THE EFFECTS OF WEIGHT LOSS INTERVENTION ON LIPOPROTEIN PROFILES AND RELATIONSHIP TO RISK FACTORS FOR CARDIOVASCULAR DISEASE

A Dissertation

by

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ABSTRACT

Previous investigations from our lab indicated that women adhering to a hypo-energetic diet combined with supervised exercise promoted reductions in low-density lipoprotein (LDL) cholesterol and maintenance of high-density lipoprotein (HDL) cholesterol. The purpose of this study was to conduct additional analyses to determine whether the diet and exercise intervention had significant effects on lipoprotein subclasses. Samples from 75 participants who were randomly assigned to a no exercise or diet control or to higher- (55% kcal for carbohydrate and 15% kcal for protein), moderate- (30% kcal for carbohydrate and 45% kcal for protein), or lower-carbohydrate (20% kcal for carbohydrate and 45% kcal for protein) hypo-energetic (1,400 kcal for week 1 and 1,500 kcal for weeks 2-24) diets while participating in a resistance-based circuit training program. Results revealed that the higher protein groups showed significant improvements in total cholesterol, LDL-cholesterol, and total to HDL-cholesterol ratio, whereas all diet and exercise groups maintained HDL-cholesterol. Baseline, 12-week, and 24-week samples were analyzed for LDL and HDL lipoprotein subclasses employing a modified ultracentrifugation technique[1]. Data were analyzed using general linear model with repeated measures and Pearson's correlation. Overall, we did not find that adherence to diet and exercise had any significant effect on lipoproteins or their respective subclasses. However, correlative data indicate that improvements in anthropometrics, body composition, cardiovascular fitness, glucose homeostasis, and blood lipids significantly correlated to larger LDL and HDL subclasses.

CONTRIBUTORS AND FUNDING SOURCES

Contributors

This work was supported by a dissertation committee consisting of Richard B. Kreider, PhD, Professor of the Department of Nutrition and Professor and Department Head of Health and Kinesiology, Rosemary L. Walzem, PhD, Professor of the Department of Poultry Science, Stephen B. Smith, PhD, Professor of the Department of Animal Science, and Stephen F. Crouse, PhD, Professor of the Department of Health and Kinesiology. The data analyzed for Chapters IV and V was provided by Professors Richard B. Kreider, PhD and Rosemary L Walzem, PhD. The analyses depicted in Chapters IV and V were conducted in part by the graduate students and assistants of the Exercise and Sport Nutrition Laboratory of the Department of Health and Kinesiology, Texas A&M University. Additional training was received by Professor Rosemary L. Walzem, PhD and additional analysis depicted in Chapters IV and V were conducted in the laboratory of Professor Rosemary L. Walzem, PhD by the student. All other work conducted for the dissertation was completed by the student independently.

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DEDICATION

I dedicate this first and foremost to my Lord and Savior Jesus Christ and to the amazing family He put in my life to be my support and strength through it all. To my mom, Norma, who was always there to help me when I was down, be my sounding board, and my support, to my brother, Peter, and to my loving husband, Albert Elrod IV, who continuously had the faith and confidence in me even when I doubted myself and thought I didn't have what it takes to accomplish my dream.

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NOMENCLATURE

CHO	Carbohydrate
DASH	Dietary Approaches to Stop Hypertension
FM	Fat Mass
FFM	Fat Free Mass
HCLF	Higher Carbohydrate, Low Fat Diet
HDL	High-density Lipoprotein
Kcal	Kilocalorie
LCHP	Lower Carbohydrate, Higher Protein Diet
LDL	Low-density Lipoprotein
MCHP	Moderate Carbohydrate, Higher Protein Diet
NED	No Exercise or Diet Control
PRO	Protein
RDA	Recommended Dietary Allowance
TRL	Triglyceride-Rich Lipoproteins
VAT	Visceral Adipose Tissue
VO_{2peak}	Max Peak Aerobic Capacity

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CHAPTER I

INTRODUCTION AND RATIONALE

Background

Cardiovascular disease (CVD) is the leading cause of death in the United States. In 1948, Congress commissioned the initiation of the Framingham Heart Study, which is a long-term study under the control of the National Heart, Lung, and Blood Institute. In this study, investigators sought to uncover factors that increase heart disease in participants from Framingham, Massachusetts to develop a deeper understanding of the causes of CVD. This study progressed to form cohorts of the spouses and offspring of individuals from the original cohort. Through studies such as the Framingham Heart Study, investigators identified CVD "risk factors" referring to traits (e.g. age, gender, blood pressure, total cholesterol, LDL-, and HDL-cholesterol levels, dyslipidemia, obesity, and smoking) that increase the likelihood of developing CVD [2]. Research also asserts that cardiovascular fitness (e.g., VO_{2peak} being a measure of cardiovascular fitness) is strongly related to CVD and can improve risk assessment when considered with established risk factors [3].

Although risk factors such as age, gender, and family history, cannot be manipulated to reduce risks of developing CVD, adopting lifestyle changes that reduce and/or maintain a healthy weight is vital for the prevention and treatment of obesity (having a BMI $\geq$30 kg/m^2) which is an independent risk factor for CVD [4]. According to the Center for Disease Control, a consensus report from 1999-2000 found that 30.5% of adults were obese, and this number has continued to increase where data from 2015-2016 indicates that 39.6% of adults and 18.5% of youth in the United States are obese [5, 6]. Additionally, research has shown that central as opposed to peripheral fat deposition referred to as visceral adipose tissue (VAT) is more indicative of health

than waist-to-hip circumference where individuals with a VAT area ≥106 cm^2 had an increased risks to CVD [7] and those with a VAT area ≥ 163 cm^2 were more likely to have elevated triglycerides, lower HDL-cholesterol, reduced HDL2 subclasses, greater two-hour fasting glucose, and fasting insulin compared to women in lowest and second lowest VAT quintiles [7]. The strong correlation between obesity and CVD led the American Heart Association to established goals for 2020 to reduce CVD in the United States by promoting lifestyle changes that include healthier eating plans and increases in physical activity [8, 9].

Previous findings from studies conducted by the Exercise and Sport Nutrition Laboratory (ESNL) showed that adopting a hypo-energetic diet with regular exercise training, regardless of macronutrient distribution, is beneficial for reducing weight and fat while preserving fat free mass [10]. Additionally, higher protein intakes show more favorable effects on risk factors for metabolic syndrome and CVD [10-13] (further discussed in Chapter II). A number of these studies conducted from our lab assessed the implementation of the Curves® program [11-13]. In a study conducted by Sanchez, et al. [12] participants were randomized into a no exercise or diet group (NED) or to one of three hypo-energetic diets: a higher carbohydrate, lower fat diet; a moderate carbohydrate, higher protein, low fat diet; or a lower carbohydrate, higher protein, moderate fat diet and were instructed to participate in resistance-based circuit training four times a week. Results at 24 weeks indicate that adherence to a reduced energy diet combined with resistance-based circuit training significantly reduced body weight, fat mass, percent body fat, and waist circumference, maintained fat-free mass, and significantly increased relative maximum peak aerobic capacity (VO_{2peak}) [12]. In a concurrent study including assessment of the Curves® program, diet and exercise groups showed a significant decrease in VAT area from baseline [13]. All subjects assigned to hypo-energetic, high protein diets, with either a moderate or low

carbohydrate intake, with exercise experienced a significant decrease in LDL-cholesterol and retention of HDL-cholesterol [12].

Considering the findings from our lab indicating effects on weight, body composition, cardiovascular fitness, glucose homeostasis, and blood lipids and the scientific literature suggesting strong relationships between CVD and lipid subclasses, we sought to conduct additional analysis of our own study to evaluate the effects of hypo-energetic diet and exercise interventions varying in macronutrient intake on lipoprotein subclasses in overweight/obese women.

Statement of Problem

Will hypo-energetic diets varying in macronutrient composition-combined with exercise alter an individual's lipoprotein profile? Secondarily, how do atherogenic and atheroprotective lipoprotein subclasses correlate with markers of health and fitness?

Purpose

The purpose of this study was to determine if macronutrient intake is important for creating a more atheroprotective lipoprotein profile.

General Study Overview

Samples were obtained from Sanchez et al. [12], a previous investigation conducted at the ESNL where participants were assigned to no diet or exercise or one of three hypo-energetic diets: a higher carbohydrate, lower fat diet (HCLF: 55%, PRO: 15%, FAT: 30%) recommended by the American Heart Association, a moderate carbohydrate, higher protein, lower fat diet (MCHP: CHO: 30%, PRO: 45%, FAT: 25%) with access to Curves® online diet plan, a lower carbohydrate, higher protein, moderate fat diet (LCHP: CHO: 20%, PRO: 45%, FAT: 35%) combined with a resistance-based circuit training program. Results from this study showed that

all intervention groups experienced a significant reduction in weight (kg), body fat (kg), and percent body fat. A secondary analysis utilizing isopycnic ultracentrifugation looked at the effect of these hypo-energetic diets varying in macronutrient intake with exercise on lipoprotein subclasses. All subjects were considered; however, only subject samples with a sufficient supply of serum at 0-, 12-, and 24-week time points were used for further analysis using high-density isopycnic ultracentrifugation. The LDL and HDL lipoprotein subclasses for each subject at 0-, 12-, and 24-week time points were measured employing modified ultracentrifugation technique [1], based on McFarlane's method [14], which utilizes fluorescent labeling of lipoproteins and isopycnic ultracentrifugation.

Through statistical analysis, lipoprotein profiles of serum from blood samples taken at 0-, 12-, and 24-weeks were compared between groups and correlated with changes in anthropometric values (e.g., weight, waist and hip circumference), DEXA analysis (e.g., VAT area), cardiovascular fitness (e.g., VO_{2peak} and time to exhaustion), insulin resistance (e.g. insulin, glucose, and HOMA-IR), and blood lipids (e.g. triglycerides, total cholesterol, LDL-cholesterol, and HDL-cholesterol).

Hypothesis

Hypothesis 1: Significant changes to LDL subclasses favoring a less atherogenic lipid panel will be observed among subjects assigned to diet and exercise interventions.

Hypothesis 2: Significant changes to HDL subclasses favoring a more cardioprotective lipid panel will be observed among subjects assigned to diet and exercise interventions.

Hypothesis 3: Changes to the lipid panel reflecting less atherogenic LDL and more atheroprotective HDL subclasses will more likely be observed among subjects assigned to the higher protein diet and exercise interventions.

Hypothesis 4: Significant correlations between lipoprotein subclasses and anthropometrics will be observed.

Hypothesis 5: Significant correlations between lipoprotein subclasses and body composition will be observed.

Hypothesis 6: Significant correlations between lipoprotein subclasses and cardiovascular fitness will be observed.

Hypothesis 7: Significant correlations between lipoprotein subclasses and markers of insulin resistance will be observed.

Hypothesis 8: Significant correlations between lipoprotein subclasses and blood lipids will be observed.

Delimitations

1. This study was limited to the participants who completed the Sanchez et al. [12] study that included 86 women (37.5 ± 13.4 years of age with a BMI of 30.5 ± 5.9 kg/m^2) recruited from the Brazos county and neighboring counties for participation.
2. All eligible participants were familiarized with the testing protocol and informed of individual requirements and responsibilities as previously described by Sanchez et al. [12]. After familiarization participants still interested in participating filled out all necessary paperwork including medical history and informed consent followed by scheduling of their initial testing session.
3. Participants did not consume ergogenic aids of any kind for at least six months prior to and throughout the study.
4. Participants did not have weight changes greater than or equal to seven pounds nor did they participate in any weight loss program for at least six months prior to the study.

5. Participants were not breastfeeding, had not born a child 12 months prior to the study, and did not plan to become pregnant in the next 12 months.
6. Participants were healthy in that they had no uncontrolled metabolic condition.
7. Participants did not exercise 48 hours prior to each testing session.
8. Participants did not consume NSAIDs or alcohol 24 hours prior to baseline testing.
9. Samples used for further analysis of lipoprotein subclasses using isopycnic ultracentrifugation included 75 women (24-52 years of age with a BMI of 24-36 kg/m^2) of the 82 subjects from the previous study by Sanchez et al. [12], and although all subjects were considered, only samples with serum available at 0-, 12-, and 24-week time points were used for quantification of lipoprotein subclasses using isopycnic ultracentrifugation.

Limitations

1. Participant recruitment was limited to the Brazos County and individuals from cities in local counties. Therefore, the applicability of the findings from this study may not represent the general population.
2. Food log data was self-reported presenting a possible source of inaccuracies. Participants may forego reporting all foods consumed or under-/over-report the quantity of intake. In like manner, participants may estimate the incorrect quantity of food intake.
3. Results from this study reflect the efforts of individuals willing to comply with changes to both diet and exercise possibly affecting the application of these findings to individuals from the general population who may not be as willing to undergo these lifestyle changes.

4. Participants were given access to resistance exercise machines as part of the Curves® Program at no cost to them; however, members of the general population may not have easy access to equipment at little to no cost affecting the application of these findings to the general population.
5. Participants were given access to a private environment to exercise and were provided with exercise training and dietary advice by ESNL staff throughout the entire program. This affects applicability of results to the general population where individuals in the general population may not have outside encouragement and support.
6. Ethnicity will not be taken into consideration regarding correlative data; however, ethnicity has been found to play a role in CVD risks.
7. Laboratory equipment calibration and use for data collection and analysis may serve as a source of error.
8. Lipoprotein quantification using ultracentrifugation technique for measurement of lipoprotein subclasses is a source of possible error because lipoprotein layering is dependent on the precision and accuracy of the individual applying the density gradient solution, ceramide, and hexane layer.
9. Intrinsic error can arise from camera equipment used for capturing and generating lipoprotein pixel data.

Assumptions

1. Accuracy in answering screening questions and reporting dietary intake in food logs was monitored throughout the study [12].
2. Participants complied with assigned diet throughout the entire length of time enrolled in the study [12]

3. Participants fasted for 12 hours prior to each testing session.
4. Participants did not exercise for 48 hours prior to each testing session.
5. All participants were provided with equal access to exercise equipment, exercise training, and dietary advice.
6. Participants exerted maximal effort for endurance and strength tests.
7. Normal distribution was expressed among the population.
8. Equal variability exists between each of the respective diet groups.
9. All laboratory equipment was properly calibrated by trained laboratory personnel prior to each testing session throughout the study.
10. All required training was completed prior to conducting isopycnic ultracentrifugation for proper handling of solutions and use of laboratory equipment.
11. The standard operating procedure for isopycnic ultracentrifugation was carried out correctly, which includes preparation of NaBiEDTA/serum/ceramide mixture.
12. Each sample number and testing session was correctly noted and assigned to respective data output.
13. Samples were analyzed with appropriate software.

CHAPTER II

REVIEW OF THE LITERATURE

Reflecting on the previous studies from our lab, the effects of weight loss intervention programs improve body composition, cardiovascular fitness, and risk factors of CVD including total- and LDL-cholesterol CVD [10-13, 15]. We would additionally like to consider individual LDL and HDL subclasses to determine whether implementing exercise and hypo-energetic diets of varying macronutrient distribution decreased deleterious LDL subclasses while increasing atheroprotective HDL subclasses.

The purpose of this literature review is to briefly discuss CVD and lipids. We will consider low- (LDL) and high-density lipoproteins with their respective lipoprotein subclasses with focus on their role in CVD and relationship with CVD risk factors. This review will also cover the influence of energy intake, macronutrient distribution, and exercise on lipids and their respective lipoprotein subclasses.

Cardiovascular Disease

Cardiovascular disease continues to be a major threat to health. More recent data estimated that 800,937 of 2,596,993 deaths (30.8% of deaths; 402,851 males and 398,086 women) were linked to CVD in 2013 [16]. Cardiovascular disease includes diseases that affect the heart (e.g., cardiomyopathy, heart failure, valvular heart disease, and rheumatic heart disease) and blood vessels (e.g., aortic aneurysm, cerebrovascular diseases, coronary artery disease, and peripheral arterial disease) [17]. Alarmingly, data from 2009-2012 indicate that over 100,000,000 individuals over the age of 20 have a plasma level of total cholesterol that exceeds 200 mg/dL. According to the American Heart Association, 79.7% US children, 12-19 years of age, have ideal total cholesterol (<170 mg/dL), and 63.7% adults 20-49 years of age and 30.1%

of adults over the age of 50 have ideal total cholesterol (< 200 mg/dL) [8]. Research has consistently shown the direct relationship between LDL-cholesterol [18-20] and CVD and the indirect relationship between HDL-cholesterol [2, 21] with CVD where each acts independently to increase and decrease CVD, respectively [18, 22]. This relationship has been simplified by referring to LDL-cholesterol as "bad" cholesterol and HDL-cholesterol as "good" cholesterol. Correspondingly, the prevention and treatment of CVD has been focused on lowering "bad" and raising "good" cholesterol carrying lipoproteins. However, laboratory techniques revealed LDL and HDL subclasses differing in structure. The elucidation of which spurred investigations as to whether these subclasses had differing effects on risks for CVD [23-26]. A summary of some of the observations in research investigating the relationship between lipoproteins, respective subclasses included, and CVD will be briefly discussed.

Cardiovascular Disease, Lipoproteins, and Lipoprotein Subclasses

Low-density Lipoproteins and their Subclasses

LDLs are further subdivided into five different subspecies based on density (1.019-1.063 g/mL) [27]. The identification of different LDL subclasses through ultracentrifugation and gradient gel electrophoresis enabled investigators and clinicians to categorize individuals into one of two patterns of LDL subclass expression: Pattern A, characterized by a higher percent of larger (20.6-22.0 nm), less dense LDL particles and Pattern B, characterized by a greater percent of smaller (19.0-29.5 nm), denser LDL particles [27]. Further research identified the positive relationship between CVD and smaller, denser LDL particles [27-29] where individuals expressing a Pattern B LDL profile had a three-fold increase for developing coronary heart disease [30].

The development of atherosclerosis is one mechanism that partially explains the potential

damaging effects of LDLs. Although several mechanisms describing the origin of atherosclerosis exist, mounting evidence points to the response-to-retention hypothesis, which emphasizes the relationship between LDLs and CVD [31, 32]. The general mechanism involves small, dense LDL particles that are easily removed from the lumen by subendothelial cells due to their high binding affinity for proteoglycans and glycosaminoglycans located within the arterial wall [31, 33]. These LDL particles incur oxidative damage within the arterial wall releasing chemical signals that recruit macrophages for phagocytosis. Macrophages will then form foam cells within the arterial wall and combine with accumulating smooth muscle cells embedded in the subendothelium narrowing the lumen and leading to further release of cytokines [27, 28, 33, 34]. The clinical practice of administering LDL-cholesterol lowering medication is plausible since treatments have been shown to effectively lower LDL-cholesterol to be within normal ranges [28, 35]; however, "residual risks" may still be present while a significant level of small, dense LDL particles that are more atherogenic remain [36]. Currently, standard cholesterol panels do not differentiate LDL subclasses. Clinicians may better address the health of their patients by implementing diagnostic tools that quantify and differentiate LDL subclasses along with the development of treatments/strategies (e.g., pharmaceuticals, lifestyle changes) that effectively reduce small, dense LDL-cholesterol [28, 35].

High-density Lipoproteins and their Subclasses

The inverse relationship that exists between HDL and CVD [21, 37-40], prompted investigators to develop a better understanding of HDL structure and function. HDL serves as a vehicle that carries cholesterol through the bloodstream and to the liver via reverse cholesterol transport. Individuals with HDL-cholesterol < 35 mg/dL experienced an eight fold increase in the incidence of coronary heart disease compared to those with HDL-cholesterol ≥ 65 mg/dL [18].

Gofman et al. [24] reported that analytical ultracentrifugation had been used to divide HDL into two HDL subclasses, the less dense HDL2 (1.063-1.125 g/mL) particles and the denser HDL3 (1.125-1.21 g/mL) particles [24]. Other methods of HDL separation have been implemented allowing for the further division of HDL subclasses; however, the development of numerous methods that separate HDL-cholesterol subfractions led to a number of different classifications where some methods such as analytic ultracentrifugation separates HDL into two subclasses based on density (HDL2 and HDL3), while other methods such as nondenaturing polyacrylamide gradient gel electrophoresis separate HDL into 5 subclasses based on particle size (e.g. HDL2b, HDL2a, HDL3a, HDL3b, and HDL3c) [41]. This paper will refer to the five distinct HDL subclasses isolated using isopycnic ultracentrifugation, the gold standard method used to separate LDL and HDL subclasses in the lab [41, 42]. As described by non-denaturing polyacrylamide gradient gel electrophoresis, isopycnic ultracentrifugation also identifies 5 distinct HDL subclasses based on density [43] which are listed according to size in Table 2.1.

Table 2.1: HDL subclasses and respective sizes (nm)

HDL Subclass	Diameter (nm)
HDL2b	9.7-12.0
HDL2a	8.8-9.7
HDL3a	8.2-8.8
HDL3b	7.8-8.2
HDL3c	7.2-7.8
HDL subclass designation upon separation via isopycnic ultracentrifugation. High-density lipoprotein, HDL. Nanometers, nm.	

Although the mechanism by which HDL is cardioprotective remains to be elucidated, studies suggest that larger HDL subclasses are atheroprotective [37, 44-47]. For instance, Gilmore et al. [35] noted that increases in the HDL2b subclass were related with a reduced risk for CVD [35], and Salonen et al. [48] found that individuals with a serum HDL2 level less than 25 mg/dL increased risks for myocardial infarction four-fold. The strength of this relationship

was shown in Finnish families with low HDL-cholesterol by assessing carotid intima media thickness, a means of quantitatively evaluating atherosclerosis. In this population, intima-media thickness was negatively correlated with HDL-cholesterol ($R = -0.23$, $p<0.05$), HDL particle size (nm) ($R = -0.26$, $p<0.05$), and HDL2b ($R = -0.31$, $p<0.01$) with the HDL2b subclass having the highest negative correlation with intima-media thickness [47]. The degree of involvement of individual HDL subclasses in reverse cholesterol transport and CVD is complex requiring further investigation, but mounting evidence supports the inverse relationship between larger HDL subclasses and CVD suggesting strategies that increase larger HDL subclasses, such as HDL2b, may be more effective for reducing CVD compared to methods that merely increase HDL-cholesterol [41].

Energy Balance and Lipoprotein Subclasses

Weight loss, shown to be related to changes in triglycerides and total cholesterol, is an important means of improving blood lipids to reduce risks to CVD [49]. Adjusting diet to reduce energy intake and/or adding exercise to increase energy expenditure creates a negative energy balance that is key to weight loss.

Reducing energy intake and increasing energy expenditure have also been shown to increase HDL2 mass, increase LDL particle diameter, and decrease small LDL [50], yet evidence indicates that the effects of diet and exercise differ. For instance, Pedersen et al. [1] conducted a study in sedentary, overweight individuals diagnosed with coronary artery disease and compared the effects of low energy diet (800-1,000 kilocalories per day [kcal/day]; n=29) to those of aerobic interval training (85-90% peak heart rate three times a week; n=26) on the lipid profile. Quantification of AUC showed that subjects in both the low energy diet and aerobic interval training group significantly decreased low-density lipoprotein (LDL) cholesterol (19.4% change,

p=0.001 and 12.5% change, p=0.004, respectively), and although LDL5 decreased in both groups, LDL5 was more significantly decreased for subjects in the low energy diet (32.4% change, p<0.001) than for those in the aerobic interval training group (12.8% change, p=0.025). Similarly, subjects in the low energy diet significantly reduced smaller HDL subclasses and triglyceride-rich lipoproteins (TRL)s (HDL3a: 18.2% change, p<0.001; HDL3b: 21.2% change, p<0.002; HDL3c: 19.3% change, p<0.001; TRL: 37.2% change, p<0.002; respectively) more significantly compared to aerobic interval training (HDL3a: 14.8% change, p=0.028; HDL3b: 3.4% change, p=0.659; HDL3c: 0.1% change, p=0.990; TRL: 21.6% change, p=0.077; respectively). Investigators concluded from this study that the effects of diet and exercise effect lipoproteins independently from each other [1].

The importance of macronutrient intake within hypo-energetic diets has also been considered. Meckling et al. [51] compared the effects on subjects assigned to four groups: no diet and no exercise control (CHO: 2.20 g/kg/day; PRO 0.70 g/kg/day; FAT: 0.67 g/kg/day), exercise control (CHO: 1.99 g/kg/day; PRO: 0.73 g/kg/day; FAT: 0.52 g/kg/day), higher protein control (CHO: 1.51 g/kg/day; PRO: 1.0 g/kg/day; FAT: 0.71 g/kg/day), and higher protein with exercise (CHO: 1.28 g/kg/day; PRO: 1.33 g/kg/day; FAT: 0.40 g/kg/day). Caloric intake was reduced by 500 kcal/day for all diet groups. Exercise groups performed alternating 60-second bouts of endurance exercise for 36 minutes at 65% maximum heart rate three days a week for three weeks and increased to 80% maximum heart rate by 12 weeks. Subjects assigned to the exercise control, higher protein control, and higher protein with exercise experienced a significant reduction in weight (kg) (p<0.001), fat mass (kg) (p<0.001), and percent body fat (p<0.001) from baseline values, and all groups retained lean mass (kg). Those assigned to the higher protein control and exercise control groups experienced significant decreases in total cholesterol, but

only the higher protein control experienced a significant decrease in LDL-cholesterol (41.5% change, 130±124 mg/dL at baseline to 76±37 mg/dL at 12 weeks, $p<0.05$). Additionally, only those assigned to a higher protein diet with exercise experienced significant reductions in triglycerides (29.8% change, 154±69 mg/dL at baseline to 108±28 mg/dL at 12 weeks, $p<0.05$) [51]. No group experienced a significant change in HDL-cholesterol. As we will mention in more detail under "Prior Weight Loss Research", in an investigation from our lab, the comparison of hypo-energetic diets either higher in carbohydrates or higher in protein intake showed that only subjects assigned to the higher protein groups experienced significant reductions in LDL-cholesterol [12].

Numerous studies focus on weight reduction by instructing overweight individuals to reduce energy intake and increase physical activity. Although the adoption of either lifestyle change will show benefits, diet and exercise appear to alter blood lipids independently from each other [1, 35]. Findings support that the implementation of weight loss programs incorporating both diet and exercise are more promising than adopting either of these lifestyle changes alone in order to modify lipids in a way that reduces risks to CVD [1, 35]. Some studies suggest that higher protein diets may offer additional benefit when following a hypo-energetic diet [12, 51].

Macronutrient Intake and Lipid Subclasses

The following paragraphs will look at the individual and combined effects of diet and exercise on lipoproteins and their subclasses. Diet is a key area to implement changes, and although several diets have been designed to reduce weight, research has yet to conclude if there is an ideal macronutrient intake that more effectively reduces weight and improves parameters of health. In general, exercise is beneficial for reducing risks to CVD, and studies support that the effects of endurance and resistance exercise on lipoproteins differ. Further discussion regarding

the effects of increasing and/or decreasing specific macronutrients and the effects of endurance versus resistance exercise on lipoproteins and their subclasses follow.

High Protein

The amount of protein intake necessary to induce positive changes in health has been debated. The estimated average requirement for protein intake is 0.66 g/kg/day and the recommended dietary allowance (RDA) for protein intake is 0.80 g/kg/day). According to recent analysis in the National Health and Nutrition Examination Survey (2001-2014), Americans consume well above the RDA for protein intake where the relative protein intake in the US averages to 1.10±0.01 g/kg of ideal body weight for adults ≥71 years and 3.63±0.07 g/kg of ideal body weight for children two to three years of age [52]. This report may suggest that the RDA for protein intake should be increased. Research provides further support for greater protein intakes. For example, Pasiakos et al. [53] found that diets with protein intakes above RDA (1.0-1.5 g/kg) were associated with a greater concentration of HDL-cholesterol [53]. Values for protein intake recommended by MyPlate (1.48-1.86 g/kg/day) and the reported beneficial intakes reported by Pasiakos exceed the RDA and fall safely within the AMDR (1.05-3.67 g/kg/day) [54]. Although the RDA is only an estimate of the protein intake necessary to maintain health, based on data reporting average protein consumption and accumulating research elucidating the benefits of diets higher in protein some investigators suggest the RDA be increased [55]. Further studies must be conducted to establish a need for increasing the RDA.

Protein intakes should be reported consistently across studies to properly define the protein "dosage" linked to reduced risks to diseases such as CVD. When comparing high protein diets to higher carbohydrate diets, we must draw consensus as to what is considered a high protein diet. Antonio et al. [6] recommends reporting protein intake as grams per kilogram of

body weight instead of percentages of macronutrient intake. Defining protein intake in terms of percentages can be misleading. For example, 45% of a 1,200-kcal diet versus a 2,000-kcal diet allows for a large range of variation; moreover, it does not consider individual body mass.

Antonio, et al. [6] suggests high protein diets be defined as the daily consumption of protein that exceeds 2.0 g/kg/day. Based on this definition, when referring to protein intakes, we will consider protein intakes above 2.0 g/kg/day as high protein diets. Studies, including our own, that report protein intakes greater than comparable diets, but below 2.0 g/kg/day will be referred to as "higher protein" diets. Another thing to consider is the reporting of protein intake as g/kg/day for overweight subjects. Some argue that considering protein intakes as g/kg/day may underestimate protein needs in overweight subjects compared to that of healthy weight subjects. Thus, when considering protein assignment and their effects across studies, which may include overweight and/or healthy weight subjects, we need to consider if there is a need to prescribe and report intakes per body weight, as most studies in this review have done or per ideal body weight, as performed in Berryman et al. [52]. Overall, studies investigating the effects of macronutrient intake on health parameters report intakes as g/kg/day so for this study, we will simply report in this manner to avoid entering the debate over prescribing intakes per lean mass. In doing so we hope to add to the bulk of literature that seeks to determine an ideal "dosage" of macronutrient intakes that best support overall health [6].

Studies investigating the effects of consuming diets high in protein compared to carbohydrates support that increasing protein consumption is beneficial. The Nurses' Health Study provided evidence that replacing carbohydrates with protein reduced risks for ischemic heart disease in women [56]. Higher protein diets were not only recommended for improving the lipid profile, but also for preserving lean muscle in individuals adopting a reduced energy diet to

lose weight [57]. We find that adopting a higher protein diet with exercise has beneficial short- and long-term effects on the lipid profile. In a 10-week clinical trial, Layman et al. [57] investigated the effects of a higher protein diet (125 g PRO/d; 1.6 g PRO/kg/day) to a higher carbohydrate diet (68 g PRO/d; 0.8 g PRO/kg/day) on body composition and blood lipids in overweight women. Both groups experienced a significant reduction in weight where the higher protein diet group lost 7.53±1.44 kg (8.9% change) and the higher carbohydrate diet group lost 6.96±1.36 kg (8.1% change) with no significant difference between groups. Although total cholesterol was reduced in the higher protein and higher carbohydrate diet groups, the higher protein groups with and without exercise retained HDL-cholesterol levels (nonsignificant 4.2% increase and 2.3% decrease, respectively) while the higher carbohydrate groups with and without exercise experienced decreases in HDL-cholesterol levels (nonsignificant 5.9% decrease and a significant 7.7% decrease, $p<0.05$). Investigators observed that the higher protein group and the higher protein with exercise group expressed a significant reduction in triglycerides (21.1% and 25.2% respectively) [57]. In a follow up to their 10-week study, Layman et al. [58] addressed the chronic effects of diet and exercise intervention comparing a higher protein diet (40% CHO, 30% PRO, 30% FAT; 1.6 g PRO/kg/day) to a higher carbohydrate diet (55% CHO, 15% PRO, and 30% FAT; 0.8 g PRO /kg/day) in 130 obese men and women (48±8 years of age) for a total period of 12 months (4 weeks weight loss followed by 8 months maintenance). Lipid values (LDLs, HDLs, and triglycerides) were obtained at 0, 4, and 12 months from the day of dietary intervention. Both groups significantly reduced weight and percent body fat. Regarding lipids, the higher protein and higher carbohydrate groups experienced positive changes in HDL-cholesterol and triglycerides. The higher protein and higher carbohydrate group experienced a significant increase in HDL-cholesterol at 4 months and again at 12 months, and the increase in

HDL-cholesterol for the higher protein group was significantly greater than that of the higher carbohydrate group ($p<0.05$). Both groups experienced a reduction in triglycerides at 4 months that was maintained at 12 months, and the higher protein group maintained a significantly greater reduction in triglycerides compared to the higher carbohydrate group at both time points ($p<0.05$). Data support that reducing carbohydrates by replacing them with proteins enhanced the beneficial changes observed for HDL-cholesterol and triglycerides in this study [59].

Carbohydrates

Research including epidemiological studies has elicited that low-fat, high-carbohydrate diets are associated with higher fasting plasma triglycerides [60-62]. Having a high level of plasma triglycerides is a risk factor for CVD where hypertriglyceridemia is associated with myocardial infarction, ischemic heart disease, and death [63]. Higher carbohydrate diets have also shown negative effects on HDL-cholesterol. In a cross-over control designed study, nine healthy men were assigned to consume either a higher carbohydrate (65% calories from carbohydrates) or lower carbohydrate (15% calories from carbohydrates) diet for three weeks followed by a second three-week period where men initially assigned to a lower carbohydrate diet switched to a higher carbohydrate diet, and men initially assigned to a higher carbohydrate diet switched to a lower carbohydrate diet. Men initially assigned to the higher carbohydrate diet experienced a rise in triglycerides at one and two weeks followed by a return to baseline values. The HDL2 to HDL3 ratio during the time of higher carbohydrate consumption was significantly lower than the time of lower carbohydrate consumption indicating that smaller, less mature HDL subclasses were significantly greater in the higher carbohydrate group. The reduction of beneficial/more mature HDL subclasses led investigators to conclude that the higher carbohydrate diet implemented in this study did not reduce risks to CVD [64]. Similarly, other

investigators found that individuals consuming a higher carbohydrate diet experienced a greater reduction in HDL2 to HDL3 ratio compared to those on a lower carbohydrate diet [65-68]. Moreover, the isoenergetic replacement of saturated fatty acids with carbohydrates increased fasting plasma triglycerides and the total to HDL-cholesterol ratio [62]. Other studies observed this inverse relationship between plasma triglycerides and HDL-cholesterol [60, 64, 69] where reduced levels of HDL-cholesterol often accompanied elevated levels of triglycerides.

Although numerous studies support that higher carbohydrate diets increase plasma triglycerides and insulin resistance [70], studies also support that higher carbohydrate diets reduce risks to CVD [71]. To help clarify the discrepancies among the effects of high- versus low-carbohydrates, Liu et al. [72] made a clear distinction between the effects of carbohydrates from whole grains and carbohydrates from refined starches/added sugars. His investigation found that replacing 5% of energy intake from saturated fatty acids with carbohydrates from whole grains reduced risks to coronary heart disease by 9%; whereas, replacing 5% saturated fatty acids from refined carbohydrates/added starches had no significant association with coronary heart disease; thus, findings concluded that the type of carbohydrate consumed determines whether carbohydrate consumption reduces risks to CVD [73]. The American Heart Association recognizes the relationship between simple carbohydrates and plasma triglycerides, stating that the consumption of specific carbohydrates, namely fructose and simple sugars within refined grains, contributes to increases in plasma triglycerides [14]. Considering the observed effects of high-carbohydrate diets and the study by Liu et al. [72], reducing dietary carbohydrate intake from refined starches/added sugars is a means of reducing risks to CVD. All carbohydrates are not equal in effect on triglycerides and blood lipids; thus, the type of carbohydrates consumed in the high-carbohydrate diets implemented in studies that seek to determine their effects on health

parameters need to be carefully recorded and reported to differentiate between the effects of simple versus complex carbohydrate intake.

Dietary Fat Intake

Initially, governing bodies such as the American Heart Association recommended reducing dietary intake of saturated fatty acids as a healthy means to lose weight [62, 72]. Investigators supported that the implementation of a low-fat, high-carbohydrate diet reduced risk factors to CVD by reducing total cholesterol and LDL-cholesterol [37, 74, 75]; however, investigators also observed that low-fat, high-carbohydrate diets reduced HDL-cholesterol in men and women [74, 76] and negatively affected insulin resistance [77, 78].

Although replacing 10% dietary fat intake with carbohydrates while maintaining caloric intake reduces weight [62], low-fat diets combined with a higher intake of carbohydrates were poorly effective in reducing risks to CVD [79]. In the DELTA study, the reduction of dietary total fat and saturated fatty acids reduced total cholesterol and LDL-cholesterol, but also reduced beneficial HDL-cholesterol [80]. Other findings elucidated that implementation of low-fat diets reduced beneficial HDL-cholesterol [37, 62, 74, 75, 81], and increased plasma triglycerides [76, 82]. In a 16-week trial comparing a low fat, low polyunsaturated fatty acid diet to a moderate fat, high polyunsaturated fatty acid diet, both diets reduced total cholesterol; however, subjects who adopted the low fat, low polyunsaturated fatty acid diet experienced a reduction in HDL-cholesterol and an increase in fasting VLDL triglycerides compared to subjects assigned to the moderate fat, high polyunsaturated fatty acid diet. It is important to note that the low fat diet was also high in carbohydrates [82].

