHO reduces HDL-C levels when exchanged for dietary SFAs in the traditional isocaloric Western diet. Mensink et al. (1989) explored the effects of a LCHF MUFA-enriched diet using oleic acid-rich olive oil versus a HCLF diet enriched in complex CHO diet on apolipoproteins and subfractions of HDL-C under isocaloric conditions within an outpatient adult population for five weeks using a parallel study design. Solid food meals were prepared in a metabolic kitchen and supplied to the study participants. Both dietary interventions reduced LDL-C to the same extent and in agreement with the results of Grundy (1986). Prior to this study, Grundy had received
criticism for using liquid formula diets to compare a high-SFA diet versus a high-MUFA (high oleic safflower oil) diet versus a high glucose diet for three four-week periods under metabolic ward conditions. Thus, the research community adopted the belief that the observed lipid lowering effects of MUFA s on LDL-C are probably due to dietary oleic acid rather than to some unknown factor in olive or safflower oil.

Numerous subsequent studies have supported the findings of Grundy (1986) and Mensink et al. (1987), i.e., an isocaloric HCLF diet induces a decrease in TC, LDL-C and HDL-C, with a concomitant increase in VLDL-TG, in contrast to an isocaloric LCHF MUFA-enriched diet which reduces TC, LDL-C, and VLDL-TG with little or no effect on HDL-C (Baggio et al., 1988; Garg, Bonanome, Grundy, Zhang, & Unger, 1988; Ginsberg et al., 1990; Berry et al., 1992). The magnitude of the response to dietary MUFA s in these aforementioned studies is variable, which Berry et al. (1992) suggests may be modulated by the macronutrient composition of the preceding isocaloric diet consumed by the study participants. However, the experimental design and duration of the studies also differ. Additionally, investigators employ different kinds of participants (e.g., students, prison inmates, patients in mental institutions, hyperlipidemic patients, and patients with diabetes mellitus) of variable ages and subject them to different kinds of diets, i.e., liquid formula versus solid meals. Studies reported in the literature might be performed in metabolic units or in free-living settings with meals provided to participants.

The metabolic unit studies involving HCLF versus LCHF (MUFA-enriched) dietary interventions have featured crossover designs under isocaloric conditions, with or without randomization, small sample sizes (approximately n = 10),
and predominantly males. Length of time exposed to the metabolic ward dietary interventions has ranged from three to six weeks, whereas studies in free-living settings have been typically ten weeks in duration per dietary intervention period. These studies have utilized a level of fat in the high-MUFA dietary interventions ranging from 33 to 50% of total calories, with a fatty acid profile ranging between 4-10% SFA, 17-33% MUFA, and 5-11% PUFA, and a level of fat on the high-CHO diets ranging from 18-31%. Additionally, Yu, Derr, Etherton and Kris-Etherton (1995) noted the neutral response to MUFAs previously reported by investigators could be due to the variable amount of cholesterol in the experimental diets.

Hopkins and Barter (1986) suggest an explanation for the dietary CHO induced fall in HDL-C under isocaloric conditions. These investigators noted a rise in hepatic secretion of VLDL-TG, as observed on isocaloric HCLF diets, and fractional catabolism of plasma apoA-I.

The mechanism by which isocaloric LCHF (MUFA-enriched) diets reduce LDL-C is not clear. Berry et al. (1992) noted the LDL-receptor activity of circulating monocytes at the end of a LCHF (MUFA-enriched) diet using olive oil and 100 g almonds/d was approximately 20% higher than at the end of a HCLF dietary intervention under isocaloric conditions. This difference in LDL-receptor activity could have caused accelerated LDL-C clearance from the circulation and thus a lower plasma LDL-C concentration.

Mensink and Katan (1992) evaluated 27 controlled trials published between 1970 and 1991 using meta-analysis and found that the replacement of dietary CHO with cis-MUFA or PUFA fat sources lowered serum TG under isocaloric
conditions, independent of the nature of the fat. These investigators also found that the replacement of dietary SFAs by unsaturated fatty acids typically raises the HDL-C to LDL-C ratio, whereas replacement by dietary CHO has no effect on increasing HDL-C. However, caution must be exerted with extrapolation of this data to free-living populations because if high fat diets promote obesity, then their favorable effects on serum lipids will be lost.

3. **Polyunsaturated Fatty Acids Versus Monounsaturated Fatty Acids**

Mattson and Grundy (1985) found smaller decreases in HDL-C using an isocaloric 40% total fat formula diet with 28% MUFAs, as compared to PUFAs, using participants within a metabolic unit setting with four-week dietary interventions. Mensink and Katan (1989) subsequently compared the effects of an isocaloric diet enriched with MUFAs or PUFAs on levels of LDL-C and HDL-C in healthy adults for a five-week duration using a randomized parallel study design. The PUFA-enriched dietary intervention did not reduce the levels of HDL-C or apoA-I, in contrast to the findings of Mattson and Grundy (1985). These investigators noted that previous studies that demonstrated a decline in the HDL-C level featured very high P/S ratios (2.0 to 6.5) and/or other changes in the dietary intervention, i.e., an increase in CHO, a decrease in cholesterol, or both. Hence it is possibly immaterial whether dietary SFAs are replaced by a mix of MUFA and PUFA, or by PUFA alone, in the isocaloric Western diet; both will have the same effect on the HDL-C as long as extremely large amounts of PUFAs (13% of energy) are avoided.

Wahrburg, Martin, Sandkamp, Schulte, & Assmann (1992) used a crossover study design to investigate the effects of the American Heart Association
(AHA) Step 1 diet versus a MUFA-enriched diet (with equivalent total fat under isocaloric conditions) after a change from the high SFA intake characteristic of the Western diet. Serum TC and LDL-C values were reduced with equal efficacy, however significant reductions in HDL-C were found on both the Step 1 and MUFA-enriched dietary interventions. Mensink and Katan (1987) observed only slight decreases in HDL-C concentrations on their high fat (40%) isocaloric diets with moderate amounts of PUFA (12% of energy) or MUFA (15% of energy).

Using a randomized blind crossover study design, Wardlaw and Snook (1990) compared serum lipid and apolipoprotein concentrations in 20 men consuming 40% of energy as fat from isocaloric diets based on corn oil, high-oleic sunflower oil, and butter. Each phase of the crossover design included two weeks of a butter-based dietary intervention followed by five weeks of the designated vegetable oil intervention with a seven-week washout period between phases. The vegetable oil intervention reduced serum TC by 16-21%, LDL-C by 16-21%, TG by 10-21%, and apoB by 22-29% without a concurrent change in serum HDL-C or apoA-I levels. Thus even an isocaloric diet containing 40% energy from fat can allow for a substantial reduction in blood lipids if the intake of SFAs is minimized. In a subsequent study, the same group examined the effects of PUFA or MUFA-enriched (canola oil) isocaloric diets, each with 40% total fat, compared with a SFA-enriched isocaloric diet under closely supervised feeding conditions using a randomized parallel design among male volunteers (Wardlaw, Snook, Lin, Puangco, & Kwon, 1991). Replacing much of the dietary SFA with either MUFA or PUFA reduced serum LDL-C levels by ≥ 10-20%,
with a trend towards larger and more consistent changes in LDL-C on the PUFA intervention. However, HDL-C remained almost neutral on both interventions.

Berry et al. (1991) evaluated the effects of a MUFA-enriched diet using almonds versus a PUFA-enriched diet using walnuts under isocaloric conditions in a randomized crossover design featuring 12-week study periods in free-living male students. Adherence to the dietary interventions was monitored by changes in erythrocyte membrane composition, thus strengthening the validity of the results. High PUFA versus high MUFA intake resulted in a somewhat lower reduction in TC (16% versus 10%), however HDL-C remained neutral on both dietary interventions. Further investigations are warranted using free-living females and for determination of whether serum lipid concentrations would be maintained or drift back to the initial values over a longer time period.

Sirtori et al. (1986) utilized a randomized crossover study design to evaluate the activity of isocaloric low-fat (30% of total calories) diets using olive oil or corn oil on serum lipids and platelets in 23 subjects with high atherosclerotic risk factors over an eight week time period. Plasma TC was reduced with corn oil, however HDL-C levels fell lower with corn oil and were unchanged, or raised, by olive oil. Plasma apoB levels were reduced equally by both dietary interventions, and both the apoA-I and the apoA-I/apoB ratio rose only with the olive oil. Platelet studies performed provided evidence that the two interventions exerted similar favorable effects.

Yu, Derr, Etherton, and Kris-Etherton (1995) performed a meta-analysis of controlled feeding studies published between 1970 and 1993 in which the effects of
dietary fatty acid manipulations on plasma lipids and lipoproteins were reported. Their study suggests that MUFA-enriched isocaloric diets significantly decrease serum TC and LDL-C, and increase HDL-C concentrations in both men and women. These investigators speculate that the magnitude of the effect of MUFAs is dependent on the amount of SFAs (and specifically the amount of hypercholesterolemic SFA, i.e. 12:0-16:0 SFAs) in the dietary intervention.

An alternative substitution for dietary PUFA and MUFA containing oils is the inclusion of avocado. Colquhoun, Moores, Somerset and Humphries (1992) conducted a randomized crossover trial to compare a high MUFA diet enriched with avocado with an AHA Step 3 diet (low fat high-complex-CHO) in females (n=15) with three three-week dietary intervention periods under isocaloric conditions. The avocado intervention was more effective in lowering TC, LDL-C and apoB, and had no deleterious effects on HDL-C or apoA-I levels. The investigators noted that avocado contains no squalene, which is found in appreciable amounts in olive oil. These investigators also reported an easy adjustment by participants to an avocado-enriched dietary intake, i.e., avocado was reportedly easier to tolerate by study participants than the high-complex CHO dietary intervention.

Lichtenstein et al. (1993) compared a typical Western dietary intake with the use of canola, corn and olive oils incorporated into a National Cholesterol Education Program Step 2 dietary intervention in female and male participants for four 32-day study phases under isocaloric conditions. None of the oils providing 20% of the total calories offered a significant advantage against each other, however a 15% reduction in LDL-C resulted in comparison to the Western diet.
Epidemiological evidence supports the known safety of isocaloric MUFA-enriched diets, which are advantageous with respect to preserving apoA-I levels. Since there are no known adverse effects under isocaloric conditions, an increase in the intake of MUFAs as a replacement for dietary SFAs may be generally recommended to the public. Additionally, the use of MUFA-rich foods increases the variety in meal planning and may improve long-term compliance in TC and LDL-C lowering dietary interventions.

4. Energy Restriction and Serum Lipids

Several investigators have studied the effects on serum lipids of weight loss by energy restriction. The sequential effects of an isocaloric AHA Step 1 diet for a two month period and subsequent hypocaloric AHA Step 1 diet for six months was evaluated in 48 normocholesterolemic and hypercholesterolemic obese postmenopausal women who were free of comorbid diseases (Nicklas, Katzel, Bunyard, Dennis, & Goldberg, 1997). The use of a sequential study design permitted the independent analysis of the relative contribution of dietary change and weight reduction to changes in serum lipids. The hypocaloric AHA Step 1 diet reduced HDL-C levels in all women, with the largest decreases occurring in women with the highest baseline HDL-C concentrations. Weight loss partially corrected this decline, however a net decrease in HDL-C levels was observed in these women. These findings are analogous to the findings of Leenen et al. (1993) who observed the effects of a low-fat isocaloric diet and weight loss to be additive on reducing TC, whereas the HDL-C lowering effect of the isocaloric low-fat diet was counteracted by the weight loss which induced an increase in the HDL-C among the middle-aged obese men and women.
The clinical importance of the aforementioned study (Nicklas et al., 1997) is found in noting the differential lipid responses to the isocaloric AHA Step 1 diet and weight loss which result on the basis of the participant’s baseline lipid concentrations. Women with hypercholesterolemia had significant decreases in TC, LDL-C and TG, however no significant lipid changes occurred among normocholesterolemic or mildly hypercholesterolemic women. A major study limitation was that the women were still significantly overweight at the completion of the six-month weight loss phase. Also, the effects on lipoproteins of weight loss alone cannot be assessed, i.e., weight loss without a low-fat isocaloric diet may increase HDL-C above baseline levels while also lowering TC, LDL-C and TG. Lastly, the effects of the intervention on other coronary artery disease risk factors, i.e., glucose tolerance, fibrinolysis and blood pressure, were not examined.

Weinsier et al. (1992) observed that TC, LDL-C, and TG were reduced independently by both energy restriction and weight loss utilizing a four phase study design to separate the effects of energy restriction and weight loss on lipid and insulin concentrations of 24 obese postmenopausal women. The hypocaloric diet provided contained 55% CHO, 22% fat, and 23% protein with a P/S ratio of 0.7. The energy-restriction effect was determined by comparing average values in stable-weight phases (I and IV) with low-energy phases (II and III) and demonstrated lower levels of TG, TC, LDL-C and LDL-C/HDL-C ratio, and an increased HDL-C. The weight loss effect was determined by comparing values in obese phases I and II with reduced weight phases III and IV, with resulting reductions in TG, TC and LDL-C without a change in the HDL-C or LDL-C/HDL-C ratio. These findings suggest that reducing to a weight-
steady nonobese state significantly lowers TG, TC, and LDL-C but does not improve HDL-C or the LDL-C/HDL-C ratio using a hypocaloric HCLF dietary regimen.

These studies reflect the nutritional dilemma which exists in the treatment of obesity, i.e., a decline in HDL-C concentration in response to a HCLF diet is potentially harmful for older women who are at a heightened risk for coronary artery disease (CAD). This adverse effect may be nullified by the concomitant reduction in the concentration of the atherogenic LDL-C. However, only women with elevated baseline LDL-C levels are likely to show significant decreases in their LDL-C and their LDL-C/HDL-C ratio. Thus, an isocaloric HCLF without substantial weight loss may not be beneficial for improving lipoprotein lipid risk factors in obese, postmenopausal women with normal lipid profiles.

Zambon et al. (1999) studied the effects of a CHO-enriched versus an olive oil-enriched (MUFA) hypocaloric diet on plasma lipoprotein levels in 20 mildly obese premenopausal women. Both diets were effective in reducing body weight, however at steady weight conditions, only the high MUFA diet improved LDL-C and HDL-C subclasses. HDL-C increased significantly in the high-MUFA group, whereas a decreased level was observed in the high CHO group. Future studies should evaluate changes in lipids and lipoproteins in participants who lose comparable amounts of weight, but do so by adhering to diets that differ significantly in their macronutrient content and using whole foods.

Dattillo and Kris-Etherton (1992) quantified the effects of weight loss by dieting on lipids and lipoproteins by performing a meta-analysis of 70 studies from 1966 to 1989. Results indicated that weight reduction was associated with significant
decreases and correlations for TC ($r = 0.32$), LDL-C ($r = 0.29$), VLDL-C ($r = 0.38$) and TG ($r = 0.32$). For every kilogram decrease in body weight, a 0.009 mmol/L increase in HDL-C concentration occurred for participants at a stabilized reduced weight, and a 0.007 mmol/L decrease for participants actively losing weight. Thus, one may conclude that weight reduction, when maintained, has beneficial effects on the lipid profile of obese individuals.

Gumbiner, Low, and Reaven (1998) utilized a randomized parallel group design to determine whether the lipoprotein response to weight loss in obese male and female subjects with type 2 diabetes could be improved by using a MUFA-enriched hypocaloric formula [10% CHO, 70% FAT, 20% protein (PRO)] as compared to the commonly prescribed low-fat high-CHO hypocaloric diet (70% CHO, 10% FAT, 20% PRO) for a six-week period. Both diets produced decreases in TC, LDL-C, HDL-C, TG, and apoA-I and apoB. However, the MUFA-enriched group manifested a greater decrease in TC, TG, and apoB and a smaller decrease in HDL-C and apoA-I than the high-CHO group. Hence this study demonstrated that macronutrient composition of hypocaloric diets is an important determinant of the lipoprotein profile in a type 2 diabetic population. Since the sample size in the two groups was small, i.e., eight participants in the high-CHO group and nine participants in the MUFA-enriched group, additional studies are warranted with observations of longer duration to allow for a more complete assessment of MUFA-enriched hypocaloric diets.

Research on the effects of dieting on serum lipids performed among females is scant, and the literature search dwindles further when looking at studies involving obese females. Studies designed to solely involve this homogeneous study
population limit generalization of the findings, however the increasing prevalence of obesity among US women deserves heightened attention to their inclusion into future clinical trials.

E. Protective Effect of Nuts on Cardiovascular Risk Factors

Nuts possess several known nutrients that are postulated to provide a protective effect against coronary heart disease (Sabaté & Fraser, 1993). Recent epidemiological research findings from Mediterranean countries and the Adventist Health Study have stimulated an interest in evaluating nuts and in promoting novel methods of incorporating the frequent consumption of nuts into the US mainstream (Fraser, Sabaté, Beeson, & Strahan, 1992). Berry et al. (1991) inadvertently were the pioneers of nut research with their initial Jerusalem Nutrition Study that evaluated the benefits of a high-PUFA versus high-MUFA dietary intervention in male students under isocaloric conditions. However, Abbey et al. (1994) was the first group to directly compare the effects of nuts of different fatty acid compositions on plasma lipids. Using a nine-week supplemental field study design with 16 normolipidemic males, participants consumed a background diet with 18% of calories from fat in combination with an additional 18% fat being provided during three three-week periods to create either a Western dietary profile, a MUFA-enriched (86g/d of raw almonds) profile, and lastly a PUFA-enriched (68 g/d of raw walnuts) profile under isocaloric conditions. Almond supplementation produced significant reductions in TC and LDL-C, 7% and 10%, respectively, and walnut supplementation yielded 5% and 9% reductions respectively. This group commented that almonds may confer an advantage over walnuts in lowering cholesterol and in reducing the risk of coronary heart disease in that they (Abbey, Noakes, Beling, 22
& Nestel, 1993) and others (Berry et al., 1991; Reaven et al., 1991) have demonstrated that LDL-C from participants consuming diets rich in PUFAs is more prone to oxidation than is LDL-C from participants consuming MUFA-enriched diets under isocaloric conditions.

Spiller et al. (1990) compared the plasma cholesterol lowering properties of almonds (100g/d) and olive oil against a control group under isocaloric conditions using hypercholesterolemic males and females for a four-week period using a parallel group study design. Compared to the control group, both the almond and the olive oil dietary interventions caused a reduction in TC with no change in HDL-C and TG. These investigators speculated that the greater effect on the TC found in the almond group may be due to the different dietary P/S ratios (almond = 3.5; 0.47 = olive oil), the vegetable protein of the almond (Kritchevsky et al., 1981), or the fiber within the almond (Brown, Rosner, Willett, & Sacks, 1999).

