The Effects Of Modifiable Lifestyle Behaviors On Lipoprotein Particle Concentration And Size

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THE EFFECTS OF MODIFIABLE LIFESTYLE BEHAVIORS ON LIPOPROTEIN PARTICLE CONCENTRATION AND SIZE

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ABSTRACT

Lipoprotein concentrations are well established biological markers associated with cardiovascular disease (CVD) risk. Recent research has placed great importance on the various functions of the different lipoprotein subfractions (small and large low-density lipoprotein particles [LDL-P]; small, medium and large high-density lipoprotein particles [HDL-P]). Modifiable lifestyle behaviors such as exercise, diet, and sleep have been shown to have direct effects on CVD risk, in part by altering blood lipid and lipoprotein profiles. The overall goal of this dissertation was to determine if specific modifiable lifestyle behaviors were associated with the concentration and size of lipoprotein subfractions.

Two studies, both utilizing a longitudinal study design, were conducted to 1) investigate the effects of 16-weeks of higher-dose (14 kilocalories per kilogram body weight per week [KKW]) compared to lower-dose (8 KKW) exercise training on blood lipid and lipoprotein particle concentrations and size and 2) investigate the effects of sleep restriction (SR) during 8-weeks of a caloric restriction (CR) diet compared to CR alone on blood lipid and lipoprotein particle concentrations and size. Data for the first study were collected from the WEWALK study, a clinical exercise trial involving older women. Data for the second study were collected from the WORDS study, a clinical diet and sleep trial involving overweight men and women. For both studies, lipoprotein profiles were analyzed pre- and post-intervention utilizing nuclear magnetic resonance (NMR) spectroscopy.
In the WEWALK study, the lower-dose exercise group displayed a decrease in total HDL particle (HDL-P) concentration (p=0.001), while the higher-dose group displayed an increase in mean LDL-P size (p<0.05). Both exercise doses were found to significantly increase mean HDL particle size (p<0.05) with no significant difference between groups.

In the WORDS study, large HDL-P concentration decreased in the CR group while mean HDL-P size decreased in the CR+SR group. No between-group differences were observed comparing lipoprotein subclasses of CR and CR+SR intervention groups.

Overall, this dissertation found that alterations within modifiable lifestyle behaviors may alter the degree to which behavior change affects CVD risk in terms of lipoprotein subfractions concentration and size. Higher-dose exercise may be more beneficial than lower-dose exercise in older women, and CR may be more beneficial for overweight individuals if sleep is not restricted during CR. Due to limited existing research on these topics, further investigation is needed to reach more definitive conclusions.
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LIST OF ABBREVIATIONS