The types of fatty acids consumed in the diet may help elucidate the possible reasons for their observations. Through dietary analysis and meta-analysis such as the Nurses' Health Study,

investigators found that changing the type of dietary fats consumed resulted in reduced risks to CVD. Investigators from the Nurses' Health study described an inverse relationship between the intake of polyunsaturated fatty acids and coronary heart disease risks and identified a direct relationship between trans-fatty acid intake and increased risks to coronary heart disease [83]. Moreover, replacing 5% of saturated fatty acids consumed with monounsaturated fatty acids and polyunsaturated fatty acids resulted in a reduction in triglycerides, total cholesterol, LDL-cholesterol, and the preservation of HDL-cholesterol [72]. Etherton and Yu [84] brought further insight through their study investigating the various effects of the dietary consumption of different fatty acids. In their paper, consumption of saturated fatty acids (specifically 12:0, 14:0, and 16:0) decreased HDL-cholesterol along with total cholesterol and LDL-cholesterol, while the addition of trans fatty acids (trans-18:1) increased total cholesterol, LDL-cholesterol, and decreased HDL-cholesterol. Interestingly, consumption of the saturated fatty acid, stearic acid (18:0), significantly lowered plasma total cholesterol and LDL-cholesterol when substituted for 12:0-16:0 saturated fatty acids and a neutral effect on HDL-cholesterol. In their elucidation of the benefits of monounsaturated fatty acids and polyunsaturated fatty acids, they found that *cis*-18:1 (oleic acid) and 18:2 (n-6) (linoleic acid) decreased total cholesterol and LDL-cholesterol, both acting independently from one another. Where *cis*-18:1 (oleic acid) increased HDL-cholesterol, 18:2 (n-6) reduced total cholesterol and LDL-cholesterol [84]. Findings suggest focusing on reducing daily intake of specific saturated fatty acids, such as lauric (12:0), myristic (14:0), and palmitic acid (16:0), and replacing them with dietary sources of unsaturated fats such as monounsaturated fatty acids and polyunsaturated fatty acids instead of decreasing total dietary fat intake [77, 84]. Thus, the consumption of dietary fat is essential for maintaining healthy levels of HDL-cholesterol while altering the types of fatty acids consumed in the diet can

be an important means of adjusting triglyceride, LDL-cholesterol, and HDL-cholesterol levels [84].

Exercise and Lipid Subclasses

The Framingham Heart Study was one of the primary studies that sought to discover factors that reduce risks to CVD. Findings from this massive study brought support for the beneficial effects of exercise and helped elucidate its role in decreasing risks to CVD [2]. Studies that followed provided substantial evidence that participating in an exercise regime correlates with improvements in CVD risk factors such as blood pressure [85], VAT [86, 87], LDL-cholesterol, and HDL-cholesterol [88]. Other benefits were observed in weight loss studies. Reducing energy intake to achieve weight loss may result in a loss of lean muscle mass and HDL-cholesterol; however, participation in regular exercise has been shown to attenuate these effects [57, 59]. Currently, physical inactivity is considered second only to obesity as a major risk factor for CVD [89]. Further discussion on the effects of participating in endurance and resistance exercise to CVD will be discussed in the following paragraphs, and the importance of duration, time, and frequency of exercise will be reviewed under "Physical Activity Recommendations".

Aerobic/Endurance Exercise and Lipid Subclasses

Endurance exercise helps reduce risks to CVD [90, 91]. Leon and Sanchez's [81] meta-analysis of 51 interventions incorporating aerobic exercise performed at 50-80% VO_{2peak} or percent heart rate for 30-60 minutes, three to five times a week for at least 12 weeks (500-5,000 kcal/wk) found that subjects experienced an average 5% decrease in LDL-cholesterol, 4.6% increase in HDL-cholesterol, and 3.7% decrease in triglycerides [81, 92]. Overall, the participation in aerobic/endurance exercise reduces LDL-cholesterol [93], increases HDL-

cholesterol [94, 95], and plasma triglycerides and has been shown as an effective accompaniment to medical treatment by enhancing the effects of statins [90, 91].

Several studies suggest that aerobic/endurance exercise primarily affect HDL-cholesterol [96]. In Tambalis et al. [88] meta-analysis, increases in HDL-cholesterol occurred in 22 of 37 trials and significant reductions in LDL-cholesterol occurred in 7 out of the 35 trials that measured LDL-cholesterol supporting his conclusion that aerobic/endurance exercise primarily effects HDL-cholesterol [88]. In Kelley and Kelley's [97] meta-analysis of 19 randomized control trials investigating the effects of aerobic exercise, there was an 11% increase in HDL2 in men and women following an aerobic exercise program for a period of $\geq$8 weeks [97]. The beneficial effects of aerobic exercise on HDL-cholesterol is seen in both younger [98, 99] and older individuals that regularly engage in endurance type activities [94]. Seals et al. [94] looked at young endurance trained athletes and older endurance-trained master athletes. Both young and master athletes had a higher VO_{2peak} and a lower body fat mass compared to age matched untrained men. Endurance-trained master athletes had significantly greater HDL-cholesterol than older and younger untrained groups where the HDL-cholesterol of older endurance trained men was 47% greater than that of untrained younger men and 57% greater than that of untrained, older men from a similar age class [94].

The inverse relationship between triglycerides and HDL-cholesterol noted prior under "Carbohydrates" has also been observed when comparing trained to untrained subjects [18]. When comparing the triglyceride and HDL-cholesterol levels of endurance athletes to sedentary controls, triglyceride levels were typically 18-77 mg/dL lower and HDL-cholesterol levels were typically 4-24 mg/dL greater in endurance athletes [100]. In addition, Thompson et al. [99] conducted a study comparing the lipid profiles of male endurance athletes and sedentary men.

Endurance athletes had lower fasting triglyceride and higher HDL-cholesterol concentrations with increases in HDL2 and HDL3 subclasses. Thompson's group noted that the clearance rate of plasma triglyceride was 92% higher in endurance-trained men and was directly related to HDL, HDL2, and HDL3, and inversely related to triglyceride concentrations. From this study, investigators suggested that the enhanced clearance of triglyceride was partially responsible for increased levels of HDL-cholesterol in endurance athletes [98].

Resistance Exercise and Lipoprotein Subclasses

Resistance exercise preserves lean muscle mass upon adoption of a reduced calorie diet and has shown cardioprotective and osteogenic benefits [101]. A possible mechanism through which resistance training can produce cardioprotective benefits is through the preservation of lean muscle mass. This has been postulated since lean muscle relies on the use of glucose and triglycerides as a source of energy [102]. Since studies have reported the existence of an inverse relationship between triglycerides and HDL-cholesterol [18], preserving and/or increasing lean muscle mass by participating in resistance exercises may have beneficial effects on HDL-cholesterol as well.

Tambalis, et al. [88] noted in his meta-analysis that the primary effect observed when adopting a resistance-training program was a reduction in LDL-cholesterol [88]. Prabhakaran et al. [103] showed that premenopausal women completing 14 weeks of resistance exercise, three times a week at a high intensity equivalent to 85% 1-RM significantly reduced total cholesterol and LDL-cholesterol with no significant change to triglycerides, HDL-cholesterol, or fat mass [103]. Additionally, changes in weight or fat mass do not appear to be necessary for resistance exercise to induce positive changes in blood lipids. In a study including obese, postmenopausal women (n=21) who performed 3 sets of 10 whole body resistance exercises at 8-RM (repetition

maximum, which is the maximum amount of weight you can lift for 8 repetitions) 3 times a week for 12 weeks, subjects experienced significant reductions in total and LDL-cholesterol despite no significant change in BMI, body mass, or body composition [104]. Interpretation of these results should not lead to the conclusion that resistance exercise works exclusively on LDL-cholesterol, but research supports that resistance exercise can improve the lipid profile [88]. For instance, Hurley et al. [105] found that untrained male subjects participating in resistance exercises for 16 weeks experienced a 5% decrease in LDL-cholesterol and a 13% increase in HDL-cholesterol despite no change in VO_{2peak}, body weight, or body composition [106]. The presence of variations in results raise the question as to the direct effect of resistance exercise on the lipid panel [88, 102]. We must consider in our assessment of the effects of resistance exercise that results can differ across studies (some showing significant changes in LDL-and/or HDL-cholesterol) due to the training status of the subject, and as will be discussed under "Exercise Prescription", the duration, frequency, and intensity of the exercise being employed to induce changes.

Endurance and Resistance Exercise and Lipid Subclasses

Different forms of exercise rely on aerobic and anaerobic energy systems to varying degrees, the practical application of the effects of aerobic and resistance exercise on LDL- and HDL-cholesterol can be seen in trained and untrained individuals. Positive effects on LDL and HDL have been observed in athletes who engage in sports that rely to varying degrees on aerobic and anaerobic energy systems. When the cholesterol profile of elite basketball and soccer players was measured and compared, soccer players had a greater decrease in triglycerides (9.7%, 78.3 ± 6.7 to 70.7 ± 6.3, $p < 0.01$) and total cholesterol (4.2%, 179.3 ± 10.7 to 171.6 ± 9.6, $p < 0.01$) compared to elite basketball players. Both elite basketball players and soccer players experienced

a decrease in LDL-cholesterol (6.7%, 110.9 ± 8.9 to 103.5 ± 7.5, $p < 0.01$ and 7.5%, 126.8 ± 9. 5 to 117.3 ± 9.1, $p < 0.01$, respectively) and an overall 9-12% increase in HDL-cholesterol. [107].

Participating in activities that incorporate aerobic and resistance exercises alter LDL- and HDL-cholesterol serving as a useful means to effectively reduce risk factors for CVD. Additionally, the beneficial effects on LDL and HDL-cholesterol appear to be dependent on the energy system being taxed. In a cross-sectional study, Sgouraki, et al. [108] recruited 78 male athletes involved in basketball, swimming, long distance running, or wrestling, to elucidate the effects of participating in these different sports on the lipid profile. Investigators noted that all exercise groups experienced a significant increase in HDL-cholesterol, while long distance runners had the highest increase in HDL-cholesterol, a finding that aligns with Tambalis et al.'s [88] observation that aerobic exercise programs appear to primarily alter HDL-cholesterol. Investigators also considered the effects of sport on individual lipid subclasses and found that long-distance runners, basketball players, and wrestlers experienced a significant increase in HDL2 subclasses. The effects on HDL2 appear to be related to cardiovascular fitness where investigators identified a positive correlation between VO_{2peak} and HDL2 ($R = 0.37$, $p < 0.01$), and a negative correlation between VO_{2peak} and sdLDL: HDL2 ratio regardless of sport [108].

The beneficial changes from adopting an exercise program translate to untrained individuals at increased risk for CVD. Shaw et al. [93], compared the effects of varying exercise modes on the lipid panel in 38 untrained men with borderline elevated LDL-cholesterol. Men were assigned either to a control group, an aerobic training program, or a combined aerobic and resistance training program. Both exercise groups trained three times a week for 16 weeks. Aerobic training was performed for 45 minutes at 60% maximum heart rate. The combined aerobic and resistance group also trained for 45 minutes with 22 minutes devoted to aerobic

exercise performed at 60% maximum heart rate and resistance exercise including eight exercises (two sets of 15 repetitions) performed at 60% of 1-RM. Subjects who participated in either aerobic or a combined aerobic and resistance training program experienced a significant reduction in LDL-cholesterol from baseline with no significant difference between the exercise modalities [93] while subjects who did not exercise experienced no significant change in LDL-cholesterol. Although a resistance training only group was not included, we see that for untrained subjects, beneficial effects on LDL did not differ between aerobic only and a combined aerobic and resistance training program. In general, studies support the idea that both types of exercise improve LDL [88] and HDL-cholesterol as long as participation is consistent and helps maintain energy balance.

Physical Activity Recommendations

Physical fitness is defined as the ability to carry out day-to-day tasks and enjoy leisure activities while maintaining a sufficient supply of energy to meet emergency situations that may arise. Exercise is a form of physical activity that is a planned, structured body movement performed with repetition for the purpose of improving or maintaining a component of physical fitness. The intensity of physical activity can be quantified via Bruce's protocol. Bruce's protocol enables the investigator to measure oxygen consumption and peak aerobic capacity assuming subjects being tested do not have cardiovascular or pulmonary disease upon reaching their true physiologic limit. The VO_{2peak} is a measure of cardiorespiratory fitness that can be expressed as an absolute (ml/min) or relative (ml/kg/min) value. For this study, relative as opposed to absolute measures are of interest since the test was performed using modified Bruce's Protocol where participants performed a weight bearing activity by running on the treadmill. Also, since relative VO_{2peak} considers weight, it allows us to compare performance across

participants with varying body weight [109].

Despite substantial support for the positive health effects of participating in regular exercise, only 54.1% of adults over the age of 18 meet the minimum physical activity guidelines, 27.7% meet muscle-strengthening guidelines, and 24.3% meet aerobic and muscle-strengthening activity guidelines [110]. The American Heart Association recommends combining aerobic exercise and moderate intensity resistance training to preserve lean muscle. To reduce CVD risk factors (e.g., physical inactivity and obesity) the American Heart Association and American College of Sports Medicine recommend individuals perform cardiovascular exercises at a moderate intensity for 30 minutes greater than or equivalent to five days a week (≥150 minutes a week) or participate in vigorous activity for 25 minutes greater than or equivalent to three days a week (≥75 minutes a week) or some equivalent combined with resistance-type exercises performed at moderate- to high-intensity at least two days a week to reduce blood pressure and total cholesterol.

Equating a prescribed exercise dosage to a significant change in blood lipid markers for CVD is complex. Aerobic and resistance exercise consist of several components that provide a means to measure and possibly determine the necessary dosage to induce positive changes. Aerobic exercise consists of frequency, intensity, time (duration), volume (quantity), and rate of progression while resistance exercise consists of frequency, volume (sets and repetitions), intensity (weight), technique, and progression/maintenance [96, 111]. Unfortunately, these components are also a source of variability across study protocols and can contribute to the inconsistent findings across studies especially when different exercise modalities are being examined [88].

Numerous studies have assessed the importance of frequency, intensity, and duration for

altering blood lipids. Ullrich, et al. [112] showed that men participating in a weight-training program for a frequency of three times a week for eight weeks significantly increased HDL-cholesterol and decreased LDL-cholesterol. Investigators reviewing 37 studies that investigated the effects of exercise mode on the lipid panel concluded that intensity was the most important factor where lipids were positively affected by exercise that exceeded an energy expenditure of seven kcal/min [88]. To elucidate the importance of exercise intensity on cholesterol, three intensity groups and a control were compared. The exercise groups included subjects assigned to one of the following: a high-amount of high-intensity aerobic exercise (equivalent to an energy expenditure of 23 kcal/kg/week; 17.4 miles/wk), a low-amount of high-intensity aerobic exercise (equivalent to an energy expenditure of 14 kcal/kg/week; 11 miles/wk), or a low-amount of moderate-intensity activity (equivalent to an energy expenditure of 14 kcal/kg/week; 11.1 miles/wk) while subjects assigned to the control continued normal activities. All exercise groups experienced a significant increase in LDL particle size while subjects assigned to the high-amount of high-intensity aerobic exercise achieved the greatest increase in LDL particle size. Only subjects assigned to a high-amount of high-intensity aerobic exercise experienced a significant reduction in LDL-cholesterol and increase in HDL-cholesterol concentration and HDL particle size. Investigators concluded that beneficial effects were due to the amount of activity and not weight change, intensity, or increases in fitness [45]. Tambalis et al. [88] showed that men (n=8,499) participating in a resistance training program greater than four hours per week reduced risks of developing hypercholesterolemia [88]. Duration has been a strong predictor where HDL-cholesterol increased 1.4 mg/dL (0.036 mmol/L) for every 10 minutes added to an exercise session [113]. Again, we find that frequency, intensity, and duration were not the primary predictors of lipid changes in a review of the effects of resistance exercise on

HDL-cholesterol where the positive effects on CVD risk factors were attributed primarily to caloric expenditure [100]. Additionally, in a meta-analysis of 25 randomized control trials, frequency and intensity were unrelated to changes observed for HDL-cholesterol while a minimal volume equivalent to 900 kcal/wk (120 minutes of exercise/wk) equated to a mean change of 2.53 mg/dL (0.065 mmol/L) HDL-cholesterol.

Caloric expenditure is an excellent means of measuring work performed at each exercise session and can provide a less varied means for investigators to compare results across studies. [96]. In a cross-sectional analyses, a significant reduction in triglycerides (5-38 mg/dL) and increase in HDL-cholesterol (2-8 mg/dL) was achieved above 15 to 20 miles/wk of walking or jogging, which equated to a caloric expenditure of 1,200 to 2,200 kcal/wk [100]. Considering results from previous studies performed at our lab, reductions in weight and risk factors for metabolic syndrome were independent of macronutrient distribution as long as subjects adhered to a reduced energy intake [10]. Mann et al. [96] sums it up in concluding from his own research that caloric expenditure transcends exercise mode (aerobic versus resistance exercise). He also added that aerobic exercise performed at a high intensity is preferred while resistance exercise performed at a high volume is ideal to expend the necessary amount of energy that can induce significant changes to the lipid profile [96]. Although an exercise dose linked to significant measurable improvements in health has not been determined, when we consider results from our own lab and outside independent studies, the common finding is that consistent participation in exercise, which increases energy expenditure, produces beneficial health effects.

Prior Weight Loss Research

The ESNL has conducted a number of studies on the role of exercise and nutrition on weight loss and body composition since the early 1990's. In 2003, the ESNL launched the

Women's Health and Fitness Initiative in collaboration with Curves® International (Waco, TX). At the time, Curves® was one of the fastest franchise companies in the world that grew to over 5 million active members at 10,000 sites on over 78 countries worldwide. ESNL researchers developed and/or modified the exercise and weight management programs that have literally been used by millions of women to improve health and fitness. As a result, researchers from the ESNL have published a number of studies evaluating how different types of exercise and/or diet programs affect health, fitness, and quality of life.

The initial study published in this series compared the effects of a high energy, higher carbohydrate diet with exercise (1,846.6 kcal/day; CHO: 55%, PRO: 15%, FAT: 30%) to three hypo-energetic diets varying in macronutrient intake with exercise. The carbohydrate intake for these three diets was replaced with protein to varying degrees as follows: a very low carbohydrate, higher protein diet with exercise (1,653.6 kcal/day; CHO: 41%, PRO: 29%, FAT: 30%), a lower carbohydrate, moderate protein diet with exercise (1,653.6 kcal/day; CHO: 50%, PRO: 20%, FAT: 30%), and a higher carbohydrate, lower protein diet with exercise (1,653.6 kcal/day; CHO: 55%, PRO: 15%, FAT: 30%). Controls included a no diet with no exercise group and an exercise only group [114]. All subjects who adopted a hypo-energetic diet with higher (1.1 g PRO/kg/day), moderate (0.9 g PRO/kg/day), and lower (0.71 g PRO/kg/day) protein intakes combined with a resistance-based circuit training program reduced weight (5.2% change, -5.6 kg, $p<0.001$; 7.1% change, -6.5 kg, $p<0.01$; 4.6% change, -4.0 kg, $p<0.001$, respectively), fat mass (9.1% change, -4.2 kg, $p<0.001$; 7.8% change, -2.9 kg, $p<0.001$; 8.3% change, -2.9 kg, $p<0.001$, respectively), and percent body fat (-2.0%, $p<0.005$; -1.7%, $p<0.001$; -2.0%, $p<0.001$, respectively). Reductions for weight and fat mass were significantly different from the no exercise or diet group. Only the reduced calorie, moderate protein diet with exercise showed a

significant reduction in triglycerides (22% change, $p<0.05$). Subjects assigned to the reduced calorie, lower protein diet with exercise significantly lowered total cholesterol (3% change, $p<0.05$) while those assigned to the higher energy, higher carbohydrate diet (0.74 g PRO/kg/day) with exercise did not show significant changes in weight (1.4% change, $p=0.26$) or total cholesterol (2.8% change, $p=0.46$) [114].

Since this preliminary study showed that all reduced calorie diet groups significantly lost weight with no significant difference between groups, this study was repeated with some modifications to investigate the impact of decreasing the carbohydrate to protein intake ratio on health. In comparison to the preceding study, reduced calorie diets were modified as follows: phase 1 (reduced energy intake to 1,200 kcal/day) was implemented for one week instead of two and phase 2 (increased caloric intake to 1,600 kcal/day) was implemented for nine instead of eight weeks. Also, the macronutrient intakes were adjusted for the higher protein and moderate protein diet with exercise groups (CHO: 15%, PRO: 55%, FAT: 30%) and the higher energy diet group (CHO: 40%, PRO: 30%, FAT: 30%). Finally, phase 3 (increased caloric intake to 2,600 kcal/day) was implemented for four weeks where all four diet groups followed the high energy, higher carbohydrate diet (CHO: 55%, PRO: 15%, FAT: 30%). No significant changes occurred for triglycerides ($p=0.95$) or for total- ($p=0.67$), HDL- ($p=0.90$), or LDL-cholesterol ($p=0.63$) in this study [15].

In a retrospective analysis including 661 sedentary overweight or obese women (BMI $\geq$25.0-62.0 kg/m^2; 46±11 years) from eight studies conducted between 2002-2014 in accordance with IRB approvals from Baylor and Texas A&M Universities. Subjects participated in hypo-energetic diets (week 1: 1,200 kcal/day; weeks 2-9: 1,600 kcal/day) either higher in protein (1.14 g PRO/kg/day) or carbohydrates (2.2 g CHO/kg/day) with a low-fat intake (35 g FAT/kg/day)

combined with aerobic and resistance training exercises 30 minutes a day, three times a week for ten weeks. Although both groups significantly reduced weight, fat mass, and percent body fat, subjects in the higher protein group exhibited significantly greater weight loss compared to the higher carbohydrate group. Both groups significantly reduced caloric intake by 176±300 kcal/day, $p<0.01$). Only the higher protein group consumed a diet significantly ($p<0.01$) different from baseline macronutrient intake, 35% carbohydrate intake (1.41±0.49 g CHO/kg/day) and 29% protein intake (1.14±0.49 g PRO/kg/day). Both diets were equally effective in reducing weight, triglycerides, and HDL-cholesterol from baseline ($p<0.05$) with no significant difference between groups. This retrospective analysis concluded that reducing energy intake through diet and increasing energy expenditure through exercise precedes macronutrient assignment in weight loss. Furthermore, reductions in weight and risk factors for metabolic syndrome were independent of macronutrient assignment [10].

This retrospective analysis was followed with a study comparing the following weight loss programs: Curves® complete 90-day Challenge (1,342 kcal/day; CHO: 36%, PRO: 25%, FAT: 38%), Weight Watchers Points Plus (1,280 kcal/day; CHO: 44%, PRO: 22%, FAT: 32%), Jenny Craig At Home (1,250 kcal/day; CHO: 54%, PRO: 20%, FAT: 26%), Nutrisystem Advance Select (1,036 kcal/day; CHO: 40%, PRO: 26%, FAT: 33%), and control (1,742 kcal/day; CHO: 43%, PRO: 18%, FAT: 38%), on weight loss and risk factors of metabolic syndrome, subjects who adopted programs that included diet with exercise for 12 weeks significantly lost weight (Curves®: 5.0% change, -4.32 kg, Weight Watchers: 5.0% change, -4.31 kg, Jenny Craig: 6.2% change, -5.34 kg, and Nutrisystem: 5.5% change, -5.03 kg) compared to the control group (0.2% change, +0.16 kg) with no significant difference between groups. At 12 weeks, those assigned to the Curves® diet, a higher protein (0.96 g PRO/kg/day) and relatively

moderate fat (0.67 g FAT/kg/day) diet among all four diet and exercise groups, appeared to experience the most beneficial effects on the lipid panel where LDL-cholesterol and triglycerides were significantly reduced ($p<0.001$), and HDL-cholesterol was significantly increased from baseline ($p<0.001$). By 12 weeks, subjects assigned to Weight Watchers having a protein intake that met RDA (0.81 g PRO/kg/day) and a relatively low-fat intake (0.55 g FAT/kg/day) also had a significant reduction in triglycerides, but these participants did not experience a significant decrease in LDL- or increase in HDL-cholesterol by week 12. High-carbohydrate diets without exercise are associated with increases in plasma triglycerides [60-62, 64], and this study found that following a reduced calorie diet and exercise program with protein intakes below RDA showed similar results. Subjects assigned to Jenny Craig and Nutrisystem, which were lowest in protein intake (0.69 g PRO/kg/day and 0.74 g PRO/kg/day, respectively) and fat intake (0.43 g FAT/kg/day and 0.41 g FAT/kg/day, respectively) among all the groups including the control (0.79 g PRO/kg/day and 0.79 g FAT/kg/day), had significant reductions in HDL-cholesterol and increases in triglycerides at 12 weeks, and this reduction in triglycerides was significant only for the Jenny Craig diet. Subjects assigned to Nutrisystem significantly reduced total and LDL-cholesterol by 12 weeks. In this study, the higher protein, moderate fat diet with exercise (Curves® complete 90-day Challenge) showed the most beneficial effects on blood lipids similar to Meckling et al., [51] who showed that only the higher protein group had significant reductions in LDL-cholesterol and triglycerides. Additionally, the observation that lower fat diets reduced HDL-cholesterol in this study agrees with previous research from other investigators that following a reduced calorie, low fat diet significantly decreases total-, LDL-, and HDL-cholesterol [82].

This study was followed with an investigation evaluating the effects of hypo-energetic

diets varying in macronutrient intake with exercise. Subjects were randomized into a no exercise or diet group or to one of three hypo-energetic diet and exercise groups where subjects were asked to consume 1,400 kcal/day during week one and 1,500 kcal/day during weeks 2-24. Subjects assigned to a diet and exercise group either followed a higher carbohydrate diet (CHO: 55%, PRO: 15%, FAT: 30%; 2.4 g CHO/kg/day, 0.9 g PRO/kg/day, 0.7 g FAT/kg/day) or one of two higher protein diets, which consisted of either a moderate carbohydrate intake (CHO: 30%, PRO: 45%, FAT: 25%; 1.7 g CHO/kg/day, 1.2 g PRO/kg/day, 0.7 g FAT/kg/day) or a lower carbohydrate intake (LCHP; CHO: 20%, PRO: 45%, FAT: 35%; 1.4 g CHO/kg/day, 1.5 g PRO/kg/day, 0.8 g FAT/kg/day). Subjects assigned to one of the hypo-energetic diets also participated in a resistance-based circuit training program performed at 65-85% maximum heart rate, three times a week for 24 weeks. Results indicate that adherence to a reduced energy diet combined with resistance-based circuit training significantly ($p<0.05$) reduced body weight, fat mass, percent body fat, and waist circumference while maintaining fat-free mass and significantly improving relative maximum peak aerobic capacity (VO_{2peak}). Total cholesterol was reduced from baseline values at 24 weeks for both higher protein groups either moderate (12% change, $p<0.05$) or lower in carbohydrate intake (8% change, $p<0.05$). All diet and exercise groups experienced a reduction in triglycerides from baseline values at 24 weeks; however, reductions in triglyceride levels were only significant for the lower carbohydrate, higher protein group (14% change, $p<0.05$) at 12 weeks and for the moderate carbohydrate, higher protein group (29% change, $p<0.05$) at 24 weeks. Low-density lipoprotein cholesterol was reduced from baseline at 24 weeks for both higher protein diet groups on a moderate (33% change, $p<0.05$) and lower carbohydrate intake (24% change, $p<0.05$) [12]. Thus, subjects assigned to a hypo-energetic diet with exercise were more likely to show improvements in the lipid profile when

their diet was higher in protein.

The observed improvement in the lipid profile was supported in a concurrent study in our lab where two higher protein diets, either moderate or lower in carbohydrate consumption, were compared. Subjects assigned to the moderate carbohydrate, higher protein diet (1.19 g CHO/kg/day, 1.17 g PRO/kg/day, 0.54 g FAT/kg/day) experienced a reduction in HDL-cholesterol (1.3% change, -0.8 mg/dL) and triglycerides (20.5% change, -34.5 mg/dL) while those assigned to the lower carbohydrate, higher protein diet (1.02 g CHO/kg/day, 1.20 g PRO/kg/day, 0.55 g FAT/kg/day) experienced an increase in HDL-cholesterol (13.2% change, +6.9 mg/dL) and decrease in triglycerides (9% change, -10.6 mg/dL) [13]. Although both showed significant changes in blood lipid parameters, more favorable improvements occurred when carbohydrate intake was lower. However, since the difference in carbohydrate and protein intake was not significant between these groups, we would need to run a follow up investigation.

Overall, studies consistently support the positive effects of adopting a hypo-energetic diet combined with aerobic and resistance exercise [115] for overweight and/or obese individuals to lose weight and improve cardiovascular fitness [8, 10]. Furthermore, studies conducted in our lab show that diets favoring higher protein intakes show some additional benefits over higher carbohydrate intakes [12, 13]. The purpose of further analysis, which is the topic of this paper, was to determine whether lipid subclasses were influenced by the implementation of a hypo-energetic diet and exercise and to assess whether these effects are dependent on macronutrient intake, namely high protein compared to higher carbohydrate diets. Since we observed a reduction in LDL-cholesterol for high protein groups, we might expect to find changes in LDL subclass distribution. Although there were no significant changes in HDL-cholesterol, it is possible there were changes to individual HDL subclasses, which is a question that will be

presented and discussed in Chapters IV and V of this paper, respectively. Furthermore, by measuring lipoprotein subclasses in our study, we seek to identify any correlations that exist between lipid subclasses and parameters of risk factors of CVD (e.g., weight, total cholesterol, and blood glucose).

CHAPTER III

METHODS

Experimental Design

This was a randomized parallel, prospective, comparative effectiveness trial conducted at the ESNL at Texas A&M University, College Station, TX and approved by the Texas A&M University Institutional Review Board, #2013-0737F. Overall, results from this study showed that adherence to a hypo-energetic diet and exercise program regardless macronutrient intake reduced weight and improved body composition, cardiovascular fitness, muscle strength and endurance, and metabolic health markers.

We ran a secondary analysis on available serum samples (n=75) obtained from this cohort to evaluate the impact of adherence to a hypo-energetic diet and resistance-based circuit training program on lipoproteins. The primary focus of these secondary analyses is to evaluate the effect of adopting a diet and exercise program on lipoproteins and their respective subclasses. Secondarily, we will report the relationship observed between lipoproteins and their respective subclasses and parameters of energy intake (kcal/kg) and/or macronutrient intake [PRO (g/kg), CHO (g/kg), FAT (g/kg)], anthropometric measures [weight (kg), waist and hip circumference (cm), and waist to hip ratio], body composition [fat mass, fat free mass, percent body fat, VAT area (cm^2)], cardiorespiratory fitness [VO_{2peak} (L/min and ml/kg/min) and time to exhaustion], glucose homeostasis [glucose (mg/dL), insulin (μIU/ml), and calculated HOMA-IR], and blood lipids [triglyceride (mg/dL), total- (mg/dL), LDL- (mg/dL), and HDL-cholesterol (mg/dL)].

Diet Intervention

Participants (n=86) were randomized into one of the following groups for 24 weeks [12]: a no exercise or diet control (NED), a higher carbohydrate, lower fat diet (HCLF; 55%, PRO:

15%, FAT: 30%) recommended by the American Heart Association, a moderate carbohydrate, higher protein, lower fat diet (MCHP; CHO: 30%, PRO: 45%, FAT: 25%) with access to Curves® online diet plan, or a lower carbohydrate, higher protein, moderate fat diet (LCHP; CHO: 20%, PRO: 45%, FAT: 35%). Participants assigned to NED were asked to maintain normal dietary habits. Energy intake for diet groups consisted of two phases: phase 1 where 1,400 kcals/d were consumed during week 1 and phase 2 where 1,500 kcals/d were consumed during weeks 2-24. The HCLF and LCHP diets were provided with meal plans in booklet format along with the Diabetic Exchange List [116] while MCHP had access to online instruction. Participants assigned to MCHP and LCHP diet groups met with a registered dietician and/or exercise physiologist at the ESNL, prior to commencing their meal plan and weekly to ensure dietary compliance.

Exercise Intervention

Subjects were assigned to a diet group and asked to participate in resistance-based circuit training, while those assigned to the NED group were asked to continue normal daily activities without any exercise program. Subjects assigned to diet groups were instructed and trained to perform Curves® circuit-type exercises on Curves® hydraulic machines located in the ESNL as described in Sanchez et al. [12]. Each participant was provided with a Polar watch compatible with Polar heart rate monitor and asked to maintain 60-80% of age predicted maximum heart rate (heart rate = 220 - age). Although heart rate data was not analyzed in this study, this is mentioned to indicate how we ensured subjects reached the proper exercise intensity of 60-80% according to American College of Sports Medicine and NSCA guidelines [109, 117].

Subjects completed four training sessions weekly. Three sessions consisted of a 26-minute workout in which participants completed all Curves® machines throughout the circuit

twice, with 30-second intervals performing calisthenic exercises (e.g., jumping jacks and lunges) between each machine, and one weekly session that included Zumba where participants moved through the circuit once performing 1 minute of Zumba exercises, led by a certified Zumba instructor, after 1 minute of resistance exercise on each Curves® machine. After each exercise session, participants completed whole body stretches for four minutes. For the three non-training days, participants were provided with a pedometer and instructed to reach 10,000 steps/d. Trained ESNL staff members knowledgeable in fitness exercises supervised all exercise sessions. At the end of each workout, attendance was noted on a spreadsheet to ensure >75% compliance.

Participants

As previously described by Sanchez et al. [12]. Subjects (n=267) were screened and excluded (n=70) if presenting any of the following: 1) uncontrolled metabolic or cardiovascular disorder such as a history of hypertension, arrhythmias, diabetes, thyroid disease, hypogonadism, musculoskeletal, autoimmune, or neurological disease, 2) taking prescribed medications for thyroid disease, hyperlipidemia, or weight loss for three months prior to the study, 3) uncontrolled hypertension or androgenic conditions, 4) had been pregnant within the past year or were interested in becoming pregnant in the next year, 5) had participated in a regular exercise program within three months prior to familiarization, 6) had no physician clearance to participate in the study. Participants (n=197) that did not fall under any of these categories were eligible and randomized into groups. Following is a consort diagram (Figure 3.1) expressing the number of subjects assessed for eligibility, number of subjects recruited and randomized into groups, number of subjects that completed the study, and the number of subject's samples available for further lipoprotein analysis based on there being a sufficient sample volume from all three, 0-, 12-, and 24-week, time points.