Sabaté et al. (1993) compared the effects on serum lipids of a diet rich in walnuts (20% of total calories) to a control diet (AHA Step 1) under isocaloric conditions using a controlled, randomized crossover design in 18 normocholesterolemic males over two four-week periods. Reduced lipid levels were seen on both dietary interventions, however the walnut-enriched diet further decreased TC and LDL-C by 12% (22.4 mg/dl) and 16% (18.2 mg/dl) respectively. A 2.3 mg/dl reduction in HDL-C was observed in the walnut-enriched diet, however a significant decrease in the LDL-C/HDL-C ratio occurred.

The inclusion of 60 to 120 g/d of raw almonds, cashews and peanuts into a vegetarian isocaloric diet was explored to determine the effect on serum lipids in ten
healthy male and female participants using a randomized crossover design (control diet = 30% fat) and produced significant improvements in the serum lipid profile after 2 weeks (Jenkins et al., 1997). However one is unable to separate the effects of the nuts versus the vegetables from the study design.

O'Byrne, Knauft and Shireman (1997) studied post-menopausal hypercholesterolemic women who were previously consuming a 34% fat diet and placed them onto an isocaloric low-fat (26% fat) MUFA-enriched diet incorporating high-oleic peanuts for a 6-month period. Women already consuming low-fat isocaloric diets were used as controls to monitor the variation in serum lipids due to seasonal variations. Serum TC and LDL-C levels decreased 10% and 12% respectively in the peanut intervention. Additionally, the peanut intervention showed a trend toward beneficial changes in the LDL-C/HDL-C and apoA-I/apoB ratios as compared to the control group.

Rajaram, Burke, Connell, Myint and Sabaté (2001) recently investigated the effects of a 20% isoenergetic replacement of an AHA Step I diet using pecans rich in monounsaturated fat in adults with normal to moderately high serum cholesterol. This single-blind, randomized, controlled, crossover feeding study found that the pecan-enriched diet decreased serum TC and LDL-C by 6.7 and 10.4%, respectively and TG by 11.1% beyond the Step I diet, while increasing HDL-C by 2.5 mg/dl. Thus the weight of evidence may be considered in favor of the use of nuts to reduce the prevalence of dyslipidemia among obese adults.
F. Satiation and Satiety

Both satiation and satiety can be defined by measurable events. Satiation develops during the course of a meal and eventually brings the meal period to an end (Blundell, Lawton, Cotton, & Macdiarmid, 1996). Satiety is the state in which further eating is inhibited and follows the end of an eating episode, i.e., arises as a consequence of food ingestion. The intensity of satiety is measured by the duration of time that elapses until eating is recommenced, or by the amount consumed at the next meal (Blundell et al., 1996).

The regulation of food intake involves the complex processes of hunger, appetite and satiation. The presence of physiologic hunger initiates food-seeking behavior, however the sensation of hunger may be modified by many factors, including the cephalic phase of appetite, which is the response to the thought, sight, taste, or smell of food (Anderson, 1996). Appetite, the desire for food, may lead to the specific intake of energy to satisfy body energy deficits, specific nutrient requirements, or to meet a hedonic desire for a specific taste (Anderson, 1996).

Upon food ingestion, complex physiological and psychological responses occur which lead to satiation and the termination of food intake. Under hypocaloric conditions, hunger sensations can be decreased by the intake of energy containing macronutrients or by meeting one’s nutrient specific appetite. Dietary bulk, macronutrient composition, rate of absorption, and metabolic responses affect the time frame in which satiety ultimately occurs. The duration of satiety and the interval to the next ingestion of food depend on a complex system of neuronal responses integrated in
the central nervous system (Anderson, 1996). Additionally, the presence of others eating, social factors and hedonic factors contribute to the relative state of satiation.

1. **Leptin**

Obesity is positively correlated with increased leptin levels, insulin resistance, dyslipoproteinemia, and hypercoagulability. Adipocytes secrete leptin, a protein molecule with a secondary cytokine structure whose concentrations correlate with the amount of adipose tissue. Increased leptin levels downregulate appetite and increase sympathetic activity and thermogenesis in the hypothalamus (Haynes, Morgan, Walsh, Mark, & Sivitz, 1997). One mechanism through which leptin acts in regulating food intake is inhibition of the synthesis and release by the hypothalamus of neuropeptide-Y (NPY) (Stephens et al., 1995). Diet-induced weight loss reduces adipose TNF-alpha expression and serum leptin levels and is associated with improved insulin sensitivity and lipid metabolism.

Havel, Townsend, Chaump and Teff (1999) found high-fat meals reduced 24-hour circulating leptin concentrations in normal weight women under isocaloric diet conditions. These investigators previously reported that adipocyte glucose utilization is involved in insulin-induced leptin secretion in vitro. However, there is a nocturnal increase of leptin that is related to the insulin response to meals. Theoretically, LCHF meals, which induce smaller insulin and glucose responses, would produce lower leptin concentrations than HCLF meals. Consumption of LCHF meals resulted in lowered 24- hour circulating leptin concentrations, which the authors suggest may be a consequence of decreased adipocyte glucose metabolism.
2. Influence of Macronutrients

There is agreement among researchers that protein is the most satiating of all the macronutrients (Hill & Blundell, 1986; Rolls, Heatherington, & Burley, 1988). Calorie for calorie, amino acids contribute more than carbohydrate and fat to the suppression of hunger in the post-absorptive period (Booth & Chase, 1970). Thus there may be a direct effect of protein, or of its amino acid content in regulating satiety (Anderson, Li, & Glanville, 1984). Animal studies and human studies have found that protein ingestion leads to less food intake in the next meal than can be accounted for by protein energy content alone (Li, 1982; Barkeling, Rossner, & Bjorvell, 1990).

There is controversy about how fat affects hunger, satiety and the regulation of food intake. High fat intakes may contribute to the process of satiation through a variety of mechanisms. Specific brain neuropeptides (e.g., galanin) are proposed to regulate fat intake (Akabayashi et al, 1994), and fat ingestion leads to the release of cholecystokinin, which slows gastric emptying and provides a direct satiation signal to central food intake regulatory mechanisms (Read, French, & Cunningham, 1994). However, fat has a weaker effect than carbohydrate on satiety in humans and experimental animals (Rolls et al., 1994).

Carbohydrates are efficient appetite suppressants and contribute markedly to the satiating efficiency of food (Rogers & Blundell, 1989). Carbohydrate-specific appetites are attributed to low levels of the neurotransmitter serotonin in the brain (Wurtman, et al., 1985). NPY has recently been identified as a regulator of carbohydrate intake in rats (Leibowitz, 1994). Additionally, the type of carbohydrate, i.e., simple versus complex, influences the level of satiety in humans. Complex
carbohydrates that are slowly absorbed and cause a small but sustained increase in blood glucose have greater satiation than rapidly absorbed carbohydrates (Raben, 1994).

3. **Control Mechanisms of Food Intake**

Diet-induced thermogenesis (DIT) and the release of pre- and post-absorptive signals are detected by the central nervous system and function in the process of satiation. Strominger and Brobeck (1953) proposed the original thermostatic hypothesis of feeding control which states that during food consumption heat released through DIT increases body core temperature and results in termination of feeding, however the role of DIT in regulating satiety remains to be established. The brain regulates food intake through stimulation of the ventromedial and the lateral hypothalamus, however several other anatomical sites in the brain participate in regulating food intake through complex neural networks, neurotransmitters, and medial hypothalamus-related neuropeptides, i.e., NPY, galanin, opioids and growth hormone releasing factor (Anderson, 1994).

Mechanical, secretory, and receptive elements within the gastrointestinal system contribute to the regulation of feeding behavior (Read et al., 1994). Preabsorptive signals in the gut arise with food intake and may be transmitted to the brain via the vagus nerve due to physical, chemical, osmotic, or hormonal responses (Read et al., 1994). Low rates of gastric emptying occur with the consumption of high total fat and high complex carbohydrate intakes, thus have the potential to influence satiety. Release of food into the small intestine leads to the release of cholecystokinin (Read et al., 1994), which slows gastric emptying and may interact with neural inputs to
feeding centers in the brain. Lastly, digestion of macronutrients provides signals to the brain via the vagus nerve through chemoreceptors in the wall of the small intestine.

Postabsorptive signals occur after food absorption due to the entry of nutrients into the portal vein, or by fluctuations of nutrient concentrations in plasma or the brain (Anderson, 1994). Peripheral glucose and amino acid levels have led to the glucostatic and aminostatic theory of feeding, respectively. Mayer’s glucostatic theory proposes that blood glucose reflects the availability of energy to the brain and other tissues, thus controls food intake (1955). Human studies have shown an association between declines in blood glucose and increased hunger (Campfield, 1992).

Insulin has been studied to determine it’s metabolic effects and direct influence on the brain. Peripheral to the central nervous system, hyperinsulinemia is associated with increased hunger, food intake and obesity (Heller, 1994), however cerebral insulin action leads to the suppression of food intake (McGowan, Andrews, & Grossman, 1992) in rats. Schwartz et al. (1993) found that insulin in the arcuate nucleus is believed to inhibit the synthesis of hunger-related NPY due to reduced levels of NPY mRNA, with subsequent reductions of NPY in the paraventricular nucleus. Thus it is proposed that caloric deprivation reduces circulating and brain insulin, which permits the increased production of NPY, and in turn stimulates feeding.

4. Measurement of Satiety

Silverstone (1982) validated the use of 100-mm visual analog scales (VAS) for the subjective measurement of hunger and food intake. Numerous researchers have since utilized VAS for the subjective measurement of appetite, hunger, thirst, nausea, prospective consumption, and satiety (Blundell & Burley, 1987;
Gielkens, Verkijk, Lam, Lamers, & Masclee, 1998; Rolls, Bell, Castellanos, Chow, Pelkman, & Thorwart, 1999) before and after meals.

G. Future Research and Conclusions

Long-term controlled trials for weight reduction featuring hypocaloric HCLF diets have been disappointing, i.e., the loss of body weight insufficient to compensate for the HDL-lowering effect of the diet has occurred (Jones, Judd, Taylor, Campbell, & Nair, 1987). Hence, an alternative dietary approach might include the use of MUFA-enriched hypocaloric diets using either a high-oleic oil, or a nut, to potentiate a favorable lipoprotein response during energy restriction. Zambon et al. (1999) found a significant difference in lipoprotein subclass levels comparing an olive oil enriched hypocaloric diet to a CHO-enriched hypocaloric diet. Since isocaloric almond feeding trials have demonstrated hypocholesterolemic effects without changing HDL-C levels significantly among hypercholesterolemic men and women (Spiller et al., 1990), the incorporation of almonds into a medically supervised hypocaloric dietary regimen is worth investigating. If the use of nuts produces favorable weight reduction and lipid responses in the background of a hypocaloric diet, we will have an additional effective strategy for treating the obese population worldwide.

There is no dispute that ample research exists showing that dietary fat can play a role in the development of obesity. Since a reduction in dietary fat can significantly reduce the difference between total energy intake and total energy expenditure, it is the targeted macronutrient of choice to restrict in weight reduction programs. Willett (1998) has noted that the dietary intake of fat and the prevalence of obesity has not been positively correlated within geographic areas of similar economic development. The
literature abounds with the results of short-term randomized trials that show that a modest reduction in body weight occurs in individuals randomly assigned to diets with a lower percentage of energy derived from fat. However, compensatory mechanisms appear to operate as fat consumption within the range of 18-40% of energy appears to have little if any effect on body fatness in clinical trials lasting greater than one year (Willett, 1998).

Since the early 1970s, Americans' average fat intake has decreased from over 40% of total calories to 34%, yet there has not been a concurrent improvement in health (Taubes, 2001). The NIH started to encourage dietary fat restriction among the US population in 1984, however Americans have failed to replace fat calories with fruits, vegetables and legumes. Willett (1998) argues that the decline in the percentage of energy from fat consumed during the past two decades in the US has corresponded with a massive increase in the prevalence of obesity. Thus, high fat diets do not appear to be the primary cause of the high prevalence of excess body fat among the US population, and reductions in percent of energy from fat should not be promoted as the magic bullet cure for obesity.

Almonds are 75% total fat by analysis, of which 72% is MUFA, mainly oleic acid (C 18:1, n-9). Additional research is necessary to compare the effects of the unique nutrient profile of almonds on plasma lipids and other cardiovascular risk factors in obese adults under hypocaloric conditions. Future research should also explore the effects of MUFA-enriched hypocaloric diets incorporating nuts versus conventional HCLF diets on total body weight loss and compliance, through the measurement of
satisfaction (i.e., satiety, taste and texture) during participation in comprehensive medically supervised weight reduction programs.

In conclusion, clinicians treating obese adults become quickly frustrated with the lack of novel dietary intervention strategies, as well as the high rate of recidivism, in medically supervised weight reduction programs. The obese program participants that are prescribed a partial or complete liquid protein supplement for protein-sparing effects complain about insufficient food variety and lack of “crunch” at meal and/or snack times. The incorporation of nuts into weight reducing diet plans has been avoided in the past due to an inherent fear of the clinician in recommending the consumption of a calorically dense food item within the construct of a low calorie diet. Thus, to discount the fallacy that nuts are fattening, and to appease the desire of the obese client for more options with crunchy texture, in dietary interventions incorporating nuts must be tested and validated in the free-living population.
CHAPTER 3

METHODS

A. Subjects and Design

The study sample was a sample of convenience, as research participants must have been concurrently enrolled in the 24-week D & CVRRP at COH. Prospective research participants were identified during the D & CVRRP screening process with the cooperation of the D & CVRRP Program Coordinator and Program endocrinologists. Once the Program Coordinator or Program endocrinologist referred the patient to the Principal Investigator (PI), the PI approached the patient, verified their willingness to participate in the research study, and obtained written informed consent.

Potential research participants had the opportunity to take the COH Institutional Review Board (IRB) approved Informed Consent form home with them before deciding to participate in the study to enable them to discuss participation in the study with their family, friends or other confidants prior to signing the study consent form. The PI followed up within 24 hours of explaining the Informed Consent form to the potential research participants.

All research participants followed standard informed consent procedures as identified by the COH Research Subjects Protection Office. Notification of the intent to do research at COH using a COH IRB approved Informed Consent was provided in writing to the Loma Linda University IRB according to the Multiple Project Assurance M-1295 guidelines.

Research participants were given a copy of the Experimental Subjects' Bill of Rights to read and sign in accordance with California State Law. Research participants
were given a written copy of the study Informed Consent to read and sign and were
given a signed copy to keep. A witness signed and dated the study Informed Consent
form to attest that the requirements for informed consent were satisfied. A copy was
given to the person who attested to the form. Once signed consent had been obtained,
research participants were randomized and entered into the study.

Research participant recruitment was open to all minorities and both genders.
Based on the previous seven years of D & CVRRP operation, the sample size was
anticipated to include 70% women. Females of childbearing potential and/or pregnant
women were excluded from the study as the effects of very low calorie (protein sparing
modified fast) diets on the fetus are unknown. In 1998, the COH total population
included 39% ethnic minorities of which 20% were Hispanic (predominant minority
group).

A statistical analysis of the data of patients previously enrolled in the D &
CVRRP during 1998 and 1999 (males, pre-menopausal women and post-menopausal
women not receiving hormone replacement therapy) revealed a baseline mean HDL-C
of 43.9 mg/dl with a standard deviation of 12.2 mg/dl. No significant difference in
HDL-C at baseline was found across gender or menopausal status in these patients.

The research study featured a randomized two group pretest-posttest
experimental design. The notation for this research design is shown below (Cook and
Campbell, 1979).

\[
R \ O_1 \ X_1 \ O_2 \\
R \ O_1 \ X_2 \ O_2
\]
Randomization of subjects is denoted by R, observation at baseline and 24 weeks is denoted by $O_1$ and $O_2$ respectively, and, the dietary interventions HCLF and LCHF are denoted by $X_1$ and $X_2$ respectively.

Research participants were randomized into the almond or complex carbohydrate (CHO) group according to the registration procedures in Appendix A. The COH Department of Biostatistics utilized a Fortran software program developed at Stanford University entitled Random Number Generation (RNDGEN). The randomization schema featured stratification according to gender and presence or absence of diabetes across the two study groups.

The two study groups had different levels of monounsaturated fat due to the distinct dietary components prescribed. However, both groups were equally balanced on total calories and featured the use of the same amount of prescribed liquid protein supplement. Table 1 below shows the prescribed supplement, vitamins and foods for the complex carbohydrate and almond group.

Table 1. Prescribed Supplement, Vitamins and Foods for the Complex Carbohydrate and Almond Group

<table>
<thead>
<tr>
<th>Complex carbohydrate group</th>
<th>Almond group</th>
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<tbody>
<tr>
<td>4 packages of Health Management Resources (HMR) 70 Plus</td>
<td>4 packages of Health Management Resources (HMR) 70 Plus</td>
</tr>
<tr>
<td>2 HMR vitamin/mineral supplements</td>
<td>2 HMR vitamin/mineral supplements</td>
</tr>
<tr>
<td>Salad with 2 cups lettuce, 1 cup fresh tomato, 1 cup fresh celery, vinegar/lemon juice</td>
<td>Salad with 2 cups lettuce, 1 cup fresh tomato, 1 cup fresh celery, vinegar/lemon juice</td>
</tr>
<tr>
<td>2 teaspoons standard safflower oil</td>
<td>3 ounces (84 grams) almonds</td>
</tr>
<tr>
<td>Complex carbohydrates (see Appendix B)</td>
<td></td>
</tr>
</tbody>
</table>

Health Management Resources (HMR) 70 Plus is a commercially available protein sparing formulation prescribed during energy restriction to ameliorate the loss
of lean body mass (LBM). HMR 70 Plus contains minimal simple CHO (sucrose and fructose) for palatability, however is not a significant dietary source of complex CHO.

The amount of HMR 70 Plus prescribed was individually tailored to the requirements of the participant to achieve a daily intake of 1.0 to 1.4 grams of protein per kilogram ideal body weight (IBW). All research participants were required to consume their prescribed HMR 70 Plus formula daily to spare LBM loss.