ABCA1 ........................................ ATP-binding cassette transporter A-1
ADF............................................................. Alternate Day Fasting
AHA .......................................................... American Heart Association
ANOVA ...................................................... Analysis of Variance
apoA........................................................ Apolipoprotein A
apoB ........................................................ Apolipoprotein B
APOE ........................................................ Apolipoprotein E Study
AT/RT .................................................. Aerobic Training versus Resistance Training
BMI .......................................................... Body Mass Index
CAD ........................................................ Coronary Artery Disease
CAV1 .................................................. Caveolin-1
CES-D .............................................. Center for Epidemiologic Studies Depression
CHD ........................................................ Coronary Heart Disease
CR .......................................................... Caloric Restriction
CR+SR .................................................. Caloric Restriction plus Sleep Restriction
CRF ........................................................ Cardiorespiratory Fitness
CVD ........................................................ Cardiovascular Disease
DREW .................................................. Dose-Response to Exercise in Women
ECG..............................................................Electrocardiogram
EPIC..................................................European Prospective Investigation into Cancer and Nutrition
ESS......................................................Epworth Sleepiness Scale
FOSQ ........................................................Functional Outcomes of Sleepiness
GERS .........................................................Gene Exercise Research Study
GXT ..........................................................Graded Exercise Test
HDL ....................................................................High-density Lipoprotein
HDL-C ............................................................High-density Lipoprotein Cholesterol
HDL-P ............................................................High-density Lipoprotein Particle
HR ......................................................................Heart Rate
HRmax ...........................................................Maximal Heart Rate
HRR .............................................................Heart Rate Reserve
IDL ..................................................................Intermediate-density Lipoprotein
IDL-P ..........................................................Intermediate-density Lipoprotein Particle
LDL ....................................................................Low-density Lipoprotein
LDL-C ............................................................Low-density Lipoprotein Cholesterol
LDL-P ............................................................Low-density Lipoprotein Particle
LPL ..................................................................Lipoprotein Lipase
MESA ................................................Multi-Ethnic Study of Atherosclerosis
MRFIT ....................................................Multiple Risk Factor Intervention Trial
NMR .............................................................Nuclear Magnetic Resonance
PCSK9 ............................................................Proprotein convertase subtilisin/kexin type 9
POMS ..........................................................Profile of Mood States
PPARγ ..........................................................Proliferator-activated receptor gamma
PROCAM ....................................................Prospective Cardiovascular Muenster
PSQI ............................................................... Pittsburgh Sleep Quality Index
PVT ................................................................. Psychomotor Vigilance Test
RCT ............................................................... Reverse Cholesterol Transport
RMR ............................................................... Resting Metabolic Rate
RPE ................................................................. Rating of Perceived Exertion
RR ................................................................. Relative Risk
SD ................................................................. Standard Deviation
SF-36 ............................................................. Short Form Health Survey
SOL ............................................................... Sleep Onset Latency
SR ................................................................. Sleep Restriction
STRRIDE ... Studies of a Targeted Risk Reduction Intervention through Defined Exercise
TC ................................................................. Total Cholesterol
TG ................................................................. Triglyceride
TIB ................................................................. Time in Bed
TST ................................................................. Total Sleep Time
US ................................................................. United States
VA-HIT ......................................................... Veterans Affairs High-Density Lipoprotein Intervention Trial
\( \dot{V} \text{CO}_2 \) .................................................. Carbon Dioxide Production
VLDL ............................................................. Very Low-density Lipoprotein
VLDL-P .......................................................... Very Low-density Lipoprotein Particle
\( \dot{V} \text{O}_2 \) ......................................................... Oxygen Consumption
\( \dot{V} \text{O}_2 \text{peak} \) .............................................. Peak Oxygen Consumption
WASO .................................................................. Wake time After Sleep Onset
WEWALK ......................................................... Women’s Energy Expenditure in Walking Programs
WORDS ............................................................ Weight Outlooks by Restriction of Diet and Sleep
CHAPTER 1

INTRODUCTION

One’s lifestyle is the sum of many modifiable behaviors. Many of our modifiable behaviors have been demonstrated to have direct effects on our health, and many of these behaviors affect multiple health outcomes. Some modifiable behaviors include smoking, diet, physical activity, and sleep. These four behaviors alone affect respiratory health, cardiovascular health, mental health, hypertension, obesity, diabetes, and hypercholesterolemia, just to name a few (Byrne et al., 2016). Since there are many modifiable lifestyle behaviors, and numerous health outcomes associated with such behaviors, this dissertation focused on the effects of physical activity, dietary restriction, and sleep on blood lipid and lipoprotein particle concentrations and size.

Blood lipid and lipoproteins are of interest because of their association with cardiovascular disease (CVD) in individuals irrespective of age, race, nationality, or sex (Austin, Hokanson, & Edwards, 1998; Austin & Hokanson, 1994; Benjamin et al., 2017; Cullen, 2000; Durstine, 2004; Mykkänen, Kuusisto, Haffner, Laakso, & Austin, 1999). In 2015 alone, worldwide deaths associated with ischemic heart disease totaled 8.76 million (World Health Organization, 2017). In 2012, the cost associated with CVD and stroke was an estimated $316.1 billion (Benjamin et al., 2017). By 2030, Benjamin et al. (2017) projects that nearly 40% of the United States (US) population will have some form of CVD resulting in a $918 billion annual cost.
Though elevated blood lipids and lipoproteins are associated with chronic disease, they are essential to maintain life. Lipids are circulated in the blood, in conjunction with various proteins referred to as lipoproteins, and are delivered to the cells for routine cellular function and structure. Lipoproteins are transporters of triglyceride (TG) and cholesterol, which are utilized for multiple essential processes within the body (Ginsberg, 1998). Only when blood lipid and lipoproteins reach abnormal levels do these lipid transporters become associated with chronic disease (Austin et al., 1998; Brewer, 1999). Modifiable behaviors such as physical activity, exercise, diet, and sleep have been demonstrated to be modifiers of blood lipid and lipoproteins (Bjorvatn et al., 2007; Durstine & Haskell, 1994; Franklin, Durstine, Roberts, & Barnard, 2014; Haskell, 1986; Keys, 1980; Wirth, Diehm, Kohlmeier, Heuck, & Vogel, 1983), thereby changing CVD risk without the necessity of pharmacological intervention.