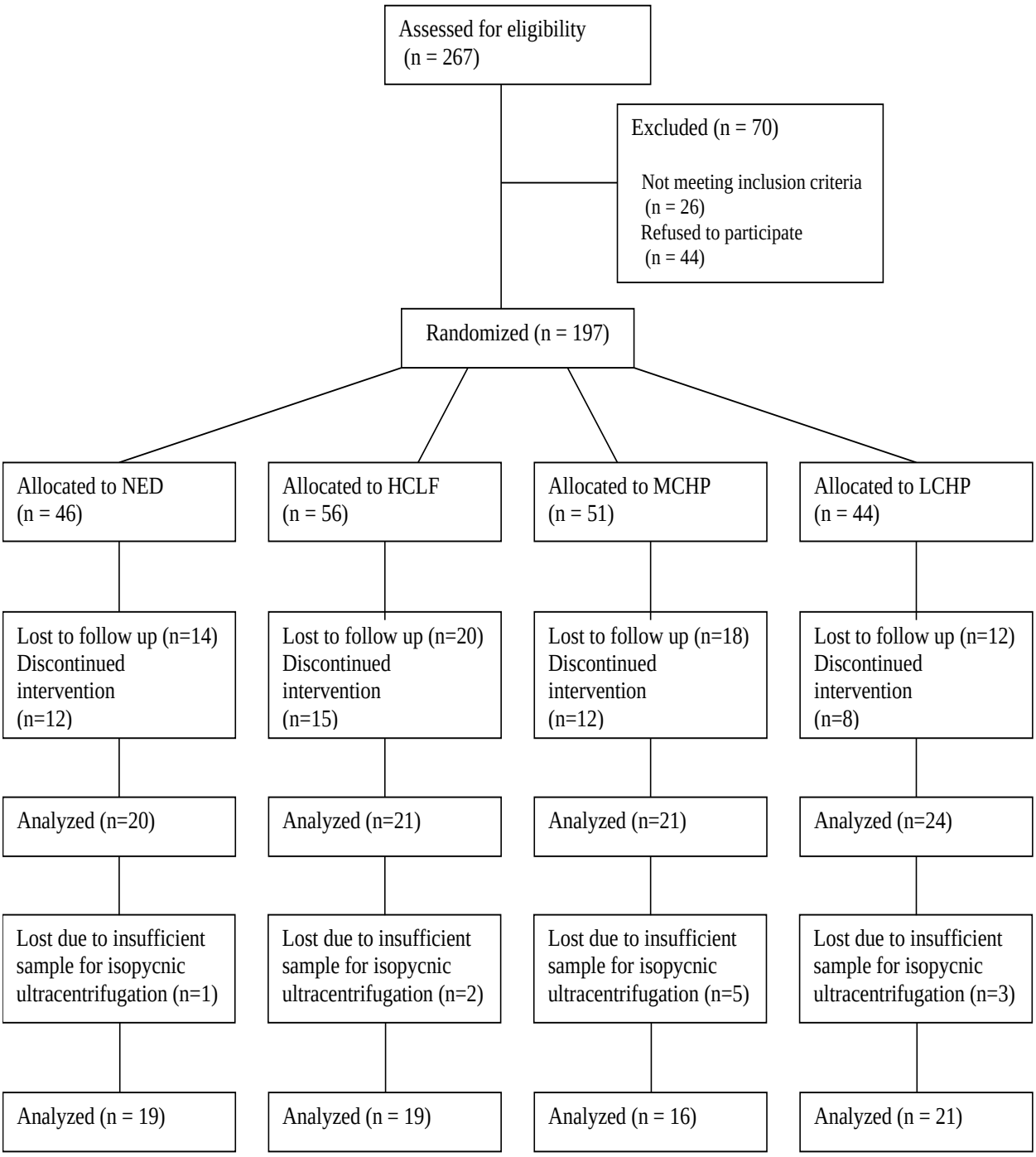

Figure 3.1: Consort Diagram for Samples

Procedures

Dietary Assessment

Prior to each testing session, participants recorded food and energy containing beverages consumed for three weekdays and one weekend day on food logs. Participants met weekly with a registered dietitian and were informed how to measure and record food and beverage intake. Food logs, checked for accuracy by ESNL staff including registered dieticians, were analyzed using ESHA Food Processor (*Version 8.6, 2006, ESHA Research Inc., Salem OR*).

Anthropometric Measures

Weight and height data were obtained using a Health o meter Professional Scale Model 500KL (*Pelstar LLC, Alsip, IL, USA*) with a precision of ±0.02kg. Waist and hip circumference were measured using a tension-controlled tape measure in compliance with guidelines established by the American College of Sports Medicine [111].

Body Composition/DEXA

Whole body bone density, body composition (e.g., fat mass, fat free mass, percent body fat, and VAT area) were determined with the Hologic Discovery W QDR series Dual-Energy X-ray Absorptiometry system (*DEXA; Hologic Inc., Waltham, MA, USA*) and analyzed with APEX Software (*APEX Corporation Software, Pittsburgh, PA, USA*). Quality control calibration was performed using the spine phantom (*Discovery W-CALIBER Model DPA/QDR-1 anthropometric spine phantom*). Coefficient of variation (CV) for total bone mineral content and total fat free mass was 0.31-0.45% and mean intraclass correlation was 0.985 [118]. Visceral adipose tissue (cm^2) analysis required manual manipulation of the VAT area box using APEX software where the top and bottom borders of the box were extended to include lumbar region L3-L5 and the left and right borders were extended to the left and right side of the soft tissue to

include the participant's entire abdominal region. Values were entered into a data sheet to be analyzed by SPSS software.

Cardiovascular Fitness

ESNL staff conducted cardiopulmonary exercise tests using a modified version of the Bruce's protocol described in the American College of Sports Medicine's *Guidelines for Exercise Testing and Prescription* [111]. Participants performed a graded treadmill test on the Trackmaster TMX425C treadmill (*JAS Fitness Systems, Newton, KS*) at 0-, 12-, and 24-week time points. The Quinton 710 ECG (Quinton Instruments, Bothell, WA) and the ParvoMedics 2400 True Max Metabolic Measurement System (*ParvoMedics, Inc., Sandy, UT*) were used to conduct cardiopulmonary stress test and indirectly measure gas exchange, respectively. Quality control calibration was conducted daily prior to testing for gas and flowmeter calibration (calibrated with the Hans Rudolph series 5530 three-liter syringe, Hans Rudolph Inc., Kansas City, MO) with a CV of ±2% for each calibration.

An ECG printout was made using the Nasiff Cardio Card electrocardiograph (*Nasiff Associates, Inc., Central Square, NY, USA*) when the participant was in the supine position before and on the treadmill. The participant was properly instructed to place a mouthpiece sterilized with Metrizyme dual enzymatic detergent (*Metrex* ™) into their mouth. Headgear was place over the head and adjusted, and a nose clip was positioned on the nose. Participants were instructed to keep hands to their side during the testing and told to grab onto the handrails to indicate they wanted to stop the test.

After completing the modified two-minute warm-up on the treadmill at 2.0 mph and 0% grade, the graded exercise test was initiated. The protocol consisting of sequential three-minute stages each increasing in grade (incline), and speed was executed as described in Sanchez et al.

[12]. Blood pressure, heart rate, rate of perceived exertion (indicated by the participant using the Borg Scale[109] located on the treadmill), and an ECG were recorded/printed prior to testing, at the end of each stage of the testing session, at peak heart rate, and during the active and passive three-minute recovery phases. Time from beginning to end of testing session was recorded as time to exhaustion. The flowmeter and oxygen/carbon dioxide analyzers measured expiration to generate ventilation, CO_2 production, O_2 consumption, and respiratory quotient values of interest. After the participant grabbed the handrails to indicate they could no longer continue, a staff member initiated the cool-down phase and removed the nose clip and headgear. For the active and passive recovery phases, the participant was instructed to continue walking on the treadmill for three minutes followed by instruction to remain seated in a chair for three minutes.

Blood Collection and Analysis

Sample Collection, Storage, and Analysis for Blood Lipids, Insulin, and HOMA-IR

Participants fasted at least 10 hours and did not exercise 48 hours prior to blood draw. Using standard phlebotomy procedures through venipuncture of antecubital vein, 20-24 mL of venous blood was collected into BD Vacutainer EDTA and two SST™ tubes (*BD Franklin Lakes, NJ, USA*). Serum used for analysis was obtained from blood collected in SST™ tubes centrifuged in MegaFuge 40R (*Unity Lab Services Thermo Fisher Scientific, Asheville, NC, USA*) at 3,500 x g for 10 minutes, aliquoted into micro centrifuge tubes, and stored in Innova U725 Ultra-Low Temperature Freezer (*USA Scientific, Ocala, FL, USA*) set to -80°C. Serum samples were removed from the freezer and thawed prior to running serum chemistry analysis. Fasting glucose, triglycerides, and total-, LDL-, and HDL-cholesterol were obtained using COBAS® c-111 analyzer (*ROCHE Diagnostics, Basel, Switzerland*). Intra-and inter-assay coefficient of variation for these tests was less than 3% and 2%, respectively [119]. Fasting

insulin was assayed using Enzyme Linked Immunosorbent assay (*ELISA*) kit (*No. 80-INSITU-E10, ALPCO, Salem, NH*) and measured using BioTek ELX-808 Ultra-microplate reader (*BioTek instruments Inc., Winooski, Vermont*) set to a detection of 450 nm using known standard with BioTek Gen 5 Analysis Software (*BioTek Instruments Inc., Winooski, VT*). The intra- and inter-assay coefficient of variation ranges from 2.9-6.2% and 5.4% to 8.6% (ALPCO, *Salem, NH*) respectively. Glucose and insulin data were used to calculate the Homeostasis model assessment insulin resistance (HOMA-IR) using the following formula as described in Sanchez et al., [12]:

$$\text{HOMA-IR} = \frac{\text{glucose } (\frac{\text{mg}}{\text{dL}}) \text{ x insulin}}{405}$$

Transport and Sample Preparation for Isopycnic Ultracentrifugation

Samples from 0-, 12-, and 24-week time points representing 75 different subjects were available for isopycnic ultracentrifugation. These samples were included in the description of the statistical analysis, results, and conclusion of this paper. Participants were kept in the same groups as assigned in the previous study [12] leaving us with the following: NED (n=19), HCLF (n=19), MCHP (n=16), and LCHP (n=21). Samples were placed in a sealed primary container, which was housed in a sealed secondary container marked with appropriate biosafety hazard labels and transported from the ESNL to the laboratory of Dr. Rosemary L. Walzem where samples were stored prior to isopycnic ultracentrifugation analysis.

Preparation of 0.18 M NaBiEDTA and Ceramide Solution

A 500 mL dH_2O solution of NaBiEDTA (molecular weight: 520.18) was prepared at least one day prior to sample preparation and ultracentrifugation. Initially, 500 grams of dH_2O (1 mL dH_20 = 1 gram) and a magnetic stir bar was added to a volumetric flask, and after the flask was placed on a magnetic stir plate, 46.8162 g NaBiEDTA was added. Once the solution was cleared,

the refractometer was calibrated with dH_2O (R = 1.33333) to ensure refraction for the 0.18 M NaBiEDTA solution was 1.3465. One ml DMSO (*SIGMA-ALDRICH, Lewis, MO, USA*) was added to 1 mg C-6 NBD Ceramide (*Cayman Chemical Company, MI, 48108 USA*), which was then used for sample preparation.

Application of Isopycnic Ultracentrifugation

Samples and ceramide were removed from the freezer and allowed to thaw for 5-10 minutes. During this time, the ultracentrifuge was turned on and ten microtubes (1.5 mL capacity) with snap caps were numbered "1" to "10", and 1,284 µL 0.18 M NaBiEDTA (*Toshiba Kita-ku TCI Tokyo, Japan*) were added to each microtube. Each sample was vortexed 10-15 seconds, and 6 µL of sample was added to its respective microtube. After C-6 NBD Ceramide dye (*Cayman Chemical Company, MI, 48108 USA*) was vortexed for 10-15 seconds, 10 µL of ceramide dye was added to each microtube followed by vortexing for 10-15 seconds to ensure mixing and incubation for at least 10 minutes. Ten 11 x 34 mm polycarbonate centrifuge tubes (*Beckman Coulter, Inc. Brea, CA, 92821 USA*) were observed for any scratches and marked using a sharpie marker. The rim of each polycarbonate centrifuge tube was marked with 1-10 tick marks corresponding with the side where scratches were evident. From each microtube 1,150 µL of the NaBiEDTA/serum/ceramide mixture were transferred to its corresponding labeled polycarbonate centrifuge tube to ensure proper balancing within the ultracentrifuge. Samples were weighed to ensure each tube weighed within 0.03 g of each other. Samples were carefully placed in the MLA-130 rotor so that the "trill line" was aligned with the scratch markings, previously marked with tick marks. For each run a total of 10 tubes including nine samples and one "standard" were placed in the rotor. The lid of the rotor was sealed and secured prior to placement in the drive hub of the vacuum container inside the ultracentrifuge. Settings

for the ultracentrifuge were checked to ensure speed = 120,000 RPM, time = 6 hours, temperature = 4° C, acceleration = 5, and deceleration = 5. After securing the rotor and closing the centrifuge door, the "VACUUM" button was pressed and allowed to reach at least 30 microns before pressing "START". The rotor was observed until it reached >100,000 RPM before leaving the ultracentrifuge unattended for six hours.

Lipoprotein Data Collection

Before samples completed ultracentrifugation, a fluorescent light beneath the photo box station was turned on and allowed to warm up for 20 minutes. Picture Frame software was opened, and exposure time adjusted (200-300 mS) to obtain a photo of an empty ultracentrifuge tube and labeled "BLANK #" (# being the number of the run). The exposure time was returned to 2.93 seconds with a target intensity equal to 30% and gain equal to 1.0000. After six hours of ultracentrifugation at 120,000 RPM, the vacuum was turned off and the rotor was carefully removed from the ultracentrifuge. Samples were carefully removed from the rotor with tongs so that the layered medium was undisturbed, inserted into the transportable rack, and placed in the imaging photo box. Samples were placed one at a time into the tube holder angled so that the portion of the tube facing the camera was void of scratches. Prior to imaging, 90 µL of 95% anhydrous hexane (*SIGMA-ALDRICH® Milwaukee, WI, USA*) was added slowly to the top of the surface of each sample three times (total of 270 µL of hexane). The room light was turned off and the curtain closed before each photo was taken. The fluorescent light (Dolan-Jenner's Fiber-Lite® MH-100 Metal Halide Machine Vision Illuminator) emitted light onto the sample. The varying intensities of light reflected depended on the degree of ceramide saturation, which is based on lipoprotein density, to reveal the layers of different lipoprotein subclasses. Snapshots of each sample were taken using a digital microscope camera and PictureFrame Software™

Application 3.0. Each image was labeled "C2013, Participant Code #, Testing Session #"). This process was repeated for each sample. All images were saved in a file on the desktop and on a flash drive. Intra- and inter- assay CV were determined using the average values of the standard samples present at each sample run. Intra-assay %CV (AUC) and %CV (%AUC) was 36.7% and 14.85%, respectively while inter-assay %CV(AUC) and %CV (%AUC) was 6.70% and 2.71%, respectively.

Lipoprotein Data Analysis

Different lipoprotein subclasses separated along the density gradient created by the NaBiEDTA solution according to their respective densities and photographed. Origin Pro 2015 Analysis and 7.0 Graphing Software, Version 92E was used to digitally convert each photo image into numerical data. The AUC for lipoproteins and their subfraction areas were determined for each sample by measuring the pixels along the length of each ultracentrifuge tube. Pixel data represent numerical values corresponding to the intensity of light reflected by each layer within a sample. Pixels were categorized according to its location along the length of the tube where tube coordinates corresponded to a specified density. The density region for each lipoprotein subclass was known allowing for the categorization of pixels into individual subfraction areas.

Eleven columns were selected from the center of the matrix (columns 160 to 170) representing 6-33 mm in length from the center of the sample tube. Data was copied and pasted into a prepared template labeled "0.18M NaBiY Template", which includes the following formula:

"((col(160)+col(161)+col(162)+col(163)+col(164)+col(165)+col(166)+col(167)+col(168)+col(169)+col(170))/11/16-16"

This formula averages out 11 columns of pixel data (corresponding to the total intensity of light reflected by the lipoproteins) from the center of the tube to generate the lipoprotein density profile for the entire sample and total AUC, which consists of the following subfraction areas: TRL, LDL1, LDL2, LDL3, LDL4, LDL5, HDL2b, HDL2a, HDL3a, HDL3b, and HDL3c.

After pasting raw pixel data into "0.18M NaBiY Template", formulas generate total AUC and the AUC for each lipoprotein subclass. With this information it is possible to calculate each lipoprotein subclass as a percent of total AUC and as a percent of total LDL AUC or total HDL AUC as shown in the following formulas. The following formula represents the calculation for TRL as percent of total AUC:

TRL as percent of total AUC = (AUC for TRL) / (AUC for TRL + LDL1 + LDL2 + LDL3 + LDL4 + LDL5 + HDL2b + HDL2a + HDL3a + HDL3b + HDL3c)

The following formula represents the calculation for LDL as percent of total AUC, which was repeated for HDL:

LDL as percent of total AUC = (AUC for LDL1+ LDL2+ LDL3 + LDL4 + LDL5) / (AUC for TRL + LDL1 + LDL2 + LDL3 + LDL4 + LDL5 + HDL2b + HDL2a + HDL3a + HDL3b + HDL3c)

The following formula represents the calculation for HDL2b as percent of total AUC, which was repeated for each of the remaining LDL and HDL subfraction areas:

HDL2b as percent of total AUC = (AUC for HDL2b) / (AUC for TRL + LDL1 + LDL2 + LDL3 + LDL4 + LDL5 + HDL2b + HDL2a + HDL3a + HDL3b + HDL3c)

The following formula represents the calculation for LDL1 as percent of total LDL AUC, which was repeated for LDL2, LDL3, LDL4, and LDL5:

LDL1 as percent of total LDL AUC = (AUC for LDL1) / (AUC for LDL1 + LDL2 + LDL3 + LDL4 + LDL5)

The following formula represents the calculation for HDL2b as percent of total HDL AUC, which was repeated for HDL2a, HDL3a, HDL3b, and HDL3c:

HDL2b as percent of total HDL AUC = (AUC for HDL2b) / (AUC for HDL2b + HDL2a + HDL3a + HDL3b + HDL3c)

Statistical Analysis

For these statistical analyses, samples from 75 participants who completed a 24-week weight-loss intervention program incorporating a resistance-based circuit training program and dietary changes were used. Series mean method were employed to replace missing data points only for reported food logs.

Variables consist of the AUC for each lipoprotein subclass where each layer within a sample is separated into subfraction areas due to differences in density. Thus, the following lipoprotein subfraction areas were represented: TRL, LDL1, LDL2, LDL3, LDL4, LDL5, HDL2b, HDL2a, HDL3a, HDL3b, and HDL3c using isopycnic ultracentrifugation. As mentioned in the formulas previously presented, LDL and HDL were reported as percent of total AUC, each LDL and HDL subfraction area was reported as percent of total AUC and as percent of total LDL and HDL AUC, respectively.

The statistical analysis was performed using SPSS (*Version 25, IBM Corporation, Armak, NY*). Participant baseline demographic data was analyzed by one-way Analysis of Variance (ANOVA). Dependent and independent variables were analyzed by General Linear Model (GLM) with repeated measures on time. General Linear Model Wilks' Lambda time and group x time p-values and univariate group effects were reported. The Greenhouse-Geisser

univariate test for time, group x time, and group effect was reported for each variable in the GLM with repeated measures. Significant group, time, and group x time interaction effects were followed with Tukey's LSD post hoc analysis to determine where significant differences lie and significant p-values were reported. Changes were calculated for 12- and 24-week time points by subtracting the baseline testing session from the 12-week testing session and by subtracting the baseline testing session from the 24-week testing session, respectively. These values were analyzed by GLM with repeated measures to normalize differences in baseline values and to identify significant changes. The percent change from baseline was calculated for 12- and 24-week time points by subtracting the baseline testing session from the 12-/24-week testing session, dividing by the baseline value, and multiplying by 100. Effect size was reported from GLM with repeated measures output as partial eta-squared (η^2) with adherence to the following designations outlined by Cohen: small = 0.01, medium = 0.06, and large = 0.14 [120]. Pearson's correlation was conducted to detect significant correlations between changes to lipoprotein subclasses and changes to anthropometrics, body composition, cardiovascular fitness, glucose homeostasis, blood lipids. Data were considered statistically significant when the probability of type I error was $p<0.05$. Data were represented as mean ± standard deviation (X±SD).

CHAPTER IV

RESULTS

Baseline Demographics

Seventy-five healthy, sedentary women (age: 38.3±13.9 yrs., height: 163.8±7.0 cm, weight: 81.1±16.1 kg, BMI: 30.1±5.8 kg/m^2, fat mass: 31.1±9.5 kg, waist circumference: 88.0±13.6 cm, and VAT area: 138.2±72.2 cm^2) completed a 24-week intervention. Samples used for additional analyses represent the women randomly assigned to no exercise or diet (NED, n=19), or to one of three diet and exercise interventions, a higher carbohydrate, low fat diet (HCLF, n=19), a moderate carbohydrate, higher protein diet (MCHP, n=16), or a lower carbohydrate, higher protein diet (LCHP, n=21). Analyses from one-way ANOVA indicate that there were no significant differences between groups at baseline for age, height (cm), weight (kg), body mass index (BMI, kg/m^2), fat mass (kg), waist circumference (cm), or VAT (cm^2). The statistical analysis, results, and conclusion of this paper are based on the data and results from serum drawn at 0-, 12-, and 24-week time points.

Table 4.1: Baseline demographics

Group	N	Age	Height (cm)	Weight (kg)	BMI (kg/m^2)	FM (kg)	Waist (cm)	VAT Area (cm^2)
NED	19	36.6 ± 14.1	165.8 ± 7.7	79.1 ± 17.9	28.7 ± 5.6	28.1 ± 10.180	85.6 ± 12.5	120.1 ± 63.9
HCLF	19	39.1 ± 13.6	162.6 ± 6.4	80.5 ± 16.2	30.4 ± 5.8	31.6 ± 9.350	88.5 ± 16.6	141.6 ± 70.0
MCHP	16	40.4 ± 13.8	163.0 ± 5.0	81.6 ± 16.3	30.8 ± 6.3	32.0 ± 9.300	90.1 ± 13.9	150.8 ± 74.0
LCHP	21	37.4 ± 14.7	163.8 ± 8.2	83.2 ± 14.9	30.6 ± 5.8	32.8 ± 9.030	87.9 ± 11.7	142.0 ± 81.5
Mean	75	38.3 ± 13.9	163.8 ± 7.0	81.1 ± 16.1	30.1 ± 5.8	31.1 ± 9.460	88.0 ± 13.6	138.2 ± 72.2
p-value		0.86	0.51	0.88	0.68	0.43	0.80	0.63

Data represented as mean±standard deviation. Significance level ($p<0.05$). No exercise or diet, NED (n=19). Higher carbohydrate/lower fat, HCLF (n=19). Moderate carbohydrate/higher protein, MCHP (n=16). Lower carbohydrate/higher protein, LCHP (n=21). Visceral Adipose Tissue, VAT, as area (cm^2) measured via Dual Energy X-ray Absorptiometry, DEXA. Body Mass Index, BMI. Fat Mass, FM.

Energy and Macronutrient Intake

Table 4.2 displays the energy intake expressed as kilocalories per day (kcal/day) along with carbohydrate (%CHO), protein (%PRO), and fat intake (%FAT) as a percent of kcal/day. General linear model (GLM) with repeated measures was run on nutrient intake at 0-, 12-, and

24-week time points. Results from GLM representing dietary intake revealed an overall Wilks' Lambda time effect, ($p<0.001$, $\eta_p^2=0.294$), time by group interaction (T×G) ($p<0.001$, $\eta_p^2=0.165$), and group effect ($p<0.001$, $\eta_p^2=0.265$). Univariate analysis revealed a significant time effect for energy intake (kcal/day) ($p<0.001$, $\eta_p^2=0.120$), %CHO ($p<0.001$, $\eta_p^2=0.126$), and %PRO ($p<0.001$, $\eta_p^2=0.368$). There was a significant T×G interaction for %CHO ($p<0.001$, $\eta_p^2=0.267$), and %PRO ($p<0.001$, $\eta_p^2=0.308$). Univariate analysis revealed a significant group effect for energy intake ($p<0.001$, $\eta_p^2=0.242$), %CHO ($p<0.001$, $\eta_p^2=0.330$), %PRO ($p<0.001$, $\eta_p^2=0.378$), and %FAT ($p=0.135$, $\eta_p^2=0.075$).

Table 4.2: Total energy intake (kcal/day) and macronutrient intake (% kcal/day)

Variable	Group	0 Weeks	12 Weeks	24 Weeks	Group (SEM)		P-level	η^2
Energy Intake	NED	2136.0 ± 536.0	1952.0 ± 616*	1658.0 ± 381*†	1915.0 ± 69	T	<0.001	medium
(kcal/day)	HCLF	1832.0 ± 531.0	1557.0 ± 567^{a}	1514.0 ± 283*	1635.0 ± 69^{a}	G	<0.001	large
	MCHP	1529.0 ± 536^{a}	1326.0 ± 306^{a}	1473.0 ± 366	1443.0 ± 76^{a}	T×G	0.335	small
	LCHP	1790.0 ± 365^{a}	1495.0 ± 304^{*a}	1525.0 ± 503*	1603.0 ± 66^{a}			
	Mean	1833.0 ± 526.0	1591.0 ± 516*	1545.0 ± 394*				
CHO	NED	42.6 ± 10.4	42.6 ± 9.5	44.3 ± 10.3	43.2 ± 1.5	T	<0.001	medium
(%kcal/day)	HCLF	45.6 ± 9.0	49.2 ± 6.9^{a}	48.6 ± 6.2	47.8 ± 1.5^{acd}	G	<0.001	large
	MCHP	43.9 ± 9.8	35.2 ± 6.4^{*ab}	37.3 ± 7.6^{*ab}	38.8 ± 1.6^{b}	T×G	<0.001	large
	LCHP	46.1 ± 6.8	30.5 ± 10.4^{*ab}	32.3 ± 10.3^{*ab}	36.3 ± 1.4^{ab}			
	Mean	44.6 ± 9.0	39.3 ± 11.2*	40.6 ± 10.8*				
PRO	NED	16.8 ± 5.8	19.7 ± 5.6	20.5 ± 8.4	19.0 ± 1.2	T	<0.001	large
(%kcal/day)	HCLF	18.1 ± 3.9	17.7 ± 3.2	22.4 ± 10.7*†	19.4 ± 1.2	G	<0.001	large
	MCHP	19.5 ± 5.9^{d}	29.6 ± 8.0^{*abd}	27.1 ± 8.2^{*a}	25.4 ± 1.3^{ab}	T×G	<0.001	large
	LCHP	16.2 ± 3.5	35.2 ± 8.7^{*abc}	31.5 ± 8.4^{*ab}	27.6 ± 1.1^{ab}			
	Mean	17.5 ± 4.9	25.6 ± 9.9*	25.5 ± 9.9*				
FAT	NED	39.5 ± 7.9	37.7 ± 7.4	37.1 ± 10.5	38.1 ± 1.4	T	0.098	small
(%kcal/day)	HCLF	35.4 ± 6.6	32.5 ± 7.3^{a}	33.3 ± 5.9	33.7 ± 1.4^{a}	G	0.135	medium
	MCHP	35.2 ± 8.3	34.3 ± 8.1	35.2 ± 7.4	34.9 ± 1.5	T×G	0.889	small
	LCHP	37.5 ± 7.6	34.3 ± 7.1	36.9 ± 7.8	36.2 ± 1.3			
	Mean	37.0 ± 7.6	34.7 ± 7.5	35.7 ± 8.1				

Data presented as means ± standard deviation and group ± standard error of the mean (SEM). N=75; No exercise or diet, NED (n=19). Higher carbohydrate/lower fat, HCLF (n=19). Moderate carbohydrate/higher protein, MCHP (n=16). Lower carbohydrate/higher protein, LCHP (n=21). Time effect, T. Group effect, G. Time × Group interaction, T×G. Carbohydrate intake, CHO. Protein intake, PRO. Fat intake, FAT. *significant time effect from baseline ($p<0.05$). †significant time effect from 12 weeks ($p<0.05$). Letter superscripts indicate significance ($p<0.05$) from post hoc LSD analyses: ^{a}significantly different from NED ($p<0.05$), ^{b}significantly different from HCLF ($p<0.05$), ^{c}significantly different from MCHP ($p<0.05$), ^{d}significantly different from LCHP ($p<0.05$). Partial eta-squared (η^2) reported with adherence to the following designations outlined by Cohen: small = 0.01, medium = 0.06, and large = 0.14 [120].

Subjects assigned to any of the three diet and exercise groups significantly reduced energy intake. Post hoc tests showed that energy intake for subjects assigned to HCLF (-280.6

kcal/day; 95% CI, -476.4, -84.8), MCHP (-472.5 kcal/day; 95% CI, -677.3, -267.8), and LCHP (-312.2 kcal/day; 95% CI, -503.2, +121.1) was significantly lower than that of NED. Although subjects did not meet recommended macronutrient intakes, macronutrient intakes were significantly different than baseline values. Percent protein intake for subjects assigned to MCHP and LCHP was significantly greater than that for subjects assigned to NED and HCLF, and percent carbohydrate intake for subjects assigned to HCLF was significantly greater than that for subjects assigned to NED, MCHP, and LCHP.

Table 4.3 displays the nutrient intake expressed as kilocalories per kilogram per day (kcal/kg/day), along with CHO, PRO, and FAT intake as grams per kilogram of body weight per day (g/kg/day) at 0, 12, and 24 weeks. General Linear Model revealed an overall Wilks' Lambda time effect ($p<0.001$, $\eta_p^2=0.238$), T×G interaction ($p<0.001$, $\eta_p^2=0.140$), and group effect ($p<0.001$, $\eta_p^2=0.232$). Univariate analysis revealed a significant time effect for energy intake kcal/kg/day ($p=0.001$, $\eta_p^2=0.090$), CHO (g/kg/day, $p<0.001$, $\eta_p^2=0.164$), PRO (g/kg/day, $p<0.001$, $\eta_p^2=0.137$), and FAT (g/kg/day, $p=0.001$, $\eta_p^2=0.097$). There was a significant T×G interaction for CHO ($p=0.007$, $\eta_p^2=0.119$) and PRO ($p<0.001$, $\eta_p^2=0.206$) with no significant interaction for FAT ($p=0.114$, $\eta_p^2=0.070$). Univariate analysis indicated there was a significant group effect for energy intake as kcal/kg/day ($p=0.003$, $\eta_p^2=0.176$), CHO ($p<0.001$, $\eta_p^2=0.270$), PRO ($p=0.020$, $\eta_p^2=0.129$), and FAT ($p=0.001$, $\eta_p^2=0.198$).

Post hoc tests revealed that total energy intake for HCLF (-3.63 kcal/kg/day; 95% CI, -7.04, -0.24), MCHP (-6.60 kcal/kg/day; 95% CI, -10.16, -3.05), and LCHP (-4.80 kcal/kg/day; 95% CI, -8.11, -1.48) was significantly less than that of NED. CHO for HCLF was not significantly different from baseline values at 12 and 24 weeks, while CHO for MCHP at 12 weeks (-0.567±0.249, $p=0.026$) and for LCHP at 12 and 24 weeks (-1.088±0.217, $p<0.001$

and -1.006±0.207, p<0.001, respectively) was significantly decreased from baseline values. According to post hoc analysis both higher protein groups, MCHP and LCHP, increased PRO at 12 and 24 weeks compared to baseline values, and PRO for LCHP remained significantly greater than NED (+0.461 g/kg/day; 95% CI, 0.066, 0.856) and HCLF (+0.42 g/kg/day; 95% CI, 0.03, 0.82) at 24 weeks. Although, FAT for HCLF, MCHP, and LCHP was significantly lower than NED at 0 and 12 weeks, group differences were no longer present at 24 weeks. Results indicate FAT for NED at 24 weeks was significantly reduced from baseline (-0.383±0.098, p<0.001).

Table 4.3: Total caloric (kcal/kg/day) and macronutrient intake (g/kg/day)

Variable	Group	0 Weeks	12 Weeks	24 Weeks	Group (SEM)		P-level	η^2
Energy Intake	NED	28.3 ± 9.1	25.9 ± 10.4	21.6 ± 5.9*†	25.3 ± 1.2	T	0.001	medium
(kcal/kg/day)	HCLF	23.5 ± 7.4	20.4 ± 5.7^{a}	21.0 ± 6.1	21.6 ± 1.2^{a}	G	0.003	large
	MCHP	19.2 ± 6.3^{a}	17.3 ± 4.3^{a}	19.5 ± 4.8	18.7 ± 1.3^{a}	T×G	0.084	medium
	LCHP	22.3 ± 6.7^{a}	19.2 ± 4.4^{a}	19.9 ± 6.4*	20.5 ± 1.2^{a}			
	Mean	23.5 ± 8.0	20.8 ± 7.3*	20.5 ± 5.8*				
CHO	NED	3.0 ± 1.1	2.8 ± 1.5	2.4 ± 1.1*	2.8 ± 0.2	T	<0.001	large
(g/kg/day)	HCLF	2.7 ± 1.0	2.5 ± 0.7	2.6 ± 0.9	2.6 ± 0.2	G	<0.001	large
	MCHP	2.1 ± 0.8^{a}	1.5 ± 0.5^{*ab}	1.8 ± 0.6^{ab}	1.8 ± 0.2^{ab}	T×G	0.007	medium
	LCHP	2.6 ± 0.8	1.5 ± 0.6^{*ab}	1.6 ± 0.6^{*ab}	1.9 ± 0.2^{ab}			
	Mean	2.6 ± 1.0	2.1 ± 1.08*	2.1 ± 0.91*				
PRO	NED	1.2 ± 0.4	1.2 ± 0.4	1.1 ± 0.6	1.2 ± 0.1	T	<0.001	medium
(g/kg/day)	HCLF	1.1 ± 0.4	0.9 ± 0.3^{a}	1.2 ± 0.6	1.0 ± 0.1	G	0.020	medium
	MCHP	0.9 ± 0.3^{a}	1.3 ± 0.4^{*b}	1.3 ± 0.5*	1.2 ± 0.1	T×G	<0.001	large
	LCHP	0.9 ± 0.3^{a}	1.7 ± 0.5^{*abc}	1.6 ± 0.8^{*ab}	1.4 ± 0.1^{ab}			
	Mean	1.0 ± 0.4	1.3 ± 0.5*	1.3 ± 0.6*				
FAT	NED	1.3 ± 0.5	1.1 ± 0.5	0.9 ± 0.3*†	1.1 ± 0.1	T	0.001	medium
(g/kg/day)	HCLF	0.9 ± 0.3^{a}	0.8 ± 0.3^{a}	0.8 ± 0.3	0.8 ± 0.1^{a}	G	0.001	large
	MCHP	0.8 ± 0.4^{a}	0.7 ± 0.2^{a}	0.8 ± 0.2	0.7 ± 0.1^{a}	T×G	0.114	medium
	LCHP	0.9 ± 0.4^{a}	0.7 ± 0.2^{*a}	0.8 ± 0.4	0.8 ± 0.1^{a}			
	Mean	1.0 ± 0.4	0.8 ± 0.4*	0.8 ± 0.3*				

Data presented as means ± standard deviation and group ± standard error of the mean (SEM). N=75; No exercise or diet, NED (n=19). Higher carbohydrate/lower fat, HCLF (n=19). Moderate carbohydrate/higher protein, MCHP (n=16). Lower carbohydrate/higher protein, LCHP (n=21). Time effect, T. Group effect, G. Time × Group interaction, T×G. Carbohydrate intake, CHO. Protein intake, PRO. Fat intake, FAT. *significant time effect from baseline (p<0.05). †significant time effect from 12 weeks (p<0.05). Letter superscripts indicate significance (p<0.05) from post hoc LSD analyses: ^{a}significantly different from NED (p<0.05), ^{b}significantly different from HCLF (p<0.05), ^{c}significantly different from MCHP (p<0.05), ^{d}significantly different from LCHP (p<0.05). Partial eta-squared (η^2) reported with adherence to the following designations outlined by Cohen: small = 0.01, medium = 0.06, and large = 0.14 [120].

Anthropometrics

Table 4.4 displays anthropometrics including weight (kg), waist and hip circumference (cm), and waist-to-hip ratio. General linear model with repeated measures on time revealed a

Wilks' Lambda time effect (p<0.001, η_p^2=0.214) and T×G interaction (p<0.001, η_p^2=0.109) with no significant differences between groups (p=0.722, η_p^2=0.041). Univariate tests revealed significant time effects for weight (p<0.001, η_p^2=0.358), waist circumference (p<0.001, η_p^2=0.128) and hip circumference (p<0.001, η_p^2=0.241). There was a significant T×G interaction for weight (p<0.001, η_p^2=0.237), waist (p=0.024, η_p^2=0.101) and hip circumference (p<0.001, η_p^2=0.224).