The nutrient analysis for the information in the tables below were performed using Food Processor for Windows (Version 7.11). Table 2 compares the nutritional profile of four packages of the HMR 70 Plus product and 2 HMR vitamin/mineral supplements versus one package of HMR 70 Plus.
Table 2. Nutritional Analysis of HMR 70 Plus and Vitamin/Mineral Supplementation

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>4 packages HMR 70 Plus + 2 HMR vitamin/mineral supplements</th>
<th>1 package HMR 70 Plus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy, kcal</td>
<td>416</td>
<td>104</td>
</tr>
<tr>
<td>Protein, g</td>
<td>56</td>
<td>14</td>
</tr>
<tr>
<td>Carbohydrate, g</td>
<td>50</td>
<td>12.6</td>
</tr>
<tr>
<td>Total, g</td>
<td>0.8</td>
<td>0.2</td>
</tr>
<tr>
<td>Saturated fat, g</td>
<td>Negligible</td>
<td>Negligible</td>
</tr>
<tr>
<td>Fiber, g</td>
<td>2.8</td>
<td>0.7</td>
</tr>
<tr>
<td>Cholesterol, mg</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Sodium, mg</td>
<td>764</td>
<td>156</td>
</tr>
<tr>
<td>Potassium, mg</td>
<td>688</td>
<td>172</td>
</tr>
<tr>
<td>Manganese, mg</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Calcium, mg</td>
<td>1200</td>
<td>300</td>
</tr>
<tr>
<td>Phosphorus, mg</td>
<td>800</td>
<td>200</td>
</tr>
<tr>
<td>Magnesium, mg</td>
<td>444</td>
<td>36</td>
</tr>
<tr>
<td>Zinc, mg</td>
<td>22.5</td>
<td>0</td>
</tr>
<tr>
<td>Copper, mg</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Vitamin A, I.U.</td>
<td>5000</td>
<td>0</td>
</tr>
<tr>
<td>Vitamin D, I.U.</td>
<td>340</td>
<td>60</td>
</tr>
<tr>
<td>Vitamin E, I.U.</td>
<td>45</td>
<td>0</td>
</tr>
<tr>
<td>Vitamin C, mg</td>
<td>84</td>
<td>0</td>
</tr>
<tr>
<td>Folic acid, mcg</td>
<td>400</td>
<td>0</td>
</tr>
<tr>
<td>Thiamin, mg</td>
<td>1.8</td>
<td>0</td>
</tr>
<tr>
<td>Riboflavin, mg</td>
<td>1.7</td>
<td>0.34</td>
</tr>
<tr>
<td>Niacin, mg</td>
<td>30</td>
<td>0</td>
</tr>
<tr>
<td>Vitamin B6, mg</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Vitamin B12, mcg</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Pantothentic acid, mg</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>Biotin, mcg</td>
<td>450</td>
<td>0</td>
</tr>
<tr>
<td>Iodine, mcg</td>
<td>150</td>
<td>0</td>
</tr>
<tr>
<td>Iron, mg</td>
<td>27</td>
<td>0</td>
</tr>
<tr>
<td>Vitamin K, mcg</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Selenium, mcg</td>
<td>150</td>
<td>0</td>
</tr>
<tr>
<td>Chromium, mcg</td>
<td>150</td>
<td>0</td>
</tr>
<tr>
<td>Molybdenum, mcg</td>
<td>300</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 3 compares 2 teaspoons safflower oil + 1 ½ cup peas + 15 Triscuits™ versus 84 grams of almonds. These are the uncommon dietary elements of the two treatments, thus this represents the difference in nutrients in the two groups. The
imbalance in vitamin A, vitamin E and folic acid was noted and a rationale for supplementation was investigated. In light of no known interactions of these macronutrients on the metabolic and anthropometric parameters under investigation in this study, vitamin supplementation was not pursued.

Table 3. Nutrient Composition of the Almond Supplement and the Complex Carbohydrate Dietary Counterpart of the Study Diets

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Complex carbohydrate group (2 teaspoons safflower oil + 1 ½ cup peas + 15 wheat crackers)</th>
<th>Almond group (84 grams almonds)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy, kcal</td>
<td>508</td>
<td>495</td>
</tr>
<tr>
<td>Protein, g</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>Carbohydrate, g</td>
<td>75</td>
<td>17</td>
</tr>
<tr>
<td>Total Fat, g</td>
<td>18</td>
<td>44</td>
</tr>
<tr>
<td>Saturated fat, g</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Fiber, g</td>
<td>20</td>
<td>9</td>
</tr>
<tr>
<td>Cholesterol, mg</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sodium, mg</td>
<td>532</td>
<td>9</td>
</tr>
<tr>
<td>Potassium, mg</td>
<td>485</td>
<td>615</td>
</tr>
<tr>
<td>Manganese, mg</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Calcium, mg</td>
<td>72</td>
<td>223</td>
</tr>
<tr>
<td>Phosphorus, mg</td>
<td>337</td>
<td>437</td>
</tr>
<tr>
<td>Magnesium, mg</td>
<td>112</td>
<td>249</td>
</tr>
<tr>
<td>Zinc, mg</td>
<td>3</td>
<td>2.4</td>
</tr>
<tr>
<td>Copper, mg</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>Vitamin A, I.U.</td>
<td>1603</td>
<td>0</td>
</tr>
<tr>
<td>Vitamin D, I.U.</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Vitamin E, I.U.</td>
<td>9.7</td>
<td>30</td>
</tr>
<tr>
<td>Vitamin C, mg</td>
<td>24</td>
<td>1</td>
</tr>
<tr>
<td>Folic acid, mcg</td>
<td>157</td>
<td>49</td>
</tr>
<tr>
<td>Thiamin, mg</td>
<td>1</td>
<td>0.2</td>
</tr>
<tr>
<td>Riboflavin, mg</td>
<td>0.42</td>
<td>0.65</td>
</tr>
<tr>
<td>Niacin, mg</td>
<td>6.3</td>
<td>2.8</td>
</tr>
<tr>
<td>Vitamin B6, mg</td>
<td>0.4</td>
<td>0.1</td>
</tr>
<tr>
<td>Vitamin B12, mcg</td>
<td>0.6</td>
<td>0</td>
</tr>
<tr>
<td>Pantothenic acid, mg</td>
<td>0.9</td>
<td>0.4</td>
</tr>
<tr>
<td>Biotin, mcg</td>
<td>1</td>
<td>54</td>
</tr>
<tr>
<td>Iodine, mcg</td>
<td>4.8</td>
<td>1.7</td>
</tr>
<tr>
<td>Iron, mg</td>
<td>5.8</td>
<td>3</td>
</tr>
<tr>
<td>Selenium, mcg</td>
<td>11</td>
<td>4</td>
</tr>
</tbody>
</table>
A comparison of the nutrient profile of the two prescribed dietary interventions is shown in Table 4 below.

Table 4. Nutrient Composition of the Study Diets

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Complex carbohydrate group</th>
<th>Almond group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy, kcal</td>
<td>1015 kcal</td>
<td>1012 kcal</td>
</tr>
<tr>
<td>% kcal from fat</td>
<td>18%</td>
<td>39%</td>
</tr>
<tr>
<td>% kcal from carbohydrate</td>
<td>53%</td>
<td>32%</td>
</tr>
<tr>
<td>Fat</td>
<td>22 grams</td>
<td>46 grams</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>144 grams</td>
<td>85 grams</td>
</tr>
<tr>
<td>Protein</td>
<td>78 grams</td>
<td>78 grams</td>
</tr>
<tr>
<td>Fiber</td>
<td>32 grams</td>
<td>20 grams</td>
</tr>
<tr>
<td>Saturated fat</td>
<td>3 grams (3%)</td>
<td>3 grams (3%)</td>
</tr>
<tr>
<td>Monounsaturated fat</td>
<td>6 grams (5%)</td>
<td>28 grams (25%)</td>
</tr>
<tr>
<td>Polyunsaturated fat</td>
<td>9 grams (11%)</td>
<td>10 grams (9%)</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>4 mg</td>
<td>4 mg</td>
</tr>
<tr>
<td>Magnesium</td>
<td>556 mg</td>
<td>693 mg</td>
</tr>
<tr>
<td>Arginine</td>
<td>1.16 grams</td>
<td>2.23 grams</td>
</tr>
<tr>
<td>Lysine</td>
<td>4.76 grams</td>
<td>4.47 grams</td>
</tr>
<tr>
<td>Lysine/arginine ratio</td>
<td>4.1</td>
<td>2</td>
</tr>
<tr>
<td>Vitamin E-alpha equivalent</td>
<td>8.4 mg</td>
<td>18.7 mg</td>
</tr>
</tbody>
</table>

Research participants were instructed to discontinue their intake of all non-prescription vitamin/mineral supplements one month prior to study initiation to allow a wash out period. Research participants were also instructed to take two HMR vitamin/mineral supplement tablets two weeks prior to study initiation to allow a two-week run-in period.

During week one of the 24-week program/study, research participants were given explicit guidelines on their prescribed daily HMR formula, HMR vitamin/mineral supplements, food and fluid intake. Appendix C provides a description of the General Dietary Guidelines provided to the research participants.
Nutrition education, behavior modification and exercise classes were provided weekly on a rotating basis to facilitate weight loss and maintenance. Nutrition education and behavior modification classes were taught by the Program Dietitian (PI) and Program Psychologist, respectively, to enable the patient to integrate new behaviors that facilitate weight reduction and maintenance into his/her current lifestyle. Exercise classes were taught by a Registered Physical Therapist to enable the research participants to develop a personalized exercise program.

To assure the safety of their health, research participants signed a D & CVRRP contract (Appendix D) ensuring the consumption of all the formula/food prescribed, and agreed to attend scheduled appointments for the duration of the program. Two consecutive scheduled weekly absences or five non-consecutive visits or portions of visits during the 24-week program cycle were grounds for discharge from the program and study.

Compliance to the dietary regimen and body weight were carefully monitored. Research participants had a time commitment in the outpatient clinic every Wednesday from 3:45 p.m. to 6:00 p.m. for a brief physician visit that was required after measurements of blood pressure, weight, blood ketones and blood glucose were taken. The Program Dietitian, Program Psychologist and Physical Therapist were available for short consultations upon research participant or physician request. Failure to achieve a one to two pound per week average weight loss, inability to maintain a mild ketotic state, or, a high blood glucose (in type 2 diabetes research participants) were potential signs of dietary and exercise non-compliance. These situations warranted the
scheduling of an intermediate-length consultation with the Program Dietitian and/or the Program Psychologist.

In addition to the blood ketone and blood glucose measures of dietary compliance, research participants completed daily food and exercise records for weekly review by the Program Dietitian. Daily reporting of the following was recorded by research participants using the D & CVRRP calorie and exercise record book: HMR formula intake, fluid intake, intake of prescribed and/or non-prescribed foods, time of day meals or snacks were consumed, computation of daily total caloric intake according to the Program Dietitian’s guidelines, and, type and duration of exercise (minutes). Additionally, research participants were asked to subjectively evaluate and record the acceptability of their dietary regimen in terms of satiety, palatability and texture using a questionnaire at baseline, week 8, 16 and 24 (Appendix E). Validation of the instrument is discussed in Section H.1.

Research participants who failed to bring their weekly record book to clinic were advised to mail the book to the Program Coordinator within 24 hours. Research participants who failed to perform the daily food and exercise recording received a warning from the physician and were scheduled for private counseling sessions with the Program Psychologist to improve record-keeping compliance.

B. Research Variables

Almonds and complex CHO were the sources of the macronutrients manipulated in this study, hence are the independent variables. The outcome (dependent) variables were TC, HDL, TG, HDL-C, HDL-TG, LDL-C, LDL-TG, VLDL-C, VLDL-TG, glucose, insulin, HgbAlc, blood ketones, total body weight loss, waist-to-hip ratio
(WHR), waist circumference, percent body fat, fat mass (FM), fat free mass (FFM), total body water (TBW) and blood pressure.

**C. Anthropometric and Body Composition Methodology**

To determine if an individual is obese versus simply overweight due to increased muscle mass, multiple techniques and standards for quantitating body weight, body fat, and distribution of body fat should be utilized. Anthropometric measurements were performed including height and weight and circumferences of the waist and hip. Height and weight measurements provide an estimate of the degree of obesity, whereas the WHR and waist circumference provide practical measures for estimation of regional fat distribution. Bioelectrical impedance analysis (BIA) using the Tanita® TBF-300 body composition analyzer scale was performed to derive measurements of body composition.

1. **Weight and Height Measurements**

Fat-free mass or lean body mass (LBM) comprises 70-90% of body weight, hence LBM and weight should be related (Forbes, 1996). Likewise, in an obese population with widely variable fat content, body fat and weight should also be related. The outpatient clinic assistant measured body weight twice to the nearest 0.05 kg at each weekly clinic visit using the COH Outpatient Clinic Digital Scaletronic™ scale. The average of the two measurements was recorded in the medical record. Clinic scales were calibrated weekly by the COH Biomedical Instrumentation Systems division. Weights were taken with the patient wearing minimal street clothing and no shoes. Height was measured on the first clinic visit using the outpatient clinic wall mounted
stadiometer. Patients were measured twice without shoes and the average of the two measurements was recorded.

2. Girth Measurements

Waist circumference is an index of regional fat distribution and is related to fat-free mass. When used in a ratio with the buttock (hip) circumference, waist circumference is an indicator of the degree of the android distribution of adipose tissue. Above normal ratios are considered to constitute an increased risk of heart disease and type 2 diabetes. The buttocks (hip) circumference is a measurement of the external pelvic size that reflects the amount of adipose tissue in the region. Adipose tissue in this region is largely subcutaneous and relates to the lower segment of the body.

Waist and buttocks (hip) circumference measurements were taken according to guidelines in the Anthropometric Standardization Reference Manual (Lohman, Roche, & Martorell, 1991). Two measurements of the waist and buttocks (hip) were taken at baseline and at 24 weeks.

Research participants were advised to wear loose-fitting clothing so that the tape measure could be correctly positioned and were advised to stand erect with abdomen relaxed, arms at the sides and feet together. The PI faced the research participant and placed the inelastic tape around them, in a horizontal plane, at the level of the natural waist (narrowest part of the torso) as seen from the anterior aspect. The measurement was taken at the end of a normal expiration, without the tape compressing the skin, and recorded to the nearest 0.1 cm. In some research participants it was difficult to identify a waist narrowing. In such cases, the smallest horizontal circumference was measured in the area between the ribs and iliac crest. To determine the hip circumference the PI
squatted at the side of the patient so that the level of maximum extension of the buttocks could be seen. An inelastic tape was placed around the buttocks in a horizontal plane at this level without the tape compressing the skin and the measurement was recorded to the nearest 0.1 cm.

3. Bioelectrical Impedance Analysis

Bioelectrical impedance analysis (BIA) was performed by the PI on the research participants at baseline and 24-weeks during pre-scheduled appointments in the COH GCRC clinic. BIA was performed using the Tanita® TBF-300 body composition analyzer/scale which utilizes "foot-to-foot" pressure contact electrode technology to determine internal body composition (Nunez, 1997). BIA technology is based upon the principle that lean tissues contain a high electrolyte and water content which provides a good electrical pathway as compared to fat mass which contains a lower percentage of body water (Heyward, 1996). First, a measurement of the baseline resistance to the electrical current flow is made by inducing a low energy, high frequency, electrical signal (50 kHz, 500 microamp). The current is then passed through the anterior electrode on the platform of the Tanita® scale and the drop in voltage is measured on the scale’s posterior electrode. The volume of the conductor correlates with the resistance measurement which is used to determine TBW, FFM, and FM. A proprietary formula derives the percent body fat by combining impedance and weight measurements with height, gender, age and physical activity level.

The manufacturers’ instructions were followed for the estimation of percent body fat at baseline and week 24. Research participants were instructed to
avoid alcohol 48 hours before the test, fast for 12 hours, avoid intense exercise 12 hours before the test, and to empty their bladder 30 minutes before the test.

D. Lipoprotein Quantification (LPQ) and Lipid Panel

Fasting blood was collected at weeks 0, 8, 16 and 24. Plasma variables were determined from venous blood drawn after a 12-hour fast. Venous blood was collected in appropriately treated tubes and plasma was obtained by use of a Beckman Allegra™ 21 centrifuge at 2500 revolutions per minute for 15 minutes at 4°C by the GCRC technician at COH. The Lipids Laboratory within the School of Pharmacy, Department of Molecular Pharmacology and Toxicology at the University of Southern California performed the Lipid Panel and Lipoprotein Quantification analysis using a TL100 Beckman Ultracentrifuge and a Cobas MIRAS analyzer (Roche). See Appendix F for collaborating laboratory procedures for the separation of LPQ, total cholesterol and triglycerides analysis using the Cobas machine, and HDL analysis by the precipitation method.

E. Blood Ketone and Blood Pressure Testing

Using fingerstick methodology, COH outpatient clinic nurses took blood ketone measurements during weekly clinic visits using a calibrated Hemocue™ monitor. Blood pressure measurements were taken by outpatient clinic nurses using automated blood pressure devices in the clinic.

F. Additional Testing

The GCRC technician produced the aliquots for additional tests and aliquots were stored at -70°C. Plasma insulin levels were determined by human specific radioimmunoassay (Linco kits) methodology at COH, and plasma glucose levels were
determined by a Stat Glucose/Lactate Analyzer Model 2000 (YSI, Yellow Springs, Ohio).

G. Instrumentation

1. Development of Satiety and Satisfaction Questionnaire

A questionnaire was developed to measure the research participant’s level of satiety and satisfaction to the “diet” prior to the start of the D & CVRRP (baseline) and at weeks 8, 16 and 24. In addition to three questions which addressed self-reported feelings of hunger and fullness one to two hours after breakfast, lunch and dinner, research participants were also asked to rate how satisfied they were with the taste and texture of their prescribed diet.

Three months prior to study enrollment, the preliminary questionnaire was discussed with Dr. Marsha Grant of the COH Department of Nursing Research. Dr. Grant has an extensive background in instrument validation. The preliminary tool was found to address the construct of satiety and to assess dietary taste and texture. Twenty-two D & CVRRP patients were asked to fill out the preliminary questionnaire for two consecutive days to provide for a test-retest analysis. Thus, the tool was validated among the same population for which it was designed. Using the criteria that if the difference is less than 1.0, there is agreement in the “raw agreement score” on day 1 and day 2, the average of the agreements within patients on day 1 and day 2 were found to be as follows: question 1, $r = 0.692$; question 2, $r = 0.692$; question 3, $r = 0.769$; question 4, $r = 0.846$; and, question 5, $r = 0.846$. Thus, all five questions were retained for the instrument.
H. Data Collection

Data was collected for database entry using data collection forms (Appendix G) for demographic information, lipid analysis data, non-lipid analysis data, BIA/anthropometric data and questionnaire data. Source documents included the research participants medical record, lipid analysis spread sheets from the USC Lipids Laboratory, data provided from the COH lab technicians, and printed output from the Tanita® body composition analyzer/scale and questionnaires.

