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A Comparison of the Impact of a Heart-Healthy Diet Versus Paleolithic Nutrition on Hyperlipidemia in Adults

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A Comparison of the Impact of a Heart-Healthy Diet Versus Paleolithic Nutrition on Hyperlipidemia in Adults

by

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DEDICATION

For Angelo.

ABSTRACT

Paleolithic nutrition can optimize serum lipids in adults with hyperlipidemia who have been on a heart-healthy diet based on the current recommendations by the Academy of Nutrition and Dietetics (the Academy) or the American Heart Association (AHA). Twenty subjects (10 male and 10 female) age 40 to 62 were selected based on the criteria of a diagnosis of hypercholesterolemia. Subjects were not taking any cholesterol-lowering medication and had followed a calorie-controlled diet, based on either the dietary principles of the Academy or the AHA, for at least four months followed by a Paleolithic diet for four months. Multivariate analysis using repeated measures ANOVA was performed using SPSS version 21. The data reveal that the mean total cholesterol ($P < .001$), LDL ($P < .001$) and triglycerides ($P < .001$) were significantly lowered and HDL ($P < 0.001$) significantly elevated following four months of a Paleolithic diet relative to the Academy/AHA diet.

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Chapter 1: Introduction

Paleolithic nutrition has been successfully used to reduce risk factors of cardiovascular disease in type 2 diabetics and reduce serum lipids in healthy nonobese subjects (1,2). The objective of the current study is to reveal the effect of Paleolithic nutrition in non-diabetic adults with hyperlipidemia that have followed a four-month heart-healthy diet such as the current cholesterol lowering recommendations of the Academy of Nutrition and Dietetics (the Academy) or the American Heart Association (AHA).

Research Statement

Paleolithic nutrition can optimize serum lipids in adults with hyperlipidemia that have been on a heart-healthy diet based on the current recommendations by the Academy or the AHA.

Study Objective

The study objective is to determine the effect of a Paleolithic diet on serum lipids (total cholesterol, HDL, LDL, and triglycerides) in criteria-meeting adults (age range 40 to 62) with hyperlipidemia, who have been following the Academy or AHA diet for cardiovascular disease (CVD) prevention or treatment.

Justification and Significance of the Study

According to the Centers for Disease Control and Prevention (CDC), one out of every six US adults has hyperlipidemia and 71 million American adults have high low-density lipoprotein (LDL) (3). The average blood cholesterol concentration in the United States is 200 mg/dL, which is considered borderline high (4). Hyperlipidemia is a cause of cardiovascular disease (CVD), the leading cause of death in the United States (3). Fewer than half of Americans with high LDL get treatment (4).

Hunter-gatherer populations followed indigenous lifestyles, consuming varying proportions of pre-agricultural foods consisting of wild plants and animals (5). Studies have revealed that hunter-gatherers living well into their sixties have normal lipids and no sign of CVD (6). Current AHA recommendations for the dietary management of hypercholesterolemia are to replace saturated fats with carbohydrate and reduce total cholesterol intake in a caloric appropriate diet for the individual (1). However, there is evidence that such a diet may result in unfavorable lipid ratios with reduced high-density lipoprotein (HDL) and an elevation of triglycerides and very low-density lipoprotein (VLDL) (2).

Table 1 depicts desirable lipid levels set forth by the CDC.

Table 1 - Centers for Disease Control and Prevention Desirable Cholesterol Levels (3).

Desirable Cholesterol Levels	
Total cholesterol	Less than 200 mg/dL
LDL ("bad" cholesterol)	Less than 100 mg/dL
HDL ("good" cholesterol)	40 mg/dL or higher for men, 50mg/dL or higher for women
Triglycerides	Less than 150 mg/dL

CDC.gov [Internet]. Centers for Disease Control and Prevention. Frequently Asked Questions About High Cholesterol. [updated January 30, 2012; cited January 27, 2013]. Available from http://www.cdc.gov/cholesterol/faqs.htm#6

Interestingly, a study of twentieth century hunter-gatherers from five continents averaged total cholesterol levels of 100 to 140 mg/dL and LDL of 50 to 70 mg/dL (6). Such LDL levels are associated with atheroma regression in CVD patients and typically are only attained with aggressive prescription medication protocols (7). Randomized statin trials in patients with recent acute coronary syndromes and stable coronary artery disease have demonstrated that cardiovascular events are reduced and cardiovascular survival optimized when LDL cholesterol is reduced to <70 mg/dl (7). A 2004 update to the National Cholesterol Education Program (NCEP) Adult Treatment Panel (ATP) III recommended a low-density lipoprotein concentration (LDL-C) of <70 mg/dl for established CVD management (7).

Benefits of the Research

The governing benefit of this research is the addition to the scientific literature on the topic of hyperlipidemia. The characterization of a Paleolithic diet based on twentieth-century hunter-gatherers and their absence of CVD warrant study into therapeutic diets that reduce the risk for CVD today. Further, this study can lead to a larger clinical trial that may provide another method of Medical Nutrition Therapy (MNT) for CVD in the patient population that does not respond to traditional Academy or AHA nutrition intervention and would prefer to avoid lipid-lowering medication. It may also be of benefit for the percentage of the population with hyperlipidemia that does not tolerate lipid-lowering medications.

Research Method

This study is a repeated measures study examining existing data sets from a clinical records review of clients in the researcher’s clinical nutrition practice. Twenty subjects (10 male and 10 female) age 40 to 62 were selected based on the criteria of a diagnosis of hypercholesterolemia. Subjects were not taking any cholesterol-lowering medication and have followed a calorie-controlled diet based on either the dietary principles of the Academy or the AHA for at least four months followed by a Paleolithic diet for four months. Exercise routines remained constant during both diet periods. All 10 female subjects did not take any supplements or medication except for calcium citrate (500 mg) and vitamin D3 (800IU) daily and all 10 male subjects only consumed vitamin D3 (800IU) daily during the full time of the data collection period.

A review by the Eastern Michigan University College of Health and Human Services Human Subjects Review Committee (CHHS-HSRC) revealed that informed consent is not necessary for obtaining previously recorded data since all subjects completed a HIPAA form that allows the use of their health information in research and no Protected Health Information (PHI) was compromised in the data collection process. Privacy has been maintained for all subjects by not disclosing names, social security numbers, or any personal information and assigning subject identifiers (1f to 10f for women and 11m to 20m for men). Only the sex, age, race, and lipid levels have been used as part of the study. Age and race were recorded next to the subject identifiers in an Excel spread sheet.

Heart-Healthy Diets

The AHA has made recommendations for diet as part of its public health efforts to reduce the incidence of CVD, which are summarized in Table 2 (8).

Table 2 - American Heart Association Diet Lifestyle Recommendations for Cardiovascular Disease Reduction (8).

- Balance calorie intake and physical activity to achieve or maintain a healthy body weight.
- Consume a diet rich in vegetables and fruits.
- Choose whole-grain, high-fiber foods.
- Consume fish, especially oily fish, at least twice a week.
- Limit your intake of saturated fat to <7% of energy, *trans* fat to <1% of energy, and cholesterol to <300 mg per day by — choosing lean meats, selecting fat-free (skim), 1%-fat, and low-fat dairy products; and — minimizing intake of partially hydrogenated fats.
- Minimize your intake of beverages and foods with added sugars.
- Choose and prepare foods with little or no salt.
- If you consume alcohol, do so in moderation.
- When you eat food that is prepared outside of the home, follow the AHA Diet and Lifestyle Recommendations.

Lichtenstein AH, Lawrence JA, Brands M, Carnethon M, Daniels S, Franch HA, Franklin B, Kris-Etherton P, Harris WS, Howard B, Karanja N, Lefevre M, Rudel L, Sacks F, Van Horn L, Winston M, Wylie-Rosette J. Diet and Lifestyle Recommendations Revision 2006: A scientific statement from the American Heart Association Nutrition Committee. Circulation. 2006;114:82-96.