Until recently, CVD risk was determined utilizing only a few blood markers (TG, total cholesterol [TC], high-density lipoprotein cholesterol [HDL-C], and low-density lipoprotein cholesterol [LDL-C]). Development of new technology and techniques have allowed for more detailed exploration, particularly related to lipoprotein subfractions. Utilizing a technique called nuclear magnetic resonance (NMR) spectroscopy, lipoprotein concentrations are analyzed based on lipoprotein particle size. Lipoprotein subfraction concentrations analyzed by NMR spectroscopy include: total very low-density lipoprotein particles (VLDL-P); small, medium and large VLDL-P; intermediate-density lipoprotein (IDL) particles (IDL-P); total LDL particles (LDL-P); small and large LDL-P; total HDL particles (HDL-P); and small, medium and large HDL-P. NMR spectroscopy also analyzes average VLDL, LDL, and HDL particle sizes.
Recent research has established that it is not simply the cholesterol associated with LDL and HDL that increase and decrease CVD risk. The function of lipoprotein particles is important in the pathophysiology of CVD. Investigation of lipoprotein subfractions has generally concluded that elevated concentrations of small LDL-P and small HDL-P are more atherogenic than their larger counterparts (Asztalos et al., 2004; Cheung, Brown, Wolf, & Albers, 1991; Després, 2007; Krauss, 2010). Smaller mean LDL and HDL size are also associated with greater CVD risk compared to larger mean LDL and HDL size (Harchaoui et al., 2009; Lamarche et al., 1997). Since the ability to analyze lipoprotein concentrations is relatively new, detailed investigation of how to most effectively modulate lipoprotein subfractions concentration and size via lifestyle is still needed.

Well established by observational and clinical research, increased physical activity and exercise do contribute to a more favorable blood lipid and lipoprotein profile by decreasing TG concentrations and increasing total HDL-C concentration (Durstine et al., 2001). Utilizing NMR spectroscopy, recent studies have demonstrated that exercise can impact lipoprotein subfractions (Brown et al., 2009; Halverstadt, Phares, Wilund, Goldberg, & Hagberg, 2007; Kraus et al., 2002; Seip et al., 2006; Shadid, LaForge, Otvos, & Jensen, 2006). A meta-analysis by Sarzynski et al. (2015) concluded that exercise training decrease of large VLDL-P, SLP, and medium HDL-P concentrations, as well as mean VLDL size. Exercise training increases for large LDL-P and large HDL-P concentrations, as well as increased mean LDL size (Sarzynski et al., 2015).

Clearly, exercise effects the lipoprotein profile, but which particles are significantly affected varies from study to study. One possible explanation for these
inconsistent results is the wide variety of exercise training protocols utilized across studies. In particular, few studies have thoroughly investigated whether the dose of exercise influences lipoprotein subfractions. One study demonstrated that high exercise dose had greater effect on 10 of 11 lipoprotein outcomes compared to lower dose exercise of similar intensity (Kraus et al., 2002). Prior to lipoprotein subfraction analysis, multiple cross-sectional studies demonstrated higher exercise dose resulted in greater decrease in TG and TG:HDL-C ratio, and greater increase in HDL-C concentration (Drygas et al., 1988, 2000; Durstine et al., 2001; Kokkinos et al., 1995; Lakka & Salonen, 1992; Williams, 1996, 1997, 1998). Though present studies demonstrate that lipoprotein cholesterol respond differently to different exercise doses, to our knowledge, no studies have published results concerning the effect of exercise dose on lipoprotein subfractions.