I. Data Analysis

The primary study hypothesis was as follows: There will be a significant difference in HDL-C, hence the LDL-C/HDL-C ratio in obese adults consuming a MUFA-enriched hypocaloric diet using almonds versus a complex CHO-enriched hypocaloric diet during a medically supervised 24-week weight reduction program.

Sample size and power calculations were performed utilizing UnifyPower Macro for SAS. To detect a 10% effect size in HDL-C with power = 80%, and $\alpha = .05$ (two-sided), 42 participants were required in each group for a total of 84 participants. Allowing for a 10% dropout rate (historical), we needed approximately 92 participants, 46 per group. Considering the specified exclusion criteria (see Appendix H), dropout rate, and, the present rate of D & CVRRP patient accrual, total target accrual should have been achieved within 12 months of COH Institutional Review Board approval.

The analysis that was planned to be used to test the primary hypothesis was an independent 2-sample t-test to compare differences between lipids at baseline and at 24 weeks across groups, as well as a paired t-test (post- vs. pre-) to compare differences across time between lipids at 24-weeks and baseline. Multiple baseline measurements
were to be pooled to deflate the effect of intra-personal variation on the blood profiles. ANOVA was to be used for the analysis of the multiple comparisons at baseline, 8, 16 and 24 weeks (repeated measures). If the assumption of equal variances was not met, the Mann-Whitney rank sum test would be used. Time to response curves were to be evaluated as a component of the descriptive statistics. The primary analysis was to be based on intent-to-treat, however a secondary “as treated” analysis would also be performed.

Data was entered into a JMP database (Version 4.0.5) and statistical analysis was performed using SAS (Version 8.2). Due to the longitudinal structure of the data, a repeated measures mixed model with an autoregressive covariance structure of lag 1 was chosen to test all hypotheses, including those involving multiple covariates. This type of model would model the covariance structure within subjects, incorporating submatrices corresponding to each subject in each group. This would allow the within subject error to be accounted for and to be separated from the overall error, allowing for a more accurate and sensitive test of each hypothesis. Autoregressive (order 1) covariance structure specifies that the covariance between two measurements w time intervals apart is $\sigma^2 \rho$. The parameter $\sigma^2$ represents the variance of an observation, $\text{Var}(y_{ijk}) = \sigma^2$. The parameter $\rho$ represents the correlation between adjacent observations on the same subject. Thus, the correlation between measurements at times one and two is $\rho$, between measurements at times one and three is $\rho^2$, and between measurements at times one and four is $\rho^3$.

All models were adjusted for baseline measurements of the variable of interest, and all four time points (0, 8, 16 and 24 weeks) were included in the analysis.
Descriptive statistics were used to summarize the patient population. A table summarizing the effect of all major endpoints was created, with the least squares means, standard error, percent change and absolute change given for time points 0 and 24 weeks, with p-values given for the overall model, which included all time points. All p-values are based on Type III tests of fixed effects, which ensures that the addition of the variable of interest was added into the models after controlling for all other variables. Visual display of the effect of each treatment on each endpoint over time was achieved through plotting the least squares mean and 95% confidence interval (CI) by treatment group over time for each endpoint.
CHAPTER 4
RESULTS

A. Enrollment

Patients (n=57) were randomized into the 2 intervention groups according to gender and presence or absence of diabetes mellitus (Figure 1). The tight eligibility criteria produced 68% of the total targeted accrual over a 24-month period. The patients completing the 24-week study (n=46) were evenly distributed across the two groups. The proportions of withdrawals from the almond and complex carbohydrate (CHO) group were similar, 6 from the almond group and 5 from the CHO group. Primary reasons for dropping out of the study included work conflicts and worsening plasma lipid levels necessitating the resumption of pre-study anti-hyperlipemic agents. 36 patients were Caucasian, 10 Hispanic, 6 African American, and 5 Asian. Demographic, anthropometric, and baseline characteristics were similar in the two intervention groups after randomization (Table 5). The pre-program clinical characteristics of the research participants were not statistically significant at baseline with the exception of a higher number of hypertensive patients having been assigned to the CHO group compared to the almond group.

B. Anthropometric Measurements and Insulin Sensitivity

Using an intent-to-treat analysis, Table 6 shows the means and standard error of the means (SEM) for the anthropometric parameters in research participants enrolled in the study at baseline and week 24. During the 24-week treatment period, mean weight loss was 15.73 kg (14%) for the almond group (95% CI, -30.73 to -0.73 kg) and 11.64 kg (11%) for the CHO group (95% CI, -27.36 to 0 kg). The mean plasma insulin,
plasma glucose, insulin:glucose ratio (IGR) and HgbA1c values are shown in Table 7. Table 8 shows the mixed model results for the anthropometric and metabolic parameters for both groups. There was a statistically significant difference in weight loss in the almond group as compared with the CHO group over time, -18.02% in the almond group vs. -10.35% in the CHO group (P < 0.0001). Improvement in anthropometric parameters occurred due to the changes in body weight and contributed to greater improvements in the cardiovascular disease risk profile in the almond group as compared to the CHO group over time. Research participants in the almond group also achieved a statistically significant difference in reduction in BMI, waist circumference and fat mass, -17.53% in the almond group vs. -10.67% in the CHO group (p < 0.0001), -14.06% in the almond group vs. -9.29% in the CHO group (p = 0.0255) and −30.32% in the almond group vs. −19.53% in the CHO group (p = 0.0205) respectively.

Both groups experienced a reduction in the waist:hip ratio, percent fat, fat free mass and total body water over time, -3.26% and -3.26% (p = 0.0003), -15.76% and -11.09% (p<0.0001), -8.21% and -3.85% (p<0.0001), and, -8.10% and -1.33% (p = 0.0034) for the almond and CHO group respectively. However, no statistically significant difference in any of these parameters was found between the two groups.

Table 9 shows the results of evaluating the outcome variable weight among the two groups after single adjustment for either gender, diabetes or hypertension in contrast to the group by time effect. Gender of the research participant or influence of chronic disease (diabetes mellitus, hypertension) were not independently responsible for the weight difference found between the two groups.
Both groups demonstrated a reduction in insulin, glucose, insulin:glucose ratio (IGR) and HgbA1c over time, -57.83% and -34.61% (p<0.0001), -18.80% and -14.07% (p = 0.0006), -47.06% and -27.03% (p = 0.0008) and -14.85% and -12.41% (p<0.0001) for the almond and CHO group, respectively. However, no statistically significant difference in any of these parameters was found between the two groups.

C. Cardiovascular Risk Factors

Tables 10 and 11 show the mean plasma lipid levels in the almond and CHO groups at weeks 0, 8, 16 and 24, respectively. Plasma lipids at baseline (week 0) were not statistically significantly different between the two groups. The mean TC:HDL ratio declined from 4.65 to 3.90 in the almond group and 4.77 to 4.21 in the CHO group during the 24-week study period.

Summary statistics for the plasma lipids are shown in Table 12. No statistically significant difference between the groups was found for any lipid parameter that was measured. However, both groups demonstrated a statistically significant reduction over time in TC (p < 0.0001), TG (p = 0.0024), VLDL-C (p = 0.0386), LDL-C (p< 0.0001), VLDL-TG (p = 0.0028), TC:HDL (p = 0.0022) and LDL-C:HDL-C (p = 0.0268). There was no significant difference over time in HDL (p = 0.7848), HDL-TG (p = 0.5140) or HDL-C (p = 0.2004).

Table 13 shows the mean blood pressure levels observed at week 0 and 24 in the two groups. Mixed model results (Table 14) reflected a statistically significant reduction in both groups over time for systolic (p = 0.0506) and diastolic (p = 0.0371) blood pressure. However, no statistically significant difference in blood pressure was found between the two groups.

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D. Other Factors

Table 8 shows the summary statistics for blood ketone levels in the two groups. Ketone levels were statistically significantly increased in the almond group (+250%) as compared to no change within the CHO group (p = 0.0230).

The research participants did not differ in their self-reported evaluation of the acceptability of their dietary regimen in terms of satiety, palatability and texture using a 10-point scale at baseline and weeks 0, 8, 16 and 24. The responses to the questionnaire (Appendix E) were evaluated according to meal, i.e. breakfast, lunch, dinner, across the two study groups over the 24-week study period. Table 15 shows a summary of the questionnaire scoring results for the three questions evaluating satiety. Non-significant results were found across the two study groups over time. In comparison to the research participant’s baseline diet, this finding reflects the ability of either almonds or complex carbohydrates to maintain satiety 1 to 2 hours after meals over at least 24 weeks. Additionally, taste and texture of the two distinct interventions did not significantly change from baseline over the 24-week study. Table 16 shows a summary of the questionnaire scoring results for the two questions evaluating texture and taste satisfaction.
58 Patients Eligible for D & CVRRP

1 Did Not Meet Lipid Criteria

57 Patients Were Randomly Assigned

Almond Diet (28 Patients)

6 Withdrew
5 Work Conflict
1 Returned to Anti-lipemic Medication

22 Completed Trial

28 Included in Intention-to-Treat Analysis
22 Included in per Protocol Analysis

Complex CHO Diet (29 Patients)

5 Withdrew
4 Work Conflict
1 Returned to Anti-lipemic Medication

24 Completed Trial

29 Included in Intention-to-Treat Analysis
24 Included in per Protocol Analysis

Figure 1. Flow of Patients Throughout the Trial
Table 5. Pre-Program Clinical Characteristics of the Research Participants\(^a\)

<table>
<thead>
<tr>
<th></th>
<th>Almond group</th>
<th>CHO group</th>
</tr>
</thead>
<tbody>
<tr>
<td>n(F/M)</td>
<td>28(16/12)</td>
<td>29(18/11)</td>
</tr>
<tr>
<td>Type 2 diabetes (Y/N)</td>
<td>20/8</td>
<td>21/8</td>
</tr>
<tr>
<td>Hypertension (Y/N)</td>
<td>11/17</td>
<td>19/10</td>
</tr>
<tr>
<td>Age (years)</td>
<td>53 ± 2</td>
<td>55 ± 2</td>
</tr>
<tr>
<td><strong>Anthropometrics</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>113.48 ± 5.24</td>
<td>103.6 ± 5.21</td>
</tr>
<tr>
<td>Weight (lbs)</td>
<td>249.65 ± 11.53</td>
<td>227.92 ± 11.47</td>
</tr>
<tr>
<td>BMI(kg/m(^2))</td>
<td>38.45 ± 1.48</td>
<td>35.92 ± 1.34</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.93 ± 0.02</td>
<td>0.91 ± 0.01</td>
</tr>
<tr>
<td>Waist circumference (in)</td>
<td>48.33 ± 1.56</td>
<td>45.85 ± 1.52</td>
</tr>
<tr>
<td>% body fat</td>
<td>41.59 ± 1.39</td>
<td>42.56 ± 1.30</td>
</tr>
<tr>
<td>Fat mass (lbs)</td>
<td>104.67 ± 6.83</td>
<td>97.97 ± 6.63</td>
</tr>
<tr>
<td>Fat free mass (lbs)</td>
<td>144.97 ± 7.00</td>
<td>129.17 ± 6.65</td>
</tr>
<tr>
<td>Total body water (lbs)</td>
<td>106.13 ± 5.12</td>
<td>92.39 ± 5.73</td>
</tr>
<tr>
<td>Insulin (μU/ml)</td>
<td>50.41 ± 9.05</td>
<td>49.89 ± 6.59</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>161.4 ± 13.59</td>
<td>153.17 ± 13.70</td>
</tr>
<tr>
<td>Insulin:Glucose</td>
<td>0.34 ± 0.05</td>
<td>0.37 ± 0.05</td>
</tr>
<tr>
<td>HgbA1C</td>
<td>8.60 ± 0.30</td>
<td>8.58 ± 0.37</td>
</tr>
<tr>
<td>Ketones</td>
<td>0.10 ± 0.02</td>
<td>0.10 ± 0.01</td>
</tr>
<tr>
<td>BPs (mmHg)</td>
<td>144.13 ± 3.24</td>
<td>135.55 ± 3.72</td>
</tr>
<tr>
<td>BPd (mmHg)</td>
<td>77.93 ± 2.20</td>
<td>77.11 ± 2.66</td>
</tr>
<tr>
<td><strong>Plasma lipids(^b)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>194.55 ± 7.67</td>
<td>215.75 ± 8.16</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>44.41 ± 1.92</td>
<td>46.28 ± 2.01</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>178.33 ± 19.11</td>
<td>203.25 ± 20.86</td>
</tr>
<tr>
<td>Total cholesterol:HDL</td>
<td>4.65 ± 0.23</td>
<td>4.77 ± 0.25</td>
</tr>
<tr>
<td>VLDL-C (mg/dl)</td>
<td>40.52 ± 3.23</td>
<td>48.00 ± 4.59</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>97.85 ± 5.01</td>
<td>105.43 ± 5.53</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>31.93 ± 1.68</td>
<td>32.87 ± 1.69</td>
</tr>
<tr>
<td>LDL-C:HDL-C</td>
<td>3.17 ± 0.16</td>
<td>3.41 ± 0.23</td>
</tr>
<tr>
<td>VLDL-TG (mg/dl)</td>
<td>118.41 ± 15.57</td>
<td>135.83 ± 15.37</td>
</tr>
<tr>
<td>LDL-TG (mg/dl)</td>
<td>29.81 ± 2.02</td>
<td>33.47 ± 1.70</td>
</tr>
<tr>
<td>HDL-TG (mg/dl)</td>
<td>11.11 ± 0.33</td>
<td>12.13 ± 0.43</td>
</tr>
</tbody>
</table>

\(^a\)Characteristics were taken at week 0.

\(^b\)To convert from mg/dl to mmol/l, divide cholesterol values by 38.67 and triglyceride values by 88.54.

Note: Data are means ± SE unless otherwise indicated

SE = standard error
Table 6. Change in Anthropometric Parameters in the Research Participants

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Almond group</th>
<th>CHO group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 0 (n = 28)</td>
<td>Week 24 (n = 22)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>113.48 ± 5.24</td>
<td>97.75 ± 5.26†‡</td>
</tr>
<tr>
<td>Weight (lbs)</td>
<td>249.65 ± 11.53</td>
<td>215.05 ± 11.58†‡</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>38.45 ± 1.48</td>
<td>32.84 ± 1.45†‡</td>
</tr>
<tr>
<td>Waist:Hip ratio</td>
<td>0.93 ± 0.02</td>
<td>0.90 ± 0.01†</td>
</tr>
<tr>
<td>Waist circumference (in)</td>
<td>48.33 ± 1.56</td>
<td>42.59 ± 1.34†‡</td>
</tr>
<tr>
<td>% Body fat</td>
<td>41.59 ± 1.39</td>
<td>34.96 ± 2.04†</td>
</tr>
<tr>
<td>Fat mass (lbs)</td>
<td>104.67 ± 6.83</td>
<td>76.86 ± 7.66†‡</td>
</tr>
<tr>
<td>Fat free mass (lbs)</td>
<td>144.97 ± 7.00</td>
<td>138.19 ± 7.23†</td>
</tr>
<tr>
<td>Total body water (lbs)</td>
<td>106.13 ± 5.12</td>
<td>101.15 ± 5.30†</td>
</tr>
</tbody>
</table>

†p< 0.05, for the contrasts from week 0 to 24 for the changes over time within groups
‡p< 0.05, for the contrasts from week 0 to 24 for the changes over time between groups
Note: Data are means ± SEM. SEM = standard error of the mean.
<table>
<thead>
<tr>
<th></th>
<th>Almond group</th>
<th>Carbohydrate group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 0 (n = 28)</td>
<td>Week 8 (n = 26)</td>
</tr>
<tr>
<td></td>
<td>Week 16 (n = 23)</td>
<td>Week 24 (n = 22)</td>
</tr>
<tr>
<td><strong>Insulin (μU/ml)</strong></td>
<td>50.41 ± 9.05</td>
<td>26.37 ± 2.52</td>
</tr>
<tr>
<td></td>
<td>21.89 ± 1.87</td>
<td>21.54 ± 1.87†</td>
</tr>
<tr>
<td><strong>Glucose (mg/dl)</strong></td>
<td>161.4 ± 13.59</td>
<td>133.76 ± 9.57</td>
</tr>
<tr>
<td></td>
<td>119.96 ± 7.95</td>
<td>123.45 ± 7.99†</td>
</tr>
<tr>
<td><strong>Insulin:Glucose ratio</strong></td>
<td>0.34 ± 0.05</td>
<td>0.21 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>0.19 ± 0.01</td>
<td>0.18 ± 0.02†</td>
</tr>
<tr>
<td><strong>Ketones (mmol/L)</strong></td>
<td>0.01 ± 0.04</td>
<td>0.28 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>0.33 ± 0.05</td>
<td>0.38 ± 0.05†‡</td>
</tr>
<tr>
<td><strong>HgbA1c (n = 20)</strong></td>
<td>8.61 ± 0.30</td>
<td>Not performed</td>
</tr>
<tr>
<td></td>
<td>Not performed</td>
<td>Not performed</td>
</tr>
<tr>
<td></td>
<td>7.35 ± 0.43†</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Week 0 (n = 29)</td>
<td>Week 8 (n = 27)</td>
</tr>
<tr>
<td></td>
<td>Week 16 (n = 24)</td>
<td>Week 24 (n = 24)</td>
</tr>
<tr>
<td><strong>Insulin (μU/ml)</strong></td>
<td>49.89 ± 6.59</td>
<td>35.29 ± 4.41</td>
</tr>
<tr>
<td></td>
<td>27.81 ± 3.10</td>
<td>32.33 ± 5.46</td>
</tr>
<tr>
<td><strong>Glucose (mg/dl)</strong></td>
<td>153.17 ± 13.70</td>
<td>138.21 ± 11.68</td>
</tr>
<tr>
<td></td>
<td>138.54 ± 13.72</td>
<td>130.67 ± 12.02†</td>
</tr>
<tr>
<td><strong>Insulin:Glucose ratio</strong></td>
<td>0.37 ± 0.05</td>
<td>0.30 ± 0.37</td>
</tr>
<tr>
<td></td>
<td>0.23 ± 0.03</td>
<td>0.27 ± 0.05</td>
</tr>
<tr>
<td><strong>Ketones (mmol/L)</strong></td>
<td>0.10 ± 0.04</td>
<td>0.11 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>0.14 ± 0.05</td>
<td>0.11 ± 0.05‡</td>
</tr>
<tr>
<td><strong>HgbA1c (n = 21)</strong></td>
<td>8.58 ± 0.37</td>
<td>Not performed</td>
</tr>
<tr>
<td></td>
<td>Not performed</td>
<td>Not performed</td>
</tr>
<tr>
<td></td>
<td>7.62 ± 0.37†</td>
<td></td>
</tr>
</tbody>
</table>