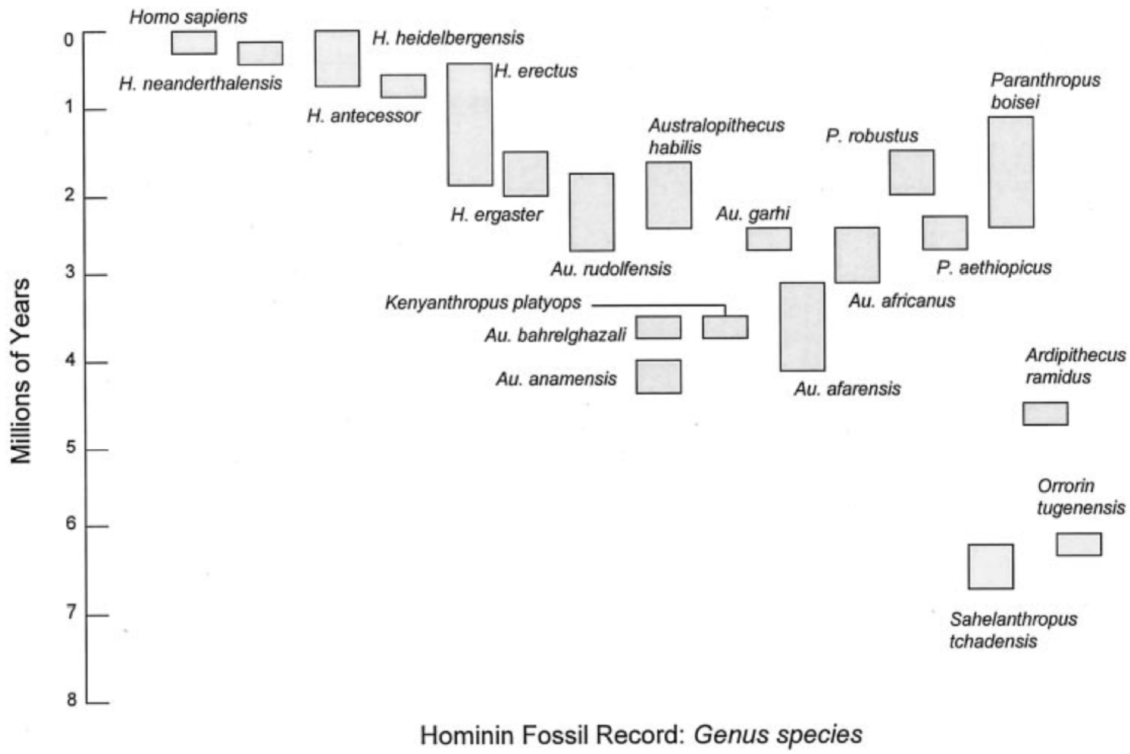
Examining these recommendations in-depth, saturated fatty acids should comprise less than 7% of energy in the diet (8). Trans fatty acids should not exceed 1% of energy, which is approximately 3 grams per day based on a 2000 kcal diet. Fish should be consumed twice per week. The majority of the daily calorie intake should come from whole grains.

The Academy considers a heart-healthy diet to be low in sodium, saturated fats, trans fatty acids, cholesterol, added sugars, and refined grains and to increase intake of fruits, vegetables, whole grains, fat-free and low-fat dairy products, and seafood (9). Specifically, the Academy recommends the 2010 Dietary Guidelines for Americans, which include consuming less than 300 mg per day of dietary cholesterol, consuming less than 10 percent of calories from saturated fats, reduce trans fatty acids as low as possible, reduce intake of sugars and refined carbohydrates, reduce sodium intake to less than 2300 mg and further reduce intake to 1500 mg among persons who are 51 and older and those of any age who are African American or have hypertension, diabetes, or chronic kidney disease (10). The diet should be based on grains (half of which should be whole grains), vegetables, fruits, and low-fat or fat-free dairy products (10).

Definition of Paleolithic Nutrition

Humans evolved during the Paleolithic period, approximately 2.6 million to 10,000 years ago (Figure 1). Animal source foods played a critical role in human evolution (11). It is clear that no universal diet existed during human evolution (11). Diets varied by climate and geographic location (11). However, the evidence seems to indicate there are similar characteristics in that plant foods and animal foods were the basis for nutrition throughout human evolution and studies of diverse twentieth century hunter-gatherer populations indicate that plants and animal foods were the basis of the diet (11).

Figure 1: The Hominin Fossil Record (11)



The Paleolithic diet is a diet based on foods that were available during the Paleolithic era, which includes animal proteins (meat, poultry, eggs and fish), nuts, vegetables and fruit (4). It is void of dairy, legumes, and grains (4). The Paleolithic diet, when compared to the standard American diet, contains three times more fiber, potassium, polyunsaturated and monounsaturated fats, four times more omega-3 fatty acids, and five times less sodium (11,13,14). Paleolithic diets are higher in protein than standard Academy or AHA diets but rich in vegetables and fruit (4,12,13).

One could argue that the relatively lower life expectancy of hunter-gatherers discounts an examination of CVD risk factors, as these are commonly associated with age. There have been studies on twentieth century hunter-gatherers living into their late sixties and upon examination; they are virtually void of CVD risk factors (5,6), a feat that would not easily be replicated with a random sample of the same age group of our current population today (4). Further, even those hunter-gatherer societies that did have an average life expectancy of 40 years, one must factor in injury, infection, and infant mortality, and, in doing so, an examination of the data clearly indicates they still lived longer than agrarian civilizations of the 1600s and late 18th century (18 years and 25 years, respectively) (15). Moreover, risk factors for adult diseases such as CVD and type 2 diabetes are appearing in younger patient populations (16).

Another potential argument is that energy expenditure must have been far greater in hunter-gatherer societies compared to modern societies and that alone must account for historically low lipid concentrations of hunter-gatherer societies. In a 2012 study using doubly-labeled water, measurements of total energy expenditure (TEE) among the Hadza hunter-gatherers compared to their Western counterparts resulted in similar TEE, suggesting that differences in energy intake rather than expenditure may be the main factor in obesity and abnormal lipid concentrations (17).

Studies on the human genome suggest very little has changed in our DNA during the past 10,000 years (12). Research seems to indicate that we are genetically almost identical to our Stone Age ancestors (5,11,12,18). It is possible to replicate the nutritional template with which we evolved, and this may reduce the incidence of CVD for the at-risk population (13).

Chapter 2: Review of the Literature

Comparison of Paleolithic, Academy, and AHA Diets

Studies of hunter-gatherers themselves have revealed them to be lean throughout their lives with a low incidence of cardiovascular disease (19). A review of 229 hunter-gatherer societies indicates the macronutrient ratios differ from the Academy and AHA diets for cardiovascular disease prevention and treatment (20). The combined AHA and the Academy recommendations for heart health limit cholesterol consumption to less than 300 mg per day, restrict calories from saturated fats to less than 7 to 10%, reduce trans fatty acids as low as possible with a recommendation to not exceed 1% of energy, increase seafood consumption to twice per week, reduce sodium, reduce sugars and refined carbohydrates but base the diet on a foundation of grains (half of which should be whole grains), vegetables and low fat or fat-free dairy products (8,10). True hunter-gatherer societies consume 45 to 65% of energy from animal foods yielding 19 to 35% of energy from protein, with carbohydrate sources from plants providing 22 to 40% of energy (20). In 97% of the world's hunter-gather societies, fat intake exceeded 30% of total energy (2). It has been shown that diets rich in lean protein and fish rich in long-chain omega-3 polyunsaturated fatty acids (PUFA) content, result in a serum lipid profile thought to be protective against atherosclerosis (2,21,22).

Paleolithic Diet and CVD

With one out of every six US adults having hypercholesterolemia, it is not known how many individuals can normalize serum lipids using a heart-healthy diet due to the impossibility of being able to predict individualized lipid response (3,23,24).

One high-risk population for cardiovascular disease is type 2 diabetics. Paleolithic nutrition has been found to have a favorable impact on cardiovascular disease risk factors in type 2 diabetics (25). Type 2 diabetics not treated with insulin were educated to consume a Paleolithic diet basing their meal planning on foods that were available during the Paleolithic era, which are lean meat, fish, vegetables, fruits, eggs, and nuts or a standard diabetes diet based on current American Diabetes Association guidelines in a randomized crossover study. In the Paleolithic diet group, dried fruit, potatoes and wine were limited to one serving each daily and whole eggs were limited to two daily. There were no limitations on animal protein and vegetables. There was no advice given to control calories. The Paleolithic diet resulted in lower triglycerides (-7.2 mg/dL, $p = 0.003$) and waist circumference (-4 cm, $p = 0.02$), with an increase in HDL (+1.44 mg/dL, $p = 0.03$) when compared to the American Diabetes Association diet group after three months.

Previous data include a statistically significant reduction in total cholesterol, LDL and triglycerides in a 31-day short trial of a 21-day "ramp-up" diet and a 10-day Paleolithic diet in non-obese sedentary healthy volunteers (26). The "ramp-up" diet increased potassium and fiber and led up to the switch to a full Paleolithic diet for the last 10 days of the study. The Paleolithic diet component of the study consisted of the same foods and exclusions as the aforementioned type 2 diabetes trial, except for potatoes, which were excluded. Caloric intake was increased if weight decreased by three pounds. Total cholesterol decreased by 16% and LDL decreased by 22%. This short trial exceeded lipid-lowering effects of the Academy, AHA and Omni-Heart diets.

Data on twentieth century hunter-gatherers supports the proposition that although a Paleolithic diet is a meat-based diet with up to 65% of all intake coming from animal foods, it is non-atherogenic (2). From 1960 to 1996 data has been published on 13 twentieth century hunter-gatherer societies consuming 65% of the total diet from animal food yet revealed an absence of cardiovascular disease (2). Autopsy studies on the Inuit and other hunter-gatherers from 1960 to 1993 reveals a low rate of cardiovascular disease (2). Anthropological research over the last 64 years reveals low total cholesterol and triglyceride levels in hunter-gatherers consuming a Paleolithic diet (2). When hunter-gatherers transitioned to a grain-based agricultural diet, cardiovascular disease risk factors began to appear such as abnormal lipid levels (19).

When Westernized former hunter-gatherer Australian Aborigines with hyperlipidemia and type 2 diabetes were returned to their normal hunter-gatherer diet, there was either great improvement or complete normalization of serum lipids and type 2 diabetes after just seven weeks (2).