Table 4.4: Weight, waist, and hip circumference, and waist to hip ratio

Variable	Group	0 Weeks	12 Weeks	24 Weeks	Group (SEM)		P-level	η^2
Body Weight (kg)	NED	79.1 ± 17.9	79.6 ± 18.5	80.1 ± 18.5	79.6 ± 3.7	T	<0.001	large
	HCLF	80.5 ± 16.2	77.4 ± 16.2*	75.8 ± 16.1*†	77.9 ± 3.7	G	0.96	<0.01
	MCHP	81.6 ± 16.3	77.9 ± 14.2*	76.5 ± 13.8*†	78.7 ± 4.0	T×G	<0.001	large
	LCHP	83.2 ± 14.9	79.6 ± 14.5*	78.7 ± 14.8*	80.5 ± 3.5			
	Mean	81.1 ± 16.1	78.7 ± 15.7*	77.8 ± 15.7*†				
Waist Circumference (cm)	NED	85.6 ± 12.5	86.8 ± 11.4	85.9 ± 12.6	86.1 ± 2.9	T	<0.001	medium
	HCLF	88.5 ± 16.6	86.1 ± 14.2*	83.9 ± 14.8*†	86.2 ± 2.9	G	0.926	<0.01
	MCHP	90.1 ± 13.9	87.4 ± 12.3*	88.0 ± 11.8	88.5 ± 3.2	T×G	0.024	medium
	LCHP	87.9 ± 11.7	85.3 ± 11.1*	84.8 ± 12.1*	86.0 ± 2.8			
	Mean	88.0 ± 13.6	86.3 ± 12.0*	85.5 ± 12.7*				
Hip Circumference (cm)	NED	17.0 ± 1.9	17.2 ± 1.8	17.3 ± 1.9	17.2 ± 0.4	T	<0.001	large
	HCLF	17.3 ± 2.1	17.1 ± 1.8	16.6 ± 1.9*†	17.0 ± 0.4	G	0.887	<0.01
	MCHP	17.6 ± 1.7	16.8 ± 1.6*	16.8 ± 1.7*	17.1 ± 0.4	T×G	<0.001	large
	LCHP	17.8 ± 1.4	17.3 ± 1.4*	17.2 ± 1.7*	17.4 ± 0.4			
	Mean	17.5 ± 1.8	17.1 ± 1.6*	17.0 ± 1.8*				
Waist-to-Hip Ratio	NED	5.0 ± 0.4	5.0 ± 0.4	5.0 ± 0.5	5.0 ± 0.1	T	0.858	<0.01
	HCLF	5.1 ± 0.4	5.0 ± 0.4	5.0 ± 0.4	5.0 ± 0.1	G	0.375	small
	MCHP	5.1 ± 0.5	5.2 ± 0.5	5.2 ± 0.5	5.2 ± 0.1	T×G	0.279	small
	LCHP	4.9 ± 0.5	4.9 ± 0.4	4.9 ± 0.4[c]	4.9 ± 0.1			
	Mean	5.0 ± 0.4	5.0 ± 0.4	5.0 ± 0.4				

Data presented as means ± standard deviation and group ± standard error of the mean (SEM). N=75; No exercise or diet, NED (n=19). Higher carbohydrate/lower fat, HCLF (n=19). Moderate carbohydrate/higher protein, MCHP (n=16). Lower carbohydrate/higher protein, LCHP (n=21). Time effect, T. Group effect, G. Time × Group interaction, T×G. *significant time effect from baseline (p<0.05). †significant time effect from 12 weeks (p<0.05). Letter superscripts indicate significance (p<0.05) from post hoc LSD analyses: [a]significantly different from NED (p<0.05), [b]significantly different from HCLF (p<0.05), [c]significantly different from MCHP (p<0.05), [d]significantly different from LCHP (p<0.05). Partial eta-squared (η^2) reported with adherence to the following designations outlined by Cohen: small = 0.01, medium = 0.06, and large = 0.14 [120].

Post hoc tests indicated at 24 weeks that weight for HCLF (-4.71±0.99, p<0.001), MCHP (-5.11±1.08, p<0.001), and LCHP (-4.47±0.94, p<0.001); waist circumference for HCLF (-4.63±1.23 cm, p<0.001) and LCHP (-3.08±1.18 cm, p=0.011); and hip circumference for HCLF (-0.74±0.17, p<0.001), MCHP (-0.81±0.18, p<0.001), and LCHP (-0.64±0.16, p<0.001) were significantly lower than their respective baseline values. Overall, percent change in weight from baseline for HCLF, MCHP, and LCHP was significantly greater than NED at 12 and 24

weeks. We did not observe a significant change in weight, waist and hip circumference, or waist-to-hip ratio for NED at 12 or 24 weeks.

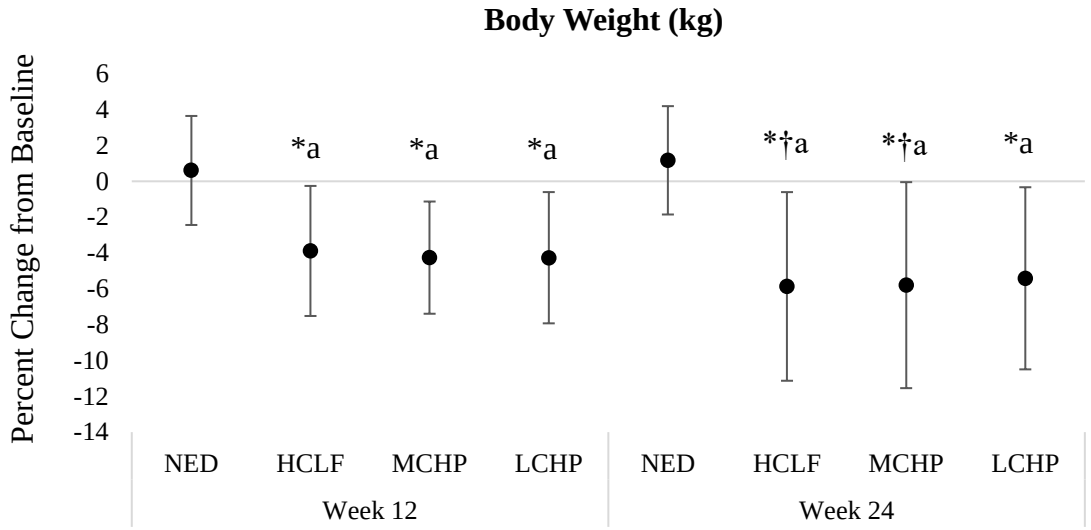

Figure 4.1: Percent change from baseline for body weight. Data presented as mean change ± standard deviation from baseline at 12 and 24 weeks. N=75; no exercise or diet, NED (N=19), higher carbohydrate/low fat, HCLF (N=19), moderate carbohydrate/higher protein, MCHP (N=16), and lower carbohydrate/higher protein, LCHP (N=21).*significant percent change, $p<0.05$. †significantly different from 12 weeks, $p<0.05$.

Body Composition

General linear model with repeated measures on time for fat mass (kg), fat free mass (kg), percent body fat, and VAT (cm^2) revealed a Wilks' Lambda time effect ($p<0.001$, $\eta_p^2=0.404$) and T×G interaction ($p<0.001$, $\eta_p^2=0.167$) with no significant difference between groups ($p=0.628$, $\eta_p^2=0.046$) (Data shown in Table 4.5 for VAT). Univariate analysis revealed a significant time effect for fat mass ($p<0.001$, $\eta_p^2=0.456$), percent body fat ($p<0.001$, $\eta_p^2=0.364$), and VAT area ($p<0.001$, $\eta_p^2=0.297$, Table 4.5) and T×G interaction for fat mass ($p<0.001$, $\eta_p^2=0.307$), percent body fat ($p<0.001$, $\eta_p^2=0.193$), and VAT area ($p<0.003$, $\eta_p^2=0.138$).

Figures 4.2, 4.3, 4.4, and 4.5 display percent change from baseline for fat mass, fat free mass, percent body fat, and VAT area, respectively, at 12 and 24 weeks. Post hoc tests showed that there were significant ($p<0.001$) reductions in fat mass, percent body fat, and VAT area for

Table 4.5: Visceral adipose tissue area

Variable	Group	0 Weeks	12 Weeks	24 Weeks	Group (SEM)		P-level	η^2
VAT	NED	120.1 ± 63.9	122.0 ± 65.7	115.0 ± 56.6	119.0 ± 14.9	T	<0.001	large
(cm^2)	HCLF	141.6 ± 70.0	121.2 ± 67.4*	118.0 ± 70.4*	126.9 ± 14.9	G	0.915	<0.01
	MCHP	150.8 ± 73.9	124.4 ± 54.3*	116.2 ± 59.9*	130.5 ± 16.3	T×G	0.003	medium
	LCHP	142.0 ± 81.5	103.3 ± 65.7*	106.4 ± 70.3*	117.2 ± 14.2			
	Mean	138.2 ± 72.2	117.1 ± 63.2*	113.6 ± 63.8*				

Data presented as means ± standard deviation and group ± standard error of the mean (SEM). N=75; No exercise or diet, NED (n=19). Higher carbohydrate/lower fat, HCLF (n=19). Moderate carbohydrate/higher protein, MCHP (n=16). Lower carbohydrate/higher protein, LCHP (n=21). Time effect, T. Group effect, G. Time × Group interaction, T×G. Visceral adipose tissue, VAT. *significant time effect from baseline ($p<0.05$). †significant time effect from 12 weeks ($p<0.05$). Letter superscripts indicate significance ($p<0.05$) from post hoc LSD analyses: [a]significantly different from NED ($p<0.05$), [b]significantly different from HCLF ($p<0.05$), [c]significantly different from MCHP ($p<0.05$), [d]significantly different from LCHP ($p<0.05$). Partial eta-squared (η^2) reported with adherence to the following designations outlined by Cohen: small = 0.01, medium = 0.06, and large = 0.14 [120].

HCLF, MCHP, and LCHP from their respective baseline values at 12 and 24 weeks. At 24 weeks, fat mass for HCLF (-4.12±0.77, $p<0.001$), MCHP (-4.81±0.84, $p<0.001$), and LCHP (-4.75±0.74, $p<0.001$); percent body fat for HCLF (-3.55±0.71, $p<0.001$), MCHP (-3.45±0.77, $p<0.001$), and LCHP (-4.25±0.67, $p<0.001$); and VAT area (cm^2) for HCLF (-23.67 cm^2, $p<0.003$), MCHP (-34.53 cm^2, $p<0.001$), and LCHP (-35.59 cm^2, $p<0.001$) were significantly lower than their respective baseline values. Percent change from baseline for weight, fat mass, percent body fat, and VAT area from baseline for all diet and exercise groups were significantly greater than NED at 24 weeks.

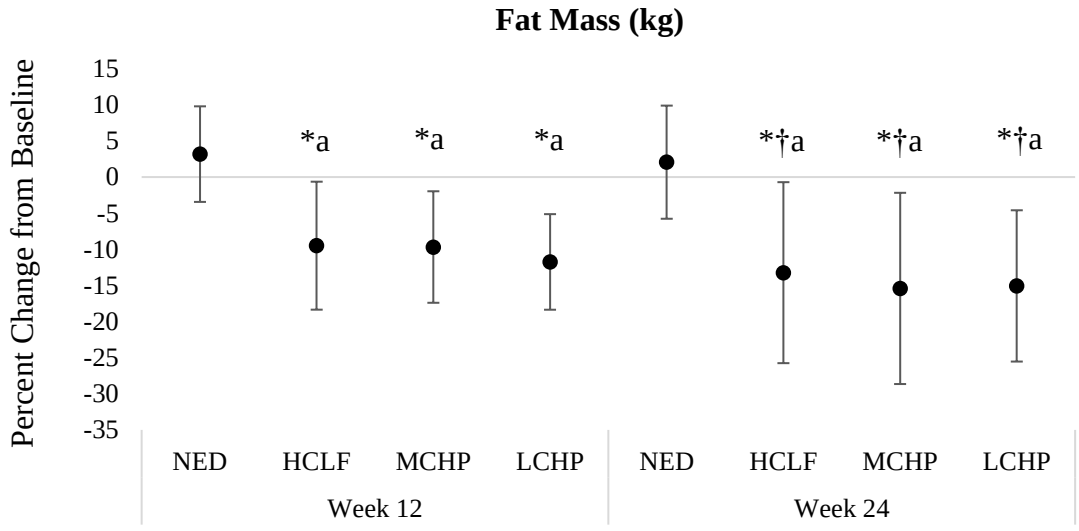

Figure 4.1: Percent change from baseline for fat mass. Data presented as mean change ± standard deviation from baseline at 12 and 24 weeks. N=75; no exercise or diet, NED (N=19), higher carbohydrate/low fat, HCLF (N=19), moderate carbohydrate/higher protein, MCHP (N=16), and lower carbohydrate/higher protein, LCHP (N=21).*significant percent change, $p<0.05$.†significantly different from 12 weeks, $p<0.05$.

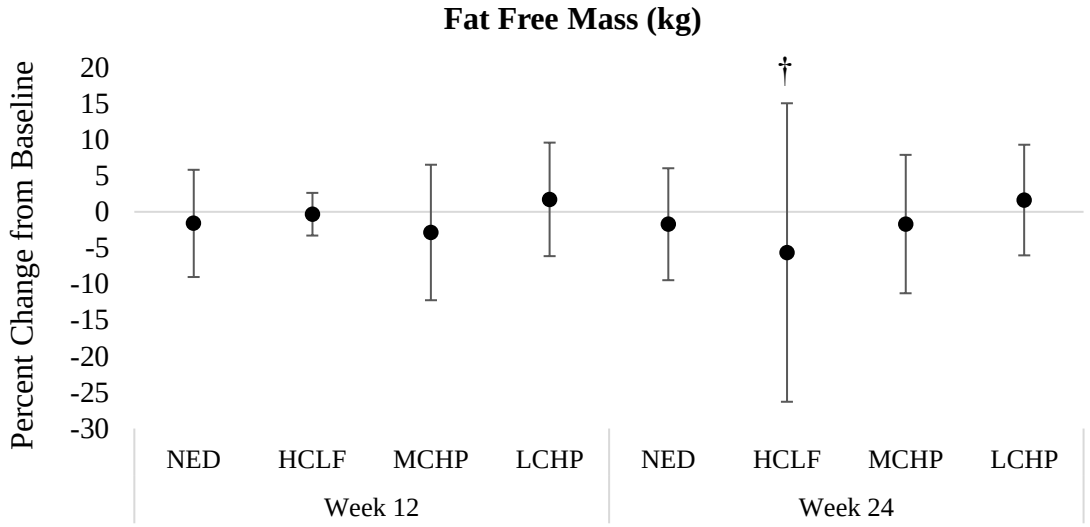

Figure 4.3: Percent change from baseline for fat free mass. Data presented as mean change ± standard deviation from baseline at 12 and 24 weeks. N=75; no exercise or diet, NED (N=19), higher carbohydrate/low fat, HCLF (N=19), moderate carbohydrate/higher protein, MCHP (N=16), and lower carbohydrate/higher protein, LCHP (N=21).*significant percent change, $p<0.05$. †significantly different from 12 weeks, $p<0.05$.

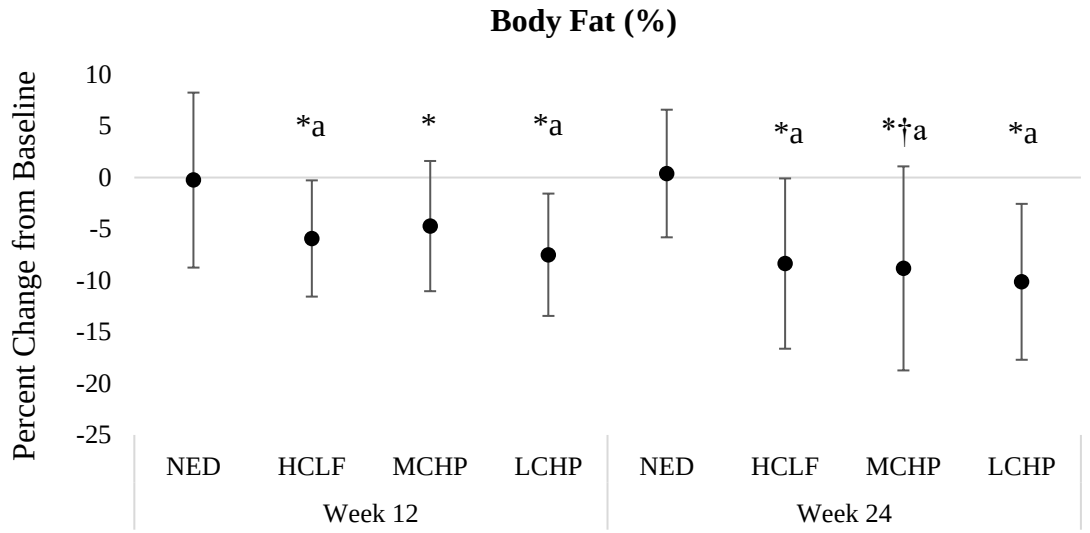

Figure 4.4: Percent change from baseline for percent body fat. Data presented as mean change ± standard deviation from baseline at 12 and 24 weeks. N=75; no exercise or diet, NED (N=19), higher carbohydrate/low fat, HCLF (N=19), moderate carbohydrate/higher protein, MCHP (N=16), and lower carbohydrate/higher protein, LCHP (N=21).*significant percent change, $p<0.05$. †significantly different from 12 weeks, $p<0.05$.

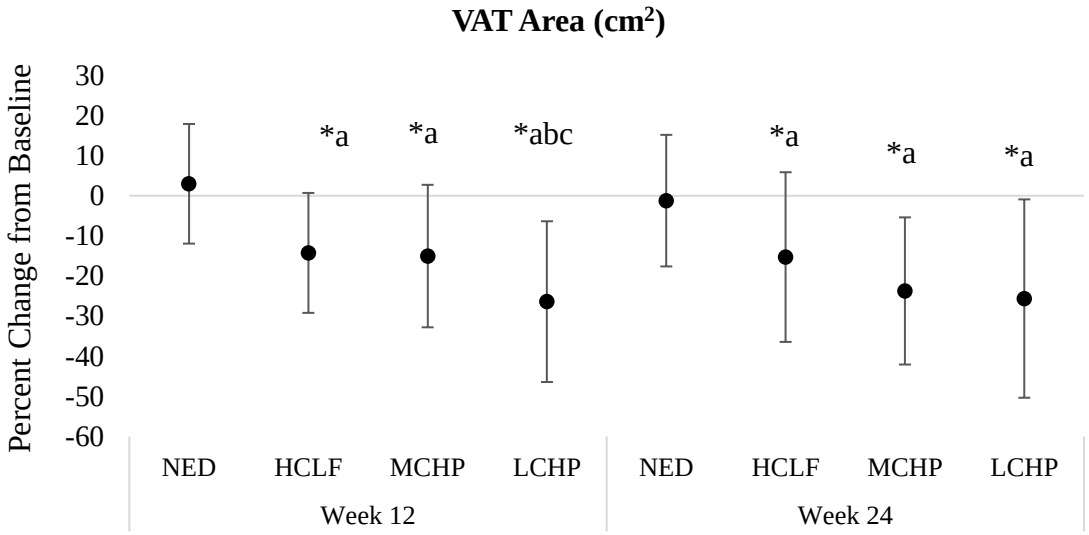

Figure 4.5: Percent change from baseline for visceral adipose tissue (VAT) area (cm^2). Data presented as mean change ± standard deviation from baseline at 12 and 24 weeks. N=75; no exercise or diet, NED (N=19), higher carbohydrate/low fat, HCLF (N=19), moderate carbohydrate/higher protein, MCHP (N=16), and lower carbohydrate/higher protein, LCHP (N=21).*significant percent change, $p<0.05$. Letter superscripts indicate significance ($p<0.05$) from post hoc LSD. †significantly different from 12 weeks, $p<0.05$. ^{a}significantly different from NED, ^{b}significantly different from HCLF, ^{c}significantly different from MCHP, ^{d}significantly different from LCHP.

Cardiorespiratory Fitness

Table 4.6 displays VO_{2peak} and time to exhaustion, measures of cardiorespiratory fitness, at 0, 12, and 24 weeks. General Linear Model with repeated measures on time revealed a Wilks' Lambda time effect ($p<0.001$, $\eta_p^2=0.257$), T×G interaction ($p<0.001$, $\eta_p^2=0.101$) and no significant difference between groups ($p=0.785$, $\eta_p^2=0.026$). Univariate analysis revealed a Wilks' Lambda time effect for VO_{2peak} (L/min) ($p=0.001$, $\eta_p^2=0.098$), VO_{2peak} (ml/kg/min) ($p<0.001$, $\eta_p^2=0.374$), and time to exhaustion (secs) ($p<0.001$, $\eta_p^2=0.234$) and a Wilks' Lambda T×G interaction for VO_{2peak} (L/min) ($p=0.049$, $\eta_p^2=0.088$), VO_{2peak} (ml/kg/min) ($p<0.001$, $\eta_p^2=0.204$), and time to exhaustion (secs) ($p=0.034$, $\eta_p^2=0.093$).

Post hoc tests showed that VO_{2peak} (L/min) for HCLF (+0.16±0.05, p=0.001), MCHP (+0.13±0.05, p=0.02), and LCHP (+0.19±0.05, p<0.001); VO_{2peak} (ml/kg/min) for HCLF (+4.17±0.78, p<0.001), MCHP (+3.71±0.85, p<0.001), and LCHP (+4.37±0.74, p<0.001); and

Time to exhaustion (secs) for HCLF (+45.47±14.03, p<0.002), MCHP (+61.50±15.29, p<0.001), and LCHP (+56.95±13.35, p<0.001) at 24 weeks were significantly greater than their respective baseline values while that of NED was not significantly different from baseline (-0.002±0.05, p=0.97; -0.08±0.78, p=0.91; and +5.79±14.03, p=0.68, respectively).

Table 4.6: Cardiorespiratory fitness

Variable	Group	0 Weeks	12 Weeks	24 Weeks	Group (SEM)		P-level	η^2
VO_{2peak}	NED	2.0 ± 0.5	1.9 ± 0.5	2.0 ± 0.5	2.0 ± 0.1	T	0.001	medium
(L/min)	HCLF	1.9 ± 0.3	2.1 ± 0.3*	2.1 ± 0.3*	2.0 ± 0.1	G	0.831	small
	MCHP	1.8 ± 0.3	1.9 ± 0.4	2.0 ± 0.4*	1.9 ± 0.1	T×G	0.049	medium
	LCHP	1.9 ± 0.4	2.0 ± 0.4*	2.1 ± 0.4*	2.0 ± 0.1			
	Mean	1.9 ± 0.4	2.0 ± 0.4*	2.0 ± 0.4*				
VO_{2peak}	NED	26.3 ± 6.0	25.7 ± 5.9	26.2 ± 6.1	26.1 ± 1.2	T	<0.001	large
(ml/kg/min)	HCLF	23.8 ± 4.5	27.4 ± 5.3*	28.0 ± 5.1*	26.4 ± 1.2	G	0.908	<0.01
	MCHP	23.2 ± 5.1	25.6 ± 6.0*	26.9 ± 7.0*	25.2 ± 1.3	T×G	<0.001	large
	LCHP	23.2 ± 4.6	25.9 ± 5.9*	27.6 ± 5.4*†	25.5 ± 1.2			
	Mean	24.1 ± 5.1	26.2 ± 5.7*	27.2 ± 5.8*†				
Time to Exhaustion	NED	555.9 ± 107.3	553.2 ± 94.6	561.7 ± 90.9	556.9 ± 20.2	T	<0.001	large
(seconds)	HCLF	527.6 ± 92.7	557.5 ± 92.9*	573.1 ± 101.7*	552.7 ± 20.2	G	0.785	small
	MCHP	498.2 ± 81.2	532.3 ± 98.0*	559.7 ± 85.8*†	530.0 ± 22.0	T×G	0.034	medium
	LCHP	520.6 ± 88.8	570.9 ± 80.6*	577.5 ± 105.6*	556.3 ± 19.2			
	Mean	526.5 ± 93.6	554.8 ± 90.4*	568.6 ± 95.3*†				

Values are presented as means ± standard deviation. Standard deviation of the mean, SEM. N=75; No exercise or diet, NED (n=19). Higher carbohydrate/lower fat, HCLF (n=19). Moderate carbohydrate/higher protein, MCHP (n=16). Lower carbohydrate/higher protein, LCHP (n=21). Time effect, T. Group effect, G. Time × Group interaction, T×G. Maximal oxygen consumption, VO_{2peak}. *significant time effect from baseline (p<0.05). †significant time effect from 12 weeks (p<0.05). Letter superscripts indicate significance (p<0.05) from post hoc LSD analyses: [a]significantly different from NED (p<0.05), [b]significantly different from HCLF (p<0.05), [c]significantly different from MCHP (p<0.05), [d]significantly different from LCHP (p<0.05). Partial eta-squared (η^2) reported with adherence to the following designations outlined by Cohen: small = 0.01, medium = 0.06, and large = 0.14 [120].

Blood Analysis

Glucose Homeostasis

Table 4.7 displays fasting blood glucose at 0, 12, and 24 weeks. Fasting insulin and calculated HOMA-IR at 0 and 24 weeks are also shown. General linear model with repeated measures on time revealed an overall Wilks' Lambda time effect (p<0.001, η_p^2=0.102) with no T×G interaction (p=0.117, η_p^2=0.057) or group effect (p=0.568, η_p^2=0.036). Univariate analysis revealed a significant time effect for insulin (p<0.001, η_p^2=0.171).

Post hoc tests showed that glucose for MCHP at 24 weeks was significantly less than

baseline and 12-week values (-10.15±4.85, p=0.04 and -8.29±3.60, p=0.02, respectively). Insulin for HCLF (-1.86±0.81, p=0.03) and LCHP (-2.75±0.77, p<0.001) and HOMA-IR for LCHP (-0.73±0.36, p=0.04) at 24 weeks were significantly reduced from their respective baseline values.

Table 4.7: Fasting blood glucose, insulin, and calculated homeostatic model

Variable	Group	0 Weeks	12 Weeks	24 Weeks	Group (SEM)		P-level	η^2
Glucose	NED	96.9 ± 17.8	101.8 ± 34.7	102.5 ± 45.6	100.4 ± 4.2	T	0.781	<0.01
(mg/dL)	HCLF	89.8 ± 13.0	89.0 ± 10.8	93.0 ± 9.8	90.6 ± 4.2	G	0.438	small
	MCHP	99.6 ± 15.0	97.8 ± 16.6	89.5 ± 7.3*†	95.6 ± 4.6	T×G	0.078	medium
	LCHP	97.4 ± 10.2	94.2 ± 10.9	94.4 ± 14.2	95.3 ± 4.0			
	Mean	95.8 ± 14.3	95.6 ± 20.8	95.0 ± 24.8				
Insulin	NED	12.0 ± 7.4	-	11.0 ± 7.2	11.5 ± 1.4	T	<0.001	large
(μIU/mL)	HCLF	10.8 ± 6.1	-	8.9 ± 7.0*	9.9 ± 1.4	G	0.577	small
	MCHP	9.0 ± 6.4	-	8.2 ± 6.2	8.6 ± 1.5	T×G	0.285	small
	LCHP	10.8 ± 5.7	-	8.1 ± 4.6*	9.5 ± 1.3			
	Mean	10.7 ± 6.4	-	9.1 ± 6.3*				
HOMA-IR	NED	3.0 ± 2.5	-	3.2 ± 4.7	3.2 ± 0.5	T	0.050	small
	HCLF	2.4 ± 1.6	-	2.1 ± 1.9	2.3 ± 0.5	G	0.456	small
	MCHP	2.3 ± 1.9	-	1.8 ± 1.4	2.1 ± 0.5	T×G	0.368	small
	LCHP	2.6 ± 1.4	-	1.9 ± 1.1*	2.3 ± 0.5			
	Mean	2.6 ± 1.9	-	2.3 ± 2.7				

Values are presented as means ± standard deviation. SEM, standard deviation of the mean. N=75; No exercise or diet, NED (n=19). Higher carbohydrate/lower fat, HCLF (n=19). Moderate carbohydrate/higher protein, MCHP (n=16). Lower carbohydrate/higher protein, LCHP (n=21). Time effect, T. Group effect, G. Time × Group interaction, T×G. Homeostatic model assessment of insulin resistance, HOMA-IR. *significant time effect from baseline (p<0.05). †significant time effect from 12 weeks (p<0.05). Letter superscripts indicate significance (p<0.05) from post hoc LSD analyses: ^{a}significantly different from NED (p<0.05), ^{b}significantly different from HCLF (p<0.05), ^{c}significantly different from MCHP (p<0.05), dsignificantly different from LCHP (p<0.05). Partial eta-squared (η^2) reported with adherence to the following designations outlined by Cohen: small = 0.01, medium = 0.06, and large = 0.14 [120].

Blood Lipids

Table 4.8 displays fasting blood lipids at 0, 12, and 24 weeks. General linear model with repeated measures on time revealed an overall Wilks' Lambda time effect (p<0.001, η_p^2=0.142), T×G interaction (p=0.058, η_p^2=0.061), and no significant group effect (p=0.209, η_p^2=0.090). Univariate analysis found a significant time effect for total cholesterol (p=0.002, η_p^2=0.085), LDL-cholesterol (p=0.001, η_p^2=0.119), and HDL-cholesterol (p=0.003, η_p^2=0.081). Univariate analysis detected a significant T×G interaction for triglycerides (p=0.024, η_p^2=0.109) and LDL-cholesterol (p=0.004, η_p^2=0.143).

Table 4.8: Fasting blood lipids

Variable	Group	0 Weeks	12 Weeks	24 Weeks	Group (SEM)		P-level	η^2
Triglycerides	NED	120.1 ± 66.2	125.7 ± 64.7	113.8 ± 56.2	119.9 ± 13.1	T	0.061	small
(mg/dl)	HCLF	107.3 ± 49.7	107.7 ± 56.0[a]	111.3 ± 57.5	108.8 ± 12.4	G	0.484	small
	MCHP	153.2 ± 84.0[b]	125.0 ± 64.4[*]	114.3 ± 69.0[*]	130.8 ± 13.6	T×G	0.083	medium
	LCHP	112.0 ± 60.3	97.9 ± 45.1	104.8 ± 47.1	104.9 ± 11.8			
	Mean	121.7 ± 66.1	112.9 ± 57.3	110.7 ± 56.1[*]				
Total	NED	201.8 ± 56.8	191.4 ± 41.4	198.6 ± 53.1	197.3 ± 9.6	T	0.000	medium
Cholesterol	HCLF	209.9 ± 41.8	190.1 ± 37.1[*]	205.3 ± 40.6[†]	201.8 ± 9.1	G	0.603	small
(mg/dl)	MCHP	196.7 ± 45.2	187.1 ± 39.6	179.0 ± 38.8[*]	187.6 ± 9.9	T×G	0.398	small
	LCHP	199.1 ± 46.9	177.6 ± 37.9[*]	185.0 ± 42.7[*]	187.2 ± 8.7			
	Mean	202.0 ± 47.1	186.1 ± 38.5[*]	192.1 ± 44.4[*]				
LDL	NED	126.9 ± 53.7	119.2 ± 39.4	130.9 ± 46.0	125.6 ± 11.6	T	0.001	medium
Cholesterol	HCLF	142.1 ± 45.9	143.6 ± 54.4	133.5 ± 45.0	139.7 ± 10.9	G	0.337	small
(mg/dl)	MCHP	174.9 ± 72.4[a]	168.3 ± 70.3[a]	122.1 ± 57.0[*†]	155.1 ± 11.9	T×G	0.007	medium
	LCHP	147.3 ± 60.6	134.1 ± 51.5[*]	118.4 ± 45.7[*]	133.3 ± 10.4			
	Mean	147.3 ± 59.6	140.6 ± 56.1[*]	126.0 ± 47.7[*†]				
HDL	NED	56.1 ± 19.9	53.0 ± 14.6	56.9 ± 16.7[†]	55.3 ± 3.8	T	0.008	medium
Cholesterol	HCLF	65.1 ± 17.8	56.9 ± 16.6[*]	63.4 ± 17.6[†]	61.8 ± 3.6	G	0.328	small
(mg/dl)	MCHP	55.0 ± 17.7	54.8 ± 15.4	54.9 ± 16.1	54.9 ± 3.9	T×G	0.570	small
	LCHP	63.4 ± 18.1	59.3 ± 14.2	64.1 ± 19.9	62.3 ± 3.4			
	Mean	60.3 ± 18.5	56.2 ± 15.1[*]	60.2 ± 17.8[†]				
Total to HDL	NED	3.8 ± 1.3	3.8 ± 1.0[*]	3.7 ± 1.2	3.8 ± 0.2	T	0.009	medium
Cholesterol	HCLF	3.4 ± 0.9	3.5 ± 0.9	3.4 ± 0.8	3.4 ± 0.2	G	0.303	small
Ratio	MCHP	3.9 ± 1.5	3.6 ± 1.2	3.5 ± 1.1[*]	3.7 ± 0.3	T×G	0.257	small
	LCHP	3.3 ± 1.2	3.1 ± 0.9[a]	3.1 ± 0.9[*]	3.2 ± 0.2			
	Mean	3.6 ± 1.2	3.5 ± 1.0[*]	3.4 ± 1.0[*†]				

Values are presented as means ± standard deviation. Standard deviation of the mean, SEM. N=73; No exercise or diet, NED (n=17). Higher carbohydrate/lower fat, HCLF (n=19). Moderate carbohydrate/higher protein, MCHP (n=16). Lower carbohydrate/higher protein, LCHP (n=21). Time effect, T. Group effect, G. Time × Group interaction, T×G. Low density lipoprotein, LDL. High density lipoprotein, HDL. [*]significant time effect from baseline ($p<0.05$). [†]significant time effect from 12 weeks ($p<0.05$). Letter superscripts indicate significance ($p<0.05$) from post hoc LSD analyses: [a]significantly different from NED ($p<0.05$), [b]significantly different from HCLF ($p<0.05$), [c]significantly different from MCHP ($p<0.05$), [d]significantly different from LCHP ($p<0.05$). Partial eta-squared (η_p^2) reported with adherence to the following designations outlined by Cohen: small = 0.01, medium = 0.06, and large = 0.14 [120]. Two samples from NED were removed prior to running GLM due to the presence of outliers.

Post hoc analysis showed that both higher protein groups significantly improved in total cholesterol, LDL-cholesterol, and total to HDL cholesterol ratio. At 24 weeks, total cholesterol for MCHP and LCHP (-17.74±7.77 mg/dL, p=0.025 and -14.16±6.78 mg/dL, p=0.04), LDL-cholesterol for MCHP and LCHP (-52.78 ±12.26 mg/dL, p<0.001 and -28.93 ±10.70 mg/dL, p=0.009, respectively), and total to HDL cholesterol ratio for MCHP and LCHP (-0.44±0.15, p=0.004 and -0.29±0.13, p=0.024, respectively) were significantly reduced from their respective baseline values. Post hoc analysis showed that the total to HDL cholesterol ratio for LCHP was significantly different from NED (-0.73; 95% CI, -1.36, -0.10; p=0.02) at 24 weeks. Figures 4.6 and 4.7 indicate a significant change in LDL cholesterol at 24 weeks for LCHP and a significant change in the total to HDL cholesterol ratio for MCHP and LCHP at 24 weeks.

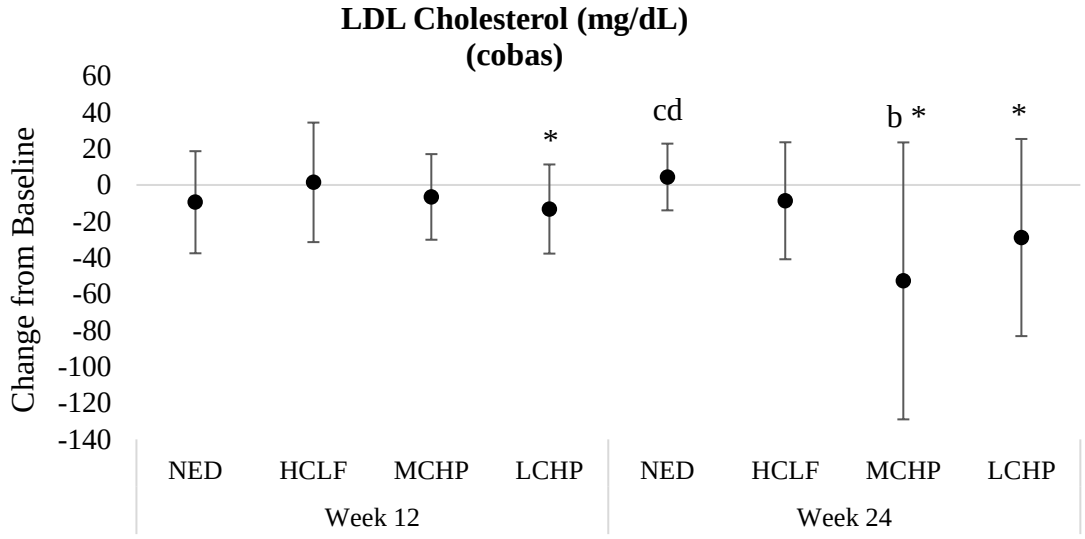

Figure 4.6: Change from baseline for low-density lipoprotein (LDL) cholesterol (mg/dL). Data presented as mean change ± standard deviation from baseline at 12 and 24 weeks. N=75; no exercise or diet, NED (N=19), higher carbohydrate/low fat, HCLF (N=19), moderate carbohydrate/higher protein, MCHP (N=16), and lower carbohydrate/higher protein, LCHP (N=21).*significant percent change, $p<0.05$. [†]significantly different from 12 weeks, $p<0.05$. Letter superscripts indicate significance ($p<0.05$) from post hoc LSD. [a]significantly different from NED, [b]significantly different from HCLF, [c]significantly different from MCHP, [d]significantly different from LCHP.