†p < 0.05, for the contrasts from week 0 to 24 for the changes over time within groups
‡p < 0.05, for the contrasts from week 0 to 24 for the changes over time between groups

Note: Data are means ± SEM
SEM = standard error of mean
Table 8. Summary Statistics for Anthropometric and Metabolic Parameters

<table>
<thead>
<tr>
<th>Variable</th>
<th>Week</th>
<th>Almond LSM (SE) (n = 28)</th>
<th>Carbohydrate (CHO) LSM (SE) (n = 29)</th>
<th>% change = week 24 - week 0</th>
<th>Absolute change = week 24 - week 0</th>
<th>P value† group x time effect</th>
<th>P value† time effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (lbs.)</td>
<td>0</td>
<td>246.16 (1.92)</td>
<td>245.79 (1.92)</td>
<td>-18.02</td>
<td>-44.37</td>
<td>&lt;0.0001*</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>201.79 (2.35)</td>
<td>220.36 (2.31)</td>
<td>-10.35</td>
<td>-25.43</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>0</td>
<td>37.60 (0.41)</td>
<td>37.38 (0.42)</td>
<td>-17.53</td>
<td>-6.59</td>
<td>&lt;0.0001*</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>31.01 (0.47)</td>
<td>33.39 (0.46)</td>
<td>-10.67</td>
<td>-3.99</td>
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<td></td>
</tr>
<tr>
<td>Waist (in.)</td>
<td>0</td>
<td>47.43 (0.46)</td>
<td>47.13 (0.47)</td>
<td>-14.06</td>
<td>-6.67</td>
<td>0.0255*</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>40.76 (0.53)</td>
<td>42.75 (0.52)</td>
<td>-9.29</td>
<td>-4.38</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Waist:hip</td>
<td>0</td>
<td>0.92 (0.01)</td>
<td>0.92 (0.01)</td>
<td>-3.26</td>
<td>-0.03</td>
<td>0.5656</td>
<td>0.0003*</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>0.89 (0.01)</td>
<td>0.89 (0.01)</td>
<td>-3.26</td>
<td>-0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% fat</td>
<td>0</td>
<td>42.20 (0.60)</td>
<td>42.20 (0.60)</td>
<td>-15.76</td>
<td>-6.65</td>
<td>0.1263</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>35.55 (0.68)</td>
<td>37.52 (0.60)</td>
<td>-11.09</td>
<td>-4.68</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat mass (lbs.)</td>
<td>0</td>
<td>102.61 (2.20)</td>
<td>102.30 (2.20)</td>
<td>-30.32</td>
<td>-31.11</td>
<td>0.0205*</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>71.50 (2.48)</td>
<td>82.10 (2.42)</td>
<td>-19.53</td>
<td>-19.93</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat free mass (lbs.)</td>
<td>0</td>
<td>138.11 (1.39)</td>
<td>137.31 (1.40)</td>
<td>-8.21</td>
<td>-11.34</td>
<td>0.0453*</td>
<td>&lt;0.0001*</td>
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<tr>
<td></td>
<td>24</td>
<td>126.77 (1.58)</td>
<td>132.02 (1.54)</td>
<td>-3.85</td>
<td>-5.29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total body water (lbs.)</td>
<td>0</td>
<td>100.30 (1.45)</td>
<td>98.88 (1.45)</td>
<td>-8.10</td>
<td>-1.33</td>
<td>0.0312*</td>
<td>0.0034*</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>92.18 (1.64)</td>
<td>97.56 (1.60)</td>
<td>-1.33</td>
<td>-1.32</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin (µU/ml)</td>
<td>0</td>
<td>50.42 (5.07)</td>
<td>49.79 (4.98)</td>
<td>-57.83</td>
<td>-29.16</td>
<td>0.5966</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>21.26 (5.52)</td>
<td>32.56 (5.29)</td>
<td>-34.61</td>
<td>-17.23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>0</td>
<td>161.30 (11.43)</td>
<td>152.12 (11.66)</td>
<td>-18.80</td>
<td>-30.33</td>
<td>0.4093</td>
<td>0.0006*</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>130.97 (11.78)</td>
<td>130.72 (11.68)</td>
<td>-14.07</td>
<td>-21.40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin:Glucose</td>
<td>0</td>
<td>0.34 (0.04)</td>
<td>0.37 (0.04)</td>
<td>-47.06</td>
<td>-27.03</td>
<td>0.6866</td>
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<td>0.18 (0.04)</td>
<td>0.27 (0.04)</td>
<td>-16.00</td>
<td>-10.00</td>
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</tr>
</tbody>
</table>
Table 8 (continued). Summary Statistics for Anthropometric and Metabolic Parameters.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Week</th>
<th>Almond LSM (SE) (n = 28)</th>
<th>Carbohydrate (CHO) LSM (SE) (n = 29)</th>
<th>% change = week 24 - week 0</th>
<th>Absolute change = week 24 - week 0</th>
<th>P value† group x time effect</th>
<th>P value† time effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ketones (mmol/L)</td>
<td>0</td>
<td>0.11 (0.04)</td>
<td>0.10 (0.04)</td>
<td>+250</td>
<td>0</td>
<td>0.0230*</td>
<td>0.0083*</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>0.36 (0.05)</td>
<td>0.10 (0.05)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HgbA1c (%)</td>
<td>0</td>
<td>8.62 (0.24)</td>
<td>8.62 (0.23)</td>
<td>-14.85</td>
<td>-12.41</td>
<td>0.6713</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>7.34 (0.28)</td>
<td>7.55 (0.23)</td>
<td>-1.28</td>
<td>-1.07</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

† All outcome variables were adjusted for their baseline values in the models. P values are based on mixed models with an autoregressive covariance structure of order one, with all time points (weeks 0, 8, 16 and 24) included in the model.

*Indicates statistically significant difference between groups.

LSM = least squares means
SE = standard error
<table>
<thead>
<tr>
<th>Outcome variable</th>
<th>Adjustment variable (AV)</th>
<th>Wk</th>
<th>Almond LSM (SE)</th>
<th>CHO LSM (SE)</th>
<th>% change = ( \frac{wk 24 - wk 0}{wk 0} )</th>
<th>Absolute change = ( wk 24 - wk 0 )</th>
<th>P value†</th>
<th>Group x time effect</th>
<th>AV x time effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight</td>
<td>Diabetes</td>
<td>0</td>
<td>240.30(2.82)</td>
<td>238.69(2.78)</td>
<td>-17.54</td>
<td>-10.83</td>
<td>-42.14</td>
<td>-25.84</td>
<td>0.0067*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>24</td>
<td>198.16(3.14)</td>
<td>212.85(3.02)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight</td>
<td>Hypertension</td>
<td>0</td>
<td>240.98(2.77)</td>
<td>239.48(2.75)</td>
<td>-17.59</td>
<td>-10.83</td>
<td>-42.39</td>
<td>-25.93</td>
<td>0.0065*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>24</td>
<td>198.59(3.15)</td>
<td>213.55(2.99)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight</td>
<td>Gender</td>
<td>0</td>
<td>241.17(2.77)</td>
<td>239.48(2.72)</td>
<td>-17.59</td>
<td>-10.79</td>
<td>-42.42</td>
<td>-25.84</td>
<td>0.0062*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>24</td>
<td>198.75(3.13)</td>
<td>213.64(3.00)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

† The outcome variable (weight) was adjusted for its baseline value in all models. P values are based on mixed models with an autoregressive covariance structure of order one, with all time points (weeks 0, 8, 16 and 24) included in the model.
*Indicates statistically significant difference between groups.
LSM = least squares mean
SE = standard error
Wk = week
CHO = carbohydrate
Table 10. Change in Plasma Lipids from Week 0 to Weeks 8, 16 and 24 in the Almond Group

<table>
<thead>
<tr>
<th>Plasma lipids</th>
<th>Week 0 (n = 28)</th>
<th>Week 8 (n = 26)</th>
<th>Week 16 (n = 23)</th>
<th>Week 24 (n = 22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>194.55 ± 7.67</td>
<td>175.5 ± 10.45</td>
<td>169.47 ± 8.60</td>
<td>170.41 ± 9.50</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>44.41 ± 1.92</td>
<td>42.31 ± 1.44</td>
<td>42.52 ± 1.85</td>
<td>43.54 ± 1.92</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>178.33 ± 19.11</td>
<td>161.46 ± 19.74</td>
<td>137.48 ± 15.56</td>
<td>123.36 ± 18.60</td>
</tr>
<tr>
<td>Total cholesterol:HDL</td>
<td>4.65 ± 0.23</td>
<td>4.20 ± 0.24</td>
<td>4.06 ± 0.19</td>
<td>3.90 ± 0.22</td>
</tr>
<tr>
<td>VLDL-C (mg/dl)</td>
<td>40.52 ± 3.23</td>
<td>37.88 ± 3.91</td>
<td>36.61 ± 3.26</td>
<td>31.27 ± 3.98</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>97.85 ± 5.01</td>
<td>86.88 ± 5.86</td>
<td>81.48 ± 4.87</td>
<td>83.18 ± 6.73</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>31.93 ± 1.68</td>
<td>30.73 ± 1.28</td>
<td>29 ± 1.37</td>
<td>30.72 ± 1.82</td>
</tr>
<tr>
<td>LDL-C:HDL-C</td>
<td>3.17 ± 0.16</td>
<td>2.89 ± 0.19</td>
<td>2.91 ± 0.20</td>
<td>2.86 ± 0.29</td>
</tr>
<tr>
<td>VLDL-TG (mg/dl)</td>
<td>118.41 ± 15.57</td>
<td>106.42 ± 15.21</td>
<td>87.35 ± 11.75</td>
<td>77.23 ± 14.31</td>
</tr>
<tr>
<td>LDL-TG (mg/dl)</td>
<td>29.81 ± 2.02</td>
<td>27.54 ± 1.75</td>
<td>26.65 ± 1.86</td>
<td>24.09 ± 1.33</td>
</tr>
<tr>
<td>HDL-TG (mg/dl)</td>
<td>11.11 ± 0.33</td>
<td>10.58 ± 0.64</td>
<td>10.48 ± 0.65</td>
<td>9.95 ± 0.64</td>
</tr>
</tbody>
</table>

*To convert from mg/dl to mmol/l, divide cholesterol values by 38.67 and triglyceride values by 88.54.

Note: Data are means ± SEM
SEM = standard error of the mean
Table 11. Change in Plasma Lipids from Week 0 to Weeks 8, 16 and 24 in the Carbohydrate (CHO) Group

<table>
<thead>
<tr>
<th>Plasma lipids</th>
<th>Week 0 (n = 29)</th>
<th>Week 8 (n = 27)</th>
<th>Week 16 (n = 24)</th>
<th>Week 24 (n = 24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>215.75 ± 8.16</td>
<td>192.07 ± 5.97</td>
<td>197.87 ± 7.62</td>
<td>200.33 ± 7.21</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>46.28 ± 2.01</td>
<td>44.63 ± 1.97</td>
<td>49.58 ± 2.32</td>
<td>51.5 ± 2.48</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>203.25 ± 20.86</td>
<td>177.26 ± 20.29</td>
<td>138.58 ± 13.54</td>
<td>135.79 ± 10.78</td>
</tr>
<tr>
<td>Total cholesterol:HDL</td>
<td>4.77 ± 0.25</td>
<td>4.50 ± 0.23</td>
<td>4.13 ± 0.21</td>
<td>4.21 ± 0.23</td>
</tr>
<tr>
<td>VLDL-C (mg/dl)</td>
<td>48.00 ± 4.59</td>
<td>44.93 ± 4.16</td>
<td>40.75 ± 3.40</td>
<td>39.21 ± 3.24</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>105.43 ± 5.53</td>
<td>92.78 ± 4.02</td>
<td>99.25 ± 5.47</td>
<td>98.67 ± 4.27</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>32.87 ± 1.69</td>
<td>32.85 ± 1.85</td>
<td>36.88 ± 2.20</td>
<td>38.21 ± 2.13</td>
</tr>
<tr>
<td>LDL-C:HDL-C</td>
<td>3.41 ± 0.23</td>
<td>3.03 ± 0.20</td>
<td>2.88 ± 0.20</td>
<td>2.74 ± 0.17</td>
</tr>
<tr>
<td>VLDL-TG (mg/dl)</td>
<td>135.83 ± 15.37</td>
<td>120.18 ± 16.99</td>
<td>87.17 ± 10.47</td>
<td>82.33 ± 8.07</td>
</tr>
<tr>
<td>LDL-TG (mg/dl)</td>
<td>33.47 ± 1.70</td>
<td>29.15 ± 1.39</td>
<td>29.42 ± 2.01</td>
<td>28.17 ± 1.55</td>
</tr>
<tr>
<td>HDL-TG (mg/dl)</td>
<td>12.13 ± 0.43</td>
<td>12.04 ± 0.49</td>
<td>11.79 ± 0.80</td>
<td>11.62 ± 0.59</td>
</tr>
</tbody>
</table>

*To convert from mg/dl to mmol/l, divide cholesterol values by 38.67 and triglyceride values by 88.54.

Note: Data are means ± SEM
SEM = standard error of the mean
### Table 12. Summary Statistics for Plasma Lipids

<table>
<thead>
<tr>
<th>Variable</th>
<th>Week</th>
<th>Almond LSM (SE) (n = 28)</th>
<th>Carbohydrate (CHO) LSM (SE) (n = 29)</th>
<th>% change = week 24 - week 0</th>
<th>Absolute change = week 24 - week 0</th>
<th>P value† group x time effect</th>
<th>P value† time effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC (mg/dl)</td>
<td>0</td>
<td>198.25 (8.11)</td>
<td>212.70 (8.26)</td>
<td>-11.66</td>
<td>-23.12 -14.84</td>
<td>0.8630</td>
<td>&lt;.0001*</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>175.13 (8.63)</td>
<td>197.86 (8.50)</td>
<td></td>
<td></td>
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<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>0</td>
<td>45.29 (1.39)</td>
<td>45.73 (1.40)</td>
<td>+0.24</td>
<td>+8.62 +3.94</td>
<td>0.4983</td>
<td>0.7848</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>45.40 (1.44)</td>
<td>49.67 (1.28)</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>0</td>
<td>192.07 (18.44)</td>
<td>189.93 (18.78)</td>
<td>-31.10</td>
<td>-59.74 -36.77</td>
<td>0.5672</td>
<td>0.0024*</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>132.33 (19.85)</td>
<td>153.16 (19.46)</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC:HDL</td>
<td>0</td>
<td>4.65 (0.22)</td>
<td>4.77 (0.23)</td>
<td>-11.83</td>
<td>-5.56 -5.6</td>
<td>0.7075</td>
<td>0.0022*</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>4.10 (0.25)</td>
<td>4.21 (0.25)</td>
<td></td>
<td></td>
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<td></td>
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<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VLDL-C (mg/dl)</td>
<td>0</td>
<td>43.36 (3.76)</td>
<td>45.52 (3.70)</td>
<td>-22.86</td>
<td>-9.16 -4.17</td>
<td>0.4525</td>
<td>0.0386*</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>33.45 (4.03)</td>
<td>41.35 (3.91)</td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>0</td>
<td>98.18 (5.16)</td>
<td>105.38 (5.07)</td>
<td>-13.96</td>
<td>-13.71 -10.38</td>
<td>0.7758</td>
<td>0.0001*</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>84.47 (5.55)</td>
<td>95.00 (5.37)</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>0</td>
<td>32.17 (1.52)</td>
<td>33.41 (1.48)</td>
<td>-3.11</td>
<td>+11.28 +3.77</td>
<td>0.1836</td>
<td>0.2004</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>31.17 (1.57)</td>
<td>37.18 (1.40)</td>
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<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDL-C: HDL-C</td>
<td>0</td>
<td>3.24 (0.20)</td>
<td>3.35 (0.19)</td>
<td>-10.19</td>
<td>-18.21 -0.33</td>
<td>0.7934</td>
<td>0.0268*</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>2.91 (0.22)</td>
<td>2.74 (0.21)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VLDL-TG (mg/dl)</td>
<td>0</td>
<td>129.14 (14.38)</td>
<td>126.07 (14.13)</td>
<td>-36.14</td>
<td>-23.46 -29.58</td>
<td>0.5203</td>
<td>0.0028*</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>82.47 (15.50)</td>
<td>96.49 (15.02)</td>
<td></td>
<td></td>
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</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDL-TG (mg/dl)</td>
<td>0</td>
<td>30.00 (1.67)</td>
<td>33.41 (1.64)</td>
<td>-16.33</td>
<td>-14.82 -4.95</td>
<td>0.9135</td>
<td>0.0003*</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>25.0 (1.79)</td>
<td>28.46 (1.73)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL-TG (mg/dl)</td>
<td>0</td>
<td>11.25 (0.55)</td>
<td>12.03 (0.54)</td>
<td>-9.87</td>
<td>-3.57 -1.11</td>
<td>0.9070</td>
<td>0.5140</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>10.14 (0.62)</td>
<td>11.60 (0.59)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

† All outcome variables were adjusted for their baseline values in the models. P values are based on mixed models with an autoregressive covariance structure of order one, with all time points (weeks 0, 8, 16 and 24) included in the model. 