Dietary compliance is higher with a Paleolithic diet as satiety seems to be a beneficial component of the diet, leading to a lower caloric intake (25). Healthy volunteers followed a Paleolithic diet for three weeks and satiety drove caloric intake down 36% from the baseline diet (27). Twenty-nine male patients with ischemic heart disease were divided into two groups and instructed to follow a Paleolithic diet (n=14) or Mediterranean diet (n=15) for 12 weeks (28). Leptin decreased by 31% in the Paleolithic diet group versus 18% in the Mediterranean diet group. Calorie intake was 430 kcal lower in the Paleolithic diet group (28).

Proposed Mechanisms of Action

Proposed mechanisms of action for the lipid lowering effects of the Paleolithic diet include the hypocholesterolemic effects of protein, high monounsaturated and omega-3 fatty acid intake, reduced glycemic load and glycemic index, higher plant-based compounds such as fiber and phytochemicals, reduced calorie intake via increased satiety and body fat reduction (29,30).

Increased intake of omega-3 fatty acids from plants and fish have been shown to reduce the risk of cardiovascular disease by up to 50% (31-34). Monounsaturated fatty acids, which have been shown to reduce the incidence of cardiovascular disease, made up half the fats of the diets of hunter-gatherers (19,35). The Paleolithic diet is rich in nuts and nut consumption reduces LDL cholesterol (19). Consumption of nuts is associated with a 37% reduction in cardiovascular disease (36,37).

Dietary lean red meat and a reduction of high glycemic index carbohydrates are associated with lower cholesterol and triglycerides (38). The Paleolithic diet, rich in lean protein at the expense of carbohydrate, has a hypolipidemic effect, lowering LDL and triglycerides, yet preserving and even raising HDL (2,39). Buffalo meat has been recommended in modern Paleolithic diets because it is lower in saturated fats than domesticated farm animals. Buffalo meat consumption has been associated with lower cholesterol and triglycerides (40). Buffalo meat is low in saturated fatty acids and higher in polyunsaturated fatty acids (40).

Carbohydrate choices in a Paleolithic diet are plant based, mildly starchy and thus low glycemic index (2). An intake of high glycemic index carbohydrates has an unfavorable effect on serum lipids, with an increase in total cholesterol, LDL, VLDL and triglycerides (41).

Plant sterols are found in vegetable foods and oils. It is estimated that the plant sterol intake of hunter-gatherers was at least 1000 mg daily compared to 167-437 mg daily in the standard American diet (42,43). In a meta-analysis of eighty-four trials, plant sterols have been shown to lower LDL-C by 10% with a mean intake of 2000 mg daily, over a course of eight to twelve weeks (44).

Controlling factors associated with metabolic syndrome, such as dysglycemia, insulin resistance and obesity, has been shown to reduce total cholesterol, LDL and triglycerides (45). A 12-week clinical trial of the Paleolithic diet and the Mediterranean diet revealed a 26% reduction in area under the curve (AUC) glucose measurement in the Paleolithic diet group compared to a 7% reduction in the Mediterranean diet group (46). Waist circumference decreased 5.6 cm in the Paleolithic diet group and 2.9 cm in the Mediterranean group (46).

To date, no study has moved patients from four months of a heart-healthy diet based on the recommendations of the Academy and the AHA to four months of a Paleolithic diet to manage hypercholesterolemia. This study is the first to publish such data.

Chapter 3: Materials and Methods

This is a repeated measures study using existing data sets from a clinical records review of patients in the researcher's private nutrition practice. Twenty subjects (10 male and 10 female) age 40 to 62 were selected based on the following criteria. Subjects had a diagnosis of hypercholesterolemia. Subjects were not taking any cholesterol lowering medication and had followed a calorie controlled diet, based on the dietary principles of the Academy or the AHA, for at least four months followed by a Paleolithic diet for four months. All 10 female subjects did not take any supplements or medication except for calcium citrate (500 mg) and vitamin D3 (800IU) daily and all 10 male subjects have only consumed the supplement vitamin D3 (800IU) daily during the full time of the data collection period. No other nutritional supplementation has been consumed.

Academy or AHA Diet

The Academy recommends the 2010 Dietary Guidelines for Americans, which include consuming less than 300 mg per day of dietary cholesterol, consuming less than 10 percent of calories from saturated fats, reducing trans fatty acids as much as possible, reducing intake of sugars and refined carbohydrates, reducing sodium intake to less than 2300 mg, and further reducing intake to 1500 mg among persons who are 51 and older and those of any age who are African American or have hypertension, diabetes, or chronic kidney disease (10). The Dietary Guidelines intend the foundation of the diet to be grains (half of which should be whole grains), vegetables, fruits, and low-fat or fat-free dairy products (10). An AHA diet recommends further reduction in saturated fats to <7% of energy, total trans fatty acids <1% of energy and fish consumption at least twice per week (8).

The researcher gauged adherence to dietary principles of the Academy or AHA diet by review of detailed chart notes consisting of food intake using food frequency questionnaires and diet recalls, taken monthly during nutrition counseling sessions, and by reviewing subjects' diet journals that are requested from every client in the researcher's practice. Exercise routines have remained constant. The researcher assured adherence to exercise principles by reviewing exercise journals and chart notes for consistency.

Paleolithic Diet

The Paleolithic diet used for each subject was based on vegetables, lean animal protein, nuts, and fruit, but excludes all dairy, grains, and legumes. In the four months of the Paleolithic diet period, subjects were instructed to limit white potato, wine, and dried fruit to one serving daily (1/2 cup of potato, 4 ounces of wine, 1 ounce of dried fruit). No caloric limitations or limitations of allowed foods were implemented. Adherence to the dietary principles were based on chart notes of all nutrition counseling sessions to assure they were compliant with the nutrition principles of the Paleolithic diet. Subjects were instructed to record a diet journal of all food and beverage consumption for a total of four months. Food frequency, 24 hour diet recall, diet journal and exercise journal data were collected every two weeks to monitor compliance. Prior to beginning the Paleolithic diet phase, all subjects received a sample Paleolithic meal plan, based on three meals and two snacks per day, without caloric restriction (Appendix A).

Collection of Lipid Data

Lipids reviewed included total cholesterol, HDL, LDL, and triglycerides. During the records review, the lipid values, sex, and race of the subject were recorded in an

Excel spreadsheet, using identifiers (1f to 10f for female subjects and 11m to 20m for male subjects). The Excel spreadsheet was printed and secured with a lock and key in a fireproof safe in the researcher's office. It was not shared or given to anyone. Privacy was maintained by not disclosing names, social security numbers, or any personal information on the spreadsheet. Once printed the Excel spreadsheet was deleted from the researcher's hard drive using the secure delete feature from OSX version 10.8.3.

Lipid samples were measured after four months of an Academy/AHA diet and again after four months of a Paleolithic diet. Copies of those test results became part of each subject's chart in the researcher's office. After the Academy/AHA and Paleolithic diet periods, a phlebotomist drew the subjects' blood into lavender top EDTA tubes completely and each sample was inverted to mix the EDTA with the blood. Samples were centrifuged at 4000 rpm. Samples were removed from the centrifuge and not inverted. A laboratory technician used a fresh disposable pipette to withdraw 3 ml of plasma into lavender top clear transfer tubes. During the same blood draw red/gray top serum separator tubes were filled completely and were placed upright in a rack at room temperature for no longer than 30 minutes to clot blood. These samples were then centrifuged for 15 minutes. The serum was free of hemolysis and red blood cells. The laboratory technician used a fresh disposable pipette to withdraw 3 ml of serum into the red top amber transfer tube and cap it tightly. LabCorp (Laboratory Corporation of America) performed the lipid analysis.

Statistical Methodology

Multivariate analysis using repeated measures ANOVA was performed using IBM SPSS statistics software version 21. Diet (Academy/AHA vs. Paleolithic) served as a within-subjects factor while gender (female vs. males) was a between-subjects factor. All subjects consumed the Academy/AHA diet for four months prior to four months on the Paleolithic diet. Collected data used for the statistical analysis appear in Appendix B.

Chapter 4: Results

Table 3 reveals descriptive statistics of different plasma lipids by diet. Results show that the mean total cholesterol, LDL, and triglycerides were consistently lowered following a Paleolithic diet relative to the Academy/AHA diet. The opposite was seen with HDL, where the Academy/AHA diet had lower HDL mean than the Paleolithic diet. These figures are based on descriptive statistics and proper inferential analysis is carried out further below to test if these differences are statistically significant.

Table 3 - Descriptive Statistics of Different Lipid Values by Diet

Lipid	Diet	N	Minimum	Maximum	Mean	Std. Deviation
Total Cholesterol	*Academy /AHA*	20	203	290	229	21
Total Cholesterol	*Paleolithic*	20	151	263	182	27
HDL	*Academy /AHA*	20	35	88	51	17
HDL	*Paleolithic*	20	45	92	69	14
LDL	*Academy /AHA*	20	112	206	149	22
LDL	*Paleolithic*	20	61	169	96	27
Triglycerides	*Academy /AHA*	20	45	291	142	56
Triglycerides	*Paleolithic*	20	32	149	82	29

Results of the possible effect of diet and gender on total cholesterol, HDL, LDL, and triglycerides are reported below.