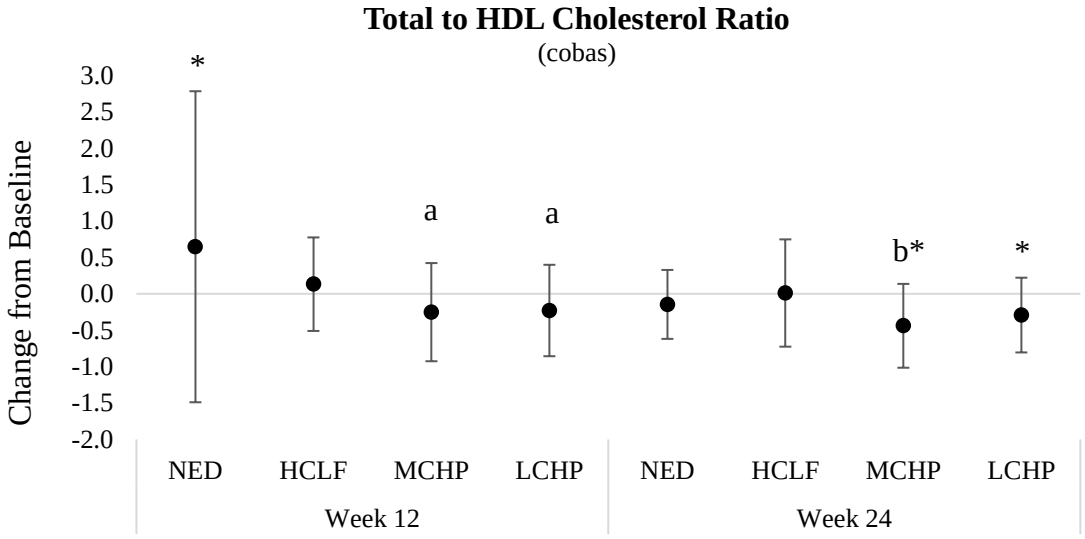

Figure 4.7: Change from baseline for total- to HDL-cholesterol ratio. Data presented as mean change ± standard deviation from baseline at 12 and 24 weeks. N=75; no exercise or diet, NED (N=19), higher carbohydrate/low fat, HCLF (N=19), moderate carbohydrate/higher protein, MCHP (N=16), and lower carbohydrate/higher protein, LCHP (N=21).*significant percent change, $p<0.05$. [†]significantly different from 12 weeks, $p<0.05$. Letter superscripts indicate significance ($p<0.05$) from post hoc LSD. [a]significantly different from NED, [b]significantly different from HCLF, [c]significantly different from MCHP, [d]significantly different from LCHP.

Lipoprotein Analysis

Table 4.9 displays Total, TRL, LDL, and HDL expressed as AUC. General linear model with repeated measures on time revealed a significant overall Wilks' Lambda time effect (p=0.003, η_p^2=0.158), no T×G interaction (p=0.577, η_p^2=0.080), and a significant group effect (p=0.011, η_p^2=0.257). Univariate analysis detected a significant time effect for LDL1 AUC (p=0.001, η_p^2=0.103); a T×G interaction for HDL3b AUC (p=0.025, η_p^2=0.096); and significant group effects for LDL (p=0.038, η_p^2=0.111), LDL4 AUC (p=0.021, η_p^2=0.128) and HDL3c AUC (p=0.001, η_p^2=0.196).

Table 4.10 displays TRL, LDL, and HDL expressed as percent of total AUC. General linear model with repeated measures on time revealed a significant overall Wilks' Lambda time effect (p<0.001, η_p^2=0.177), no T×G interaction (p=0.942, η_p^2=0.057), and a significant group effect (p=0.010, η_p^2=0.240). Univariate analysis detected a significant time effect for LDL1 as percent of total AUC (p<0.001, η_p^2=0.156) and significant group effects for LDL2 as percent of total AUC (p=0.037, η_p^2=0.112) and LDL4 as percent of total AUC (p=0.049, η_p^2=0.104).

Post hoc analysis indicated that LDL1 as percent of total AUC for NED (+0.657±0.204, p=0.002), HCLF (+0.502±0.204, p=0.016), and LCHP (+0.515±0.194, p=0.010) were significantly increased at 24 weeks compared to their respective baseline values while MCHP was not significantly different from baseline (+0.349±0.223, p=0.122). In Figure 4.8, the percent change for LDL as percent of total AUC was significant for MCHP and LCHP at 24 weeks while in Figure 4.9, the percent change for HDL as percent of total AUC was significant for NED and HCLF at 24 weeks. Figure 4.10 indicates that percent change for LDL1 as percent of total AUC for NED and LCHP was significant at 24 weeks. Figure 4.11 indicates that LDL2 as percent of total AUC for all diet and exercise groups were greater than NED while only MCHP was

significantly greater than NED (+0.42; 95% CI, 0.019, 0.819; p=0.040) at baseline. At 12 weeks, LDL2 as percent of total AUC for all diet and exercise groups was significantly greater than NED, but at 24 weeks, only that of LCHP (+0.53; 95% CI, 0.05, 1.01; p=0.03) was significantly greater than NED. Figure 4.12 considers the percent change for LDL2 as percent of total AUC and indicates that HCLF had a significant percent change at 24 weeks. In Figure 4.13, post hoc analysis showed that significant group differences occurred for LDL4 as percent of total AUC, and similar to LDL2, group differences were present at baseline where MCHP was significantly lower than HCLF (-4.342; 95% CI, -7.243, -1.441; p=0.004) and LCHP (-3.391; 95% CI, -6.228, -0.554; p=0.020). At 24 weeks, LDL4 as percent of total AUC for MCHP was significantly lower than HCLF (-3.655; 95% CI, -6.418, -0.892; p=0.01); however, as shown in Figure 4.14, percent change for LDL4 as percent of total AUC was not significant for any group.

Table 4.10 displays LDL and HDL expressed as percent of total LDL and HDL AUC, respectively. General linear model with repeated measures on time revealed a Wilks' Lambda time effect ($p<0.001$, $\eta_p^2=0.161$) with no T×G interaction ($p=0.971$, $\eta_p^2=0.036$), and a significant group effect ($p=0.004$, $\eta_p^2=0.202$). Univariate analysis showed that there was a significant time effect for LDL1 as percent of total LDL AUC ($p<0.001$, $\eta_p^2=0.128$) and HDL3a as percent of total HDL AUC ($p=0.03$, $\eta_p^2=0.053$). Univariate analysis detected a group effect for LDL2 as percent of total LDL AUC ($p=0.024$, $\eta_p^2=0.123$) and HDL3a as percent of total HDL AUC ($p=0.016$, $\eta_p^2=0.134$).

Table 4.9: Total, TRL, LDL, HDL, and their respective subfraction areas expressed as area under the density profile curve (AUC)

Variable	Group	0 Weeks	12 Weeks	24 Weeks	Group (SEM)		P-level	η^2
Total	NED	20671.1 ± 7288.9	20907.7 ± 6644.4	20601.4 ± 7132.1	20726.7 ± 1472.1	T	0.879	<0.01
AUC	HCLF	17671.0 ± 8486.6	17511.2 ± 8667.2	16125.8 ± 7515.9[a]	17102.7 ± 1472.1	G	0.064	medium
	MCHP	14898.8 ± 5413.2[ad]	16007.1 ± 7110.2[a]	15267.7 ± 6031.4[ad]	15391.2 ± 1604.2[ad]	T×G	0.303	small
	LCHP	19873.0 ± 6087.3	18785.8 ± 4842.0	20326.0 ± 5992.5	19661.6 ± 1400.2			
	Mean	18456.2 ± 7149.9	18407.7 ± 6978.0	18252.6 ± 6994.1				
TRL	NED	1359.6 ± 648.3	1691.1 ± 1309.5[*]	1344.2 ± 708.9	1464.9 ± 173.5	T	0.299	small
AUC	HCLF	1153.5 ± 684.4	1285.7 ± 831.0	1259.8 ± 1103.9	1233.0 ± 173.5	G	0.618	small
	MCHP	1227.2 ± 825.4	1293.0 ± 772.9	1185.1 ± 654.3	1235.1 ± 189.1	T×G	0.386	small
	LCHP	1381.5 ± 768.3	1400.5 ± 779.9	1633.4 ± 1080.1	1471.8 ± 165.1			
	Mean	1285.3 ± 723.2	1422.1 ± 947.4	1369.9 ± 922.6				
Total LDL	NED	8443.2 ± 3505.1	8503.1 ± 3099.5	8836.1 ± 3614.5	8594.1 ± 7266.2	T	0.857	<0.01
AUC	HCLF	7607.6 ± 3859.9	7407.4 ± 3589.0	6657.6 ± 2971.9[*a]	7224.2 ± 5896.3	G	0.038	medium
	MCHP	5798.4 ± 2528.2[ad]	6216.9 ± 2736.5[a]	5803.3 ± 2174.0[ad]	5939.5 ± 4492.5[ad]	T×G	0.185	small
	LCHP	8457.5 ± 3172.9	7847.7 ± 2350.6	8506.6 ± 3167.9	8270.6 ± 7007.5			
	Mean	7671.3 ± 3423.0	7554.3 ± 3021.5	7544.9 ± 3247.3				
LDL1	NED	370.3 ± 206.4	497.1 ± 344.4[*]	506.4 ± 361.5[*]	458.0 ± 47.5	T	0.001	medium
AUC	HCLF	287.0 ± 119.0	411.7 ± 262.4[*]	369.9 ± 259.9	356.2 ± 47.5	G	0.258	small
	MCHP	303.9 ± 164.2	370.2 ± 205.5	360.9 ± 173.6	345.0 ± 51.8	T×G	0.863	small
	LCHP	368.2 ± 200.2	444.5 ± 233.6	497.4 ± 325.3[*]	436.7 ± 45.2			
	Mean	334.4 ± 177.6	433.7 ± 266.4[*]	438.3 ± 296.0[*]				
LDL2	NED	500.4 ± 202.6	507.8 ± 196.3	503.2 ± 205.0	503.8 ± 40.3	T	0.388	small
AUC	HCLF	451.3 ± 213.8	480.9 ± 206.5	410.4 ± 167.6	447.5 ± 40.3	G	0.084	medium
	MCHP	408.9 ± 133.8[d]	467.2 ± 184.6	410.3 ± 117.4	428.8 ± 44.0	T×G	0.241	small
	LCHP	554.9 ± 221.2	544.9 ± 178.8	593.1 ± 259.3[bc]	564.3 ± 38.4[bc]			
	Mean	483.7 ± 202.4	502.7 ± 190.3	485.1 ± 209.9				
LDL3	NED	1329.3 ± 617.0	1303.9 ± 524.6	1327.3 ± 618.0	1320.2 ± 111.7	T	0.217	small
AUC	HCLF	1183.9 ± 584.8	1152.3 ± 567.4	996.0 ± 430.7[*†ad]	1110.7 ± 111.7	G	0.076	medium
	MCHP	942.6 ± 356.3[d]	1054.2 ± 459.1	943.4 ± 372.4[ad]	980.0 ± 121.7[ad]	T×G	0.259	small
	LCHP	1424.4 ± 662.4	1300.4 ± 455.4	1336.3 ± 512.5	1353.7 ± 106.2			
	Mean	1236.6 ± 593.1	1211.3 ± 504.7	1164.0 ± 519.8				
LDL4	NED	3509.0 ± 1770.5	3584.3 ± 1543.4	3699.0 ± 1773.9	3597.4 ± 315.2	T	0.646	<0.01
AUC	HCLF	3359.0 ± 1761.8	3180.7 ± 1689.2	2929.2 ± 1421.6	3156.3 ± 315.2	G	0.021	medium
	MCHP	2167.3 ± 892.2[abd]	2370.9 ± 1034.7[ad]	2204.3 ± 841.7[ad]	2247.5 ± 343.5[ad]	T×G	0.441	small
	LCHP	3731.5 ± 1741.3	3355.0 ± 1524.5	3467.6 ± 1408.8	3518.0 ± 299.8			
	Mean	3247.1 ± 1684.6	3159.0 ± 1520.0	3120.3 ± 1499.4				
LDL5	NED	2734.1 ± 1264.0	2609.9 ± 1051.3	2800.1 ± 1501.0	2714.7 ± 257.8	T	0.413	small
AUC	HCLF	2326.4 ± 1635.1	2181.8 ± 1287.3	1952.1 ± 1018.3[a]	2153.4 ± 257.8	G	0.204	medium
	MCHP	1975.7 ± 1371.7	1954.4 ± 1209.7	1884.4 ± 1015.4[a]	1938.2 ± 280.9[a]	T×G	0.133	medium
	LCHP	2378.5 ± 865.3	2202.9 ± 727.5	2612.2 ± 1294.5[†]	2397.9 ± 245.2			
	Mean	2369.5 ± 1300.3	2247.7 ± 1079.6	2337.3 ± 1273.2				

Values are presented as means ± standard deviation. Standard deviation of the mean, SEM. N=75; No exercise or diet, NED (n=19). Higher carbohydrate/lower fat, HCLF (n=19). Moderate carbohydrate/higher protein, MCHP (n=16). Lower carbohydrate/higher protein, LCHP (n=21). Time effect, T. Group effect, G. Time × Group interaction, T×G. Area under the density profile curve, AUC. Triglyceride rich lipoproteins, TRL. Low density lipoprotein, LDL. High density lipoprotein, HDL. [*]significant time effect from baseline ($p<0.05$). [†]significant time effect from 12 weeks ($p<0.05$). Letter superscripts indicate significance ($p<0.05$) from post hoc LSD analyses: [a]significantly different from NED ($p<0.05$), [b]significantly different from HCLF ($p<0.05$), [c]significantly different from MCHP ($p<0.05$), [d]significantly different from LCHP ($p<0.05$). Partial eta-squared (η^2) reported with adherence to the following designations outlined by Cohen: small = 0.01, medium = 0.06, and large = 0.14 [120].

Table 4.9: Continued...

Variable	Group	0 Weeks	12 Weeks	24 Weeks	Group (SEM)		P-level	η^2
Total HDL	NED	10906.6 ± 4221.8	10755.2 ± 3753.5	10492.6 ± 3601.2	10718.1 ± 813.5	T	0.826	<0.01
AUC	HCLF	8909.9 ± 4437.7	8818.1 ± 4582.7	8208.4 ± 4090.5	8645.5 ± 813.5	G	0.137	medium
	MCHP	7873.3 ± 3149.8	8497.2 ± 4217.5	8279.2 ± 3979.6	8216.6 ± 886.5[a]	T×G	0.370	small
	LCHP	10034.0 ± 3083.8	9537.7 ± 2850.7	10186.0 ± 2663.6	9919.2 ± 773.8			
	Mean	9509.3 ± 3867.5	9441.9 ± 3882.7	9355.9 ± 3669.9				
HDL2b	NED	2870.9 ± 1573.1	2787.6 ± 1286.4	2777.9 ± 1303.9	2812.1 ± 295.8	T	0.913	<0.01
AUC	HCLF	2237.7 ± 1458.6	2179.9 ± 1566.5	2125.5 ± 1536.3	2181.0 ± 295.8	G	0.213	medium
	MCHP	1991.6 ± 1017.6	2263.1 ± 1583.5	2190.7 ± 1359.7	2148.5 ± 322.3	T×G	0.813	small
	LCHP	2833.4 ± 1331.3	2785.1 ± 1226.8	2783.0 ± 1001.2	2800.5 ± 281.3			
	Mean	2512.4 ± 1395.9	2521.0 ± 1413.1	2488.8 ± 1316.2				
HDL2a	NED	2092.1 ± 1017.6	2039.8 ± 841.1	2014.9 ± 905.6	2049.0 ± 197.4	T	0.970	<0.01
AUC	HCLF	1747.4 ± 1044.2	1709.3 ± 1123.6	1629.0 ± 1011.9	1695.2 ± 197.4	G	0.405	small
	MCHP	1505.8 ± 745.3	1685.1 ± 1033.9	1623.0 ± 933.7	1604.6 ± 215.1	T×G	0.584	small
	LCHP	1925.3 ± 839.5	1837.4 ± 718.7	1955.9 ± 611.5	1906.2 ± 187.7			
	Mean	1833.0 ± 930.0	1823.7 ± 924.7	1817.0 ± 871.3				
HDL3a	NED	2617.8 ± 882.8	2603.0 ± 841.2	2481.0 ± 776.3[*]	2567.2 ± 181.9	T	0.321	small
AUC	HCLF	2263.1 ± 1070.2	2187.9 ± 1019.1	2016.2 ± 873.7	2155.8 ± 181.9	G	0.245	small
	MCHP	2027.4 ± 754.6[a]	2120.9 ± 874.3	2026.0 ± 893.7	2058.1 ± 198.2	T×G	0.158	medium
	LCHP	2362.3 ± 717.7	2184.3 ± 639.5	2397.8 ± 721.1[†]	2314.8 ± 173.0			
	Mean	2330.5 ± 874.8	2277.8 ± 852.8	2242.9 ± 824.3				
HDL3b	NED	2281.6 ± 678.3	2270.6 ± 704.6	2177.0 ± 593.7	2243.1 ± 142.5	T	0.529	<0.01
AUC	HCLF	1901.7 ± 837.4	1928.3 ± 833.4	1710.7 ± 686.8[*†a]	1846.9 ± 142.5	G	0.072	medium
	MCHP	1680.4 ± 533.1[a]	1736.5 ± 599.9[a]	1710.3 ± 598.9[a]	1709.1 ± 155.2[a]	T×G	0.025	medium
	LCHP	2026.2 ± 587.9	1891.7 ± 498.9	2099.1 ± 635.0[†]	2005.7 ± 135.5			
	Mean	1985.6 ± 691.7	1963.8 ± 683.4	1937.5 ± 654.7				
HDL3c	NED	1044.0 ± 335.1	1054.2 ± 366.0	1041.8 ± 333.6	1046.7 ± 62.3	T	0.605	<0.01
AUC	HCLF	760.0 ± 284.7[a]	812.7 ± 350.8[a]	727.0 ± 276.8[†ad]	766.6 ± 62.3[a]	G	0.001	large
	MCHP	668.2 ± 242.0[ad]	691.7 ± 292.4[a]	729.3 ± 267.3[ad]	696.4 ± 67.9[ad]	T×G	0.079	medium
	LCHP	886.8 ± 238.0	839.1 ± 176.3[a]	950.2 ± 306.5[†bc]	892.0 ± 59.3			
	Mean	847.9 ± 305.8	855.5 ± 323.6	869.7 ± 323.3				

Values are presented as means ± standard deviation. Standard deviation of the mean, SEM. N=75; No exercise or diet, NED (n=19). Higher carbohydrate/lower fat, HCLF (n=19). Moderate carbohydrate/higher protein, MCHP (n=16). Lower carbohydrate/higher protein, LCHP (n=21). Time effect, T. Group effect, G. Time × Group interaction, T×G. Area under the density profile curve, AUC. Triglyceride rich lipoproteins, TRL. Low density lipoprotein, LDL. High density lipoprotein, HDL. [*]significant time effect from baseline ($p<0.05$). [†]significant time effect from 12 weeks ($p<0.05$). Letter superscripts indicate significance ($p<0.05$) from post hoc LSD analyses: [a]significantly different from NED ($p<0.05$), [b]significantly different from HCLF ($p<0.05$), [c]significantly different from MCHP ($p<0.05$), [d]significantly different from LCHP ($p<0.05$). Partial eta-squared (η^2) reported with adherence to the following designations outlined by Cohen: small = 0.01, medium = 0.06, and large = 0.14 [120].

Table 4.10: TRL, LDL, HDL, and their respective subfraction areas expressed as percentage of total area under the density profile curve (AUC)

Variable	Group	0 Weeks	12 Weeks	24 Weeks	Group (SEM)		P-level	η^2
TRL:Total AUC (%)	NED	6.8 ± 2.5	8.0 ± 5.1	6.5 ± 2.2	7.1 ± 0.6	T	0.222	small
	HCLF	6.6 ± 2.6	7.2 ± 2.0	7.2 ± 3.3	7.0 ± 0.6	G	0.711	small
	MCHP	8.1 ± 3.2	8.0 ± 2.7	7.9 ± 3.2	8.0 ± 0.7	T×G	0.506	small
	LCHP	6.9 ± 3.1	7.7 ± 3.9	7.9 ± 3.7	7.5 ± 0.6			
	Mean	7.1 ± 2.8	7.7 ± 3.6	7.4 ± 3.2				
LDL:Total AUC (%)	NED	40.5 ± 7.1	40.7 ± 6.2	42.4 ± 5.6*†	41.2 ± 1.3	T	0.988	<0.01
	HCLF	42.8 ± 5.6	42.5 ± 5.2	41.9 ± 5.6	42.4 ± 1.3	G	0.356	small
	MCHP	38.9 ± 7.5	39.2 ± 7.1	38.7 ± 7.2	39.0 ± 1.5	T×G	0.212	small
	LCHP	42.0 ± 6.0	41.7 ± 6.0	41.2 ± 5.7	41.7 ± 1.3			
	Mean	41.2 ± 6.6	41.1 ± 6.1	41.2 ± 6.0				
LDL1:Total AUC (%)	NED	1.8 ± 0.9	2.3 ± 1.3*	2.5 ± 1.7*	2.2 ± 0.2	T	<0.001	large
	HCLF	1.7 ± 0.4	2.4 ± 0.7*	2.2 ± 0.7*	2.1 ± 0.2	G	0.937	<0.01
	MCHP	2.1 ± 0.9	2.3 ± 0.9	2.5 ± 0.9	2.3 ± 0.2	T×G	0.795	small
	LCHP	1.9 ± 0.7	2.4 ± 1.2*	2.4 ± 1.0*	2.2 ± 0.2			
	Mean	1.9 ± 0.8	2.4 ± 1.0*	2.4 ± 1.1*				
LDL2:Total AUC (%)	NED	2.4 ± 0.6	2.4 ± 0.5	2.4 ± 0.5	2.4 ± 0.1	T	0.148	small
	HCLF	2.7 ± 0.6	2.9 ± 0.5^{a}	2.7 ± 0.5	2.7 ± 0.1	G	0.037	medium
	MCHP	2.8 ± 0.6^{a}	3.0 ± 0.6^{a}	2.8 ± 0.6	2.9 ± 0.1^{a}	T×G	0.731	small
	LCHP	2.8 ± 0.7	3.0 ± 0.9^{a}	3.0 ± 1.2^{a}	2.9 ± 0.1^{a}			
	Mean	2.7 ± 0.6	2.8 ± 0.7*	2.7 ± 0.8				
LDL3:Total AUC (%)	NED	6.3 ± 1.3	6.2 ± 1.6	6.3 ± 1.3	6.3 ± 0.3	T	0.104	small
	HCLF	6.8 ± 1.6	6.8 ± 1.6	6.4 ± 1.3	6.6 ± 0.3	G	0.571	small
	MCHP	6.4 ± 1.3	6.7 ± 1.0	6.2 ± 1.2	6.4 ± 0.3	T×G	0.778	small
	LCHP	7.0 ± 1.8	6.9 ± 1.7	6.6 ± 1.9	6.8 ± 0.3			
	Mean	6.6 ± 1.5	6.6 ± 1.5	6.4 ± 1.5†				
LDL4:Total AUC (%)	NED	16.6 ± 4.4	17.3 ± 4.6	17.5 ± 4.0	17.1 ± 0.9	T	0.860	<0.01
	HCLF	19.1 ± 4.4	18.1 ± 4.1	18.5 ± 4.5	18.6 ± 0.9	G	0.049	medium
	MCHP	14.7 ± 4.0^{bd}	15.2 ± 3.5	14.9 ± 3.8^{b}	14.9 ± 1.0^{bd}	T×G	0.360	small
	LCHP	18.1 ± 4.4	17.5 ± 4.9	16.8 ± 4.0	17.4 ± 0.8			
	Mean	17.2 ± 4.5	17.1 ± 4.4	17.0 ± 4.2				
LDL5:Total AUC (%)	NED	13.4 ± 4.1	12.5 ± 3.0	13.7 ± 4.7†	13.2 ± 0.9	T	0.106	small
	HCLF	12.6 ± 4.3	12.4 ± 4.1	12.1 ± 2.7	12.4 ± 0.9	G	0.868	small
	MCHP	12.9 ± 5.1	12.1 ± 4.8	12.3 ± 4.2	12.4 ± 0.9	T×G	0.640	small
	LCHP	12.3 ± 3.6	11.9 ± 3.4	12.5 ± 3.9	12.3 ± 0.8			
	Mean	12.8 ± 4.2	12.2 ± 3.7*	12.7 ± 3.9				

Values are presented as means ± standard deviation. Standard deviation of the mean, SEM. N=75; No exercise or diet, NED (n=19). Higher carbohydrate/lower fat, HCLF (n=19). Moderate carbohydrate/higher protein, MCHP (n=16). Lower carbohydrate/higher protein, LCHP (n=21). Time effect, T. Group effect, G. Time × Group interaction, T×G. Area under the density profile curve, AUC. Triglyceride rich lipoproteins, TRL. Low density lipoprotein, LDL. High density lipoprotein, HDL. *significant time effect from baseline ($p<0.05$). †significant time effect from 12 weeks ($p<0.05$). Letter superscripts indicate significance ($p<0.05$) from post hoc LSD analyses: ^{a}significantly different from NED ($p<0.05$), ^{b}significantly different from HCLF ($p<0.05$), ^{c}significantly different from MCHP ($p<0.05$), ^{d}significantly different from LCHP ($p<0.05$). Partial eta-squared (η^2) reported with adherence to the following designations outlined by Cohen: small = 0.01, medium = 0.06, and large = 0.14 [120].

Table 4.10: Continued...

Variable	Group	0 Weeks	12 Weeks	24 Weeks	Group (SEM)	P-level		η^2
HDL:Total AUC	NED	52.9 ± 8.2	51.5 ± 6.7	51.5 ± 7.1	51.9 ± 1.6	T	0.406	small
(%)	HCLF	50.5 ± 5.4	50.3 ± 5.3	50.9 ± 6.1	50.6 ± 1.6	G	0.702	small
	MCHP	52.9 ± 8.8	52.8 ± 8.1	53.3 ± 8.2	53.0 ± 1.7	T×G	0.796	small
	LCHP	51.0 ± 7.5	50.6 ± 6.9	50.9 ± 7.3	50.9 ± 1.5			
	Mean	51.8 ± 7.5	51.2 ± 6.7	51.6 ± 7.1				
HDL2b:Total AUC	NED	13.3 ± 3.8	13.0 ± 3.1	13.2 ± 3.2	13.2 ± 0.8	T	0.702	<0.01
(%)	HCLF	12.3 ± 3.1	11.9 ± 2.9	12.6 ± 3.8	12.2 ± 0.8	G	0.350	small
	MCHP	13.0 ± 3.0	13.3 ± 3.5	13.6 ± 3.5	13.3 ± 0.9	T×G	0.579	small
	LCHP	14.2 ± 4.5	14.4 ± 4.3[b]	14.0 ± 4.4	14.2 ± 0.8			
	Mean	13.2 ± 3.7	13.2 ± 3.6	13.3 ± 3.7				
HDL2a:Total AUC	NED	9.9 ± 2.4	9.8 ± 2.2	9.8 ± 2.4	9.8 ± 0.6	T	0.576	<0.01
(%)	HCLF	9.8 ± 2.5	9.5 ± 2.4	9.9 ± 2.8	9.7 ± 0.6	G	0.937	<0.01
	MCHP	10.1 ± 2.8	10.2 ± 2.7	10.3 ± 2.7	10.2 ± 0.6	T×G	0.885	small
	LCHP	9.7 ± 3.0	9.6 ± 2.5	9.8 ± 2.4	9.7 ± 0.5			
	Mean	9.9 ± 2.7	9.8 ± 2.4	9.9 ± 2.5				
HDL3a:Total AUC	NED	13.0 ± 2.0	12.7 ± 1.9	12.4 ± 2.0[*]	12.7 ± 0.4	T	0.062	small
(%)	HCLF	12.9 ± 1.7	12.7 ± 1.6	12.7 ± 1.6	12.8 ± 0.4	G	0.066	medium
	MCHP	13.8 ± 2.5	13.5 ± 2.2	13.2 ± 2.0	13.5 ± 0.5[c]	T×G	0.834	small
	LCHP	12.0 ± 1.8[c]	11.7 ± 2.0[c]	11.9 ± 2.0[c]	11.9 ± 0.4			
	Mean	12.9 ± 2.1	12.6 ± 2.0	12.5 ± 1.9[*]				
HDL3b:Total AUC	NED	11.5 ± 2.1	11.0 ± 1.5	11.0 ± 1.7	11.1 ± 0.4	T	0.434	small
(%)	HCLF	11.0 ± 1.4	11.4 ± 1.6	10.9 ± 1.7	11.1 ± 0.4	G	0.202	medium
	MCHP	11.5 ± 1.6	11.3 ± 1.9	11.4 ± 1.3	11.4 ± 0.4[c]	T×G	0.316	small
	LCHP	10.4 ± 1.9	10.2 ± 1.8[b]	10.4 ± 1.9	10.4 ± 0.3			
	Mean	11.1 ± 1.8	10.9 ± 1.7	10.9 ± 1.7				
HDL3c:Total AUC	NED	5.2 ± 1.3	5.0 ± 0.9	5.2 ± 1.2	5.2 ± 0.2	T	0.320	small
(%)	HCLF	4.6 ± 0.9	4.9 ± 1.0	4.7 ± 1.0	4.7 ± 0.2	G	0.231	small
	MCHP	4.6 ± 1.0	4.5 ± 1.1	4.9 ± 0.7†	4.6 ± 0.2	T×G	0.336	small
	LCHP	4.7 ± 1.1	4.6 ± 0.8	4.7 ± 0.9	4.7 ± 0.2			
	Mean	4.8 ± 1.1	4.8 ± 1.0	4.9 ± 1.0				

Values are presented as means ± standard deviation. Standard deviation of the mean, SEM. N=75; No exercise or diet, NED (n=19). Higher carbohydrate/lower fat, HCLF (n=19). Moderate carbohydrate/higher protein, MCHP (n=16). Lower carbohydrate/higher protein, LCHP (n=21). Time effect, T. Group effect, G. Time × Group interaction, T×G. Area under the density profile curve, AUC. Triglyceride rich lipoproteins, TRL. Low density lipoprotein, LDL. High density lipoprotein, HDL. [*]significant time effect from baseline ($p<0.05$). [†]significant time effect from 12 weeks ($p<0.05$). Letter superscripts indicate significance ($p<0.05$) from post hoc LSD analyses: [a]significantly different from NED ($p<0.05$), [b]significantly different from HCLF ($p<0.05$), [c]significantly different from MCHP ($p<0.05$), [d]significantly different from LCHP ($p<0.05$). Partial eta-squared (η^2) reported with adherence to the following designations outlined by Cohen: small = 0.01, medium = 0.06, and large = 0.14 [120].

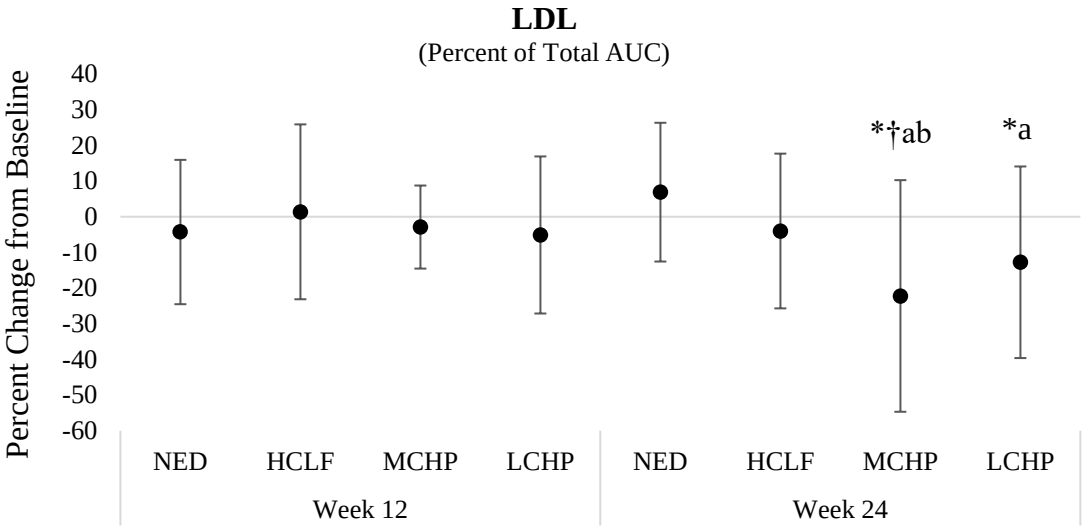

Figure 4.8: Percent change from baseline for low-density lipoprotein (LDL) as percent of total area under the density profile curve (AUC). Data presented as mean change ± standard deviation from baseline at 12 and 24 weeks. N=75; no exercise or diet, NED (N=19), higher carbohydrate/low fat, HCLF (N=19), moderate carbohydrate/higher protein, MCHP (N=16), and lower carbohydrate/higher protein, LCHP (N=21). *significant percent change, $p<0.05$. †significantly different from 12 weeks, $p<0.05$. Letter superscripts indicate significance ($p<0.05$) from post hoc LSD. [a]significantly different from NED, [b]significantly different from HCLF, [c]significantly different from MCHP, [d]significantly different from LCHP.

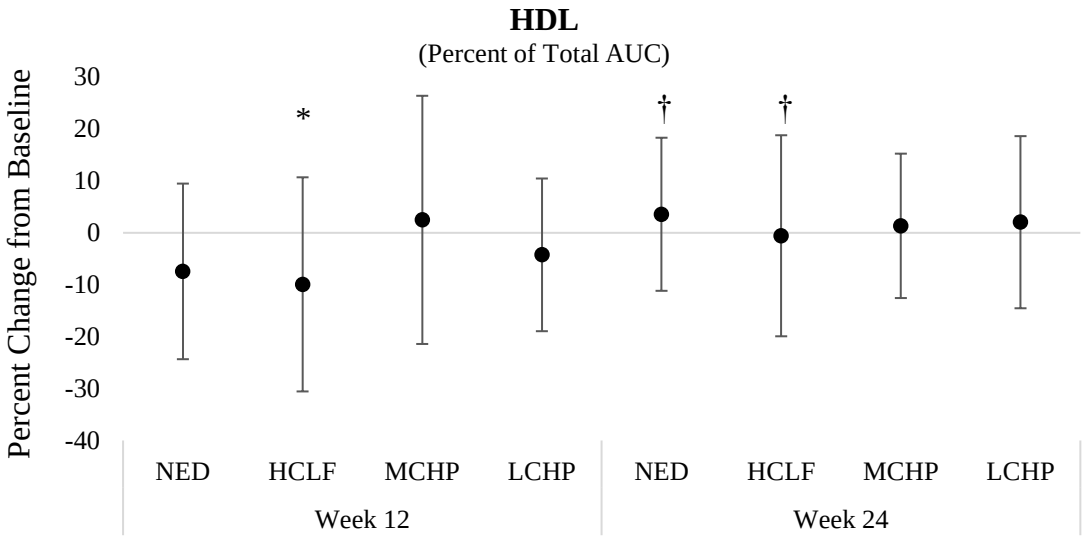

Figure 4.9: Percent change from baseline for high-density lipoprotein (HDL) as percent of total area under the density profile curve (AUC). Data presented as mean change ± standard deviation from baseline at 12 and 24 weeks. N=75; no exercise or diet, NED (N=19), higher carbohydrate/low fat, HCLF (N=19), moderate carbohydrate/higher protein, MCHP (N=16), and lower carbohydrate/higher protein, LCHP (N=21).*significant percent change, $p<0.05$. †significantly different from 12 weeks, $p<0.05$.

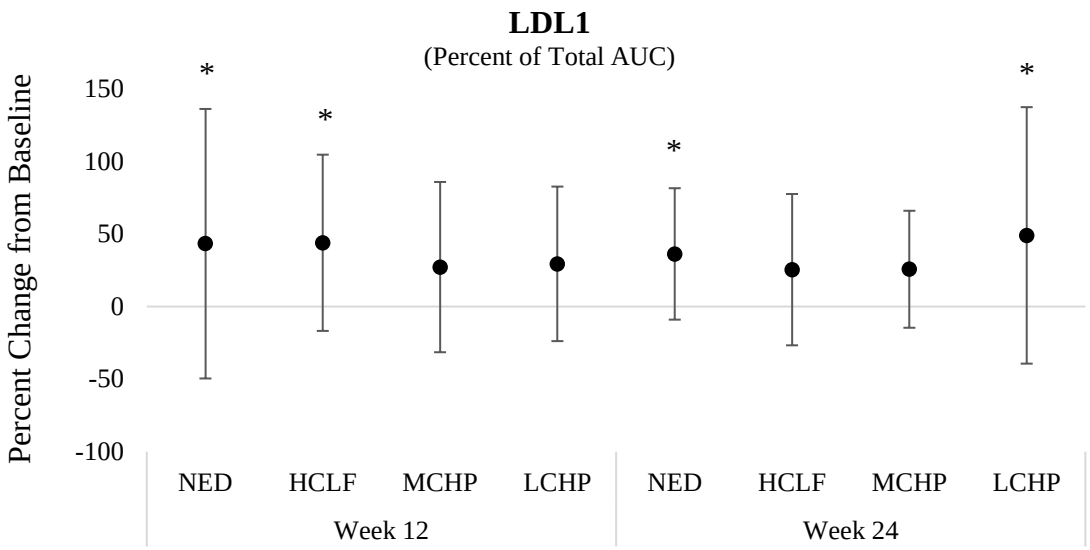

Figure 4.10: Percent change from baseline for low-density lipoprotein 1 (LDL1) as percent of total area under the density profile curve (AUC). Data presented as mean change ± standard deviation from baseline at 12 and 24 weeks. N=75; no exercise or diet, NED (N=19), higher carbohydrate/low fat, HCLF (N=19), moderate carbohydrate/higher protein, MCHP (N=16), and lower carbohydrate/higher protein, LCHP (N=21).*significant percent change, $p<0.05$. †significantly different from 12 weeks, $p<0.05$.