*Indicates statistically significant difference between groups. LSM = least squares mean; SE = standard error.
Table 13. Change in Blood Pressure from Week 0 to Week 24 in the Almond and Carbohydrate (CHO) Group

<table>
<thead>
<tr>
<th></th>
<th>Almond group</th>
<th></th>
<th>CHO group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 0 (n = 28)</td>
<td>Week 24 (n = 22)</td>
<td>Week 0 (n = 29)</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>144.14 ± 3.24</td>
<td>130.2 ± 4.78†‡</td>
<td>135.55 ± 3.72</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>77.93 ± 2.02</td>
<td>71.67 ± 2.30†</td>
<td>77.11 ± 2.66</td>
</tr>
</tbody>
</table>

†p< 0.05, for the contrasts from week 0 to 24 for the changes over time within groups  
‡p< 0.05, for the contrasts from week 0 to 24 for the changes over time between groups  
Note: Data are means ± SEM  
SEM = standard error of the mean
Table 14. Summary Statistics for Blood Pressure

<table>
<thead>
<tr>
<th>Variable</th>
<th>Week</th>
<th>Almond LSM (SE)</th>
<th>Carbohydrate (CHO) LSM (SE)</th>
<th>% change = week 24 - week 0</th>
<th>Absolute change = week 24 - week 0</th>
<th>P value† group x time effect</th>
<th>P value† time effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>0</td>
<td>144.80 (3.44)</td>
<td>134.94 (3.47)</td>
<td>-10.27</td>
<td>-14.87 -0.97</td>
<td>0.2705</td>
<td>0.0506*</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>129.93 (4.13)</td>
<td>133.97 (3.90)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>0</td>
<td>78.01 (1.86)</td>
<td>76.83 (1.88)</td>
<td>-8.15</td>
<td>-6.36 -6.04</td>
<td>0.4183</td>
<td>0.0371*</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>71.65 (2.36)</td>
<td>70.79 (2.19)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

† All outcome variables were adjusted for their baseline values in the models. P values are based on mixed models with an autoregressive covariance structure of order one, with all time points (weeks 0, 8, 16 and 24) included in the model.

*Indicates statistically significant difference between groups.

LSM = least squares means

SE = standard error
Table 15. Summary of Results of Questionnaire Scores Related to Satiety

Question #1: How strong is your wish to eat? (0 = Very weak; 10 = Very strong)

<table>
<thead>
<tr>
<th>1 to 2 hours after:</th>
<th>Breakfast</th>
<th>Lunch</th>
<th>Dinner</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group x time effect:</td>
<td>P value = 0.6412</td>
<td>P value = 0.7689</td>
<td>P value = 0.4044</td>
</tr>
<tr>
<td>Group</td>
<td>Week</td>
<td>LSM ± SE</td>
<td>LSM ± SE</td>
</tr>
<tr>
<td>Almond</td>
<td>0</td>
<td>4.7 ± 0.6</td>
<td>3.7 ± 0.5</td>
</tr>
<tr>
<td>Almond</td>
<td>8</td>
<td>3.8 ± 0.6</td>
<td>3.9 ± 0.5</td>
</tr>
<tr>
<td>Almond</td>
<td>16</td>
<td>3.9 ± 0.6</td>
<td>4.2 ± 0.6</td>
</tr>
<tr>
<td>Almond</td>
<td>24</td>
<td>3.9 ± 0.6</td>
<td>4.3 ± 0.6</td>
</tr>
<tr>
<td>CHO</td>
<td>0</td>
<td>3.9 ± 0.6</td>
<td>3.2 ± 0.5</td>
</tr>
<tr>
<td>CHO</td>
<td>8</td>
<td>4.3 ± 0.6</td>
<td>3.6 ± 0.6</td>
</tr>
<tr>
<td>CHO</td>
<td>16</td>
<td>3.4 ± 0.6</td>
<td>3.3 ± 0.6</td>
</tr>
<tr>
<td>CHO</td>
<td>24</td>
<td>3.6 ± 0.6</td>
<td>3.1 ± 0.5</td>
</tr>
</tbody>
</table>

Question #2: How hungry do you feel? (0 = Not hungry at all; 10 = Hungry as I have ever felt)

<table>
<thead>
<tr>
<th>1 to 2 hours after:</th>
<th>Breakfast</th>
<th>Lunch</th>
<th>Dinner</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group x time effect:</td>
<td>P value =0.3310</td>
<td>P value = 0.8053</td>
<td>P value = 0.5562</td>
</tr>
<tr>
<td>Group</td>
<td>Week</td>
<td>LSM ± SE</td>
<td>LSM ± SE</td>
</tr>
<tr>
<td>Almond</td>
<td>0</td>
<td>4.6 ± 0.6</td>
<td>3.7 ± 0.5</td>
</tr>
<tr>
<td>Almond</td>
<td>8</td>
<td>3.4 ± 0.6</td>
<td>3.4 ± 0.5</td>
</tr>
<tr>
<td>Almond</td>
<td>16</td>
<td>3.5 ± 0.6</td>
<td>4.2 ± 0.6</td>
</tr>
<tr>
<td>Almond</td>
<td>24</td>
<td>4.2 ± 0.6</td>
<td>3.9 ± 0.6</td>
</tr>
<tr>
<td>CHO</td>
<td>0</td>
<td>3.3 ± 0.6</td>
<td>2.8 ± 0.5</td>
</tr>
<tr>
<td>CHO</td>
<td>8</td>
<td>4.0 ± 0.6</td>
<td>3.4 ± 0.6</td>
</tr>
<tr>
<td>CHO</td>
<td>16</td>
<td>3.3 ± 0.6</td>
<td>3.4 ± 0.6</td>
</tr>
<tr>
<td>CHO</td>
<td>24</td>
<td>3.6 ± 0.6</td>
<td>3.3 ± 0.5</td>
</tr>
</tbody>
</table>
Table 15 (continued). Summary of Results of Questionnaire Scores Related to Satiety Question #3: How full do you feel? (0 = Not full at all; 10 = Very full)

<table>
<thead>
<tr>
<th>Group x time effect:</th>
<th>1 to 2 hours after:</th>
<th>Breakfast</th>
<th>Lunch</th>
<th>Dinner</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week</td>
<td>LSM ± SE</td>
<td>P value</td>
<td>LSM ± SE</td>
</tr>
<tr>
<td>Almond</td>
<td>0</td>
<td>5.1 ± 0.5</td>
<td>0.9150</td>
<td>6.0 ± 0.5</td>
</tr>
<tr>
<td>Almond</td>
<td>8</td>
<td>5.7 ± 0.5</td>
<td></td>
<td>5.7 ± 0.5</td>
</tr>
<tr>
<td>Almond</td>
<td>16</td>
<td>4.7 ± 0.6</td>
<td></td>
<td>5.2 ± 0.6</td>
</tr>
<tr>
<td>Almond</td>
<td>24</td>
<td>4.5 ± 0.6</td>
<td></td>
<td>4.8 ± 0.6</td>
</tr>
<tr>
<td>CHO</td>
<td>0</td>
<td>5.8 ± 0.6</td>
<td></td>
<td>5.7 ± 0.5</td>
</tr>
<tr>
<td>CHO</td>
<td>8</td>
<td>6.2 ± 0.6</td>
<td></td>
<td>6.6 ± 0.6</td>
</tr>
<tr>
<td>CHO</td>
<td>16</td>
<td>5.5 ± 0.6</td>
<td></td>
<td>7.0 ± 0.6</td>
</tr>
<tr>
<td>CHO</td>
<td>24</td>
<td>5.8 ± 0.5</td>
<td></td>
<td>6.7 ± 0.5</td>
</tr>
</tbody>
</table>

CHO = carbohydrate  
LSM = least squares mean  
SE = standard error
Table 16. Summary of Results of Questionnaire Scores Related to Satisfaction

**Question #4:** How satisfied are you with the texture of the diet? (0 = Not satisfied at all; 10 = Very satisfied)

<table>
<thead>
<tr>
<th>1 to 2 hours after:</th>
<th>Breakfast</th>
<th>Lunch</th>
<th>Dinner</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group x time effect:</td>
<td>P value = 0.5420</td>
<td>P value = 0.5371</td>
<td>P value = 0.1640</td>
</tr>
<tr>
<td>Group</td>
<td>Time</td>
<td>LSM ± SE</td>
<td>LSM ± SE</td>
</tr>
<tr>
<td>Almond</td>
<td>0</td>
<td>6.7 ± 0.6</td>
<td>6.7 ± 0.5</td>
</tr>
<tr>
<td>Almond</td>
<td>8</td>
<td>6.5 ± 0.5</td>
<td>6.6 ± 0.5</td>
</tr>
<tr>
<td>Almond</td>
<td>16</td>
<td>6.9 ± 0.6</td>
<td>7.1 ± 0.5</td>
</tr>
<tr>
<td>Almond</td>
<td>24</td>
<td>6.4 ± 0.6</td>
<td>6.8 ± 0.5</td>
</tr>
<tr>
<td>CHO</td>
<td>0</td>
<td>7.1 ± 0.6</td>
<td>7.8 ± 0.5</td>
</tr>
<tr>
<td>CHO</td>
<td>8</td>
<td>6.4 ± 0.6</td>
<td>7.7 ± 0.5</td>
</tr>
<tr>
<td>CHO</td>
<td>16</td>
<td>7.1 ± 0.6</td>
<td>7.1 ± 0.5</td>
</tr>
<tr>
<td>CHO</td>
<td>24</td>
<td>7.8 ± 0.6</td>
<td>7.8 ± 0.5</td>
</tr>
</tbody>
</table>

**Question #5:** How satisfied are you with the taste of the diet? (0 = Not satisfied at all; 10 = Very satisfied)

<table>
<thead>
<tr>
<th>1 to 2 hours after:</th>
<th>Breakfast</th>
<th>Lunch</th>
<th>Dinner</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group x time effect:</td>
<td>P value = 0.2184</td>
<td>P value = 0.3789</td>
<td>P value = 0.2779</td>
</tr>
<tr>
<td>Group</td>
<td>Time</td>
<td>LSM ± SE</td>
<td>LSM ± SE</td>
</tr>
<tr>
<td>Almond</td>
<td>0</td>
<td>7.0 ± 0.5</td>
<td>6.9 ± 0.5</td>
</tr>
<tr>
<td>Almond</td>
<td>8</td>
<td>7.5 ± 0.4</td>
<td>7.6 ± 0.4</td>
</tr>
<tr>
<td>Almond</td>
<td>16</td>
<td>7.9 ± 0.5</td>
<td>7.5 ± 0.5</td>
</tr>
<tr>
<td>Almond</td>
<td>24</td>
<td>7.5 ± 0.5</td>
<td>7.4 ± 0.5</td>
</tr>
<tr>
<td>CHO</td>
<td>0</td>
<td>8.2 ± 0.5</td>
<td>8.1 ± 0.4</td>
</tr>
<tr>
<td>CHO</td>
<td>8</td>
<td>7.5 ± 0.5</td>
<td>7.7 ± 0.5</td>
</tr>
<tr>
<td>CHO</td>
<td>16</td>
<td>7.5 ± 0.5</td>
<td>7.5 ± 0.5</td>
</tr>
<tr>
<td>CHO</td>
<td>24</td>
<td>8.4 ± 0.5</td>
<td>8.0 ± 0.4</td>
</tr>
</tbody>
</table>

CHO = carbohydrate; LSM = least squares mean; SE = standard error
A. Anthropometric Parameters

This randomized study found that patients consuming almonds experienced a statistically significant reduction in weight, BMI, waist circumference and fat mass (Figures 2-5, respectively) than patients consuming complex carbohydrates (CHO) during participation in the 24-week D & CVRRP. A 75% greater reduction in weight, (-18% vs. -10%), 65% greater reduction in BMI (-18% vs. -11%), 52% greater reduction in waist circumference (-14% vs. -9%), and 56% greater reduction in fat mass were observed in the almond group as compared to the CHO group. This result is inconsistent with those obtained in previous studies which found equivalent weight loss using a moderate-fat versus low-fat hypocaloric diet for weight loss in overweight adults (Golay et al., 1996; McManus, Antinoro, & Sacks, 2001). Moderate weight loss of 5 to 10% has been shown to provide benefits for obese patients by amelioration of concurrent disease states, i.e. hypertension, diabetes, dyslipidemia (Blundell, Lawton, Cotton & Macdiarmid, 1996). In this study, seventy-three percent of the patients in the almond group achieved at least a 10% weight loss, compared to only 54% in the CHO group.

In light of the group imbalance of patients with a diagnosis of hypertension at baseline, it may have been possible that diuretic effects influenced the difference noted in the weight loss that was observed between the groups. An investigation into the total body water content between the groups from the bioelectrical impedance analysis results failed to support this theory.
B. Cardiovascular Risk Factors

In the context of diminishing cardiovascular risk, the decrease in BMI, waist circumference and fat mass were more pronounced in the almond group as compared to the CHO group. Additionally, both groups experienced improved blood pressure control over time. There were a higher percentage of patients in the CHO group categorized as having hypertension at baseline. A greater reduction in systolic blood pressure was observed in the almond group, however there was no statistically significant difference in either systolic or diastolic blood pressure between the groups. Patients in both groups experienced reductions in their anti-hypertensive medications during the 24-week study. Thus, it is possible that the smaller reduction in systolic blood pressure noted within the CHO group may have been a reflection that the patients in the CHO group were on anti-hypertensive medications that led to better blood pressure control at baseline.

Some investigators have found favorable changes in lipids induced by weight reduction (Nicklas et al., 1997; Cordero-MacIntyre et al., 2000), whereas others have found no effects or deterioration in lipid indices (Weinsier et al., 1992), with the most variable lipid response being HDL-C. Our study was unable to demonstrate a statistically significant difference between the groups for any lipid parameter that was measured. Longitudinal research studies performed among the obese free-living population are challenged by the inability to control variables that impact plasma lipid concentrations, i.e. energy restriction itself, exercise and biologic/phenotype variability. Others have shown that HDL-C decreases during active weight loss but increases after the termination of dieting when body weight stabilizes (Datillo & Kris-Etherton, 1992;
Johnson et al., 1982). During acute weight reduction, tissue concentrations of lipoprotein lipase have been found to decrease by 50% to 80% (Taskinen & Nikkila, 1979), resulting in a reduction in TG-rich lipoprotein synthesis, impaired VLDL-C catabolism, and a diminished transfer of lipids to HDL-C with resulting decreased HDL-C concentrations.

Zambon et al. (1999) found that LDL-C decreased significantly in a group of obese adults consuming hypocaloric diets enriched in oleic acid (MUFA) and complex carbohydrates. However, with an equal magnitude of weight loss, HDL-C increased significantly in the high MUFA group and a decreased level was observed in the complex carbohydrate group (Zambon et al., 1999). Our study found both groups had a statistically significant decrease in LDL-C over time (Figure 6), however as noted above, no statistically significant difference was found between the groups. It is worth noting that the almond group experienced a 56% greater reduction in TC (-12% vs. 7%), 32% greater reduction in LDL-C (-14% vs. 10%), and a 62% greater reduction in TG (-31% vs. 19%). However, with an unequal magnitude of weight loss, HDL-C increased in the CHO group and decreased in the almond group (Figure 7). In light of the lesser reduction in TC, LDL-C and TG within the CHO group, there may have be a compensatory need to upregulate HDL-C synthesis.

Diminishing cardiovascular risk occurred in both groups. A 12% reduction in the TC:HDL ratio was observed in both groups (Figure 8). The CHO group demonstrated an increase in HDL-C at week 24 from baseline, possibly as an outcome of the lower magnitude of weight reduction or the achievement of weight stability approaching week 24. Participants in the almond group were found to have greater
decreases in TC, VLDL-C, LDL-C, and LDL-TG in the presence of a higher magnitude of weight loss, whereas research participants in the CHO group showed a greater decrease in HDL-C and HDL-TG. Again, no statistically significant difference was found between the groups in any of the aforementioned lipid parameters.

C. Further Comment

The unprecedented difference in weight loss and anthropometric parameters was not predicted due to the study design featuring a matched protein and total calorie intake. Neither gender, presence of diabetes mellitus nor hypertension were found to influence the difference in degree of weight loss found between the two groups.

The development of statistically significantly higher ketone levels among the almond group participants is consistent with the nature of very low calorie diets that feature high levels of protein and fat but little carbohydrate. The increased production of ketones (acetoacetic acid and 3-beta-hydroxybutyric acid) may have exceeded the levels in which the tissues could oxidize the fat intermediates as an energy source. Thus some calories could have been lost through ketonuria. Additionally, the absorption of the fat within the almond may be compromised by the fiber content of the nut. The 84 grams of almonds supplied 485 kcal of energy daily, of which 387 calories are derived from fat. Others have anecdotally noted that unexpected weight loss occurs in controlled feeding trials featuring the inclusion of almonds under isocaloric conditions (Sabaté, Communication). Thus, there may have been an imbalance of total utilisable calories between the groups.

The change in the hormonal milieu that occurs in the presence of high fat diets may have favorably improved the utilization of fat as an energy source versus
enhancement of adiposity. Others have found a greater improvement of fasting plasma glucose, insulin and IGR using a low carbohydrate diet enhanced with monounsaturated fat (Golay et al., 1996). Similarly, the almond group demonstrated a 69% greater decrease of plasma insulin (-58% vs. 29%), a 42% greater decrease of glucose (-20% vs. -14%) and a 60% greater decrease in the IGR (-50% vs. -30%) than the CHO group (Figures 9-11, respectively). Thus there may have been effects on how muscle and fat cells uptake fat in the context of a high fat diet, and the rate at which they burn more fat with resulting higher ketone levels. Or, perhaps the almond as a source of oleic acid may contribute to improved beta-cell efficiency.

Among the patients with diabetes mellitus in both groups, HgbA1c levels favorably decreased over time. However, no statistically significant difference was found between the groups. Diabetic patients in both groups experienced dramatic reductions, if not elimination, of their diabetes medications during the 24-week study. It is important to note that the greater reduction in insulin and glucose levels observed in the almond group compared to the CHO group did not correlate with the magnitude of change in HgbA1c in the almond as compared to the CHO group (-15% vs. -12%). Thus, it is possible that the diabetic patients in the almond group were weaned off of their diabetes medications at a faster rate than the CHO group, thus conferring a degree of clinical relevance.

Previous research has shown preferential use and oxidation of carbohydrate after a meal and the ability of carbohydrate to stimulate satiety more than fat (Blundell et al., 1996; Mayer, 1972). The macronutrient mix and the higher fiber intake consumed by research participants in the CHO group should have theoretically produced a higher
level of satiety. In the present study, self-reported hunger 1 to 2 hours after meals using a 10-point scale did not significantly differ between or within the groups at baseline or at weeks 8, 16 and 24. High carbohydrate intakes may affect metabolic rate and energy expenditure via the thermic effect of food to a greater degree than fat under isocaloric conditions, but it is unknown to what degree these factors influence satiety and compliance in the obese under hypocaloric conditions.