I) Total Cholesterol

Results of the multivariate test of within subject effect for total cholesterol show that there was a significant effect of the diet on total cholesterol ($P < .001$). That is, there was a highly significant difference in mean total cholesterol between the Academy/AHA and Paleolithic diets. Marginal mean estimates show that participants had higher total cholesterol when on the Academy/AHA diet compared with the Paleolithic diet. There was no significant interaction between diet and gender on total cholesterol (P = .579) and this is also evident from the almost parallel lines of marginal means by gender and diet presented in Figure 2.

Figure 2: Marginal means of total cholesterol: gender-by-diet

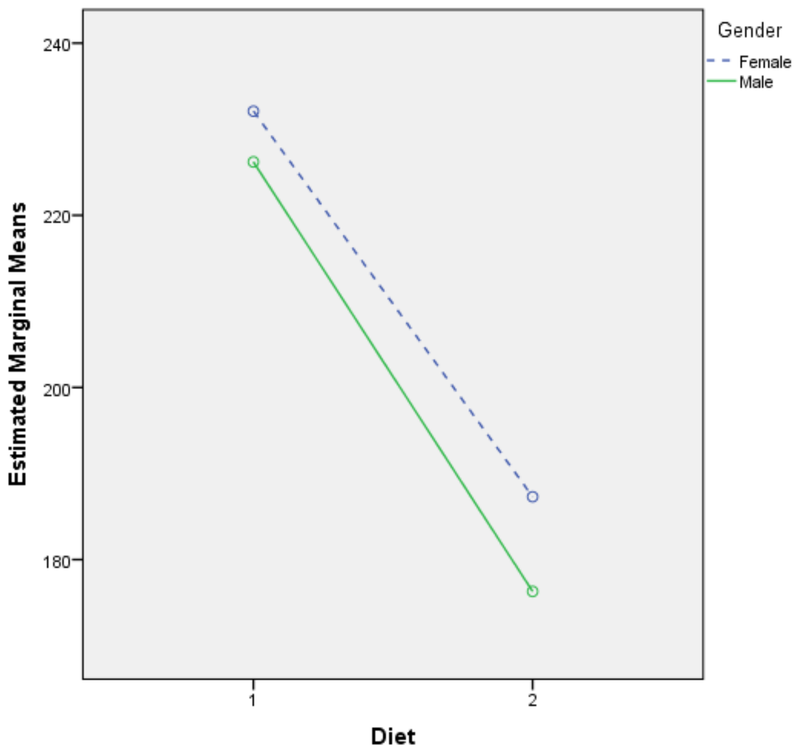
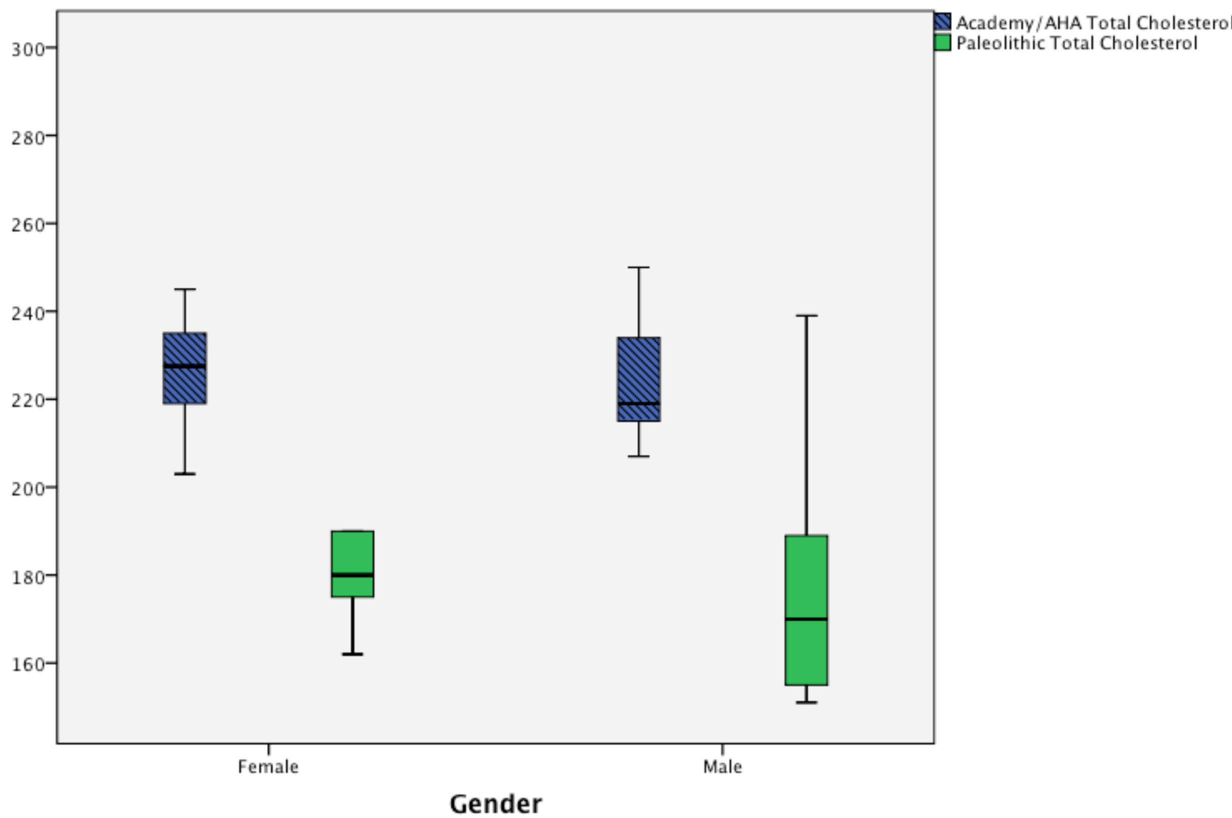


Figure 3 shows a box plot of total cholesterol by diet and gender, which graphically depicts that total cholesterol was higher for both males and females when on the Academy/AHA diet than Paleolithic diet. Results of the between subjects effects of gender on total cholesterol show that there was no significant difference in total cholesterol (P = .409) between males and females.

Figure 3: Box plot of total cholesterol by diet and gender



II) HDL

The difference between HDL levels on the two diets was statistically significant (P < 0.001) as tested using repeated measures ANOVA. In other words, there was a highly significant difference in mean HDL between the Academy/AHA diet and Paleolithic diet. Marginal mean estimates (Figure 4) show that participants had higher HDL when on the Paleolithic diet compared with the Academy/AHA diet. There was no significant interaction between diet and gender (P = .807) and this is also evident from the apparent parallel lines of marginal means by gender and diet presented in Figure 4. The absence of interaction between diet and gender indicates that the difference in HDL between diets was not different (was the same) for both males and females.