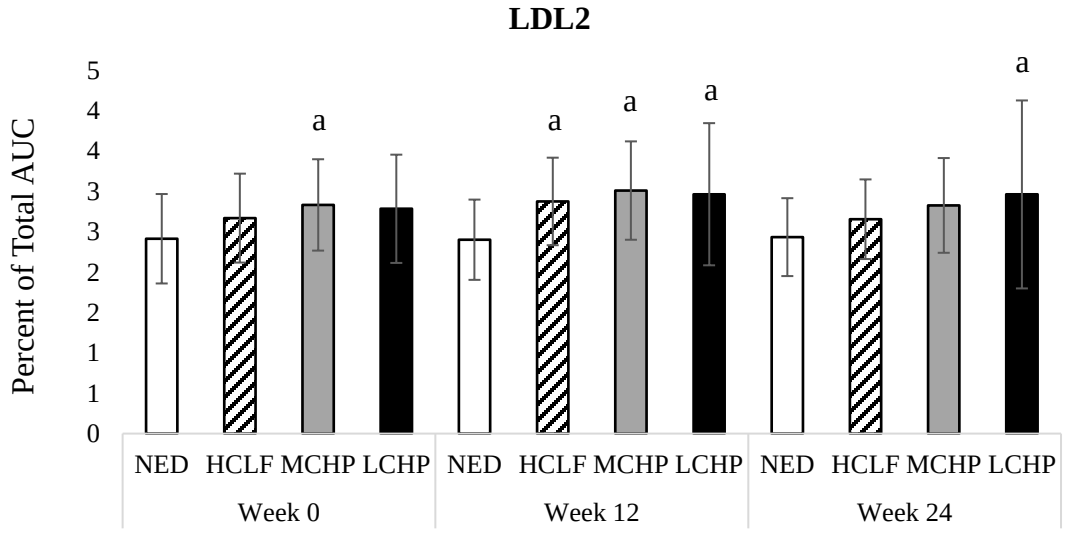

Figure 4.11: Low-density lipoprotein 2 (LDL2) as percent of total area under the density profile curve (AUC). Data presented as mean ± standard deviation at 0, 12, and 24 weeks. N=75; no exercise or diet, NED (N=19), higher carbohydrate/low fat, HCLF (N=19), moderate carbohydrate/higher protein, MCHP (N=16), and lower carbohydrate/higher protein, LCHP (N=21).*significantly different from baseline, $p<0.05$. †significantly different from 12 weeks, $p<0.05$. Letter superscripts indicate significance ($p<0.05$) from post hoc LSD. [a]significantly different from NED, [b]significantly different from HCLF, [c]significantly different from MCHP, [d]significantly different from LCHP.

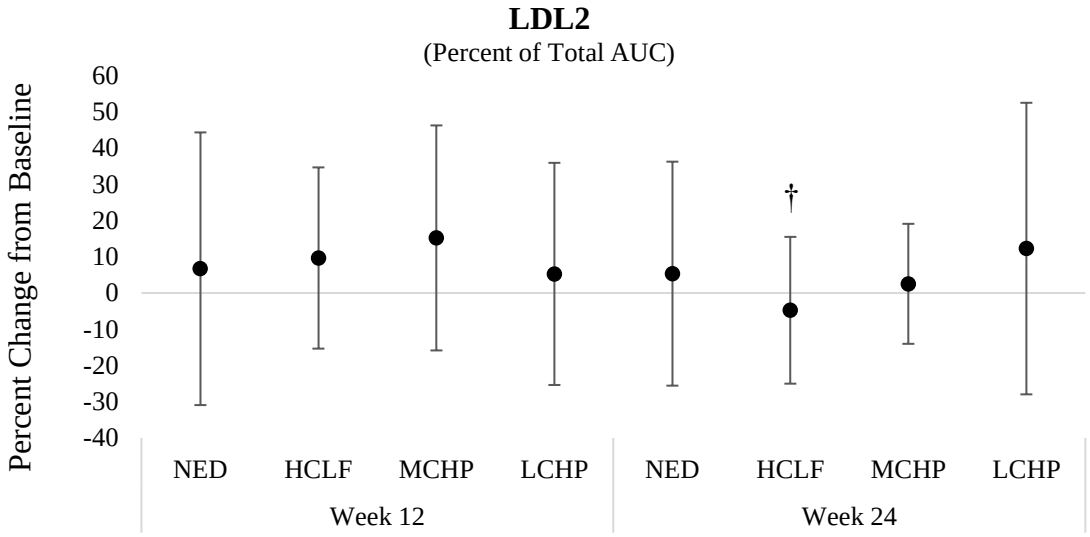

Figure 4.12: Percent change from baseline for low-density lipoprotein 2 (LDL2) as percent of total area under the density profile curve (AUC). Data presented as mean change ± standard deviation from baseline at 12 and 24 weeks. N=75; no exercise or diet, NED (N=19), higher carbohydrate/low fat, HCLF (N=19), moderate carbohydrate/higher protein, MCHP (N=16), and lower carbohydrate/higher protein, LCHP (N=21).*significant percent change, $p<0.05$. †significantly different from 12 weeks, $p<0.05$.

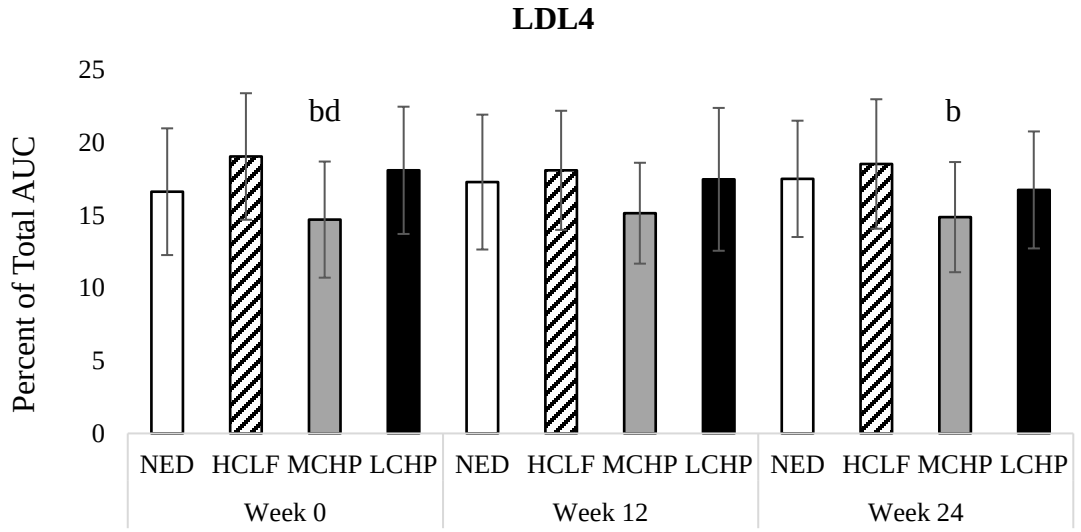

Figure 4.13: Low-density lipoprotein 4 (LDL4) as percent of total area under the density profile curve (AUC). Data presented as mean ± standard deviation at 0, 12, and 24 weeks. N=75; no exercise or diet, NED (N=19), high-carbohydrate/low fat, HCLF (N=19), moderate carbohydrate/higher protein, MCHP (N=16), and lower carbohydrate/higher protein, LCHP (N=21).*significantly different from baseline, $p<0.05$. †significantly different from 12 weeks, $p<0.05$. Letter superscripts indicate significance ($p<0.05$) from post hoc LSD. [a]significantly different from NED, [b]significantly different from HCLF, [c]significantly different from MCHP, [d]significantly different from LCHP.

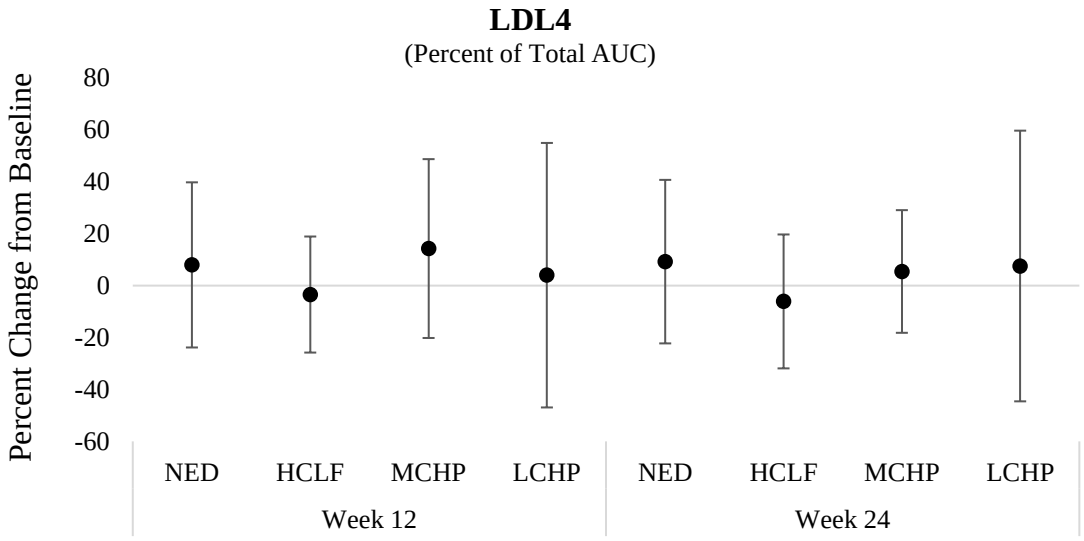

Figure 4.14: Percent change from baseline for low-density lipoprotein 4 (LDL4) as percent of total area under the density profile curve (AUC). Data presented as mean change ± standard deviation from baseline at 12 and 24 weeks. N=75; no exercise or diet, NED (N=19), higher carbohydrate/low fat, HCLF (N=19), moderate carbohydrate/higher protein, MCHP (N=16), and lower carbohydrate/higher protein, LCHP (N=21).*significant percent change, $p<0.05$. †significantly different from 12 weeks, $p<0.05$.

As shown in Table 4.11, post hoc analysis indicated that LDL1 as percent of total LDL AUC for NED, HCLF, and LCHP was significantly greater than baseline at 12 and 24 weeks, respectively. In figure 4.15, only HCLF had a percent change for LDL1 as percent of total LDL AUC that was significant at 12 and 24 weeks. Post hoc tests indicated that LDL2 as percent of total LDL AUC for MCHP was significantly greater than that of NED and HCLF (+1.34; 95% CI, 0.33, 2.35; p=0.01 and +1.09; 95% CI, 0.09, 2.10; p=0.03, respectively) at baseline. At 24 weeks, LDL2 as percent of total LDL AUC for MCHP and LCHP (+1.68; 95% CI, 0.18, 3.17; p=0.028 and +1.57; 95% CI, 0.18, 2.96; p=0.028, respectively) was significantly greater than that of NED. Although group differences occurred, as shown in Figure 4.17, the percent change for LDL2 as percent of total LDL AUC was not significant for any group. In Figure 4.18, post hoc tests show that HDL3a as percent of total HDL AUC for LCHP, was significantly lower than HCLF and MCHP at 0 weeks and significantly lower than HCLF (-1.52; 95% CI, -3.04, -0.01; p=0.049) at 24 weeks. As shown in Figure 4.19, post hoc analysis showed that percent change

for HDL3a as percent of total HDL AUC was only significant for MCHP (-1.16; 95% CI, -2.21, -0.10; p=0.034) at 24 weeks.

Table 4.11: LDL, HDL, and their respective subfraction areas expressed as percentage of total LDL and HDL area under the density profile curve (AUC), respectively

Variable	Group	0 Weeks	12 Weeks	24 Weeks	Group (SEM)		P-level	η^2
LDL1:LDL AUC (%)	NED	4.7 ± 2.5	6.0 ± 4.5*	5.9 ± 4.0*	5.5 ± 0.5	T	<0.001	medium
	HCLF	4.1 ± 1.3	5.6 ± 1.7*	5.4 ± 2.0*	5.1 ± 0.5	G	0.692	small
	MCHP	5.5 ± 2.1	6.1 ± 2.4	6.5 ± 2.6	6.0 ± 0.6	T×G	0.951	small
	LCHP	4.5 ± 2.0	6.1 ± 3.5*	5.7 ± 2.1*	5.4 ± 0.5			
	Mean	4.7 ± 2.0	5.9 ± 3.2*	5.9 ± 2.8*				
LDL2:LDL AUC (%)	NED	6.1 ± 1.4	6.1 ± 1.7	5.8 ± 1.1	6.0 ± 0.4	T	0.158	small
	HCLF	6.3 ± 1.4	6.9 ± 1.5	6.4 ± 1.3	6.5 ± 0.4	G	0.024	medium
	MCHP	7.4 ± 1.6[ab]	7.9 ± 2.0[a]	7.5 ± 1.9[a]	7.6 ± 0.4[a]	T×G	0.696	small
	LCHP	6.7 ± 1.6	7.2 ± 2.4	7.4 ± 3.5[a]	7.1 ± 0.4[a]			
	Mean	6.6 ± 1.5	7.0 ± 2.0*	6.7 ± 2.3				
LDL3:LDL AUC (%)	NED	15.6 ± 2.8	15.3 ± 3.3	14.8 ± 2.5	15.3 ± 0.7	T	0.116	small
	HCLF	15.9 ± 3.2	16.0 ± 3.4	15.2 ± 2.6	15.7 ± 0.7	G	0.420	small
	MCHP	16.6 ± 2.7	17.2 ± 2.4	16.3 ± 2.2	16.7 ± 0.7	T×G	0.966	<0.01
	LCHP	16.6 ± 3.5	16.6 ± 3.8	16.3 ± 5.6	16.5 ± 0.6			
	Mean	16.2 ± 3.0	16.3 ± 3.3	15.6 ± 3.6				
LDL4:LDL AUC (%)	NED	40.9 ± 7.4	41.9 ± 7.6	41.5 ± 9.0	41.4 ± 1.5	T	0.724	<0.01
	HCLF	44.4 ± 8.4	42.4 ± 7.2	43.9 ± 6.9	43.6 ± 1.5	G	0.150	medium
	MCHP	37.9 ± 7.5[b]	38.7 ± 5.6	38.3 ± 5.7[b]	38.3 ± 1.7[b]	T×G	0.400	small
	LCHP	42.9 ± 7.5	41.5 ± 8.2	40.5 ± 7.2	41.6 ± 1.5			
	Mean	41.7 ± 7.9	41.2 ± 7.3	41.1 ± 7.5				
LDL5:LDL AUC (%)	NED	32.8 ± 7.5	30.7 ± 4.9	32.0 ± 8.4	31.8 ± 1.6	T	0.056	small
	HCLF	29.2 ± 7.9	29.1 ± 8.1	29.0 ± 5.9	29.1 ± 1.6	G	0.513	small
	MCHP	32.6 ± 9.1	30.2 ± 8.4	31.5 ± 7.5	31.4 ± 1.7	T×G	0.727	small
	LCHP	29.3 ± 7.6	28.6 ± 6.9	30.2 ± 7.4	29.4 ± 1.5			
	Mean	30.9 ± 8.0	29.6 ± 7.0*	30.6 ± 7.3				
HDL2b:HDL AUC (%)	NED	25.0 ± 4.9	25.1 ± 4.3	25.5 ± 4.0	25.2 ± 1.0	T	0.638	<0.01
	HCLF	24.1 ± 4.6	23.5 ± 4.4	24.4 ± 5.2	24.0 ± 1.0	G	0.081	medium
	MCHP	24.5 ± 3.0	25.1 ± 4.2	25.2 ± 3.3	24.9 ± 1.1	T×G	0.538	small
	LCHP	27.4 ± 6.1[b]	28.2 ± 5.9[ab]	27.2 ± 6.1	27.6 ± 1.0[b]			
	Mean	25.3 ± 5.0	25.5 ± 5.1	25.6 ± 4.9				
HDL2a:HDL AUC (%)	NED	18.6 ± 2.4	18.8 ± 2.2	18.7 ± 2.9	18.7 ± 0.7	T	0.496	small
	HCLF	19.1 ± 3.5	18.7 ± 3.3	19.2 ± 3.7	19.0 ± 0.7	G	0.992	<0.01
	MCHP	18.7 ± 3.0	19.1 ± 2.8	19.0 ± 2.4	18.9 ± 0.7	T×G	0.859	small
	LCHP	18.6 ± 3.9	18.8 ± 3.4	19.1 ± 3.0	18.8 ± 0.6			
	Mean	18.7 ± 3.2	18.8 ± 2.9	19.0 ± 3.0				
HDL3a:HDL AUC (%)	NED	24.6 ± 2.5	24.6 ± 2.2	24.1 ± 2.1	24.4 ± 0.5	T	0.027	small
	HCLF	25.6 ± 2.3	25.2 ± 2.0	25.1 ± 2.3	25.3 ± 0.5	G	0.016	medium
	MCHP	26.0 ± 2.2	25.6 ± 2.4	24.8 ± 1.4*†	25.5 ± 0.5	T×G	0.432	small
	LCHP	23.7 ± 2.6[bc]	23.1 ± 2.7[abc]	23.5 ± 3.2[b]	23.4 ± 0.5[bc]			
	Mean	24.9 ± 2.5	24.6 ± 2.5	24.3 ± 2.4*				
HDL3b:HDL AUC (%)	NED	21.8 ± 3.3	21.5 ± 2.5	21.5 ± 3.1	21.6 ± 0.8	T	0.590	<0.01
	HCLF	22.1 ± 4.0	22.8 ± 3.8	21.8 ± 4.5†	22.2 ± 0.8	G	0.610	small
	MCHP	22.1 ± 3.0	21.6 ± 3.6	21.7 ± 2.8	21.8 ± 0.9	T×G	0.573	small
	LCHP	20.9 ± 4.7	20.6 ± 4.6	20.8 ± 4.1	20.8 ± 0.8			
	Mean	21.7 ± 3.8	21.6 ± 3.7	21.4 ± 3.7				
HDL3c:HDL AUC (%)	NED	10.0 ± 2.3	10.0 ± 2.4	10.3 ± 2.7	10.1 ± 0.5	T	0.307	small
	HCLF	9.2 ± 2.4	9.8 ± 2.3	9.5 ± 2.6	9.5 ± 0.5	G	0.455	small
	MCHP	8.8 ± 1.9	8.6 ± 2.1	9.3 ± 1.8†	8.9 ± 0.6	T×G	0.504	small
	LCHP	9.4 ± 2.7	9.3 ± 2.7	9.4 ± 2.2	9.4 ± 0.5			
	Mean	9.4 ± 2.4	9.5 ± 2.4	9.6 ± 2.4				

Values are presented as means ± standard deviation. Standard deviation of the mean, SEM. N=75; No exercise or diet, NED (n=19). Higher carbohydrate/lower fat, HCLF (n=19). Moderate carbohydrate/higher protein, MCHP (n=16). Lower carbohydrate/higher protein, LCHP (n=21). Time effect, T. Group effect, G. Time × Group interaction, T×G. Area under the density profile curve, AUC. Triglyceride rich lipoproteins, TRL. Low density lipoprotein, LDL. High density lipoprotein, HDL. *significant time effect from baseline (p<0.05). †significant time effect from 12 weeks (p<0.05). Letter superscripts indicate significance (p<0.05) from post hoc LSD analyses: [a]significantly different from NED (p<0.05), [b]significantly different from HCLF (p<0.05), [c]significantly different from MCHP (p<0.05), [d]significantly different from LCHP (p<0.05). Partial eta-squared (η^2) reported with adherence to the following designations outlined by Cohen: small = 0.01, medium = 0.06, and large = 0.14 [120].

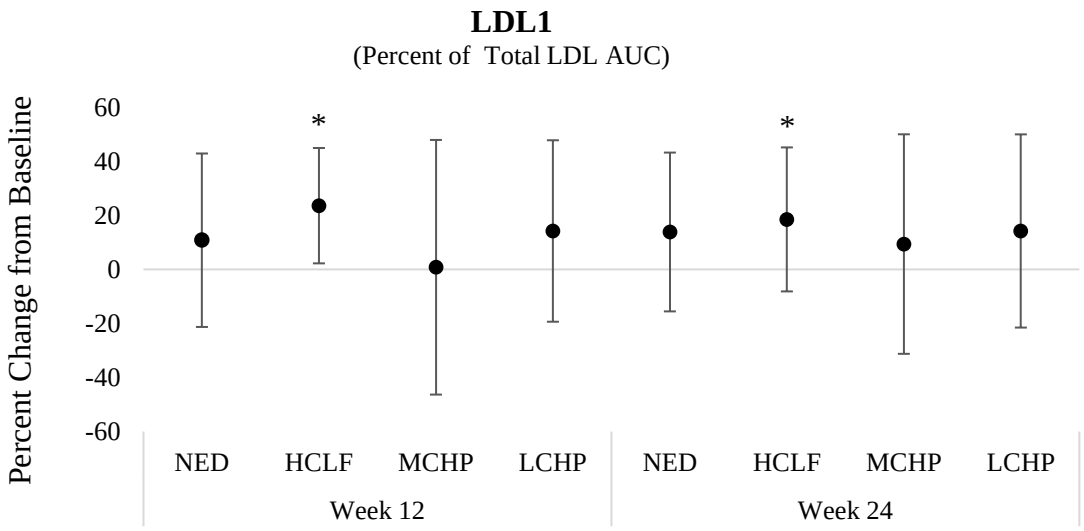

Figure 4.15: Percent change from baseline for low-density lipoprotein 1 (LDL1) as percent of total LDL area under the density profile curve (AUC). Data presented as mean change ± standard deviation from baseline at 12 and 24 weeks. N=75; no exercise or diet, NED (N=19), higher carbohydrate/low fat, HCLF (N=19), moderate carbohydrate/higher protein, MCHP (N=16), and lower carbohydrate/higher protein, LCHP (N=21).*significant percent change, $p<0.05$. †significantly different from 12 weeks, $p<0.05$.

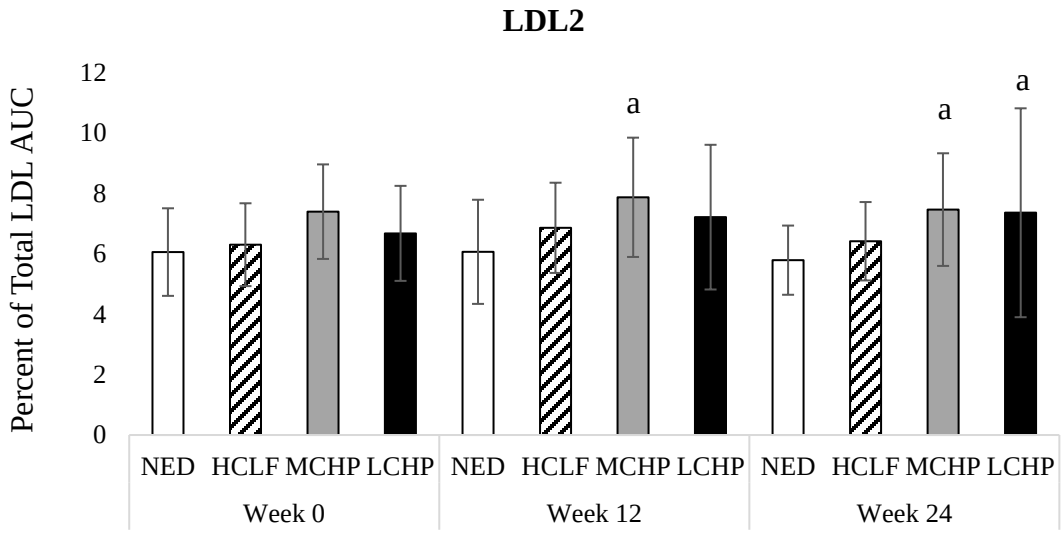

Figure 4.16: Low-density lipoprotein 2 (LDL2) as percent of total LDL area under the density profile curve (AUC). Data presented as mean ± standard deviation at 0, 12, and 24 weeks. N=75; no exercise or diet, NED (N=19), higher carbohydrate/low fat, HCLF (N=19), moderate carbohydrate/higher protein, MCHP (N=16), and lower carbohydrate/higher protein, LCHP (N=21).*significantly different from baseline, $p<0.05$. †significantly different from 12 weeks, $p<0.05$. Letter superscripts indicate significance ($p<0.05$) from post hoc LSD. [a]significantly different from NED, [b]significantly different from HCLF, [c]significantly different from MCHP, [d]significantly different from LCHP.

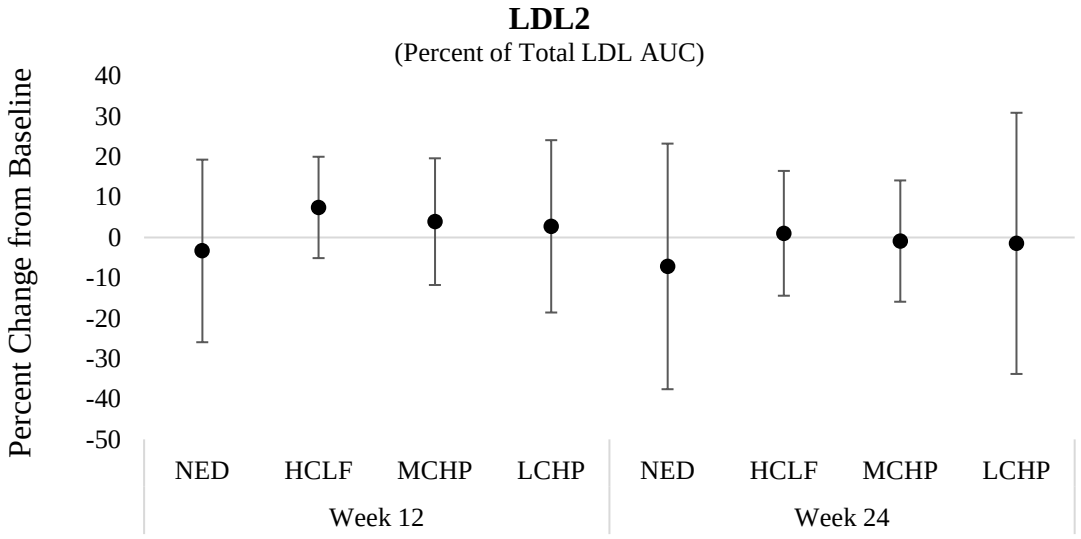

Figure 4.17: Percent change from baseline for low-density lipoprotein 2 (LDL2) as percent of total LDL area under the density profile curve (AUC). Data presented as mean change ± standard deviation from baseline at 12 and 24 weeks. N=75; no exercise or diet, NED (N=19), higher carbohydrate/low fat, HCLF (N=19), moderate carbohydrate/higher protein, MCHP (N=16), and lower carbohydrate/higher protein, LCHP (N=21).*significant percent change, $p<0.05$. †significantly different from 12 weeks, $p<0.05$.

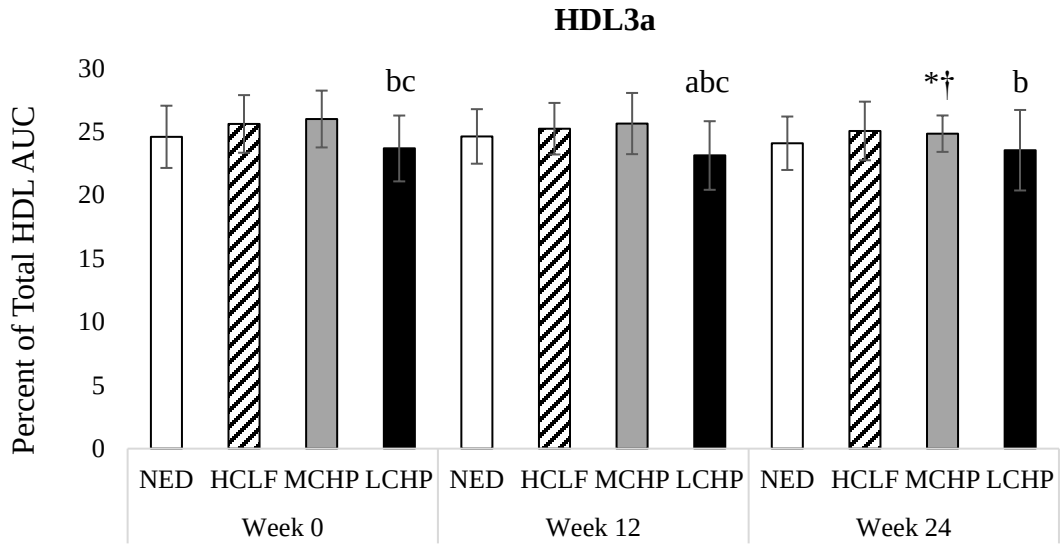

Figure 4.18: High-density lipoprotein 3a (HDL3a) as percent of total HDL area under the density profile curve (AUC). Data presented as mean ± standard deviation at 0, 12, and 24 weeks. N=75; no exercise or diet, NED (N=19), higher carbohydrate/low fat, HCLF (N=19), moderate carbohydrate/higher protein, MCHP (N=16), and lower carbohydrate/higher protein, LCHP (N=21).*significantly different from baseline, $p<0.05$. †significantly different from 12 weeks, $p<0.05$. Letter superscripts indicate significance ($p<0.05$) from post hoc LSD. [a]significantly different from NED, [b]significantly different from HCLF, [c]significantly different from MCHP, [d]significantly different from LCHP.

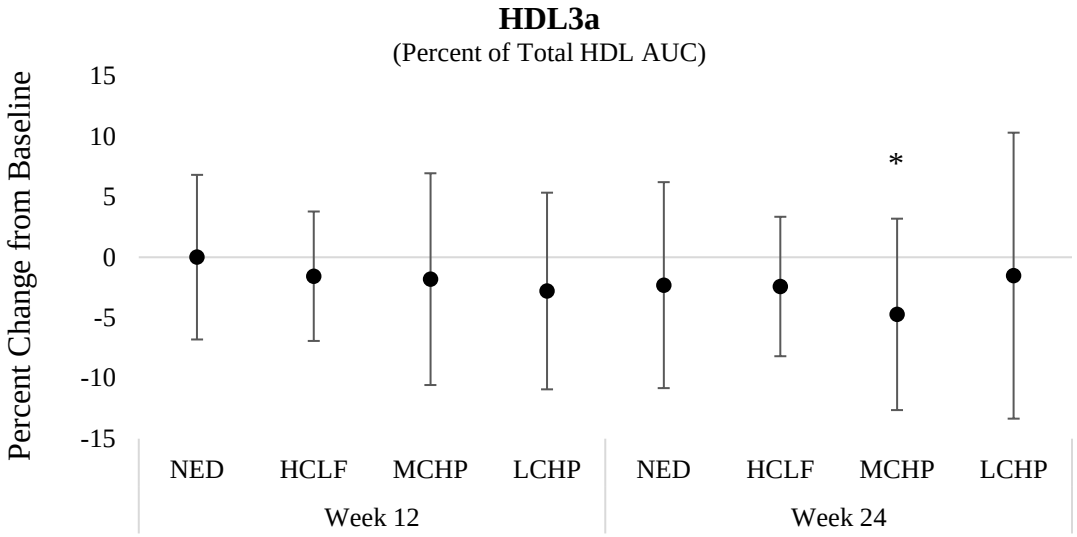

Figure 4.19: Percent change from baseline for high-density lipoprotein 3a (HDL3a) as percent of total HDL area under the density profile curve (AUC). Data presented as mean change ± standard deviation from baseline at 12 and 24 weeks. N=75; no exercise or diet, NED (N=19), higher carbohydrate/low fat, HCLF (N=19), moderate carbohydrate/higher protein, MCHP (N=16), and low-carbohydrate/higher protein, LCHP (N=21).*significant percent change, $p<0.05$. †significantly different from 12 weeks, $p<0.05$.

Correlative Analysis

We pooled data for all subjects into a group of 75 individuals at 0-, 12-, and 24-week time points. Significant positive and negative correlations were found between TRL, LDL, HDL, and their respective subclasses with dependent variables and will be presented in the following paragraphs.

The Relationship Between Lipid Subclasses and Energy and Macronutrient Intake

As shown in Table 4.12, significant correlations between lipoprotein subclasses with energy intake (kcal/day) and macronutrient intake as percent of total energy intake per day were observed; however, as shown in Table 4.13, these significant relationships were more prevalent when energy intake and macronutrient intake were expressed as kilocalorie and/or gram per kilogram per day. Primarily at 0 weeks, energy, carbohydrate, and fat intake were related positively with HDL, HDL2b, and HDL2a ($p<0.05$), and negatively with LDL2 and smaller HDL subclasses (HDL3a and/or HDL3b) ($p<0.05$). Energy and fat intake similarly showed a

negative relationship with LDL2 and LDL5 at 12 and 0 weeks, respectively. In contrast, protein intake was not significantly related with any lipoprotein subclasses at 0 weeks. Instead, protein intake (g/kg/day) was related positively with LDL1 and HDL2b ($p<0.05$) and negatively with HDL3a and HDL3b ($p<0.05$) during diet and exercise intervention.

Table 4.12: Correlations for total daily energy intake and macronutrient intake (% total kcal/day) with TRL, LDL, HDL, and their respective subfraction areas

	Energy Intake (kcal/d)			PRO (% kcal/day)			CHO (% kcal/day)			FAT (% kcal/day)		
Weeks	0	12	24	0	12	24	0	12	24	0	12	24
%TRL of Total AUC	-0.093 0.427	-0.123 0.294	0.113 0.336	0.171 0.143	-0.050 0.67	0.115 0.326	0.065 0.577	0.105 0.368	-0.162 0.165	-0.129 0.269	-0.083 0.479	0.091 0.435
%LDL of Total AUC	-0.087 0.458	0.202 0.083	0.010 0.934	0.084 0.475	-0.003 0.983	-0.057 0.629	0.038 0.744	-0.049 0.679	-0.065 0.581	-0.073 0.535	0.089 0.447	0.089 0.446
%HDL of Total AUC	0.116 0.323	-0.116 0.323	-0.055 0.638	-0.141 0.227	0.025 0.828	0.018 0.877	-0.054 0.648	-0.013 0.914	0.132 0.259	0.110 0.349	-0.031 0.794	-0.115 0.328
%LDL1 of Total AUC	0.031 0.793	-0.164 0.16	0.008 0.946	0.110 0.346	-0.076 0.516	0.355 0.002	0.116 0.323	0.117 0.319	-0.056 0.634	-0.167 0.151	-0.096 0.413	-0.029 0.807
%LDL2 of Total AUC	-0.084 0.473	-0.293 0.011	-0.116 0.323	0.156 0.182	0.192 0.099	0.078 0.504	0.041 0.728	-0.101 0.389	-0.112 0.337	-0.155 0.184	-0.143 0.22	-0.040 0.736
%LDL5 of Total AUC	-0.160 0.171	0.166 0.154	0.159 0.172	0.205 0.077	-0.100 0.394	-0.046 0.695	-0.134 0.253	0.004 0.975	-0.014 0.903	0.061 0.604	0.162 0.164	0.033 0.779
%HDL2b of Total AUC	0.210 0.07	-0.112 0.337	-0.073 0.534	-0.174 0.135	0.212 0.067	0.106 0.367	-0.080 0.493	-0.142 0.226	-0.024 0.836	0.183 0.117	-0.070 0.55	-0.006 0.958
%HDL2a of Total AUC	0.179 0.125	-0.106 0.365	-0.044 0.707	-0.154 0.188	0.028 0.814	-0.013 0.909	-0.060 0.607	0.001 0.996	0.074 0.53	0.124 0.291	-0.061 0.606	-0.009 0.94
%HDL3a of Total AUC	-0.030 0.797	-0.087 0.457	0.010 0.931	-0.019 0.873	-0.111 0.343	-0.088 0.455	-0.018 0.878	0.058 0.62	0.176 0.131	-0.003 0.981	0.047 0.687	-0.070 0.553
%HDL3c of Total AUC	-0.066 0.577	0.073 0.534	-0.043 0.716	0.018 0.88	-0.125 0.283	-0.059 0.618	0.006 0.957	0.068 0.565	0.211 0.069	-0.021 0.856	0.043 0.712	-0.289 0.012
%LDL1 of LDL AUC	0.082 0.483	-0.189 0.105	0.003 0.978	0.080 0.493	-0.064 0.588	0.369 0.001	0.096 0.413	0.114 0.328	-0.025 0.834	-0.133 0.255	-0.112 0.338	-0.060 0.609
%LDL2 of LDL AUC	-0.029 0.805	-0.350 0.002	-0.072 0.541	0.108 0.357	0.167 0.152	0.088 0.455	0.032 0.785	-0.060 0.607	-0.072 0.538	-0.126 0.282	-0.173 0.138	-0.061 0.603
%LDL5 of LDL AUC	-0.113 0.336	0.087 0.456	0.163 0.162	0.164 0.159	-0.126 0.28	-0.029 0.803	-0.175 0.133	0.027 0.819	0.042 0.719	0.128 0.274	0.163 0.161	-0.037 0.754
%HDL2b of HDL AUC	0.224 0.054	-0.082 0.485	-0.060 0.607	-0.117 0.317	0.265 0.022	0.119 0.309	-0.087 0.458	-0.178 0.126	-0.133 0.255	0.170 0.145	-0.079 0.502	0.080 0.494
%HDL2a of HDL AUC	0.230 0.047	-0.071 0.545	-0.050 0.67	-0.138 0.238	0.021 0.859	-0.042 0.722	-0.047 0.688	0.018 0.88	0.006 0.956	0.101 0.388	-0.082 0.483	0.079 0.502
%HDL3b of HDL AUC	-0.254 0.028	0.076 0.517	0.064 0.588	0.115 0.328	-0.172 0.139	-0.004 0.971	0.096 0.411	0.126 0.28	0.055 0.638	-0.164 0.16	0.055 0.637	-0.098 0.403

Values presented are p-values, p. N=75. Colored squares indicate a significant correlation, $p<0.05$. Blue indicates a positive correlation, and red indicates a negative correlation. Area under the density profile curve, AUC. Dietary protein intake, PRO. Dietary carbohydrate intake, CHO. Dietary fat intake, FAT. Triglyceride-rich lipoproteins, TRL. Low-density lipoprotein, LDL. High-density lipoprotein, HDL.