The mechanisms leading to overeating and to the obese state are multifactorial and occur over prolonged periods of time. The preponderance of previous experimental studies evaluating fuel oxidation, satiety and overeating have featured only short study periods (Willett, 1998). However, these studies universally promote the intake of low energy-dense foods over high energy-dense foods containing fat. Translation of these research findings into the complexities of food consumption patterns have been challenged by this study’s findings. The habitual underreporting of food intake among the obese population has been well documented. The obese patient receiving guidelines on how to select appropriate portion sizes of complex carbohydrates may be subconsciously translated into an unintended surplus of calories. The types of complex carbohydrates that are available at restaurants, fast food chains or in the patient’s social environment, and their ability to prepare them according to specific guidelines, may be impractical for public health focused clinicians to expect from patients over a prolonged period of time. In this study, it appears that the critical influence on total calorie intake was not the metabolic differences between macronutrients or malabsorption, but perhaps hormonal, environmental and behavioral factors.
One of the primary complaints among obese patients participating in medically supervised weight reduction programs that incorporate a liquid protein sparing formulation is the lack of chewing. Thus, motivation and compliance are difficult to sustain in long-term weight reduction programs and research studies. In this study, research participants were encouraged to consume “crunchy” complex carbohydrates, i.e. popcorn, whole wheat crackers, etc. to parallel the chewing experiences of the almond consumers.

Upon weekly questioning, patients reported satisfaction with their randomized group assignments and food options. Research participants in the almond group enjoyed the convenience of the almonds. Questions #1 and #2 on the questionnaire led to unequivocal results. However, in response to question #3 “How full do you feel”, borderline significance was found after lunch and after dinner between the two groups over time. It appeared that the almonds were losing some of their influence on satiety over time, whereas the CHO group was reporting an increasing sense of fullness over time. It can be speculated that the statistically significant difference in the magnitude of weight loss between the groups over time may have altered plasma leptin levels which could influence satiety, or perhaps the compliance among the CHO group may have diminished over time leading to larger than prescribed portion sizes of complex carbohydrates and/or intake of non-prescribed high protein foods, i.e., meat, fish, poultry. Participants in the CHO group reported satisfaction with their variety of food choices for meal and snack planning. Although there were two varieties of almonds with equivalent nutrient values (dry roasted and raw) available to reduce monotony,
over 80% of the almond group participants developed a strong preference for dry roasted almonds within two weeks of study participation.

The dietary protocol featured non-traditional weight reduction foods, i.e. popcorn, almonds, hence the foods may have been spared from monotony effects. Others (Miller et al., 2000) have found chocolate and potato chips, representing highly palatable, high-fat, energy dense snacks to be resistant to monotony effects over several weeks of daily consumption. Similarly, we found that the daily consumption of almonds and complex carbohydrates led to successful weight loss in both groups, with no dropouts in either group due to discontent with their random group assignment.

D. Limitations

The study was limited by relying on the self-reported daily food intake and exercise in the diaries of the study participants. Self-reports of dietary intakes and lifestyle behaviors may have been influenced through researcher expectancies and social desirability.

Possible confounding may have occurred due to the specific vitamin, mineral and phytochemical content in complex carbohydrates versus almonds. However, both groups were given the same wash out and run-in period of vitamin/mineral supplementation to minimize these effects.

Additional study limitations included representation, compensatory rivalry and resentful demoralization. The ethnic and socioeconomic mixture in the capture area of COH may not be representative of the general adult obese population throughout the US. Also, study participants were in contact with each other during weekly clinic visits and group classes, hence discussed differences in dietary interventions. Participants
receiving “less desirable” treatments may have been motivated to increase their weight loss effort. Resentful demoralization of participants receiving “less desirable” treatments could have played out in the weekly educational sessions or clinic visits, and these participants may have lost motivation. However, the investigator was in attendance at weekly clinic visits and group classes to provide ongoing encouragement to provide a sense of neutrality among the participants.

Lastly, the study explored the effects of only one type of tree nut versus a mixed variety of tree nuts, in a small sample size. Additional studies are warranted with larger sample sizes and using a variety of mixed tree nuts.
Figure 2. Change in Weight Over Time in the Two Study Groups (Note: Data are presented as least squares means and 95% CI)
Figure 3. Change in Body Mass Index Over Time in the Two Study Groups (Note: Data are presented as least squares means and 95% CI)

Time Effect p-value = .0001
Group Over Time Effect p-value = .0001
Figure 4. Change in Waist Circumference Over Time in the Two Study Groups (Note: Data are presented as least squares means and 95% CI)

Time Effect p-value = .0001
Group Over Time Effect p-value = .0255
Figure 5. Change in Fat Mass Over Time in the Two Study Groups  (Note: Data are presented as least squares means and 95% CI)
Figure 6. Change in Low Density Lipoprotein Cholesterol (LDL-C) Over Time in the Two Study Groups (Note: Data are presented as least squares means and 95% CI)
Figure 7. Change in High Density Lipoprotein Cholesterol (HDL-C) Over Time in the Two Study Groups (Note: Data are presented as least squares means and 95% CI)
Figure 8. Change in Total Cholesterol:High Density Lipoprotein (Total Chol:HDL) Ratio Over Time in the Two Study Groups (Note: Data are presented as least squares means and 95% CI)
Figure 9. Change in Insulin Over Time in the Two Study Groups (Note: Data are presented as least squares means and 95% CI)

Time Effect p-value = .0001
Group Over Time Effect p-value = .5966
Figure 10. Change in Glucose Over Time in the Two Study Groups (Note: Data are presented as least squares means and 95% CI)
Figure 11. Change in Insulin:Glucose Ratio Over Time in the Two Study Groups (Note: Data are presented as least squares means and 95% CI)
CHAPTER 6
SUMMARY AND CONCLUSIONS

A. Summary

This randomized study found that patients consuming almonds experienced a statistically significant reduction in weight, BMI, waist circumference and fat mass than patients consuming complex carbohydrates (CHO) during participation in the 24-week D & CVRRP. Seventy-three percent of the patients in the almond group achieved at least a 10% weight loss, compared to only 54% in the CHO group.

Improvement in metabolic parameters linked to insulin sensitivity was found over time in both groups and decreased HgbA1c levels were observed. The disparity between the increased insulin sensitivity observed in the almond group as measured by the IGR, and the similar degree of decrease in HgbA1c in both groups, leads one to speculate on the possible differences between the groups in the rates of weaning of patients from their pre-study diabetes medications.

An overall improvement in cardiovascular risk was found in both groups, however, the longitudinal study design in combination with the small sample size was unable to detect a statistically significant difference between the groups on any of the lipid parameters that were measured. Both groups experienced improved blood pressure control over time. Although there was no statistically significant difference in blood pressure reduction between the groups, anti-hypertensive medications were greatly reduced or eliminated in patients in both groups during the 24-week study. Further, this study found both groups had a statistically significant decrease in TC, LDL-C and TG over time, with greater reductions observed in the almond group.
However, with an unequal magnitude of weight loss, HDL-C increased 11% in the CHO group and decreased 3% in the almond group. The degree of improvement in the LDL-C:HDL-C was not significantly different between the groups, and both groups were in the direction of diminishing cardiovascular risk. Thus, the lower magnitude of weight reduction observed in the CHO group or the achievement of weight stability may have contributed to the overall increase in HDL-C. The lack of increase in HDL-C in the almond group as compared to the CHO group may not have any overall adverse metabolic effects in the context of an equivalent diminished lipid burden.

B. Implications of Findings

From a practical public health point of view, if clinicians are capable of sustaining motivation and adherence within their obese sector, a successful diet plan could incorporate moderate amounts of monounsaturated fat as long as anthropometric change favorably and plasma lipids do not deteriorate. Our findings support the use of almonds, as a source of MUFA, as a component of a hypocaloric diet in obese patients, as an alternative to conventional low-fat dietary foods. An almond-enriched hypocaloric diet may have unique beneficial effects compared with the conventional high carbohydrate low-fat diet approach for the treatment of the atherogenic metabolic risk profile (Krauss, 2001), and may prevent the conversion to more deleterious LDL-C subclasses. The trading of carbohydrates for MUFAs during weight reduction may be a consideration in future public health recommendations for the obese population. Many obese people enrolled in medically supervised weight control programs may find that the ingestion of almonds may be more acceptable than severe restriction of dietary fat over long periods of time. However, these results call for further study in larger
numbers of subjects and using a variety of nuts. The use of almonds or mixed nuts to prevent the recidivism after successful weight reduction also deserves consideration, as we must keep in sight the potential detrimental effects associated with weight cycling (Prentice et al., 1992; Williamson, 1996).

C. Future Research

Additional testing on plasma to evaluate changes in apolipoproteins A-1, B-100 and E, Lp(a), PAI-1-1, C-Peptide, nitric oxide (NO), magnesium and leptin would be valuable to complement and strengthen the study’s findings. Following is a summary of the justification for analytical tests that can help elucidate further benefits of almond and complex carbohydrate enriched diets during medically supervised weight reduction.

1. Plasma Free Fatty Acids

The evaluation of fasting plasma free fatty acids is imperative to help explain the differences in HgbA1c levels, glucose, and insulin levels among the research participants with type 2 diabetes mellitus and/or metabolic syndrome. Insulin resistance, hepatic glucose production and abnormal lipid production in the liver are sensitive to fasting plasma free fatty acids and evolution of the metabolic syndrome (Reaven, 1995). The adipocyte is a critical mediator between insulin and liver glucose output, and evidence that free fatty acids suppress skeletal muscle glucose uptake and insulin secretion from the beta cell supports the overall central role of the adipocyte in the regulation of glycemia (Bergman & Mittleman, 1998).

2. Apo B-100 and LDL Subclassification

Apo B-100 is the major apoprotein found within VLDL, intermediate density lipoproteins (IDL) and LDL particles, which to a great extent controls the
homeostasis of plasma LDL-C (Vrablick, Ceska, & Horinek, 2001). The atherogenic lipoprotein phenotype (ALP) is characterized by a normal cholesterol concentration with a moderate increase in plasma TG, a decrease in HDL-C, and a shift in the LDL size profile towards small dense species (Packard et al., 2000). Small dense LDL has properties that enhance its atherogenicity (De Graaf et al., 1991 & Krauss, 1994). At a given cholesterol level, large numbers of small Apo B-100-containing lipoproteins (subclass pattern B) are more atherogenic than lower numbers of large apo B-100-containing lipoproteins (subclass pattern A) (Veniant et al., 2000 & Packard et al., 2000). Due to the significantly different fat content of the dietary interventions, it would be important to investigate if the research participants in the almond group starting with subclass pattern A remained subclass pattern A, and that the research participants in the CHO group starting with subclass pattern A converted to subclass B during weight reduction.

3. Apo A-I

Apo A-I, the principle apoprotein of plasma HDL, is the major apoprotein of mesenteric lymph chylomicrons and is actively synthesized by the small intestine during lipid absorption (Davidson, Magun, Brasitus & Glickman, 1987). The ratio of apo A-1:apo B-100 is often considered the gold standard which reflects risk of developing atherosclerosis.

It would be of interest to have apo A-1 measurements performed to elucidate the mechanism by which almonds blunt the drop in HDL-C typically seen during weight reduction. It may not be deleterious to show a small reduction in HDL-C among almond consumers, in contrast to the complex carbohydrate consumers, if
there is an acceptable apo A-1:apo B-100 ratio in parallel with a greater reduction in fat mass which occurred among the almond consumers.

4. **Apo E**

Apo E is a multifunctional protein that can act as a ligand for lipoprotein receptors (Millar, Lichtenstein, Ordovas, Dolni, Kowoski & Schaefer, 2001). Interaction between genetics and diet exist with regard to apoE production rates. Diets that promote hypertriglyceridemia promote increases in small dense LDL-C particles and reduced synthesis rate of apoE (Pedreno, Hurt-Camejo, Wiklund, Badimon & Masana, 2000). High levels of apo E help clear triglycerides from plasma and inhibit platelet aggregation (Pedreno, Hurt-Camejo, Wiklund, Badimon & Masana, 2000). It would be of great interest to characterize the study population’s relationship of apo E levels with both apo B-100 levels and levels of PAI-1 (see below) to determine the net benefit of using almonds or complex carbohydrates under hypocaloric conditions.

5. **Lp(a)**

Lp(a) is a LDL-like particle that is linked to the development of atherogenesis and thrombosis. LP(a) is a cholesterol ester- and apolipoprotein B-containing particle which differs from LDL by the additional presence of a glycoprotein termed apolipoprotein(a), homologous to plasminogen (Swertfeger, 2001). Lp(a) analysis is necessary to characterize the subjects in each group and to support any antithrombotic findings caused by the consumption of almonds versus genetic influence.
6. Plasminogen Activator Inhibitor-1 (PAI-1)

The metabolic syndrome can include a procoagulant state which is known to contribute to the development of atherosclerosis. Dyslipidemia, along with hyperinsulinemia induces expression of plasminogen activator inhibitor-1 (PAI-1), which contributes to a prothrombotic state (Pandolfi et al., 2001). Others (Lopez-Segura et al., 1996) have shown that isocaloric diets rich in MUFAs decrease PAI-1 plasma activity, which is accompanied by a parallel decrease in plasma insulin levels. Due to the accelerated weight loss among the almond group and improvement in anthropometric parameters, it would be beneficial to characterize any changes in PAI-1 among the study participants.

7. C-Peptide

C-peptide levels serve as a valuable index to insulin secretion. Low C-peptide levels are to be expected where insulin secretion is diminished or suppressed as a normal response to exogenous insulin (Horwitz, Kuzuya & Rubenstein, 1976). To further support the favorable changes in plasma insulin, glucose and IGR, C-peptide is of importance.

8. Nitric Oxide (NO)

Nitric oxide (NO) performs many useful functions, including regulation of blood pressure and intercellular signaling. It would be of interest to characterize the changes in NO due to the different arginine:lysine ratios in the two dietary interventions as nut protein contains preferentially high levels of arginine, a known dietary precursor of NO, and low levels of lysine.
9. *Magnesium*

Almonds possess several known nutrients that are postulated to provide a protective effect against coronary heart disease, including magnesium. Thus, it would be of interest to characterize the effects, if any, of the two dietary interventions on plasma magnesium levels.

10. *Leptin*

High-fat meals reduce 24-hour circulating leptin concentrations under isocaloric diet conditions. Some investigators have previously reported that adipocyte glucose utilization is involved in insulin-induced leptin secretion in vitro. However, there is a nocturnal increase of leptin that is related to the insulin response to meals. High fat meals, which induce smaller insulin and glucose responses, may produce lower leptin concentrations than low fat meals. Consumption of high fat meals result in lowered 24-hour circulating leptin concentrations, which may be a consequence of decreased adipocyte glucose metabolism. It would be of interest to characterize the effects under hypocaloric diet conditions.
REFERENCES


Kiortsis, D.N., Tzotzas, T., Giral, P., Bruckert, E., Beucler, I., Valsamides, S., & Turpin, G. (2001). Changes in lipoprotein(a) levels and hormonal correlations during a


NIH Publication No. 01-3670.


APPENDIX A

REGISTRATION PROCEDURES
Registration Procedures

1. Registration for this study must be made through the Department of Biostatistics office at the City of Hope between the hours of 8:30 a.m. to 4:30 p.m., Monday through Friday (except holidays).

2. Patients must sign a COH approved Informed Consent prior to registration.

3. The investigator must confirm that the patient meets all inclusion and exclusion eligibility criteria.

If the patient qualifies, the investigator will call the Department of Biostatistics (626) 359-8111 ext. 62468 to complete the registration/randomization procedure and receive the patient’s Group assignment and study ID number.
APPENDIX B

DAILY COMPLEX CARBOHYDRATES
Daily Complex Carbohydrates

Select any one of the following combinations on a day-to-day basis

15 Triscuit crackers + 1 ½ cups boiled frozen peas
15 Triscuit crackers + 1 ½ cups boiled frozen corn
15 Triscuit crackers + 6 cups air-popped popcorn
15 Triscuit crackers + 7 ounce baked potato

1 cup boiled rice + 1 ½ cups boiled frozen peas
1 cup boiled rice + 1 ½ cups boiled frozen corn
1 cup boiled rice + 6 cups air-popped popcorn
1 cup boiled rice + 7 ounce baked potato

1 ½ cup boiled pasta + 1 ½ cups boiled frozen peas
1 ½ cup boiled pasta + 1 ½ cups boiled frozen corn
1 ½ cup boiled pasta + 6 cups air-popped popcorn
1 ½ cup boiled pasta + 7 ounce baked potato
APPENDIX C

GENERAL DIETARY GUIDELINES
General Dietary Guidelines

DAILY INTAKE:

HMR 70 Plus Formula: You must take _______ packets of HMR 70 Plus formula per day.

- Taking all formula and vitamin/mineral supplements is imperative to provide enough nutrients to maintain optimal nutritional status. Lean body mass can be depleted without adequate formula and vitamin/mineral supplement intake.
- Prepare formula by using a blender, shaker, whisk or fork. For best results, pour 6 fluid ounces of cold water into a pint container. Empty contents of formula packet into the cold water. Immediately mix with a fork or shake for 15 seconds until product is dissolved. (Do not use blender or shaker with carbonated beverages). If using a blender, leave lid slightly ajar and use quick pulses to minimize foaming. Scrape the sides of the blender to ensure complete use of the product. Be careful not to over blend.
- Refer to the recipes for cold drinks, hot drinks, and slushes provided in the program notebook for added variety.

Salad: You must consume a salad with the following ingredients:
- 2 cups chopped lettuce (any type)
- 1 cup chopped celery
- 1 cup chopped tomato
- “Dressing” may contain 2 Tbsp. Lemon Juice or Vinegar and/or pepper

Fluids: You must take a minimum of two quarts of noncaloric fluids per day in addition to the formula.

- Taking the recommended amount of fluids is essential to prevent potential lightheadedness.
- Noncaloric fluids include water, diet soda, coffee, tea, and beverage mixes that contain artificial sweeteners. Coffee and tea are allowed, although moderation (not more than the equivalent of four cups per day) is recommended to prevent physical reactions such as chronic headaches and irritability.
- Alcoholic beverages are not permitted because of the risk of medical complications.

Complex carbohydrate group research participants must additionally consume 2 teaspoons of safflower oil (to prevent gall bladder dysfunction). Complex carbohydrate intake will be achieved through the intake of a combination of complex carbohydrates. Examples of daily food combinations may be 15 whole wheat crackers (Triscuits™) plus one of the following selections for day-to-day variety: 1 ½ cup peas, 1 ½ cup corn, 6 cups air-popped popcorn or a 7-ounce baked potato.