Figure 4: Marginal means of HDL: gender-by-diet

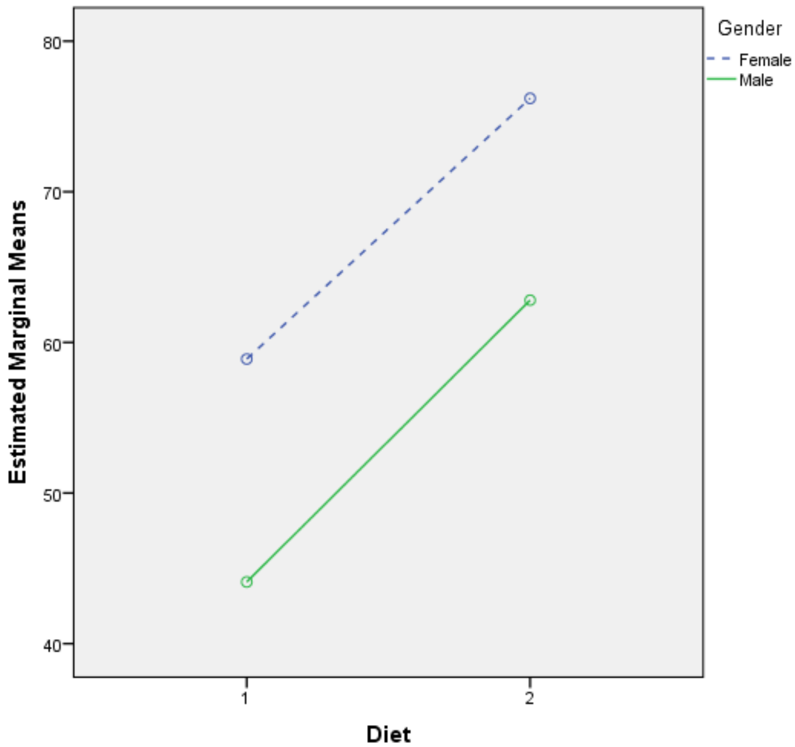
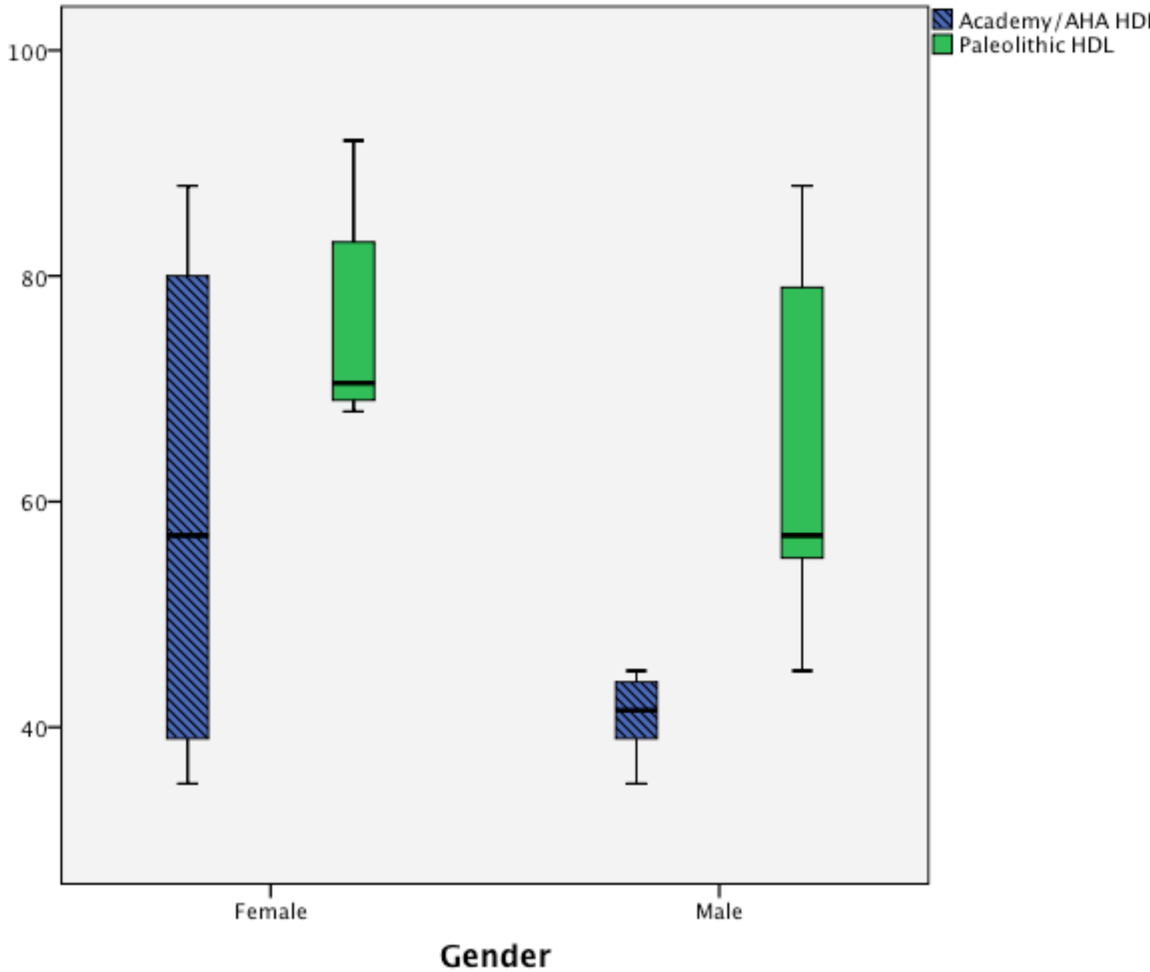


Figure 5 shows a box plot of HDL by diet and gender. The results show that HDL was higher when on the Paleolithic than the Academy/AHA diet and this was true for both males and females. Results of the between subjects effects of gender on HDL show that there was a significant difference in HDL levels between females and males. Males had lower HDL values than females for the two diets (P = .023). The variability of the data was different by gender and diet with the highest variability for females when on the Academy/AHA diet and lowest for males at the same diet.

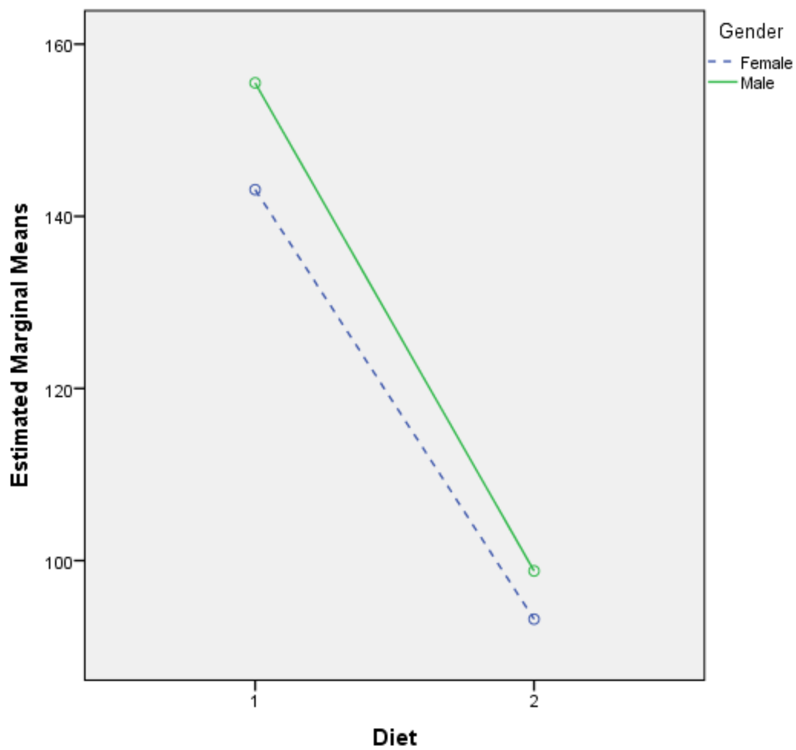
Figure 5: Box plot of HDL by diet and gender



III) LDL

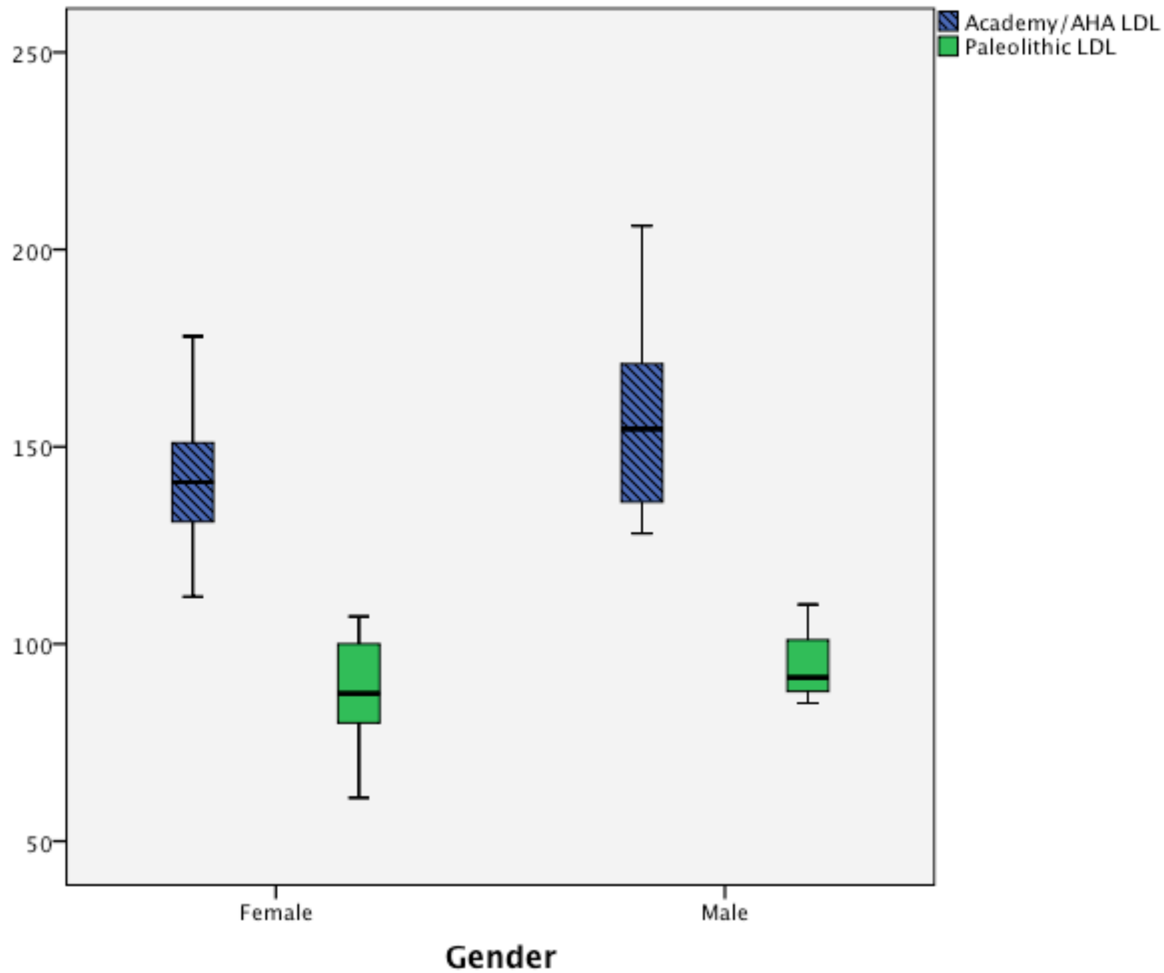
The difference between LDL levels on the two diets was statistically significant ($P < .001$). This shows that there was a highly significant difference in mean LDL between the Academy/AHA diet and Paleolithic diet. Marginal mean estimates (Figure 6) show that participants had higher LDL when on the Academy/AHA diet compared with the Paleolithic diet. There was no significant interaction between diet and gender on LDL levels ($P = .561$) and this is also evident from the almost parallel lines of marginal means by gender and diet in Figure 6. The absence of interaction between gender and diet indicates that the observed difference in LDL between diets was similar for both males and females.