Table 4.13: Correlations for energy intake (kcal/kg/day) and macronutrient intake (g/kg/day) with TRL, LDL, HDL, and their respective subfraction areas

	Energy Intake (kcal/kg)			PRO (g/kg/day)			CHO (g/kg/day)			FAT (g/kg/day)		
Weeks	0	12	24	0	12	24	0	12	24	0	12	24
%TRL of Total AUC	-0.108 0.355	-0.136 0.246	-0.017 0.882	0.029 0.804	-0.152 0.192	0.084 0.474	-0.034 0.775	-0.049 0.675	-0.159 0.173	-0.176 0.131	-0.142 0.224	0.094 0.422
%LDL of Total AUC	-0.253 0.028	0.014 0.907	-0.200 0.086	-0.159 0.174	0.010 0.935	-0.164 0.159	-0.219 0.059	-0.030 0.796	-0.208 0.073	-0.236 0.042	0.077 0.510	-0.065 0.580
%HDL of Total AUC	0.269 0.020	0.063 0.593	0.180 0.122	0.131 0.261	0.072 0.541	0.122 0.297	0.214 0.066	0.055 0.639	0.253 0.029	0.276 0.017	0.011 0.926	0.016 0.889
%LDL1 of Total AUC	0.027 0.821	0.045 0.702	0.028 0.812	0.109 0.353	-0.008 0.948	0.322 0.005	0.114 0.330	0.093 0.426	-0.050 0.670	-0.079 0.500	-0.034 0.774	0.029 0.805
%LDL2 of Total AUC	-0.144 0.216	-0.248 0.032	0.037 0.753	-0.024 0.840	0.001 0.996	0.046 0.698	-0.083 0.479	-0.234 0.043	-0.044 0.707	-0.208 0.073	-0.267 0.021	0.039 0.742
%LDL5 of Total AUC	-0.305 0.008	-0.024 0.835	-0.109 0.353	-0.125 0.286	-0.122 0.295	-0.072 0.541	-0.367 0.001	-0.019 0.871	-0.130 0.265	-0.193 0.097	0.066 0.573	-0.029 0.804
%HDL2b of Total AUC	0.311 0.007	0.082 0.484	0.162 0.165	0.138 0.236	0.287 0.012	0.210 0.071	0.249 0.031	-0.011 0.929	0.117 0.319	0.323 0.005	0.021 0.861	0.089 0.445
%HDL2a of Total AUC	0.314 0.006	0.106 0.365	0.228 0.049	0.153 0.190	0.108 0.357	0.133 0.257	0.262 0.023	0.090 0.443	0.242 0.036	0.303 0.008	0.039 0.739	0.113 0.336
%HDL3a of Total AUC	0.071 0.546	-0.012 0.916	0.147 0.208	0.050 0.670	-0.138 0.239	-0.006 0.958	0.050 0.668	0.046 0.698	0.258 0.025	0.069 0.558	-0.014 0.907	0.019 0.869
%HDL3b of Total AUC	-0.087 0.460	-0.075 0.523	-0.060 0.609	-0.055 0.639	-0.245 0.034	-0.070 0.553	-0.081 0.492	0.031 0.790	0.100 0.393	-0.069 0.554	-0.065 0.579	-0.186 0.111
%HDL3c of Total AUC	0.036 0.759	0.025 0.833	-0.084 0.472	0.055 0.636	-0.108 0.356	-0.123 0.295	0.020 0.865	0.044 0.707	0.087 0.458	0.043 0.717	0.046 0.694	-0.237 0.041
%LDL1 of LDL AUC	0.124 0.291	0.056 0.631	0.096 0.414	0.174 0.135	0.013 0.913	0.374 0.001	0.191 0.101	0.103 0.380	0.029 0.807	0.016 0.890	-0.037 0.756	0.047 0.688
%LDL2 of LDL AUC	0.018 0.878	-0.206 0.076	0.144 0.219	0.082 0.486	0.014 0.908	0.123 0.294	0.063 0.589	-0.179 0.125	0.063 0.590	-0.061 0.605	-0.258 0.025	0.089 0.448
%LDL5 of LDL AUC	-0.193 0.097	-0.042 0.718	-0.040 0.733	-0.047 0.690	-0.169 0.147	-0.013 0.914	-0.287 0.012	-0.009 0.942	-0.037 0.756	-0.072 0.540	0.038 0.743	-0.033 0.778
%HDL2b of HDL AUC	0.250 0.031	0.071 0.547	0.112 0.337	0.128 0.273	0.339 0.003	0.201 0.084	0.199 0.087	-0.048 0.685	-0.007 0.955	0.254 0.028	0.018 0.878	0.124 0.289
%HDL2a of HDL AUC	0.319 0.005	0.119 0.310	0.201 0.083	0.173 0.139	0.110 0.347	0.093 0.428	0.282 0.014	0.104 0.376	0.176 0.130	0.280 0.015	0.048 0.684	0.149 0.203
%HDL3a of HDL AUC	-0.242 0.036	-0.094 0.425	-0.034 0.774	-0.117 0.318	-0.303 0.008	-0.165 0.158	-0.198 0.088	0.009 0.937	0.047 0.689	-0.248 0.032	-0.036 0.759	-0.011 0.925
%HDL3b of HDL AUC	-0.327 0.004	-0.119 0.311	-0.187 0.108	-0.196 0.091	-0.270 0.019	-0.138 0.238	-0.272 0.018	-0.020 0.867	-0.113 0.334	-0.304 0.008	-0.068 0.564	-0.165 0.158

Values presented are p-values, p. N=75. Colored squares indicate a significant correlation, $p<0.05$. Blue indicates a positive correlation, and red indicates a negative correlation. Area under the density profile curve, AUC. Dietary protein intake, PRO. Dietary carbohydrate intake, CHO. Dietary fat intake, FAT. Triglyceride-rich lipoproteins, TRL. Low-density lipoprotein, LDL. High-density lipoprotein, HDL.

The Relationship Between Lipid Subclasses and Anthropometrics

As shown in Table 4.14, weight (kg), waist circumference (cm), and waist-to-hip ratio were related positively with LDL, LDL5, HDL3b, and HDL3c, and negatively with HDL, HDL2b, and HDL2a. Hip circumference (cm) showed similar relationships that occurred less frequently. The waist-to-hip ratio was the only parameter that was positively related with TRL.

Table 4.14: Correlations for weight, waist and hip circumference, and waist-to-hip ratio with TRL, LDL, HDL, and their respective subfraction areas

	Weight (kg)			WC (cm)			HC (cm)			Waist-to-Hip Ratio		
Weeks	0	12	24	0	12	24	0	12	24	0	12	24
%TRL of Total AUC	**0.115** 0.327	**0.082** 0.486	**0.116** 0.323	**0.141** 0.228	**0.219** 0.059	**0.217** 0.062	**0.064** 0.584	**0.035** 0.764	**0.040** 0.733	**0.195** 0.094	**0.320** 0.005	**0.319** 0.005
%LDL of Total AUC	**0.315** 0.006	**0.269** 0.020	**0.289** 0.012	**0.310** 0.007	**0.226** 0.052	**0.256** 0.026	**0.276** 0.016	**0.211** 0.069	**0.234** 0.043	**0.227** 0.050	**0.117** 0.318	**0.147** 0.208
%HDL of Total AUC	**-0.325** 0.004	**-0.290** 0.012	**-0.299** 0.009	**-0.330** 0.004	**-0.325** 0.004	**-0.320** 0.005	**-0.273** 0.018	**-0.214** 0.065	**-0.218** 0.060	**-0.273** 0.018	**-0.278** 0.016	**-0.275** 0.017
%LDL1 of Total AUC	**-0.001** 0.992	**-0.314** 0.006	**-0.047** 0.689	**0.034** 0.773	**-0.174** 0.134	**-0.069** 0.554	**0.005** 0.964	**-0.328** 0.004	**-0.109** 0.352	**0.065** 0.581	**0.078** 0.506	**0.013** 0.914
%LDL5 of Total AUC	**0.348** 0.002	**0.313** 0.006	**0.308** 0.007	**0.345** 0.002	**0.358** 0.002	**0.360** 0.002	**0.259** 0.025	**0.208** 0.073	**0.187** 0.108	**0.317** 0.006	**0.344** 0.003	**0.386** 0.001
%HDL2b of Total AUC	**-0.295** 0.010	**-0.336** 0.003	**-0.310** 0.007	**-0.289** 0.012	**-0.361** 0.001	**-0.296** 0.010	**-0.207** 0.075	**-0.215** 0.064	**-0.196** 0.093	**-0.273** 0.018	**-0.337** 0.003	**-0.269** 0.019
%HDL2a of Total AUC	**-0.363** 0.001	**-0.362** 0.001	**-0.344** 0.002	**-0.369** 0.001	**-0.419** 0.000	**-0.363** 0.001	**-0.288** 0.012	**-0.257** 0.026	**-0.251** 0.030	**-0.324** 0.005	**-0.389** 0.001	**-0.316** 0.006
%LDL1 of LDL AUC	**-0.101** 0.386	**-0.369** 0.001	**-0.149** 0.203	**-0.069** 0.559	**-0.225** 0.053	**-0.170** 0.145	**-0.092** 0.434	**-0.372** 0.001	**-0.200** 0.086	**-0.003** 0.982	**0.053** 0.653	**-0.048** 0.682
%LDL2 of LDL AUC	**-0.095** 0.420	**-0.186** 0.111	**-0.277** 0.016	**-0.073** 0.536	**-0.064** 0.584	**-0.254** 0.028	**0.002** 0.987	**-0.146** 0.212	**-0.286** 0.013	**-0.129** 0.269	**0.064** 0.587	**-0.082** 0.482
%LDL5 of LDL AUC	**0.234** 0.043	**0.244** 0.035	**0.212** 0.068	**0.250** 0.031	**0.324** 0.005	**0.297** 0.010	**0.147** 0.207	**0.153** 0.190	**0.100** 0.392	**0.278** 0.016	**0.355** 0.002	**0.385** 0.001
%HDL2b of HDL AUC	**-0.173** 0.137	**-0.272** 0.018	**-0.235** 0.043	**-0.156** 0.181	**-0.286** 0.013	**-0.213** 0.066	**-0.086** 0.464	**-0.162** 0.166	**-0.129** 0.271	**-0.177** 0.129	**-0.278** 0.016	**-0.216** 0.062
%HDL2a of HDL AUC	**-0.322** 0.005	**-0.332** 0.004	**-0.313** 0.006	**-0.317** 0.006	**-0.403** 0.000	**-0.341** 0.003	**-0.242** 0.037	**-0.227** 0.050	**-0.225** 0.052	**-0.287** 0.012	**-0.403** 0.000	**-0.316** 0.006
%HDL3b of HDL AUC	**0.293** 0.011	**0.343** 0.003	**0.322** 0.005	**0.274** 0.018	**0.388** 0.001	**0.329** 0.004	**0.202** 0.082	**0.230** 0.047	**0.226** 0.051	**0.255** 0.027	**0.370** 0.001	**0.295** 0.010
%HDL3c of HDL AUC	**0.131** 0.263	**0.231** 0.046	**0.231** 0.046	**0.129** 0.271	**0.267** 0.021	**0.239** 0.039	**0.075** 0.525	**0.141** 0.227	**0.176** 0.130	**0.144** 0.217	**0.270** 0.019	**0.202** 0.082

Values presented are p-values, p. N=75. Colored squares indicate a significant correlation, p<0.05. Blue indicates a positive correlation, and red indicates a negative correlation. Area under the density profile curve, AUC. Waist circumference, WC. Hip circumference, HC. Triglyceride-rich lipoproteins, TRL. Low-density lipoprotein, LDL. High-density lipoprotein, HDL.

The Relationship Between Lipid Subclasses and Body Composition

As shown in Table 4.15, fat mass (kg), fat free mass (kg), and VAT (cm^2) were similarly related positively with LDL5 and HDL3b, and negatively with HDL, HDL2b, and HDL2a. Additionally, both fat mass and VAT area expressed a significant positive relationship with LDL

while only VAT area was related positively with TRL and negatively with LDL4 and HDL3a. Except for a significant negative relationship between percent body fat and LDL1 as percent of total AUC at 0 weeks, percent body fat was not significantly related with LDL, HDL, or their respective subclasses.

The Relationship Between Lipid Subclasses and Cardiorespiratory Fitness

As shown in Table 4.16, except for a positive relationship with LDL5 as percent of total AUC at 0 weeks, VO_{2peak} (L/min) was not significantly related with TRL, LDL, HDL, or their respective subclasses. On the other hand, we observed significant relationships for VO_{2peak} (ml/kg/min) and time to exhaustion (seconds) at 12 and 24 weeks. Both VO_{2peak} and time to exhaustion were related negatively with LDL5, HDL3a, and HDL3b, and positively with HDL, HDL2b and HDL2a.

Since significant relationships were observed primarily after the implementation of diet and exercise, we looked at the correlation patterns for each group at the three different time points as shown in Table 4.17. Apart from a significant positive relationship observed for subjects assigned to HCLF with HDL at 24 weeks, further breakdown of the correlations by group elucidated significant relationships between VO_{2peak} and lipoprotein subclasses for NED and LCHP at 12 and 24 weeks. For subjects assigned to NED, VO_{2peak} was related, positively with HDL, HDL2b, and HDL2a and negatively with HDL3b primarily at 12 weeks ($p<0.05$), and for subjects assigned to LCHP, VO_{2peak} was related positively with HDL2b and HDL2a and negatively with HDL3a and HDL3b ($p<0.05$). This table also shows the relationships observed when all diet and exercise groups were pooled together (ED, n=56) and when all groups including NED were pooled together (ALL).

Table 4.15: Correlations for fat mass (kg), fat free mass (kg), percent body fat, and VAT with TRL, LDL, HDL, and their respective subfraction areas

	FM (kg)			FFM (kg)			%BF			VAT (cm^2)		
Weeks	0	12	24	0	12	24	0	12	24	0	12	24
%TRL of Total AUC	0.086 0.462	0.069 0.556	0.095 0.417	0.084 0.475	0.085 0.470	0.149 0.201	-0.038 0.749	-0.077 0.514	0.047 0.689	0.220 0.058	0.293 0.011	0.390 0.001
%LDL of Total AUC	0.292 0.011	0.288 0.012	0.272 0.018	0.224 0.053	0.221 0.057	0.224 0.053	-0.130 0.265	0.040 0.732	-0.038 0.743	0.368 0.001	0.323 0.005	0.296 0.010
%HDL of Total AUC	-0.296 0.010	-0.303 0.008	-0.277 0.016	-0.230 0.047	-0.246 0.034	-0.257 0.026	0.120 0.306	0.000 0.998	-0.005 0.969	-0.415 0.000	-0.455 0.000	-0.432 0.000
%LDL1 of Total AUC	-0.027 0.818	-0.297 0.010	-0.060 0.610	-0.052 0.660	-0.307 0.007	-0.003 0.980	-0.262 0.023	-0.035 0.766	-0.147 0.207	-0.051 0.666	-0.089 0.446	-0.023 0.846
%LDL5 of Total AUC	0.300 0.009	0.300 0.009	0.230 0.047	0.296 0.010	0.309 0.007	0.315 0.006	-0.032 0.784	0.145 0.216	-0.011 0.924	0.495 0.000	0.538 0.000	0.460 0.000
%HDL2b of Total AUC	-0.274 0.017	-0.337 0.003	-0.304 0.008	-0.245 0.034	-0.303 0.008	-0.223 0.055	0.152 0.192	0.001 0.992	0.061 0.605	-0.348 0.002	-0.466 0.000	-0.386 0.001
%HDL2a of Total AUC	-0.334 0.003	-0.373 0.001	-0.324 0.005	-0.276 0.016	-0.315 0.006	-0.279 0.015	0.095 0.416	-0.074 0.529	-0.023 0.844	-0.491 0.000	-0.547 0.000	-0.473 0.000
%HDL3a of Total AUC	-0.142 0.223	-0.144 0.217	-0.160 0.170	-0.098 0.404	-0.072 0.538	-0.181 0.121	0.043 0.713	-0.039 0.743	-0.104 0.374	-0.260 0.025	-0.243 0.036	-0.305 0.008
%LDL1 of LDL AUC	-0.122 0.296	-0.370 0.001	-0.157 0.177	-0.119 0.308	-0.336 0.003	-0.078 0.505	-0.191 0.101	-0.035 0.766	-0.112 0.337	-0.169 0.148	-0.183 0.115	-0.121 0.300
%LDL2 of LDL AUC	-0.075 0.525	-0.161 0.167	-0.271 0.018	-0.164 0.161	-0.209 0.072	-0.246 0.034	0.044 0.708	0.033 0.779	0.007 0.952	-0.161 0.167	-0.058 0.624	-0.223 0.055
%LDL4 of LDL AUC	-0.082 0.482	0.024 0.837	0.087 0.458	-0.100 0.395	-0.011 0.926	-0.063 0.594	-0.026 0.827	-0.065 0.579	0.069 0.556	-0.222 0.055	-0.293 0.011	-0.195 0.093
%LDL5 of LDL AUC	0.192 0.098	0.221 0.057	0.132 0.261	0.221 0.057	0.261 0.024	0.255 0.028	0.043 0.713	0.137 0.243	0.006 0.962	0.375 0.001	0.470 0.000	0.389 0.001
%HDL2b of HDL AUC	-0.166 0.156	-0.264 0.022	-0.244 0.035	-0.180 0.122	-0.256 0.027	-0.141 0.226	0.141 0.227	0.029 0.806	0.107 0.359	-0.176 0.131	-0.341 0.003	-0.258 0.026
%HDL2a of HDL AUC	-0.296 0.010	-0.334 0.003	-0.292 0.011	-0.269 0.020	-0.300 0.009	-0.251 0.030	0.032 0.783	-0.121 0.300	-0.038 0.746	-0.465 0.000	-0.521 0.000	-0.426 0.000
%HDL3b of HDL AUC	0.276 0.017	0.348 0.002	0.325 0.004	0.265 0.022	0.309 0.007	0.220 0.058	-0.082 0.484	0.080 0.494	0.001 0.994	0.378 0.001	0.477 0.000	0.400 0.000
%HDL3c of HDL AUC	0.105 0.371	0.240 0.038	0.232 0.045	0.131 0.262	0.208 0.074	0.196 0.093	-0.085 0.469	0.054 0.645	-0.014 0.903	0.221 0.057	0.406 0.000	0.326 0.004

Values presented are p-values, p. N=75. Colored squares indicate a significant correlation, $p<0.05$. Blue indicates a positive correlation, and red indicates a negative correlation. Area under the density profile curve, AUC. Fat mass, FM. Fat free mass, FFM. Percent body fat, %BF. Visceral adipose tissue, VAT. Triglyceride-rich lipoprotein, TRL. Low-density lipoprotein, LDL. High-density lipoprotein, HDL.

Table 4.16: Correlations for $VO_{2\ Peak}$ (L/min and ml/kg/min) and time to exhaustion with TRL, LDL, HDL, and their respective subfraction areas

Weeks	$VO_{2\ Peak}$ (L/min)			$VO_{2\ Peak}$ (ml/kg/min)			Time to Exhaustion (secs)		
	0	12	24	0	12	24	0	12	24
%TRL of Total AUC	**0.132** 0.258	**-0.094** 0.424	**0.042** 0.722	**-0.031** 0.792	**-0.073** 0.534	**-0.139** 0.235	**-0.050** 0.669	**-0.087** 0.456	**-0.148** 0.204
%LDL of Total AUC	**0.176** 0.131	**0.133** 0.254	**0.098** 0.401	**-0.132** 0.261	**-0.166** 0.155	**-0.204** 0.079	**-0.083** 0.477	**-0.140** 0.231	**-0.183** 0.115
%HDL of Total AUC	**-0.189** 0.104	**-0.058** 0.622	**-0.081** 0.490	**0.147** 0.209	**0.203** 0.081	**0.253** 0.029	**0.106** 0.365	**0.186** 0.110	**0.238** 0.040
%LDL1 of Total AUC	**0.202** 0.082	**-0.015** 0.900	**0.14** 0.232	**0.170** 0.145	**0.265** 0.022	**0.110** 0.350	**0.123** 0.292	**0.176** 0.130	**0.112** 0.337
%LDL5 of Total AUC	**0.246** 0.033	**0.044** 0.708	**0.077** 0.512	**-0.107** 0.359	**-0.274** 0.018	**-0.213** 0.067	**-0.143** 0.221	**-0.242** 0.036	**-0.141** 0.227
%HDL2b of Total AUC	**-0.123** 0.295	**-0.010** 0.929	**-0.001** 0.995	**0.134** 0.252	**0.257** 0.026	**0.292** 0.011	**0.119** 0.311	**0.294** 0.011	**0.263** 0.023
%HDL2a of Total AUC	**-0.182** 0.118	**-0.021** 0.861	**-0.066** 0.576	**0.160** 0.169	**0.295** 0.010	**0.307** 0.007	**0.176** 0.132	**0.271** 0.019	**0.294** 0.010
%HDL3b of Total AUC	**-0.059** 0.615	**-0.057** 0.629	**-0.092** 0.430	**-0.011** 0.927	**-0.161** 0.168	**-0.138** 0.238	**-0.099** 0.400	**-0.194** 0.095	**-0.129** 0.268
%HDL3c of Total AUC	**-0.001** 0.995	**-0.034** 0.771	**-0.049** 0.676	**0.126** 0.281	**-0.082** 0.485	**-0.097** 0.408	**0.063** 0.593	**-0.093** 0.430	**-0.083** 0.476
%LDL1 of LDL AUC	**0.135** 0.249	**-0.065** 0.579	**0.110** 0.349	**0.203** 0.080	**0.285** 0.013	**0.188** 0.106	**0.148** 0.207	**0.200** 0.085	**0.182** 0.118
%LDL5 of LDL AUC	**0.186** 0.110	**-0.033** 0.778	**0.012** 0.917	**-0.055** 0.642	**-0.257** 0.026	**-0.160** 0.169	**-0.128** 0.275	**-0.225** 0.052	**-0.093** 0.429
%HDL2b of HDL AUC	**-0.037** 0.751	**0.025** 0.830	**0.058** 0.618	**0.079** 0.503	**0.221** 0.057	**0.249** 0.031	**0.097** 0.407	**0.269** 0.020	**0.219** 0.059
%HDL2a of HDL AUC	**-0.157** 0.177	**-0.001** 0.991	**-0.062** 0.597	**0.128** 0.272	**0.286** 0.013	**0.275** 0.017	**0.189** 0.104	**0.260** 0.025	**0.262** 0.023
%HDL3a of HDL AUC	**-0.027** 0.815	**-0.065** 0.578	**-0.075** 0.524	**-0.163** 0.162	**-0.208** 0.074	**-0.157** 0.178	**-0.166** 0.155	**-0.263** 0.022	**-0.135** 0.247
%HDL3b of HDL AUC	**0.112** 0.337	**0.002** 0.987	**0.000** 0.999	**-0.127** 0.278	**-0.287** 0.013	**-0.316** 0.006	**-0.177** 0.128	**-0.295** 0.010	**-0.297** 0.010

Values presented are p-values, p. N=75. Colored squares indicate a significant correlation, $p<0.05$. Blue indicates a positive correlation, and red indicates a negative correlation. Area under the density profile curve, AUC. Peak oxygen uptake, VO_{2peak}. Triglyceride-rich lipoproteins, TRL. Low-density lipoprotein, LDL. High-density lipoprotein, HDL.

Table 4.17: Correlations by group for $VO_{2\ Peak}$ (ml/kg/min) with TRL, LDL, HDL, and their respective subfraction areas

Weeks	0						12						24					
%TRL of Total AUC	0.85	0.93	0.50	0.70	0.76	0.79	0.55	0.28	0.67	0.96	0.82	0.53	0.34	0.41	0.86	0.45	0.29	0.24
%LDL of Total AUC	0.50	0.38	0.63	0.94	0.46	0.26	0.11	0.17	0.46	0.70	0.49	0.16	0.10	0.06	0.74	0.98	0.33	0.08
%HDL of Total AUC	0.46	0.34	0.50	0.93	0.44	0.21	0.02	0.07	0.62	0.72	0.47	0.08	0.05	0.03	0.72	0.71	0.18	0.03
%LDL1 of Total AUC	0.07	0.78	0.32	0.10	0.72	0.15	0.07	0.68	0.76	0.17	0.15	0.02	0.31	0.77	0.85	0.63	0.79	0.35
%LDL5 of Total AUC	0.59	0.25	0.51	0.82	0.32	0.36	0.19	0.09	0.36	0.40	0.05	0.02	0.38	0.19	0.69	0.33	0.15	0.07
%HDL2b of Total AUC	0.18	0.96	0.96	0.54	0.68	0.25	0.02	0.29	0.67	0.22	0.18	0.03	0.04	0.15	0.96	0.07	0.08	0.01
%HDL2a of Total AUC	0.34	0.46	0.49	0.75	0.31	0.17	0.02	0.07	0.57	0.48	0.10	0.01	0.12	0.12	0.50	0.13	0.03	0.00
%HDL3b of Total AUC	0.79	0.64	0.59	0.26	0.81	0.93	0.92	0.94	0.96	0.00	0.12	0.17	0.60	0.68	0.70	0.01	0.09	0.24
%HDL3c of Total AUC	0.87	0.30	0.55	0.96	0.36	0.28	0.82	0.76	0.73	0.12	0.55	0.49	0.98	0.67	0.94	0.05	0.39	0.41
%LDL1 of LDL AUC	0.06	0.53	0.37	0.13	0.59	0.08	0.06	0.39	0.43	0.28	0.12	0.01	0.19	0.50	0.66	0.54	0.38	0.11
%LDL5 of LDL AUC	0.81	0.30	0.67	0.86	0.43	0.64	0.59	0.15	0.45	0.17	0.03	0.03	0.73	0.70	0.73	0.18	0.18	0.17
%HDL2b of HDL AUC	0.23	0.62	0.44	0.34	0.93	0.50	0.15	0.72	0.84	0.04	0.17	0.06	0.21	0.43	0.64	0.02	0.08	0.03
%HDL2a of HDL AUC	0.46	0.68	0.48	0.62	0.33	0.27	0.09	0.15	0.58	0.19	0.05	0.01	0.51	0.37	0.33	0.03	0.02	0.02
%HDL3a of HDL AUC	0.15	0.87	0.50	0.07	0.58	0.16	0.43	0.82	0.75	0.02	0.12	0.07	0.55	0.34	0.17	0.06	0.21	0.02
%HDL3b of HDL AUC	0.35	0.88	0.68	0.44	0.43	0.28	0.03	0.32	0.66	0.06	0.06	0.01	0.27	0.38	0.45	0.01	0.01	0.01
Groups	NED	HCLF	MCHP	LCHP	ED	ALL	NED	HCLF	MCHP	LCHP	ED	ALL	NED	HCLF	MCHP	LCHP	ED	ALL

Values presented are p values. No exercise or diet, NED (n=19). Higher carbohydrate, lower fat, HCLF (n=19). Moderate carbohydrate, higher protein, MCHP (n=16). Lower carbohydrate, higher protein, LCHP (n=21). Exercise plus diet groups, ED (N=56). All groups, ALL (N=75). p, p-value. AUC, area under the curve. VO_{2peak}, maximal oxygen uptake. Colored squares indicate a significant correlation, $p<0.05$. Blue indicates a positive correlation and red indicates a negative correlation.

The Relationship Between Lipid Subclasses and Glucose Homeostasis

As shown in Table 4.18, glucose, insulin, and HOMA-IR were related positively with LDL5 and negatively with HDL2a ($p<0.05$) at 0 and 12 weeks. Both insulin and HOMA-IR were related positively with LDL and negatively with HDL and HDL3a ($p<0.05$) primarily at 0 weeks prior to diet and exercise intervention.

Table 4.18: Correlations for glucose, insulin, and HOMA-IR with TRL, LDL, HDL, and their respective subfraction areas

	Glucose			Insulin			HOMA-IR		
Weeks	**0**	**12**	**24**	**0**	**12**	**24**	**0**	**12**	**24**
%TRL of Total AUC	**0.226** 0.052	**0.118** 0.313	**-0.036** 0.762	**0.124** 0.288	N/A	**0.003** 0.978	**0.126** 0.280	N/A	**-0.046** 0.697
%LDL of Total AUC	**0.016** 0.893	**0.110** 0.347	**0.174** 0.136	**0.250** 0.030	N/A	**0.215** 0.064	**0.226** 0.051	N/A	**0.233** 0.044
%HDL of Total AUC	**-0.104** 0.376	**-0.164** 0.161	**-0.132** 0.260	**-0.269** 0.020	N/A	**-0.181** 0.120	**-0.249** 0.031	N/A	**-0.177** 0.130
%LDL5 of Total AUC	**0.215** 0.063	**0.278** 0.016	**0.213** 0.067	**0.319** 0.005	N/A	**0.195** 0.093	**0.328** 0.004	N/A	**0.240** 0.038
%HDL2a of Total AUC	**-0.205** 0.077	**-0.237** 0.041	**-0.178** 0.126	**-0.295** 0.010	N/A	**-0.159** 0.174	**-0.290** 0.012	N/A	**-0.178** 0.127
%HDL3a of Total AUC	**-0.076** 0.519	**-0.078** 0.508	**-0.102** 0.385	**-0.275** 0.017	N/A	**-0.164** 0.159	**-0.256** 0.027	N/A	**-0.166** 0.154
%LDL4 of LDL AUC	**-0.303** 0.008	**-0.157** 0.179	**-0.073** 0.536	**-0.144** 0.219	N/A	**0.093** 0.427	**-0.184** 0.115	N/A	**0.035** 0.767
%LDL5 of LDL AUC	**0.270** 0.019	**0.286** 0.013	**0.152** 0.192	**0.261** 0.023	N/A	**0.122** 0.299	**0.287** 0.013	N/A	**0.154** 0.188
%HDL2a of HDL AUC	**-0.224** 0.053	**-0.250** 0.030	**-0.187** 0.108	**-0.257** 0.026	N/A	**-0.109** 0.353	**-0.257** 0.026	N/A	**-0.150** 0.199

Values presented are p-values, p. N=75. Colored squares indicate a significant correlation, $p<0.05$. Blue indicates a positive correlation, and red indicates a negative correlation. Area under the density profile curve, AUC. Homeostatic model assessment of insulin resistance, HOMA-IR. HOMA-IR was calculated by taking glucose (mg/dL) measured via COBAS® c-111 analyzer and Insulin (µIU/ml) measured via ELISA, and placed in the following formula: (glucose * Insulin)/405. Triglyceride-rich lipoproteins, TRL. Low-density lipoprotein, LDL. High-density lipoprotein, HDL.

The Relationship Between Lipid Subclasses and Blood Lipids

As shown in Table 4.19, we consider the relationship between total intensity in addition to TRL, LDL, and HDL along with their respective subclasses. Only positive correlations were observed for Table 4.19. Triglycerides were related positively with TRL and LDL5. Total cholesterol was related positively with total intensity, TRL, LDL, and with the LDL subclasses LDL2-LDL5 while HDL was related positively with total intensity, HDL and with the HDL subclasses HDL2b, HDL2a, and HDL3a.

Table 4.19: Correlations for triglycerides, total-, LDL-, and HDL-cholesterol with Total, TRL, LDL, HDL, and their respective subfraction areas expressed as area under the density profile curve (AUC)

	Triglycerides (mg/dL)			Total Cholesterol (mg/dL)			LDL Cholesterol (mg/dL)			HDL Cholesterol (mg/dL)		
Weeks	**0**	**12**	**24**	**0**	**12**	**24**	**0**	**12**	**24**	**0**	**12**	**24**
Total	**0.177** 0.135	**0.102** 0.389	**0.221** 0.060	**0.331** 0.004	**0.255** 0.029	**0.227** 0.053	**-0.067** 0.571	**-0.145** 0.222	**0.032** 0.787	**0.275** 0.019	**0.321** 0.006	**0.189** 0.109
TRL	**0.706** <0.001	**0.489** <0.001	**0.486** <0.001	**0.254** 0.030	**0.146** 0.219	**0.055** 0.643	**0.078** 0.511	**-0.035** 0.771	**-0.027** 0.819	**-0.203** 0.086	**-0.041** 0.732	**-0.141** 0.233
Total LDL	**0.208** 0.078	**0.128** 0.281	**0.265** 0.024	**0.440** <0.001	**0.326** 0.005	**0.343** 0.003	**0.058** 0.626	**-0.058** 0.625	**0.192** 0.103	**0.144** 0.225	**0.148** 0.211	**0.056** 0.636
Total HDL	**0.005** 0.970	**-0.020** 0.864	**0.060** 0.612	**0.169** 0.154	**0.173** 0.144	**0.114** 0.339	**-0.194** 0.099	**-0.208** 0.078	**-0.104** 0.381	**0.417** <0.001	**0.469** <0.001	**0.345** 0.003
LDL1	**0.342** 0.003	**0.067** 0.575	**0.177** 0.134	**0.059** 0.621	**0.041** 0.731	**-0.004** 0.972	**-0.047** 0.691	**-0.038** 0.747	**-0.042** 0.726	**-0.057** 0.630	**0.221** 0.060	**-0.071** 0.553
LDL2	**0.276** 0.018	**0.171** 0.148	**0.273** 0.019	**0.306** 0.008	**0.102** 0.391	**0.134** 0.259	**0.032** 0.791	**0.004** 0.973	**0.015** 0.897	**0.160** 0.177	**0.204** 0.083	**0.044** 0.709
LDL3	**0.161** 0.175	**0.126** 0.288	**0.233** 0.047	**0.351** 0.002	**0.256** 0.029	**0.339** 0.003	**0.030** 0.802	**-0.003** 0.978	**0.169** 0.153	**0.222** 0.059	**0.213** 0.070	**0.143** 0.226
LDL4	**-0.074** 0.534	**-0.069** 0.559	**0.082** 0.492	**0.366** 0.001	**0.300** 0.010	**0.327** 0.005	**0.011** 0.925	**-0.055** 0.641	**0.165** 0.162	**0.295** 0.011	**0.220** 0.061	**0.200** 0.090
LDL5	**0.470** <0.001	**0.346** 0.003	**0.394** 0.001	**0.472** <0.001	**0.347** 0.003	**0.337** 0.004	**0.125** 0.293	**-0.075** 0.527	**0.237** 0.043	**-0.112** 0.346	**-0.074** 0.536	**-0.132** 0.267
HDL2b	**-0.008** 0.949	**-0.025** 0.833	**0.029** 0.806	**0.200** 0.090	**0.205** 0.081	**0.130** 0.274	**-0.188** 0.110	**-0.208** 0.077	**-0.117** 0.325	**0.511** <0.001	**0.571** <0.001	**0.464** <0.001
HDL2a	**-0.122** 0.305	**-0.080** 0.499	**-0.053** 0.658	**0.116** 0.326	**0.163** 0.169	**0.078** 0.510	**-0.216** 0.066	**-0.183** 0.121	**-0.139** 0.243	**0.533** <0.001	**0.583** <0.001	**0.472** <0.001
HDL3a	**0.038** 0.752	**-0.022** 0.856	**0.066** 0.580	**0.136** 0.252	**0.119** 0.316	**0.085** 0.473	**-0.154** 0.194	**-0.148** 0.211	**-0.084** 0.479	**0.333** 0.004	**0.361** 0.002	**0.250** 0.033
HDL3b	**0.113** 0.342	**0.034** 0.775	**0.179** 0.130	**0.157** 0.185	**0.119** 0.315	**0.102** 0.393	**-0.135** 0.255	**-0.197** 0.094	**-0.052** 0.659	**0.148** 0.212	**0.191** 0.105	**0.067** 0.575
HDL3c	**0.089** 0.453	**0.079** 0.506	**0.177** 0.135	**0.120** 0.313	**0.148** 0.212	**0.126** 0.288	**-0.196** 0.096	**-0.255** 0.029	**-0.011** 0.927	**0.054** 0.649	**0.114** 0.336	**-0.010** 0.936

Values presented are p-values, p. N=73. Colored squares indicate a significant correlation, p<0.05. Blue indicates a positive correlation, and red indicates a negative correlation. Area under the density profile curve, AUC. Triglycerides, TAG. Low-density lipoprotein, LDL. High-density lipoprotein, HDL. Triglyceride-rich lipoproteins, TRL. Two samples from NED were removed prior to running Pearson's correlation for Table 4.18 due to the presence of outliers.