Weight loss by means of dietary caloric restriction (CR) is another lifestyle behavior commonly utilized to decrease CVD risk (Siri-Tarino & Krauss, 2016). CR generally decreases CVD risk by decreasing TC and LDL-C and/or elevating HDL-C (Fontana, Meyer, Klein, & Holloszy, 2004; Katzel et al., 1995; Verdery & Walford, 1998). A predominance of small LDL-P and small HDL-P are associated with overweight and obesity (Després, 2007). CR weight loss by means of daily CR and alternate day fasting (ADF) demonstrated decreased LDL-C and decreased proportion of small LDL-P among total LDL-P, as well as an increased mean LDL size (Bhutani et al., 2013; Tzotzas et al., 2011; Varady et al., 2011; Varady & Hellerstein, 2007).

Sleep is an additional modifiable behavior that may have CVD risk implications. According to the American Academy of Sleep Medicine, 1 in 5 Americans fail to get sufficient sleep. Though the health consequences of sleep deprivation are wide reaching
(Orzel-Grygolewka, 2010), like CR and exercise, evidence exists that sleep duration affects blood lipid and lipoprotein profiles. Sleep as a modifiable lifestyle behavior has only been heavily studied in relation to lipoprotein particles and CVD for the past decade, so our understanding of the relationship is limited. Population-based studies have concluded that sleep duration is associated with plasma concentrations of TG, TC, HDL-C, and LDL-C (Bjorvatn et al., 2007; Kaneita, Uchiyama, Yoshiike, & Ohida, 2008). These studies demonstrate the existence of optimal sleep duration (6-8 hour). Consistent patterns of sleeping <6 hours or >8 hours are associated with blood lipid and lipoprotein concentrations associated with high CVD risk. Only one human study has utilized NMR to analyze the effect of short term (5 days) sleep deprivation on lipoprotein subfractions and reported a decreased concentration of small, medium and large LDL-P, and small VLDL-P. There was no significant concentration change in small, medium, or large HDL-P (Aho et al., 2016). Though epidemiological observations and acute clinical trial have linked sleep deprivation with lipoprotein adaptations associated with higher CVD risk, to our knowledge, no clinical trials have analyzed the effect of chronic sleep restriction (SR) on lipoprotein subfraction concentration and size.

**Scope of the Study**

Cardiovascular disease is the primary cause of death around the world. Abnormal circulating blood lipids and lipoproteins are associated with CVD risk and can be modified via lifestyle intervention. Relatively recent evidence has brought to light the important role of lipoprotein particle subfractions in determining CVD risk. Research clearly demonstrates that exercise, diet and sleep are modifiable lifestyle behaviors that affect lipid and lipoprotein profiles, thereby affecting CVD risk. Though exercise training
dose clearly affects traditional lipoprotein profile results, lipoprotein subfraction concentration changes are yet to be investigated in response to different doses of exercise.

Dietary CR in obese individuals is demonstrated to decrease dyslipidemia associated CVD risk, while short sleep duration is a behavior clearly associated with increased CVD risk based on cross-sectional analysis of traditional lipoprotein profile results. This raises the concern that short sleep duration may negate the positive lipoprotein effects realized by those participating in a CR dietary program. The effect of long-term experimental SR on lipoprotein subfractions in individuals participating in a CR dietary program is yet to be determined.

Therefore, the overall goal of this dissertation is to 1) determine whether two different doses of a 16-week, moderate-intensity, aerobic exercise training intervention among older women differentially affect blood lipid concentrations and lipoprotein particle concentrations and size, and 2) determine and understand the effect of long-term SR on blood lipid and lipoprotein particle concentrations and size for calorically restricted individuals compared to non-sleep restricted, calorically restricted individuals.

**Specific Aim 1**

Sedentary older women underwent a 16-week aerobic exercise training intervention at one of two exercise doses; lower-dose (8 kilocalories per kilogram per week [KKW]) or higher-dose (14 KKW). Pre- and post-intervention blood samples were analyzed utilizing NMR spectroscopy. The specific aim is to investigate and compare higher-dose and lower-dose exercise training’s effects on blood lipid and lipoprotein particle concentrations and particle size. The hypothesis was that higher-dose exercise training would result in a greater increase of mean HDL-P and LDL-P size, and large HDL-P and LDL-P concentrations compared to the lower-dose exercise training. Mean
VLDL-P size, large VLDL-P, and small HDL-P and LDL-P concentrations would decrease more in the higher-dose group compared to the lower dose group.