Figure 6: Marginal means of LDL: gender by diet



Results of the between subjects effects of gender on LDL show that there was no significant difference in mean LDL levels between males and females (P = .362). Figure 7 shows a box plot of LDL by diet and gender.

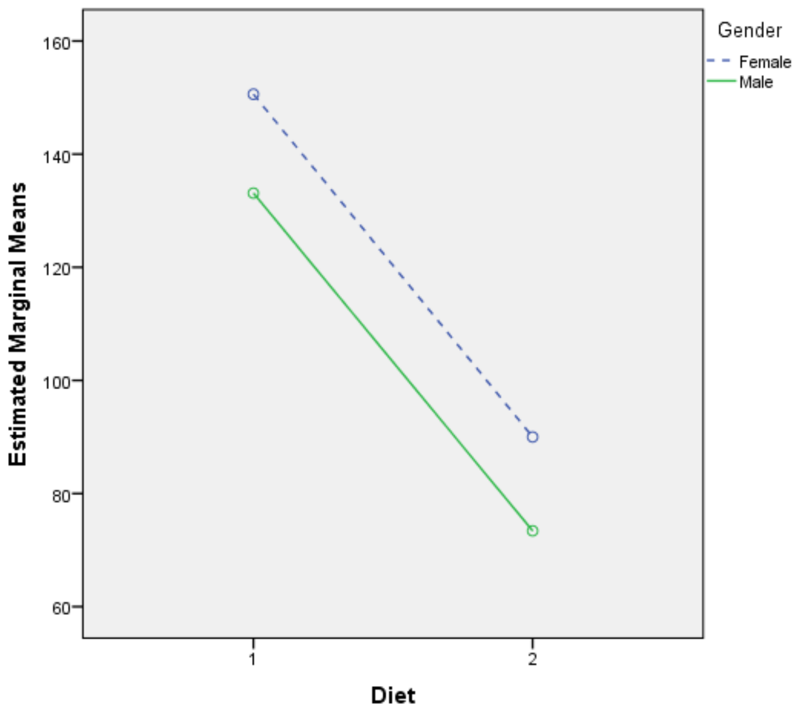
Figure 7: Box plot of LDL by diet and gender



IV) Triglycerides

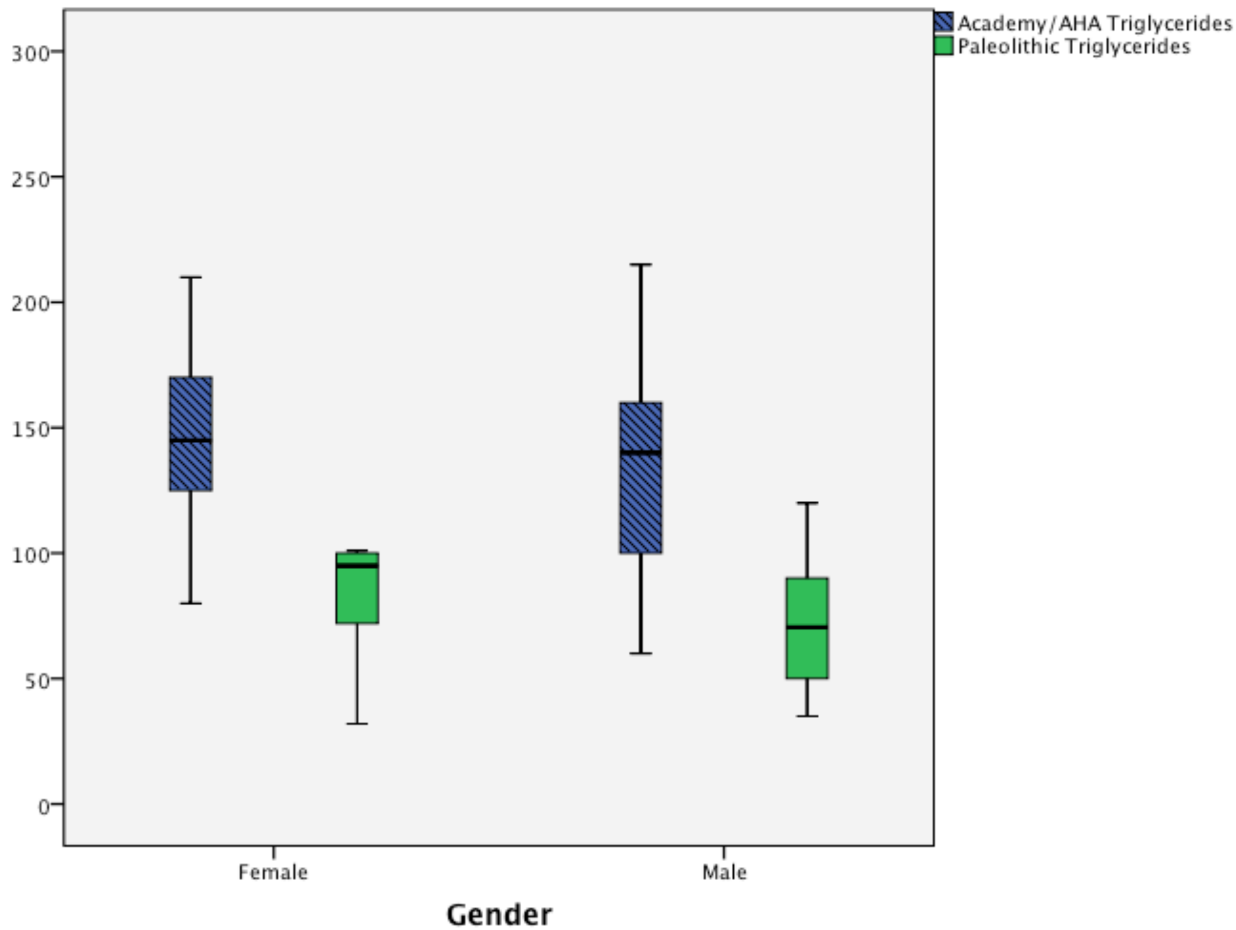
The difference between triglyceride levels on the two diets was statistically significant ($P < .001$), as tested using repeated measures ANOVA. It can be concluded that there was a highly significant difference in mean triglyceride levels between the Academy/AHA and Paleolithic diets. Marginal mean estimates (Figure 8) show that the participants had lower triglyceride levels when on the Paleolithic diet compared with the Academy/AHA diet. There was no significant interaction between diet and gender on triglyceride levels ($P = .963$) and this is also evident from the apparently parallel lines of marginal means by gender and diet in Figure 8. The absence of interaction between diet and gender indicates that the difference found in triglycerides between the two diets was the same for both males and females.

Figure 8: Marginal means of triglycerides: gender by diet



Results of the between subjects effects of gender on triglycerides show that there was no significant difference in triglyceride levels between males and females (P = .348). Figure 9 shows a box plot of triglycerides by diet and gender.

Figure 9: Box plot of triglycerides by diet and gender



Chapter 5: Discussion

The data reveal highly significant findings that the mean total cholesterol (P <.001), LDL (P <.001) and triglycerides (<.001) were lowered and HDL (P < 0.001) elevated following a Paleolithic diet relative to the Academy/AHA diet. These results are analagous to the serum lipid results from the studies on type 2 diabetics (25) and 10-day Paleolithic diet trial (26).

In the study on type 2 diabetics, 13 subjects followed a standard diabetes diet based on current American Diabetes Association guidelines for three months, followed by a Paleolithic diet for three months (25). The study participants had an average diabetes duration of nine years with a mean Hemoglobin A1C (HA1C) of 6.6 (25). Diabetes is a significant CVD risk factor. The Paleolithic diet improved HA1C (P=.01), triglycerides (P=.003) and HDL (P=.03) when compared to the American Diabetes Association diet. The application of what is universally accepted and prescribed, in the diabetic study, the conventional American Diabetes Association diet, and in the data presented in this paper, the Academy/AHA diet, were out-performed clinically by the Paleolithic diet.

In the 10-day Paleolithic diet trial, there was no significant improvement in HDL (conventional diet 1.3±0.2 versus Paleolithic diet 1.3±0.3) (26). This is potentially due in part to the very short duration of the study as the data presented herein indicates a significant improvement in all twenty subjects. Similar improvements in total cholesterol (P=.007), LDL (P=.003), and triglycerides (P=.01) were found in the 10-day Paleolithic diet trial. Worth noting is the fact that the 10-day Paleolithic diet trial moved subjects from a contemporary American diet, which appears to play a role in numerous chronic diseases, to a Paleolithic diet.