As shown in Table 4.20, serum triglycerides were related positively with TRL and negatively with LDL4. Triglycerides (mg/dL), total- (mg/dL), and LDL-cholesterol (mg/dL) were similarly, positively related with LDL5 and negatively with HDL, HDL2a, and HDL3a. Total- and LDL-cholesterol were positively related with LDL, LDL3, and LDL4 while total- and HDL-cholesterol were related negatively with HDL3c. HDL-cholesterol was the only blood lipid measure that was negatively related with HDL3a, HDL3b, and HDL3c as percent of total AUC at all three time points.

High-density lipoprotein cholesterol (mg/dL) expressed a relationship with lipoproteins and respective subclasses that contrasted with that of triglycerides, total-, and LDL-cholesterol.

For example, while triglycerides were positively related with TRL, HDL-cholesterol was negatively related with TRL ($p<0.05$), and while total- and LDL-cholesterol were related positively with LDL and LDL5, HDL-cholesterol was related negatively with LDL and LDL5 ($p<0.05$), and finally, while triglycerides, total-, and LDL-cholesterol were related negatively with HDL and larger HDL subclasses, HDL-cholesterol was related positively with HDL, HDL2b, and HDL2a ($p<0.05$) (Table 4.20).

Table 4.20: Correlations for triglycerides, total-, LDL-, and HDL-cholesterol with TRL, LDL, HDL, and their respective subfraction areas expressed as percentage of total, LDL, and HDL area under the density profile curve (AUC), respectively

	Triglycerides (mg/dL)			Total Cholesterol (mg/dL)			LDL Cholesterol (mg/dL)			HDL Cholesterol (mg/dL)		
Weeks	**0**	**12**	**24**	**0**	**12**	**24**	**0**	**12**	**24**	**0**	**12**	**24**
%TRL of Total AUC	**0.768** <0.001	**0.588** <0.001	**0.568** <0.001	**0.107** 0.367	**0.011** 0.928	**-0.123** 0.300	**0.238** 0.042	**0.149** 0.208	**-0.043** 0.717	**-0.436** <0.001	**-0.348** 0.003	**-0.406** <0.001
%LDL of Total AUC	**0.144** 0.224	**0.110** 0.353	**0.215** 0.068	**0.424** <0.001	**0.265** 0.024	**0.442** <0.001	**0.254** 0.030	**0.087** 0.463	**0.460** <0.001	**-0.221** 0.060	**-0.352** 0.002	**-0.268** 0.022
%HDL of Total AUC	**-0.420** <0.001	**-0.370** 0.035	**-0.444** <0.001	**-0.418** <0.001	**-0.240** 0.041	**-0.326** 0.005	**-0.319** 0.006	**-0.149** 0.207	**-0.377** 0.001	**0.355** 0.002	**0.473** <0.001	**0.407** <0.001
%LDL1 of Total AUC	**0.229** 0.051	**0.065** 0.582	**0.116** 0.327	**-0.143** 0.253	**-0.089** 0.452	**-0.181** 0.126	**0.064** 0.590	**0.131** 0.270	**-0.036** 0.765	**-0.241** 0.040	**-0.008** 0.949	**-0.282** 0.016
%LDL2 of Total AUC	**0.208** 0.077	**0.096** 0.417	**0.071** 0.551	**0.080** 0.502	**-0.190** 0.108	**-0.108** 0.364	**0.277** 0.018	**0.276** 0.018	**-0.011** 0.930	**-0.106** 0.372	**-0.133** 0.261	**-0.127** 0.283
%LDL3 of Total AUC	**0.075** 0.526	**0.015** 0.900	**0.061** 0.608	**0.256** 0.029	**0.085** 0.473	**0.292** 0.012	**0.238** 0.043	**0.226** 0.055	**0.273** 0.019	**0.026** 0.826	**-0.060** 0.613	**0.031** 0.795
%LDL4 of Total AUC	**-0.400** <0.001	**-0.274** 0.019	**-0.183** 0.122	**0.189** 0.109	**0.168** 0.156	**0.339** 0.003	**0.023** 0.845	**-0.013** 0.911	**0.301** 0.010	**0.120** 0.311	**-0.062** 0.603	**0.086** 0.469
%LDL5 of Total AUC	**0.548** <0.001	**0.443** <0.001	**0.457** <0.001	**0.392** 0.001	**0.256** 0.029	**0.297** 0.011	**0.240** 0.041	**-0.020** 0.865	**0.312** 0.007	**-0.425** <0.001	**-0.439** 0.001	**-0.415** <0.001
%HDL2b of Total AUC	**-0.232** 0.048	**-0.176** 0.138	**-0.271** 0.020	**-0.086** 0.471	**0.042** 0.722	**-0.068** 0.568	**-0.260** 0.026	**-0.162** 0.171	**-0.243** 0.039	**0.529** <0.001	**0.614** <0.001	**0.582** <0.001
%HDL2a of Total AUC	**-0.466** <0.001	**-0.355** 0.002	**-0.458** <0.001	**-0.295** 0.011	**-0.125** 0.293	**-0.190** 0.106	**-0.288** 0.013	**-0.077** 0.520	**-0.269** 0.021	**0.500** <0.001	**0.590** <0.001	**0.552** <0.001
%HDL3a of Total AUC	**-0.322** 0.005	**-0.341** 0.003	**-0.400** 0.000	**-0.491** <0.001	**-0.372** 0.001	**-0.382** 0.001	**-0.145** 0.221	**0.062** 0.603	**-0.272** 0.020	**0.089** 0.456	**0.095** 0.423	**0.104** 0.380
%HDL3b of Total AUC	**-0.147** 0.214	**-0.166** 0.159	**-0.124** 0.294	**-0.410** <0.001	**-0.330** 0.004	**-0.385** 0.001	**-0.090** 0.448	**-0.071** 0.550	**-0.249** 0.034	**-0.284** 0.015	**-0.243** 0.039	**-0.329** 0.004
%HDL3c of Total AUC	**-0.138** 0.244	**-0.034** 0.778	**-0.012** 0.920	**-0.295** 0.011	**-0.156** 0.188	**-0.235** 0.045	**-0.210** 0.075	**-0.249** 0.033	**-0.176** 0.135	**-0.281** 0.016	**-0.231** 0.050	**-0.376** 0.001
%LDL1 of LDL AUC	**0.181** 0.125	**0.021** 0.858	**0.052** 0.661	**-0.262** 0.025	**-0.156** 0.186	**-0.320** 0.006	**-0.012** 0.921	**0.109** 0.360	**-0.174** 0.142	**-0.148** 0.213	**0.106** 0.374	**-0.187** 0.113
%LDL2 of LDL AUC	**0.109** 0.358	**0.035** 0.769	**-0.036** 0.761	**-0.194** 0.101	**-0.300** 0.010	**-0.313** 0.007	**0.120** 0.314	**0.230** 0.050	**-0.225** 0.056	**0.043** 0.719	**0.038** 0.748	**-0.025** 0.831
%LDL4 of LDL AUC	**-0.669** <0.001	**-0.503** 0.001	**-0.430** <0.001	**-0.104** 0.381	**0.015** 0.902	**0.120** 0.312	**-0.201** 0.088	**-0.118** 0.321	**0.052** 0.664	**0.334** 0.004	**0.187** 0.113	**0.327** 0.005
%LDL5 of LDL AUC	**0.609** <0.001	**0.486** <0.001	**0.471** <0.001	**0.224** 0.057	**0.177** 0.135	**0.119** 0.315	**0.142** 0.230	**-0.081** 0.498	**0.112** 0.347	**-0.392** 0.001	**-0.309** 0.008	**-0.336** 0.004
%HDL2b of HDL AUC	**0.003** 0.982	**0.003** 0.982	**-0.083** 0.485	**0.172** 0.145	**0.200** 0.090	**0.119** 0.318	**-0.120** 0.313	**-0.110** 0.356	**-0.082** 0.491	**0.462** <0.001	**0.534** <0.001	**0.551** <0.001
%HDL2a of HDL AUC	**-0.435** <0.001	**-0.286** 0.014	**-0.401** 0.000	**-0.140** 0.238	**-0.022** 0.857	**-0.050** 0.672	**-0.177** 0.134	**0.013** 0.915	**-0.122** 0.305	**0.531** <0.001	**0.576** <0.001	**0.584** <0.001
%HDL3a of HDL AUC	**0.049** 0.681	**-0.076** 0.523	**-0.020** 0.865	**-0.170** 0.151	**-0.269** 0.022	**-0.129** 0.278	**0.207** 0.079	**0.275** 0.019	**0.092** 0.438	**-0.314** 0.007	**-0.442** 0.000	**-0.368** 0.001
%HDL3b of HDL AUC	**0.228** 0.052	**0.140** 0.236	**0.271** 0.021	**-0.004** 0.976	**-0.098** 0.411	**-0.051** 0.670	**0.164** 0.166	**0.032** 0.791	**0.090** 0.450	**-0.546** <0.001	**-0.582** <0.001	**-0.620** <0.001
%HDL3c of HDL AUC	**0.159** 0.179	**0.199** 0.092	**0.281** 0.016	**0.014** 0.907	**0.039** 0.742	**0.029** 0.808	**0.004** 0.975	**-0.120** 0.313	**0.090** 0.447	**-0.467** <0.001	**-0.442** <0.001	**-0.546** <0.001

Each square provides r from Pearson's correlation shown as the top/upper value in bold accompanied by its respective p-value, p. N=73. Colored squares indicate a significant correlation, $p<0.05$. Blue indicates a positive correlation, and red indicates a negative correlation. Area under the density profile curve, AUC. Triglycerides, TAG. Low-density lipoprotein, LDL. High-density lipoprotein, HDL. Triglyceride-rich lipoproteins, TRL.Two samples from NED were removed prior to running Pearson's correlation for Table 4.19 due to the presence of outliers.

CHAPTER V

SUMMARY AND CONCLUSION

Summary

The primary purpose of this study was to evaluate the effects of three hypo-energetic diets varying in macronutrient intake combined with a resistance-based circuit training program on lipoproteins and their respective subclasses. Additionally, this study analyzed the strength of the relationship between LDL and HDL lipoprotein subclasses with parameters of interest (e.g., anthropometrics, body composition, cardiovascular fitness, glucose homeostasis, and blood lipids. Despite observing significant changes in body weight and composition and general improvement in blood lipids and markers of glucose homeostasis in response to diet and exercise intervention, there was no significant difference in LDL or HDL AUC nor were there significant changes in their respective subclasses after implementation of diet and exercise. Correlative data show that improvements in parameters of anthropometrics, body composition, cardiovascular fitness, glucose homeostasis, and blood lipids were related positively with smaller LDL and HDL subclasses and negatively with larger LDL and HDL subclasses.

Primary Aim: Modification of Lipoprotein Subclasses

This section addresses our primary aim discussing whether we observed changes to lipoproteins and their respective subclasses based on group assignment. All diet and exercise interventions in this study promoted significant changes in weight and fat loss and improved VO_{2peak}. Our study showed that a higher protein diet combined with circuit-type, resistance training was more effective at reducing total- and LDL-cholesterol. Subjects, assigned to diet and exercise regimens that included a higher protein intake, experienced significant reductions in LDL cholesterol (mg/dL). This aligns with previously reported findings from our lab that

increasing protein intake may offer additional benefits when subjects adhere to a hypo-energetic diet with resistance-based circuit training [10, 114]. Considering the significant changes in clinical measures for LDL cholesterol observed in the previous investigation by Sanchez, et al.[12] and in this study where LDL-cholesterol for MCHP and LCHP decreased (-52.78 ±12.26 mg/dL, $p<0.01$ and -28.93 ±10.70 mg/dL, $p=0.009$, respectively), this variable showed promise for observing significant changes to lipoprotein subclasses.

Considering lipoprotein analysis, Total AUC for all groups was maintained at 12 and 24 weeks with no significant time or group effects. There were no significant effects on lipoproteins or their respective subclasses expressed as AUC or percent of AUC. Apart from a significant time effect for LDL1, the implementation of a resistance-based circuit training program and hypo-energetic diet did not have a significant effect on LDL subclasses. At 24 weeks, subjects assigned to no exercise or diet, and to the higher, and lower carbohydrate diet experienced a statistically significant increase in LDL1. The percent change for LDL1 as percent of total AUC (Figure 4.10) for no exercise or diet (+36.3±45.3%, $p=0.01$) and the lower carbohydrate diet (+49.6±88.4%, $p<0.001$) was significant at 24 weeks while that of subjects adhering to the higher (+25.4±52.1%, $p=0.08$) and moderate (+25.7±40.3%, $p=0.098$) carbohydrate diet failed to reach statistical significance. We cannot attribute the significant changes observed to the implementation of diet and exercise alone since subjects assigned to no exercise or diet experienced significant changes. Although we found differences ($p<0.05$) between diet and exercise groups for LDL2 and LDL4 at 12 and/or 24 weeks, differences between groups were present at baseline. For instance, values for LDL2 as percent of total AUC (Figure 4.11) for all diet and exercise groups were greater than the no exercise or diet group at 0, 12, and 24 weeks with all groups reaching significance at 12 weeks; however, when considering percent change for

LDL2 (Figure 4.12), only HCLF was significant at 24 weeks. Similarly, percent change for LDL4 was not significant at 12 or 24 weeks. As previously mentioned, Pedersen et al., [1] reported that subjects assigned to a low energy diet experienced a significant reduction in Total LDL, LDL2, LDL4, and LDL5 ($p<0.05$) while those assigned to aerobic interval training experienced a significant reduction in Total LDL, LDL2, LDL3, LDL4, and LDL5 ($p<0.05$) [1]. We would expect that adherence to a combined hypo-energetic diet and exercise program would have a significant effect on LDL subclasses, but this did not occur. Thus, we reject hypothesis 1 that significant changes to LDL subclasses would be observed among subjects assigned to diet and exercise. Notably, higher protein diet groups did experience significant negative percent changes from baseline for LDL as percent of total AUC.

In this study, all subjects assigned to a hypo-energetic diet with a resistance-based circuit training program experienced a preservation of HDL cholesterol levels in spite of significant reductions in weight, fat mass, and percent body fat, which is noteworthy given that research has shown that reductions in HDL-cholesterol levels often accompany weight loss [88]. In contrasts to findings from Layman et al. [59], who found that HDL-cholesterol levels were preserved only among higher protein diet groups, we did not find significant differences among diet and exercise groups to support that a higher carbohydrate or protein intake was more favorable for maintaining HDL-cholesterol. Differences between Layman et al.'s [59, 121] and our own study may partially explain results. Subjects assigned to the higher carbohydrate and higher protein diets in Layman et al. [59, 121] consumed 0.8 g PRO/kg/day and 1.6 g PRO/kg/day, respectively; while subjects assigned to higher, moderate, and lower carbohydrate hypo-energetic diets consumed 1.0±0.1, 1.2±0.1, and 1.4±0.08 g PRO/kg/day, respectively. The mean average protein intake for our study varied among groups, and only that of subjects following a lower

carbohydrate diet had a significantly greater protein intake than that of no exercise or diet and the higher carbohydrate diet at 24 weeks (+0.46 g PRO/kg/day, p=0.023 and +0.42 g/PRO/kg/day, p=0.037, respectively). Exercise type/mode was another differing factor. Participants in Layman et al. [59, 121] completed 30 minutes of walking, five days each week [59]; whereas, subjects in our study completed a resistance-based circuit training program for 30 minutes, four days a week, and were asked to complete 10,000 steps on non-training days. Our findings regarding HDL-cholesterol agree with findings from a review of previous studies from our lab concluding that an energy restricted diet combined with exercise had no significant effect on HDL cholesterol regardless of macronutrient assignment [10]. The change in HDL as percent of total AUC at 24 weeks was non-significant for NED and all diet and exercise groups (NED: +3.6±14.7, p=0.35; MCHP: +1.3±13.9, p=0.74; LCHP: +2.1 ±16.6, p=0.57; and HCLF: -0.57±19.36, p=0.88). At 24 weeks, the only notable significant effect on HDL subclasses was a reduction in HDL3a as percent of total HDL AUC for the moderate carbohydrate group at 24 weeks from its respective 0- and 12-week values. In Pedersen et al., when diet and exercise effects were considered separately, HDL as percent of total lipoprotein, was significantly increased for subjects assigned to a hypo-energetic diet and for those assigned to aerobic interval training (2.8% and 2.2%, respectively), and there was a reduction in smaller HDL subclasses that was more significant for subjects adhering to a low energy diet compared to those complying with aerobic interval training. Retention of baseline HDL levels in our study could be due to adherence to a reduced calorie diet in which fat intake was not significantly reduced from baseline values (shown in Tables 4.2 and 4.3) where low fat diets are linked to reductions in HDL-cholesterol [82], and to participation in a resistance-based circuit training program. This would support the idea that combining diet and exercise that incorporates aerobic

and resistance exercise [115] proved more beneficial than adopting either of these lifestyle changes alone [10, 122].

Significant effects of a hypo-energetic diet and exercise on fasting LDL-cholesterol (mg/dL) appear to be influenced by macronutrient assignment favoring higher protein intakes while the effects on HDL-cholesterol (mg/dL) indicate that diet, regardless of macronutrient assignment, preserved HDL-cholesterol levels, which aligns with previous investigations from our lab [10, 11, 13, 114]. These results agree with studies conducted outside of our lab. For instance, in a meta-analysis of 12 studies focusing on the effects of an energy restricted diet combined with aerobic exercise (50 to 90% maximum heart rate), showed that no significant change in HDL-cholesterol occurred despite significant improvements in triglycerides total cholesterol, LDL-cholesterol, and the total to HDL-cholesterol ratio [97]. Overall, for our study, we observed that the effects of a hypo-energetic diet and exercise on LDL and HDL subclasses were non-significant. Thus, we reject hypothesis 2 that significant changes to HDL subclasses would be observed among subjects assigned to diet and exercise.

Secondary Aims: Correlations

We found significant relationships between parameters of interest (e.g., energy intake, protein intake, carbohydrate intake, fat intake, weight, fat mass, percent body fat, VAT area, waist circumference, hip circumference, waist-to-hip ratio, VO_{2peak}, time to exhaustion, glucose, insulin, HOMA, triglycerides, total cholesterol, LDL cholesterol, HDL cholesterol) and lipoprotein subclasses. The results from these correlations will be discussed further in the following paragraphs.

Macronutrient Intake

For this population of overweight and/or obese women, reductions in energy intake

should induce reductions in body weight and in turn reduce risks to CVD by promoting healthy weight; however, we found that energy (kcal/kg/day), carbohydrate (g/kg/day), and fat intake (g/kg/day) were positively related with HDL and larger HDL subclasses, HDL2b and HDL2a, ($p<0.05$) (Table 4.11). Energy and carbohydrate intake were related negatively with LDL2, LDL5, HDL3a, and/or HDL3b ($p<0.05$) while fat intake was related negatively with total LDL, LDL2, HDL3a, HDL3b, and HDL3c ($p<0.05$). These significant relationships were observed primarily at baseline prior to the implementation of a hypo-energetic diet with exercise, with few exceptions (e.g., positive relationships between energy and carbohydrate intake with HDL2a at 24 weeks).

Higher energy and carbohydrate intake corresponding with larger HDL subclasses (Table 4.10) contrasts with our hypothesis and results from Parlesak et al.[123], who found that the carbohydrate intake among 265 healthy working male subjects was negatively related with components of HDL (e.g. cholesterol, phospholipids, and apoA1) and HDL2 (e.g. cholesterol, phospholipids, apoA1, and apoA2). The samples from our own and Parlesak et al.'s [123] investigation differed in size, gender, and activity level. In accordance with our findings, an epidemiology report including men that participated in the Framingham Study, the Honolulu Heart Study, and the Puerto Rico Heart Health Program, participants were less likely to developed myocardial infarction and coronary heart disease if they had a higher energy intake (kcal/kg). Furthermore, results from the Honolulu Heart Study and Puerto Rico Heart Health Program (not including the Framingham Study) elucidated a direct relationship between a high starch intake and a lower incidence of CVD [124]. It has been suggested that the physical activity of these subjects may provide an explanation where subjects consuming a higher carbohydrate diet may also be physically active and may not experience the same negative

effects as their inactive counterparts due to the beneficial effects of participating regularly in physical activity [124].

Protein intake showed no significant relationship with lipoprotein subclasses at 0 weeks. Significant relationships were observed after the implementation of diet and exercise. At 12 weeks, protein intake (g/kg/day) was related positively with HDL2b as percent of total AUC (+0.29, p=0.012) and as percent of total HDL AUC (+0.34, p=0.003) and negatively with HDL3a and HDL3b as percent of total HDL AUC (r= -0.30, p=0.008 and r= -0.27, p=0.02, respectively). At 24 weeks, protein intake was related positively with LDL1 as percent of total AUC (0.32, p=0.005) and as percent of total LDL AUC (0.37, p=0.001). These observations align with studies in the literature reporting a positive relationship between protein intake and larger LDL and HDL subclasses [124-126].

Overall, the presence of significant correlations appears to be related to the implementation of diet and exercise. For instance, the implementation of diet and exercise ameliorated the significant relationships observed between lipoprotein subclasses with energy, carbohydrate, and fat intake. These relationships were significant primarily at baseline and were less apparent or absent at 12 and 24 weeks. In contrast, the significant relationships observed between lipoprotein subclasses with protein intake occurred primarily after the implementation of diet and exercise.

Anthropometrics

As shown in Figure 4.12, weight, waist, and hip circumference were significantly related positively with LDL, LDL5, and smaller HDL subclasses and negatively with HDL and larger HDL2 subclasses. Thus, we fail to reject hypothesis 4 that significant correlations between lipoprotein subclasses and anthropometrics would be observed.

Although Parlesak, et al. [123] considered BMI instead of weight in his investigation, he found that BMI was related positively with an increase in smaller LDL and a decline in larger LDL while it was related negatively with HDL cholesterol and components of HDL2 (e.g. cholesterol, phospholipids, ApoA1, and ApoA2). In our study, waist circumference (cm) was positively related with HDL3c as percent of total HDL AUC at 12 and 24 weeks ($r=0.267$, $p=0.021$ and $r=0.239$, $p=0.039$, respectively). Similar to our own findings, Rosenbaum et al. [127], found that waist circumference ($r=0.479$, $p<0.001$) was positively related with HDL3c. Additionally, waist circumference, BMI, triglyceride levels, HDL-cholesterol, HOMA-IR, and fasting blood glucose ($r=+0.260$, $p=0.06$; $r=+0.241$, $p=0.011$; $r=-0.383$, $p<0.001$; $r=+0.225$, $p=0.049$, and $r=+0.240$, $p=0.012$, respectively), expressed the strongest correlation with HDL3c and was a strong independent determinant of smaller, denser lipoproteins [127].

Body Composition

Fat mass, fat free mass, and VAT area were similarly related positively with smaller LDL and HDL subclasses and negatively with total HDL and larger HDL subclasses. Thus, we fail to reject hypothesis 5 stating that there would be significant correlations between lipoprotein subclasses and body composition.

In our study we observed that VAT area and fat mass had a significant positive relationship with LDL5 ($r=+0.460$, $p<0.001$ and $r=+0.230$, $p=0.047$, respectively) and negative relationship with larger HDL subclasses, HDL2b ($r=-0.386$, $p=0.001$ and $r=-0.304$, $p=0.008$, respectively) and HDL2a ($r=-0.473$, $p<0.001$ and $r=-0.324$, $p = 0.005$, respectively) at 24 weeks. Pedersen et al. [1] did not observe a significant relationship between decreased VAT area and LDL particle size ($R^2=0.02$, $p=0.467$); however, his investigation found a significant relationship between fat mass and LDL particle size ($R^2=0.06$, $p=0.045$) [1]. Furthermore, Pedersen et al. [1]

reported that decreased values for VAT area and fat mass were significantly related with increased HDL particle size (R^2=0.37, p<0.001 and R^2=0.13, p=0.004, respectively) [1].

Research has found a strong direct relationship between central adiposity and CVD; thus, VAT area was a parameter of interest in this study as was waist circumference, which was previously described. Unlike waist circumference, VAT area expressed a significant positive relationship with TRL, but apart from this difference, we found similarities between parameters of anthropometrics and body composition. For example, VAT area, waist circumference, and waist to hip ratio were related positively with LDL5, HDL3b, and HDL3c and negatively with HDL2b and HDL2a. As research has shown, increasing weight, fat mass, and central adiposity are related with a lipid profile expressing a larger proportion of smaller lipid subclasses.

Cardiorespiratory Fitness

All diet and exercise groups significantly improved their VO_{2peak} (L/min and ml/kg/min), and time to exhaustion at 12 and 24 weeks with no significant differences between the higher carbohydrate and higher protein groups. For our study, we expected to find that increases in cardiovascular fitness would correlate with improvements in lipid subclasses, and as expected, VO_{2peak} (ml/kg/min), and time to exhaustion were related positively with LDL1, HDL2b, and HDL2a and negatively with, LDL5, HDL3a, and HDL3b. The relationship between VO_{2peak} and HDL cholesterol has been elucidated upon comparison of trained athletes to their untrained counterparts [94]. In athletes VO_{2peak} was positively related with HDL2 regardless of sport [108]. Although our population differed in that it included untrained, overweight/obese women, we found similar relationships between improvements in physical fitness and lipid subclasses. Parlesak et al., reported in a study that included healthy working men that physical activity was negatively related with components of small, dense LDL (e.g. triglycerides,

cholesterol, and apoB) and with the small, dense LDL to HDL2 cholesterol ratio; conversely, physical activity was positively related with components associated with HDL (e.g. cholesterol, phospholipids, and apoA1) [123]. Thus, we failed to reject hypothesis 6 that significant correlations between lipoprotein subclasses and cardiovascular fitness would be observed.

Significant relationships between VO_{2peak}, and time to exhaustion with lipid subclasses were not present at baseline and were observed after diet and exercise intervention. Further investigation was necessary to discern whether group assignment influenced the observed relationships between VO_{2peak} and lipoprotein subclasses. The significant relationships between VO_{2peak} and lipid subclasses based on group assignment (Figure 4.16), elucidated that subjects assigned to no exercise or diet and those assigned to the lower carbohydrate diet were responsible for the observed significant relationships present at 12 and 24 weeks. While adherence to no exercise or diet was positively related with HDL2b and HDL2a subclasses and negatively with the HDL3b subclass, adherence to the lower carbohydrate diet was positively related with the HDL2b subclass and negatively with HDL3a and HDL3b subclasses. Since we find these significant correlations among subjects assigned to no exercise or diet, we cannot attribute the occurrence of these significant correlations to diet and exercise.

Glucose Homeostasis

We found that insulin, HOMA-IR, and glucose were positively related with LDL5 (r=+0.32, p=0.01; r=+0.33, p=0.01; r=+0.29, p=0.01, respectively) and negatively with HDL2a as percent of total AUC (r=-0.26 p=0.03; r=-0.26, p=0.03; r=-0.25, p=0.03, respectively) and as percent of total HDL AUC (correlative data not shown), primarily at 12 weeks for glucose and at 0 weeks for insulin and HOMA-IR. These results support the research considering the relationship between insulin resistance and CVD. For example, Goff et al., [128] who took

1,371 men and women diagnosed with type 2 diabetes, found that lower values of insulin resistance were associated positively with LDL size ($r=+0.34$, $p<0.001$), large LDL ($r=+0.21$, $p<0.001$), HDL cholesterol ($r=+0.37$, $p<0.001$), HDL size ($r=+0.33$, $p<0.001$), and large HDL ($r=+0.31$, $p<0.001$) and negatively with small LDL ($r=-0.34$, $p<0.001$) and intermediate LDL ($r=-0.37$, $p<0.001$) [128]. Considering the diabetic population, insulin resistance was related with small LDL and inversely related with large HDL [128]. Our study made similar observations showing that baseline values for insulin and HOMA-IR were positively related with small LDL and negatively with large HDL. Thus, we failed to reject hypothesis 7 stating that significant correlations between lipoprotein subclasses and markers of insulin resistance would be observed. The relationships between lipoprotein subclasses and markers of insulin resistance provides some support for the connection between CVD and diabetes. Interestingly, at 24 weeks these correlations were absent or less frequent for insulin and HOMA-IR, respectively. This observation may be due to improvements in these parameters, a change that occurred for subjects adhering to higher, moderate, and lower carbohydrate diets.

Blood Lipids

There were significant relationships observed between clinical measures for triglycerides, total-, LDL-, and HDL-cholesterol and data derived from isopycnic ultracentrifugation technique for total, TRL, LDL, HDL and their respective subclasses expressed as AUC (Table 4.19) and with lipoproteins expressed as %AUC (Table 4.20).

Triglycerides (mg/dL) were related positively with TRL expressed as AUC and as percent of total AUC at 0, 12, and 24 weeks. We expect this relationship because triglycerides are found primarily in TRLs, primarily VLDL [129]. Triglycerides were additionally related positively LDL5. Total cholesterol (mg/dL) was related positively with LDL and LDL subclasses

expressed as AUC (LDL2-LDL5) and as percent of total AUC (LDL3-LDL5) and negatively with HDL as percent of total AUC, an observation that alludes to the function of LDL as the primary carrier of cholesterol to extrahepatic tissues [130]. We found that both LDL- and HDL-cholesterol were related with corresponding lipoproteins. LDL cholesterol (mg/dL) was related positively with LDL as percent of total AUC at 0 and 24 weeks and with LDL subclasses (LDL2-LDL5) while HDL cholesterol (mg/dL) was related positively with HDL and larger HDL subclasses expressed as AUC (HDL2b, HDL2a, and HDL3a) and as percent of total AUC (HDL2b and HDL2a) at 0, 12, and 24 weeks. Total AUC was related positively with both total- and HDL-cholesterol.

Similar to Rosenbaum et al. [127], who showed that HDL-cholesterol was negatively related with HDL3c (% of HDL), we found that HDL-cholesterol was negatively related with smaller HDL subclasses, namely HDL3a, HDL3b, and HDL3c each as percent of total HDL AUC at 0, 12, and 24 weeks and we additionally found that HDL-cholesterol was positively related with larger HDL subclasses, HDL2b and HDL2a, expressed as AUC and as percent of total HDL AUC. Unlike Rosenbaum, who showed that HDL-cholesterol was negatively related to LDL4 (% of LDL) [127], we found that HDL-cholesterol was positively related with LDL4 as percent of total LDL AUC and negatively related with LDL5 as percent of total LDL AUC. Rosenbaum's study differed from our own in that it included men and women, subjects with metabolic syndrome, and analyzed lipoprotein subclasses using gradient gel electrophoresis.

Interestingly, we observed an inverse relationship between triglycerides (mg/dL) and HDL cholesterol as percent of total AUC at 0, 12, and 24 weeks (as shown in Figure 4.18), which is a relationship that has been noted in several studies previously described [18, 60, 64, 69, 98]. Considering these observations, we fail to reject hypothesis 8 stating that significant

correlations between lipoprotein subclasses and blood lipids would be observed, respectively.

Lipoproteins

There were some additional expected observations upon comparison of lipid subclasses with each other. Larger HDL subclasses, HDL2a and HDL2b, were related positively with larger LDL subclasses, LDL1 and LDL2, and negatively with the smaller, denser LDL subclass, LDL5. Upon comparing lipoproteins within their respective subclass, larger HDL subclasses were related positively with each other and negatively with smaller HDL subclasses. For example, HDL2b of total HDL AUC was related positively with HDL2a and negatively with HDL3a, HDL3b, and HDL3c at 0, 12, and 24 weeks. Although this comparison has not been reported in other studies from our lab, researchers in the field of lipidology describe "shifts" in the lipoprotein profile to indicate that decreases in smaller LDL and HDL occur with corresponding increases in larger LDL and HDL, respectively [1, 50, 127]. Furthermore, intercorrelation mapping of HDL2 and small, dense LDL indicate that the components (e.g. cholesterol, phospholipids, triglycerides, and apolipoproteins) of HDL2 are negatively related with small dense LDL [123].

Limitations

Correlations were generated from pooling all 75 subjects, allowing for the identification of positive and negative correlations between variables regarding health outcome with lipoproteins and their subfraction areas. These correlations were not visible when individual groups (n size ranging from 16 to 21 subjects per group) were analyzed. Once subjects from all groups were pooled into a group of 75 individuals, there were more significant relationships between parameters of interest and lipoprotein subfraction areas. This was noted when conducting correlation analysis including cardiovascular fitness and lipoprotein subclasses. We

found that the direct relationship between cardiovascular fitness with HDL and larger HDL subclasses was most evident when all subjects were included in the analysis.

As with all human studies, compliance is a limitation. Although subjects met with a registered dietitian and/or exercise physiologist and were given menus to follow, it is difficult to assess compliance given our dependence on the information provided in food logs, which may or may not be an accurate record of dietary intake.

Conclusions

Similar to findings in a review of previous research from our lab [10-13, 114], all subjects assigned to diet and exercise reduced risks to CVD (e.g. reduction in weight, waist circumference, and VAT area) irrespective of macronutrient assignment; however, assignment to a higher protein diet provided an additional benefit by decreasing LDL-cholesterol (mg/dL). Data regarding lipoprotein subclasses were not reported in previous studies conducted in this lab; thus, the primary purpose of this study was to investigate the effects of implementing a hypo-energetic diet, varying in macronutrient intake, with exercise on lipoproteins and their respective subclasses, and the secondary purpose of this study was to report significant correlations between parameters of interest, namely markers of CVD risks, with lipoprotein subclasses. Considering quantitative (AUC) and qualitative data (% AUC) from the analysis of lipoproteins and their respective subfraction areas, we did not find evidence that group assignment or implementation of a diet and a resistance-based circuit training program altered LDL or HDL subclasses.

The significant correlations reported from statistical analysis from our investigation align with relationships observed in other studies where risk factors to CVD were significantly related with smaller LDL and HDL subclasses [1, 123]. Health parameters including weight, waist circumference, fat mass, VAT area, glucose, insulin, HOMA-IR, total cholesterol, and LDL-

cholesterol were related positively with smaller LDL and HDL subclasses and negatively with larger LDL and HDL subclasses while increases in VO_{2peak}, time to exhaustion, and HDL-cholesterol were similarly related positively with larger LDL and HDL subclasses and negatively with smaller LDL and HDL subclasses.

Research Recommendations

A better understanding of the effects of diet and exercise on lipoproteins can help clinicians prescribe the most effective changes for the prevention and treatment of CVD; thus, studies should focus on designing and reporting data that allows for a consistent interpretation of results across studies. Although clinical tests used to measure LDL and HDL cholesterol help doctors and patients assess risks to CVD, it is not always a complete representation of CVD risks. There is a need for research that examines the relationship of exercise, energy intake, and macronutrient partitioning on blood lipids and lipoprotein subclasses in individuals with normal and abnormal blood lipids.

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