Specific Aim 2

Obese adults in an 8-week CR intervention were randomized to one of two groups, habitual sleep or SR. Pre- and post-intervention blood samples were analyzed utilizing NMR spectroscopy.

Aim 2: To determine the effect of CR combined with SR (CR+SR) on blood lipid and lipoprotein particle concentrations and particle size compared to CR alone. The hypothesis was that a CR diet would result in an increase in HDL-P and LDL-P size, large HDL-P and large LDL-P concentrations, and a decrease in small LDL-P concentration. We further hypothesized that the addition of SR would attenuate the effects of CR alone on lipoprotein particle size and lipoprotein subfraction concentrations.

These studies will further elucidate the effects of exercise, CR, and CR+SR on the lipoprotein subfraction profile. The results from these studies will provide doctors, personal trainers, and lifestyle coaches with scientific knowledge required when prescribing lifestyle as a means to improve the lipoprotein profile and therefore decrease CVD risk.
CHAPTER 2

LITERATURE REVIEW

OVERVIEW

Cardiovascular disease (CVD) is the primary cause of death not only in the United States (US) but across the world. According to the National Vital Statistics Report published by the Centers for Disease Control, heart disease alone accounted for 23.4% of deaths in the US in 2014 (Kochanek, Murphy, Xu, & Tejada-Vera, 2016). World Health Organization reports ischemic heart disease as the leading cause of death in the world, claiming 8.76 million lives in 2015 (World Health Organization, 2017). Through decades of research, multiple risk factors have been recognized to be associated with CVD. Risk for CVD is separated into treatable and non-treatable factors. Non-treatable risk factors include family history of CVD, ethnicity, and age. Treatable risk factors include tobacco exposure, hypertension, physical inactivity, diabetes, obesity, and dyslipidemia. The focus of this literature review is on dyslipidemia and the effect of certain lifestyle interventions.

Dyslipidemia is a disorder of lipoprotein metabolism traditionally accounting for irregular plasma concentrations of lipid and lipoprotein variables. Data analyzed from the National Health and Nutrition Examination Survey 2003 - 2006 determined that approximately half the US population had at least one lipid abnormality (Parto et al., 2015). Due to the complexity and wide variety of mechanisms involved in lipoprotein
metabolism, many potential biological variables can lead to lipoprotein metabolism dysfunction. Not only are there many biological mechanisms involved in lipid metabolism, multiple lifestyle factors such as diet, sleep, and physical activity can influence the metabolic processes. These lifestyle variables are also associated with comorbidities of dyslipidemia such as obesity, metabolic syndrome, and type 2 diabetes mellitus. Poor management of diet, sleep, and physical activity can increase an individual’s risk of cardiovascular complications via dyslipidemia and/or other comorbidities.

Signs of lipid metabolism disorder are often portrayed as dyslipidemia (lipid levels outside of normal range). Uncontrolled dyslipidemia, if left unmanaged, can result in CVD or death (Anderson et al., 1987). Multiple epidemiological studies have reported abnormal plasma concentrations of these lipids and lipoproteins (to be discussed below in detail) as CVD risk factors (Anderson et al., 1987; Cui et al., 2001; Gordon et al., 1989; Park et al., 2015; Law et al., 2003). Treating dyslipidemia can reduce the risk of coronary heart disease (CHD) (a specific type of CVD) and coronary events by about 30% over a 5-year period (Goff et al., 2006; Grundy et al., 2004).

While dyslipidemia is a health concern, lipids and lipoproteins are essential to life. Lipids are utilized by cells for structure as well as an energy source for metabolic processes. Lipoproteins are complex macromolecules composed of triglycerides (TG), cholesteryl esters, phospholipids, free cholesterol and apolipoproteins (Figure 1). A traditional lipid profile obtained from a blood collection is comprised of four basic components: TG, total cholesterol (TC), high-density lipoprotein (HDL) cholesterol (HDL-C), and low-density lipoprotein (LDL) cholesterol (LDL-C). The apolipoproteins
associated with each lipoprotein, and the major functions of each apolipoprotein can be found in Table 2.