Almond group research participants must consume the prescribed 84 grams of unsalted, dry-roasted, raw or slivered almonds.
APPENDIX D

DIABETES AND CARDIOVASCULAR RISK REDUCTION (D & CVRR) PROGRAM GENERAL INFORMATION SHEET/CONTRACT
Diabetes and Cardiovascular Risk Reduction (D&CVRR)  
Program General Information Sheet/Contract

This General Information Sheet/Contract must be read completely and signed prior to entrance into the Diabetes and Cardiovascular Risk Reduction Program.

Signature on this General Information Sheet/Contract allows you to attend 8 weeks of the Diabetes & Cardiovascular Risk Reduction Program. At the end of the 8 weeks, you will be re-evaluated for continuation in the program. All rules will be strictly reinforced. Participation in this program is voluntary. No guarantees can be made with respect to the amount of weight loss you will achieve and the degree of improvement of health problems. No guarantees can be made with regards to the duration of permanence of the weight loss.

General Information

To provide individualized care during the program, a thorough medical and psychological examination is required for clearance to participate in the program. The following tests will be scheduled:

Gall bladder Ultrasound  
Stress test  
Lab test

Screening. Appointments will be made by the appointment center by telephone or mail with the specific dates and locations for these appointments.

On the third week of the program, the physician will order an exercise evaluation to be done by the physical therapist. Do Not exercise until you have this evaluation!

Program Duration. You must be willing to commit to the entire 24-month cycle of the program. The program consists of three phases; Phase 1 & 2 extends for 9 months. Phase 3 will begin immediately for 15 months duration.

Initials: _______________ Date: _______________
Witness’s Initial: _______________ Date: _______________
**Diet.** The Protein-Sparing Modified Diet (P.S.M.D.) program for weight reduction is designed for participants who have health problems known to improve with weight loss. Such problems include diabetes mellitus, high blood pressure, coronary artery disease, joint disease, and lung disease.

During the program, participants drink high-protein liquid formulas and one solid meal per day. The dietitian will determine the dietary portion of the program based upon recommendations made by the doctor at the initial evaluation prior to the program.

**Clinic Appointments.** Participants will attend clinic weekly for a brief medical evaluation by the physician.

**Program Schedule.** The D&C VRR Program weekly schedule is as follows:

- Clinic - 3:45 PM - 5:30 PM - Measurement of vital signs, weight, blood ketones and physician visit.
- Classes 6:00 PM - 7:30 PM
- Monthly activity:

Laboratory blood work will be drawn and as necessary
Monthly EKG and as necessary

- Completion of 36 weeks a stress test will be performed

**Classes.** Participants are required to attend weekly classes:

- Nutrition - Provides information to facilitate weight loss and maintenance
- Behavior modification - Enables patient to integrate new behavior into their lifestyle that facilitate weight loss and maintenance
- Exercise - Enable patient to develop a safe, effective personalized exercise plan.

Initials: ______________ Date: ______________

Witness’s Initial: ______________ Date: ______________
Participant’s Responsibilities

To assure the safety of my health, I agree to:

**Attend** all scheduled appointments for the duration of the program.

Absences, three consecutive scheduled weekly visits or seven **non-consecutive visits or portions of visits during the entire program** is grounds for discharge from the program. If you cannot attend a weekly session for any reason, please call the Program Coordinator at (626) 359-8111 ext. 5994.

**Consume** all of the formula/food prescribed on the diet.

**Consume** the prescribed amount of non-caloric fluids (2 quarts a day).

**Take** all medications as prescribed by the physician.

**Avoid** all use of Rezulin and Avandia, alcoholic beverages, illicit drugs, coumadin, antidepressants, tranquilizers, amphetamines, or other non-prescription weight-reducing medications.

**Report** all adverse reactions or side effects to the physician and/or Program Coordinator at the weekly visits.

Side effects can include sensitivity to cold, dry skin, temporary rashes, dizziness, lightheadedness, fatigue, diarrhea or constipation, muscle cramps, bad breath, change in menstrual pattern, temporary hair loss, irregular heart beats, heart palpitations, and/or excessive hunger.

Unusual side effects which occur in rare cases include numbness in the legs, loss of muscle strength in the legs, joint pain (gout) and/or gallstones. Sometimes a participant will find that as they lose weight, they will experience periods of emotional stress related to the actual weight loss. No guarantees can be made regarding the type of side effects one may experience.

Initials: ___________   Date: ___________
Witness’s Initial: ___________   Date: ___________
Special Circumstances

For participants with diabetes mellitus, home blood glucose testing is required. The diabetic participant must test blood glucose one or more times daily. For blood glucose less than 60 or greater than 250, call the Physician or Program Coordinator. The participant also must notify the physician and/or Program Coordinator whenever he/she experiences light-headedness, perspiration, trembling and nervousness. These are signs of hypoglycemia (low blood sugar).

For female participants, the effects of low calorie diet on the fetus are unknown. If a participant suspects that she is pregnant, she must notify the Physician and/or Program Coordinator immediately.

For participants with hypertension, participants must notify the Physician and/or the Program Coordinator if he/she experiences weakness or dizziness when he/she stands upright. These are signs of hypotension (low blood pressure).

I understand the following:

I may discontinue participation in the program at any time by providing the Program Coordinator with verbal or written notification.

During phase 1 & 2 the diagnosis of Diabetes, Hypertension and Hyperlipidemia will be temporarily managed by D&CVRR Program physician.

During phase 3 the medical management of medical problems related to obesity will be the responsibility of my primary physician.

I understand completely the general information regarding the program and agree to comply with the requirements of the City of Hope National Medical Center Diabetes and Cardiovascular risk Reduction Program.

If at any time I cannot meet the requirements, I will inform the Program Coordinator who will arrange for my discharge from the program. I will contact the Program Coordinator or Physicians at (626) 359-8111 ext. 2251 for any questions or problems that I encounter.

Participant’s Name/Signature ______________________________ Date ______________________________

Witness’s Name/Signature ______________________________ Date ______________________________
APPENDIX E

SATIETY AND SATISFACTION QUESTIONNAIRE
As a participant in the research study, we want to assess your overall satisfaction with your prescribed diet. Following is a list of statements regarding your appetite and dietary satisfaction. Please draw a circle at the point on the line to indicate your current feelings.

How strong is your wish to eat?

0----1----2----3----4----5----6----7----8----9----10
Very weak

Very strong

How hungry do you feel?

0----1----2----3----4----5----6----7----8----9----10
Not hungry
at all

Hungry as I have
ever felt

How full do you feel?

0----1----2----3----4----5----6----7----8----9----10
Not full
at all

Very full

How satisfied are you with the texture of the diet?

0----1----2----3----4----5----6----7----8----9----10
Not satisfied
at all

Very satisfied

How satisfied are you with the taste of the diet?

0----1----2----3----4----5----6----7----8----9----10
Not satisfied
at all

Very satisfied

Comments:
Name __________________________ 1-2 hours after lunch
Date __________________________

As a participant in the research study, we want to assess your overall satisfaction with your prescribed diet. Following is a list of statements regarding your appetite and dietary satisfaction. Please draw a circle at the point on the line to indicate your current feelings.

How strong is your wish to eat?

0-----1-----2-----3-----4-----5-----6-----7-----8-----9-----10
Very weak

How hungry do you feel?

0-----1-----2-----3-----4-----5-----6-----7-----8-----9-----10
Not hungry
at all

How full do you feel?

0-----1-----2-----3-----4-----5-----6-----7-----8-----9-----10
Not full
at all

How satisfied are you with the texture of the diet?

0-----1-----2-----3-----4-----5-----6-----7-----8-----9-----10
Not satisfied
at all

How satisfied are you with the taste of the diet?

0-----1-----2-----3-----4-----5-----6-----7-----8-----9-----10
Not satisfied
at all

Comments:
As a participant in the research study, we want to assess your overall satisfaction with your prescribed diet. Following is a list of statements regarding your appetite and dietary satisfaction. Please draw a circle at the point on the line to indicate your current feelings.

How strong is your wish to eat?

0-----1-----2-----3-----4-----5-----6-----7-----8-----9-----10
Very weak                                    Very strong

How hungry do you feel?

0-----1-----2-----3-----4-----5-----6-----7-----8-----9-----10
Not hungry                                  Hungry as I have
at all                                      ever felt

How full do you feel?

0-----1-----2-----3-----4-----5-----6-----7-----8-----9-----10
Not full                                    Very full
at all

How satisfied are you with the texture of the diet?

0-----1-----2-----3-----4-----5-----6-----7-----8-----9-----10
Not satisfied                               Very satisfied
at all

How satisfied are you with the taste of the diet?

0-----1-----2-----3-----4-----5-----6-----7-----8-----9-----10
Not satisfied                               Very satisfied
at all

Comments:
APPENDIX F

UNIVERSITY OF SOUTHERN CALIFORNIA LIPID PROCEDURES
(SEPARATION OF LPQ; HDL ANALYSIS USING
PRECIPITATION METHOD)
SEPARATION OF LPQ (LIPOPROTEIN QUANTIFICATION)

PROCEDURE:

1. Take out ten samples from the rack designated for LPQ analysis.
   
   1.1. Samples should be refrigerated only, not frozen.

2. Number 10 Polycarbonate centrifuge tubes (Beckman - 11 x 34 mm).

3. Put 0.5 ml of each sample into the centrifuge tube.

4. Add 0.5 ml 0.9% saline solution into each tube.
   
   4.1. Preparation of 0.9% saline:
   
   4.1.1. Weigh 9 grams of Sodium Chloride (NaCl) and transfer into
   
   1-liter volumetric flask.
   
   4.1.2. Q.S. to 1000 ml with double deionized (DDI) water. Mix
   
   thoroughly. Stable.

5. Load the ten tubes in the small TL 100.2 rotor.

6. Centrifuge in Beckman TL 100 centrifuge at 65,000 rpm and 5 degrees Centigrade for 24 hours (Program 6).

7. After 24 hours (22 + or - 2 hours is okay), take out the sample from the centrifuge.

8. Take out the bottom half (0.5 ml) of sample and put into another Polycarbonate centrifuge tube.
   
   8.1. With a syringe, remove 0.5 ml of the sample (HDL+ LDL) from the bottom of the tube, taking care to avoid the gel-like precipitate, and holding the syringe plunger steady while lifting it out of the tube to prevent any mixing.
   
   8.2. Empty the syringe into the corresponding centrifuge tube.
   
   8.3. Rinse the syringe 3 times with double de-ionized water.
   
   8.4. Wipe the syringe and plunger with a "Kimwipe" before proceeding to the next sample.

9. Transfer the top half (left over in the centrifuge tube) into a COBAS sample cup labeled "VLDL" with the corresponding ID number.
10. Store the "VLDL" part in the freezer.

11. To the bottom half of the sample (HDL+LDL), add 0.5 ml of 30% Sucrose Solution containing EDTA.

   11.1. Preparation of Sucrose Solution:

   11.1.1. Weigh 50 mg of EDTA and transfer to a beaker. Put about 30 ml of DDI water and mix until dissolved.

   11.1.2. Weigh 30 g of Sucrose and transfer to a 50 ml volumetric flask. Transfer the EDTA mixture into the same flask and Q.S. to 50 ml with DDI water. Stable for 2 weeks.

12. Load the ten tubes in the small TL 100.2 rotor.

13. Centrifuge at 85,000 rpm and 5°C for 24 hours (Program 8).

14. After 24 hours (22 + or -2 hours okay), transfer the bottom half into a COBAS sample cup labeled as "HDL" with the corresponding ID number.

   14.1. With a syringe, remove 0.5 ml of the sample (HDL) from the bottom of the tube, taking care to avoid the gel-like precipitate, and holding the syringe plunger steady while lifting it out of the tube to prevent any mixing.

   14.2 Repeat procedure 8.2 to 8.4.

15. Pour the leftover from the tube (upper half) to a COBAS sample tube labeled as "LDL" with the corresponding ID number.

16. Store the "HDL" and "LDL" cups in the freezer together with the "VLDL" sample cups.
HDL ANALYSIS USING PRECIPITATION METHOD

1. Number the samples you are running.

2. Number plastic microtubes (1.7 ml capacity) you will be using, accordingly.

3. Pipet 100 µl Heparin (ready to use) and 50 µl of 0.6 M MnCl₂ to all the tubes.
   
   3.1. Preparation of 0.6 M MnCl₂:
   
   3.1.1. Weigh out 11.87 g Manganese Chloride (MnCl₂)
   
   and transfer into a 100 ml volumetric flask.
   
   3.1.2. Q.S. to 100 ml with double deionized water. Mix thoroughly.
   
   Stable for 6 months.

4. Pipet 500 µl specimen into the tubes.

5. Close the lid of the tubes tightly and mix vigorously using Vortex mixer or any automatic mixer.

6. Let stand for at least 1 hour at room temperature (2 hours or more if refrigerated).
   
   6.1. Very long incubation may not give valid results.

7. Centrifuge for 15 minutes at 2,000 RPM or at maximum speed using the small Fisher centrifuge.

8. While samples are spinning, put sample cups on COBAS sample rack with the corresponding numbers as the samples.

9. Put about 4-5 drops of the supernatant into the sample cups using disposable transfer pipet.
   
   9.1. Turbid supernate should be repeated using 1:1 dilution with 0.9% saline solution.
   
   9.2. Preparation of 0.9% saline:
   
   9.2.1. Weigh 9 g of Sodium Chloride (NaCl) and transfer into a 1l volumetric flask.
10. Fill up reagent bottle with HDL reagent and put in COBAS reagent rack in the corresponding position.

10.1. Use the Cholesterol reagent (Roche) as HDL reagent.

11. Put 3-4 drops of HDL Standard (Abbott Spectrum - ready to use) and put in the corresponding position in COBAS control rack.

11.1. There are 3 levels of HDL STD. - level 1, level 2 and level 3.

12. Load the sample and reagent racks on the COBAS machine.

12.1. See manual on how to use COBAS.
APPENDIX G

DATA COLLECTION FORMS
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Data Collection Form: Demographics and body composition
Data Collection Form: Weekly weight, ketone and blood pressure

| Week | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 |
|------|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| Weight |   |   |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| Ketone |   |   |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| BPs |   |   |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| BPd |   |   |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
APPENDIX H

ELIGIBILITY CRITERIA
Eligibility Criteria

Inclusion Criteria
- Males, pre-menopausal females and post-menopausal females not receiving hormone replacement therapy are eligible.
- Must be ≥ 130% and ≤ 200% of Ideal Body Weight relative to their height and body frame according to the 1983 Metropolitan Life Insurance Company Height-Weight Table (Appendix X).
- Must undergo a complete history and physical examination performed by a COH endocrinologist or cardiologist and have one or more of the following medical problems: type 2 diabetes mellitus, mild to moderate HTN (treated), mild to moderate dyslipidemia, ischemic heart disease, coronary bypass graft, CHF, chronic obstructive pulmonary disease (COPD), and/or disabling joint disease (DJD).
- Must undergo an electrocardiogram and 24-hour ambulatory Holter monitoring with measurement of QT interval and calculation of QT corrected for heart rate and an exercise tolerance test (treadmill) to rule out evidence of active coronary artery disease.
- Patients with a history of coronary artery disease (CAD) with chest pain and/or dyspnea must have documented evidence of stabilization of the cardiac disorder.
- Patients who have sustained a cerebrovascular accident (CVA) and are without active neurological symptoms are eligible.
- Patients with type 2 diabetes mellitus must be in fair to good glycemic control with an HgbA1C level ≤ 8.0%.
- Must have the following baseline laboratory tests: CBC (hemoglobin, hematocrit, white blood cell count and red blood cell indices), SMA-18 chemistry panel (glucose, sodium, potassium, chloride, total CO₂, TC, blood urea nitrogen, creatinine, uric acid, calcium, phosphorus, SGOT, SGPT, LDH, alkaline phosphatase, bilirubin, albumin, total protein), lipid profile including HDL-C and triglycerides, thyroxine, TSH, and complete urinalysis.
- Must have a serum creatinine ≤ 1.2 mg/dl and normal liver function. Mild to moderate transaminase elevations (≤ 2x normal) at baseline are permitted.
- Must undergo a baseline body composition evaluation using bioelectrical impedance analyses (BIA) testing.
- Patients who require short courses of glucocorticoid medication to treat limited inflammatory conditions are eligible.
- Must be at least 18 years of age.
- Must sign a COH approved Informed Consent and the D & CVRRP General Information Sheet/Contract.
Exclusion Criteria

- Undergoing treatment for malignancy.
- Current smokers.
- Self-reported allergy to almonds.
- Documented history of familial hyperlipoproteinemia with TC ≥ 300 mg/dl, TG ≥ 500 mg/dl or HDL-C ≥ 80 mg/dl.
- Recent myocardial infarction within six months of program initiation or presence of severe HTN (diastolic blood pressure ≥ 115 mm Hg).
- Patients without a history of CAD but who have an electrocardiogram demonstrating frequent or multifocal premature ventricular contractions, premature atrial contractions, second degree or third degree heart block, or other arrhythmias other than a sinus arrhythmia are ineligible.
- Patients with QT interval prolongations are at increased risk for ventricular arrhythmias and are ineligible.
- Evidence of active coronary artery disease found on the pre-program exercise stress test.
- Untreated hypertension.
- Untreated hypothyroidism or hyperthyroidism, or history of total thyroidectomy receiving total thyroid hormone replacement.
- Untreated gout or hyperuricemia. If overt gout has occurred in the past, the study participant should be started on Allopurinol 300 mg daily prior to study initiation.
- Documented active ulcer disease within three months of study participation.
- Documented participation in a medically supervised weight control program within the past three months.
- No episode of phlebitis within six months of study initiation.
- Impaired renal function (creatinine > 1.2 mg/dl).
- Presence of cerebrovascular disease with neurological symptoms.
- Must not have a current prednisone requirement of ≥ 20 mg daily for more than 10 consecutive days.
- Evidence of significant psychopathology as determined by the program psychologist, including history of active bulimia and/or anorexia nervosa.
- History of substance abuse within six months of study initiation. Patients who are successfully treated for their addiction and have been stable for at least six months are eligible.
- Concurrent use of anti-hyperlipidemic medications (statins), coumadin, antidepressants, psychotropic medications (e.g. lithium), tranquilizers, amphetamines, anti-estrogen medications (Raloxifen, Tamoxifen), hormone replacement therapy, non-prescription vitamins, or other non-prescription weight-reducing medications.
- Females of childbearing potential and/or pregnant women will be excluded from the study as the effects of very low calorie diets on the fetus are unknown.
- Less than 18 years of age.