Moving subjects from what is considered and accepted as a "heart-healthy" diet to address hypercholesterolemia did not result in optimal lipid values. All twenty subjects would have required potentially lifelong statin therapy to address their abnormal lipid patterns due to the insufficient improvement the Academy/AHA diet has had on their lipids. Therefore, these data are relevant in that they represent the strong potential for avoidance of lipid lowering medication after the subjects adopted a Paleolithic diet.

Side effects of statin medication have been a clinical concern. In a retrospective cohort study, statins were discontinued by 57,292 out of 107,835 subjects due to myopathy, nausea, neuropathy and elevated liver enzymes alanine and aspartate transaminase (47). Moreover, treatment with higher potency statins, especially atorvastatin and simvastatin, have been associated with an increased risk of new onset diabetes (48).

Case series data provide powerful observational information for the clinician. Retrospective analysis can help realize novel treatments and foster the generation of a hypothesis, that can lead to focused human trials. A challenge to case series or retrospective studies is bias. However, when clear criteria of inclusion and exclusion are established bias can be virtually eliminated (49). The limitations of a retrospective case series analysis include limited generalizability to a larger population. Therefore, a larger study is recommended to verify the results of this study. Further limitations include the small sample size and the dominant Caucasian subject population of this study. Would the data extrapolate to a subject population that is not predominantly Caucasian? Such a study should be planned and implemented, because cardiovascular disease and diabetes account for one-third of the mortality difference between African Americans and

Caucasians (50). Last, CVD is viewed as a chronic inflamatory condition (51). It would be relevant to study the impact of a Paleolithic diet on markers of inflammation such as interleukin-6 (IL-6) and high sensitivity C-reactive protein (hs-CRP) (51).

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Appendix

Appendix A: Paleolithic Nutrition

Encouraged Foods

Meats (all red meat should be grass-fed)
Beef and Bison
Flank steak
Top sirloin steak
Hamburger
London broil
Chuck steak
Veal
Pork

Poultry
Chicken
Turkey breast
Game hen

Eggs (free range and organic)
Chicken
Duck
Goose

Other meats
Rabbit meat (any cut)
Goat meat (any cut)

Organ meats
Beef, lamb, pork, and chicken livers
Beef, pork, and lamb tongues
Beef, lamb, and pork marrow
Beef, lamb, and pork "sweetbreads"

Game meat
Alligator
Bear
Caribou
Deer
Duck
Elk
Emu
Goose
Kangaroo
Ostrich

Pheasant
Quail
Rattlesnake
Reindeer
Squab
Turtle
Venison
Wild boar
Wild turkey

Fish
Bass
Bluefish
Cod
Drum
Eel
Flatfish
Grouper
Haddock
Halibut
Herring
Mackerel
Monkfish
Mullet
Northern pike
Orange roughy
Perch
Red snapper
Rockfish
Salmon
Scrod
Shark
Striped bass
Sunfish
Tilapia
Trout
Tuna
Turbot
Walleye
Any other commercially available fish

Shellfish
Abalone
Clams
Crab
Crayfish

Lobster
Mussels
Oysters
Scallops
Shrimp

Fruit
Apple
Apricot
Avocado
Banana
Blackberries
Blueberries
Boysenberries
Cantaloupe
Carambola
Cassava melon
Cherimoya
Cherries
Cranberries
Figs
Gooseberries
Grapefruit
Grapes
Guava
Honeydew melon
Kiwi
Lemon
Lime
Lychee
Mango
Nectarine
Orange
Papaya
Passion fruit
Peaches
Pears
Persimmon
Pineapple
Plums
Pomegranate
Raspberries
Rhubarb
Star fruit
Strawberries
Tangerine

Watermelon
All other fruits

Vegetables
Artichoke
Asparagus
Beet greens
Beets
Bell peppers
Broccoli
Brussels sprouts
Cabbage
Carrots
Cassava root
Cauliflower
Celery
Collards
Cucumber
Dandelion
Eggplant
Endive
Green onions
Kale
Kohlrabi
Lettuce
Mushrooms
Mustard greens
Onions
Parsley
Parsnip
Peppers
Pumpkin
Purslane
Radish
Rutabaga
Seaweed
Spinach
Squash
Sweet potato
Swiss chard
Tomatillos
Tomato
Turnip greens
Turnips
Watercress
Yams

Nuts and Seeds
Almonds
Brazil nuts
Cashews
Chestnuts
Hazelnuts
Macadamia nuts
Pecans
Pine nuts
Pistachios
Pumpkin seeds
Sesame seeds
Sunflower seeds
Walnuts

LIMIT
Dried fruit - ¼ cup per day
White potatoes (any variety of potato with white flesh) – ½ cup cooked per day.
Wine one 4 oz. serving daily per day.
Whole Eggs - 2 per day (no limit on egg whites).

Foods To Avoid

Dairy Foods
All processed foods made with the milk products of all animals
Butter
Cheese
Condensed milk
Cream
Dairy spreads
Frozen yogurt
Ice cream
Ice milk
Kefir
Low-fat milk
Nonfat dairy creamer
Powdered milk
Skim milk
Whey
Whole milk
Yogurt

Cereal Grains
Amaranth
Barley

Bread/Flour products (bread, rolls, muffins, noodles, crackers, cookies, cake, doughnuts, pancakes, waffles, pasta, spaghetti, lasagna, wheat tortillas, pizza, pita bread, flat bread, and all processed foods made with the flour of any grain).
Brown Rice
Brown Rice
Buckwheat
Cornmeal
Farro/Emmer
Flaxseed
Grano
Kamut
Millet
Muesli
Oats
Oat Bread
Oat Cereal
Oatmeal
Popcorn
Whole Wheat Cereal Flakes
Rolled Oats
Quinoa
Rice (all forms)
Rye
Sorghum
Spelt
Teff
Triticale
Wheat Berries
Wild Rice

Legumes
Adzuki beans
Black beans
Black-eyed peas
Broad beans
Butter beans
Calico beans
Cannellini beans
Chickpeas
Fava beans
Field beans
Garbanzo beans
Kidney beans
Lentils
Lima beans
Mung beans

Navy beans
Peanuts
Peas
Pinto beans
Red beans
Snowpeas
Soybeans and all soybean products, including miso, tofu and tempeh
String beans
Sugar snap peas
White beans

Junk food
Candy
Soda (even diet)
Highly processed meats
Fruit juices

Paleolithic Nutrition - Sample Meal Plan

At each nutrition consultation session please bring your favorite recipes, family recipes and or cookbooks and I will adjust anything you desire to prepare into a Paleolithic meal. Below are some sample meal ideas to get you started.

Breakfast Ideas

Yam breakfast hash
1 medium yam, peeled and cut into ½ inch pieces
1 medium apple, cored and cut into ½ inch pieces
1 teaspoon lemon juice
1 tablespoon coconut oil
1 medium onion, chopped
½ cup diced, cooked, skinless turkey or chicken (white or dark meat)
1 tablespoon chopped fresh thyme, or 1 teaspoon dried
½ teaspoon of sea salt
¼ teaspoon of fresh ground black pepper
Place yam pieces in a medium saucepan, cover with water and bring to a boil. Reduce heat to medium, cover and cook for 3 minutes. Add the apple and cook until everything is just tender for 2 to 3 minutes longer.

Drain.
Transfer 1 cup of the mixture to a large bowl and mash. Stir in the lemon juice. Add the remaining unmashed mixture and stir gently to mix. Set aside.
Heat coconut oil in a large nonstick skillet over medium-high heat. Add onion and cook, stirring often, until softened, 2 to 3 minutes. Add turkey or chicken, thyme, salt and pepper; cook, stirring occasionally, until heated through, about 2 minutes.

Add the reserved yam mixture to the pan and stir to mix. Press on the hash with a wide spatula and cook about 3 minutes, until the bottom is lightly browned. Cut the hash into several rough sections; flip and cook until for 3 minutes longer until the undersides are browned. Serve immediately.

2 Salmon sausages and a bowl of fresh fruit
For convenience you may purchase salmon sausages from Vital Choice online at http://www.vitalchoice.com/shop/pc/viewCategories.asp?pageStyle=h&idCategory=272 or make your own using the following recipe.

Salmon Sausage Recipe
8 ounces of fresh salmon
1 egg
1 tablespoon of diced Vidalia onion
½ teaspoon of minced garlic
½ teaspoon of dried basil
½ teaspoon of dried oregano
1 teaspoon of chopped fresh parsley
½ teaspoon of chili powder
⅛ teaspoon cayenne pepper
⅛ teaspoon of black pepper
⅛ teaspoon of sea salt
Preheat oven to 350 degrees Fahrenheit. Chop salmon finely and mix all ingredients together thoroughly. Form four 2-ounce patties. Preheat a 10-inch sauté pan over high heat with just enough avocado oil to lightly coat the bottom of the pan. Sauté the salmon sausage patties for 1 minute per side. Transfer the sausage patties to a baking pan. Put in the oven and bake for 5 minutes.

Vegetable omelet with sliced tomato and avocado
Whisk two eggs with ¼ cup of chopped mushrooms, ½ cup of baby spinach leaves and ½ of an orange or yellow bell pepper, diced. Add 1 teaspoon of avocado oil to an 8-inch frying pan and fry your egg mixture until done to your liking. Serve folded on a plate with sliced fresh tomato and ½ of an avocado sliced.

Grilled grass-fed steak with wilted arugula
8 ounce grass-fed rib-eye
2 tablespoons of fresh lime juice
½ teaspoon of cumin
½ teaspoon of chili powder
1 clove of garlic minced
2 tablespoon of extra virgin olive oil
10 ounces of baby arugula, washed and drained well
Salt and pepper to taste

Whisk the lime juice, cumin, chili pepper, garlic and 1 tablespoon of olive oil together in a bowl. Put the steak in a large zip lock plastic bag. Pour the lime juice mixture into the bag over the steak. Seal the bag tightly and move the lime mixture all over the steak until well coated. Put in the refrigerator for 1 hour. Grill steak to desired state. As steak is grilling, place the remaining 1 tablespoon in a 10-inch skillet on medium heat and add the arugula. Stir with a spatula, flipping over the arugula until it is all evenly wilted. Serve immediately with your steak.

Pumpkin Almond smoothie – in a powerful blender such as the Vitamix (www.vitamix.com), blend the following ingredients.
⅓ cup of organic pumpkin
⅛ teaspoon of cinnamon
⅛ teaspoon of nutmeg
⅓ cup of raw or dry roasted almonds (unsalted)
1 ripe banana
12 ounces of cold water
3 ice cubes.
Blend on high until liquefied and drink.

Two poached eggs over arugula tossed in lemon, pepper and extra virgin olive oil, served with a side of sliced cantaloupe. Toss 1 cup of arugula with 2 teaspoons of fresh lemon juice, 1 tablespoon of extra virgin olive oil and ¼ teaspoon of black pepper. Top with two poached eggs.

Left overs from any lunch or dinner options make a wonderful breakfast

Lunch and Dinner Ideas

Grass-fed beef burger on a bed of romaine lettuce and tomato with grilled or steamed vegetables

Grass-fed filet mignon with sautéed broccoli and a baked sweet potato

Salmon, mixed greens with an olive oil based dressing

Citrus-thyme halibut with sautéed asparagus spears
8 to 10 ounces of halibut
3 tablespoons of fresh lime juice
1 tablespoon of fresh lemon juice
⅛ teaspoon of cayenne pepper or chili powder
1 garlic clove minced
1 tablespoon of fresh thyme chopped
1 tablespoon of extra virgin olive oil
¼ cup of water
Combine lime and lemon juices with cayenne pepper or chili powder, garlic, olive oil and ¼ cup of water to make the marinade. Place fish in a flat dish in the marinade for 15 minutes. Grill for 7 to 10 minutes each side, basting often with the marinade. Serve with asparagus spears sautéed in avocado oil with salt and pepper to taste.

Baked almond crusted chicken breast with cauliflower puree and wilted spinach
1 pound of chicken breasts
¼ cup of almond meal
½ teaspoon of oregano
½ teaspoon of basil
¼ teaspoon of thyme
⅛ teaspoon of black pepper
¼ teaspoon of sea salt
1 whole egg
Preheat oven to 350 degrees Fahrenheit. Mix all the dry ingredients together in a bowl. Whisk the egg in a separate bowl. Rise and pat dry the chicken breasts. Dip each chicken breast into the whisked egg making sure it is evenly coated. Now transfer the chicken breast to the almond meal mixture, lightly coating it to appear "breaded." Repeat with each chicken breast and transfer almond meal coated chicken breasts to a baking pan. Bake for 22 to 25 minutes or until done.

Cauliflower puree
1 head of cauliflower
½ teaspoon of sea salt
¼ teaspoon of black pepper
1 glove of garlic
1 tablespoon of grapeseed oil
1 cup of water
Remove the leaves off the cauliflower and cut out the core. Cut up the cauliflower into 1 ½ inch pieces. Put the cauliflower pieces, garlic and oil in a medium sauce pan with 1 cup of water. Bring to a boil over high heat. Reduce heat and simmer until very tender. Strain off any remaining cooking water and transfer to a blender or food processor add the salt and pepper and blend until smooth. Serve hot.

Shrimp stir fry with vegetables
2 pounds of shrimp, pealed, deveined and tails removed
2 tablespoons of avocado oil
1 clove of garlic minced
1 Vidalia onion cut into thin wedges
1 orange bell pepper cut into thin strips
2 celery stalks, chopped
½ cup of shredded carrots
2 tablespoons of fresh lemon juice
¼ teaspoon of ginger
1 small zucchini, chopped
¼ cup of sliced mushrooms

In a stir fry pan heat the avocado oil and add the shrimp and garlic and sauté the shrimp until opaque (about 3 minutes). Remove the shrimp to a plate. Add all the remaining ingredients to the stir fry pan and stir around for one minute on high heat. Stop stirring and allow the vegetables to cook for 3 more minutes on medium heat. Add the shrimp back, stir, and allow to cook for 1 more minute. Remove from heat and serve.

Oven roasted chicken with baked broccoli and cauliflower
Preheat oven to 375 degrees Fahrenheit. Cut up one half head of broccoli and one half head of cauliflower into bit size pieces and spread out on a baking sheet. Drizzle with 2 tablespoons of extra virgin olive oil and sprinkle with salt, pepper and a pinch of cayenne pepper. Bake for 20 minutes. Serve along side a pre-roasted whole chicken purchased from the market.

Burger and kale chips
1 large bunch of kale
Grass-fed beef burger
4 romaine lettuce leaves
1 heirloom tomato
½ avocado

Preheat oven to 350 degrees Fahrenheit. Wash and remove stems of kale and tear or cut leaves into large "potato chip size" pieces. Spread out on parchment paper on a baking sheet. Drizzle with 1 tablespoon of extra virgin olive oil. Sprinkle with sea salt and pepper. Bake for 20 minutes until crispy and the edges start to brown.

While the kale is baking, grill the grass-fed beef burger to the desired state of cooking (medium rare, medium, well-done) and serve wrapped in the romaine lettuce leaves with sliced tomato and avocado.

Baked salmon with lemon and chive roasted asparagus

Lamb chops with sautéed collard greens

Grilled chicken breast sliced over mixed greens with slivered almonds, extra virgin olive oil, lemon, salt and pepper

Bison burger on a bed of lettuce and tomato with roasted red and yellow bell peppers and sautéed spinach

Fresh roasted turkey breast served with roasted acorn squash and steamed spinach

Recommended cookbook:
The Paleo Diet Cookbook by Loren Cordain, PhD and Nell Stephenson. 2010. ISBN-10: 0470913045 or ISBN-13 978-0470913048. Publisher: Houghton Mifflin Harcourt.

Appendix B: Collected Data

Collected Data Academy/AHA Diet						
Identifiers	Total Cholesterol	HDL	LDL	Triglycerides	Age	Race
1F	225	81	112	160	41	Caucasian
2F	245	39	178	140	44	Caucasian
3F	235	80	130	125	45	Hispanic
4F	219	59	151	45	48	Caucasian
5F	222	37	143	210	51	Caucasian
6F	230	55	141	170	52	Caucasian
7F	235	88	131	80	58	Caucasian
8F	203	35	141	135	59	Hispanic
9F	290	67	165	291	59	Caucasian
10F	217	48	139	150	62	Caucasian
11M	234	42	171	105	42	Caucasian
12M	207	45	128	170	46	Caucasian
13M	269	43	206	100	47	Caucasian
14M	220	36	152	160	48	Caucasian
15M	215	75	128	60	51	Caucasian
16M	250	44	178	140	59	Caucasian
17M	221	35	158	140	59	Hispanic
18M	218	41	157	100	60	Caucasian
19M	218	39	136	215	61	Caucasian
20M	210	41	141	141	61	Caucasian

F = Female, M = Male

Collected Data Paleolithic Diet	Total Cholesterol	HDL	LDL	Triglycerides	Age	Race
1F	162	83	61	91	41	Caucasian
2F	190	70	100	101	44	Caucasian
3F	177	92	71	69	45	Hispanic
4F	183	70	107	32	48	Caucasian
5F	177	69	88	100	51	Caucasian
6F	190	79	91	100	52	Caucasian
7F	188	92	82	72	58	Caucasian
8F	168	68	80	99	59	Hispanic
9F	263	68	165	149	59	Caucasian
10F	175	71	87	87	62	Caucasian
11M	239	55	169	76	42	Caucasian

12M	166	57	85	120	46	Caucasian
13M	181	58	110	65	47	Caucasian
14M	153	45	88	100	48	Caucasian
15M	190	80	92	90	51	Caucasian
16M	189	88	91	50	59	Caucasian
17M	155	52	90	65	59	Hispanic
18M	151	79	63	43	60	Caucasian
19M	174	57	99	90	61	Caucasian
20M	165	57	101	35	61	Caucasian

F = Female, M = Male