More recent research has demonstrated that a combination of lipoprotein size, density, and composition allows for a more specific classification of all lipoproteins which include: chylomicrons, very low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), LDL and HDL (Table 1). With the rapid advancement of technology and blood analysis techniques, methods have been developed that allow for the analysis of lipoprotein concentrations based on particle size. The technology utilized for this dissertation, nuclear magnetic resonance (NMR) spectroscopy, enables lipoprotein particle segregation into the following: total VLDL particles (VLDL-P); small, medium and large VLDL-P; IDL particles (IDL-P); total LDL particles (LDL-P); small and large LDL-P; total HDL particle (HDL-P); small, medium and large HDL-P. NMR spectroscopy also analyzes average VLDL, LDL, and HDL particle sizes. View Table 3 for VLDL, LDL, and HDL particle size definitions.

While all functionality and mechanistic details of the segregated molecules are not completely understood, differences in composition are being studied in order to allow for a better understanding of the functional heterogeneity of these molecules. Despite not having a perfect understanding, much is known about lipoprotein functionality (Table 2) (Ginsberg, 1998; Rader et al., 1994). Chylomicrons and VLDL are triglyceride-rich molecules that deliver TG to cells in the body. VLDL is synthesized in the liver while chylomicrons are synthesized in the small intestine. Chylomicrons and VLDL release TG when they come into contact with lipoprotein lipase (LPL) which is an enzyme found on the surface of endothelial cells. LPL also breaks down TG into fatty acids and
monoglycerides, which can then diffuse into the cell for utilization in energy metabolism or storage. After VLDL is stripped off TG, the VLDL becomes the denser IDL molecule and is remodeled in the liver, becoming LDL. Cholesterol is then delivered to cells by LDL, where LDL is taken up into the cell for utilization by receptor-mediated endocytosis. Excess cholesterol in the peripheral vascular system is taken up by HDL precursor or HDL₁ (becoming HDL₂ upon uptake of cholesterol) that is synthesized and secreted by the liver and small intestine. HDL₂ transports cholesterol to the liver in a process known as reverse cholesterol transport (RCT). Once in the liver, the excess cholesterol is either secreted in bile or converted to bile salt.

**LIPIDS/LIPOPROTEINS AND CVD**

Prior to the detailed lipoprotein profiles of today, one component, elevated LDL-C of the basic lipid profile was clearly associated with greater CVD risk (Anderson, Castelli, & Levy, 1987; Cui et al., 2001; Gordon et al., 1989; Law, Wald, & Rudnicka, 2003; Park et al., 2015). Because of the well-established relationship between LDL-C and CVD, for decades, clinical practice utilized elevated LDL-C level as the blood component of greatest concern when assessing CVD risk. When noted that a large number of coronary events were occurring in individuals with low to normal serum cholesterol concentrations (Law & Wald, 2002), further investigation of mechanisms linking lipoproteins and CVD became necessary. Armed with research results based on more detailed lipoprotein outcomes, Krauss (2010) illustrated the complexity of lipoproteins and the dependent and independent association each has with coronary disease. Recent evidence suggests that TC carried by atherogenic lipoprotein particles (non HDL-C), lipoprotein subfractions, and associated components such as
apolipoprotein B (apoB) may be better markers of CVD risk (Parish et al., 2012; Mudd et al., 2007; Pencina et al., 2015; Sniderman et al., 2011; Thanassoulis et al., 2014). Other studies have demonstrated that some lipoprotein particles are more strongly associated with CVD risk based on particle size (Asztalos et al., 2004; Cheung, Brown, Wolf, & Albers, 1991; Krauss, 2010).

**Triglyceride**

A TG molecule is a molecule composed of three fatty acid chains attached to a glycerol backbone. Unutilized postprandial substrates are converted to TG and stored in adipose tissue for future utilization. While circulating TG are essential for maintaining metabolic function throughout the body, elevated concentrations (hypertriglyceridemia) are associated with increased risk of CVD. A meta-analysis of 17 population-based prospective studies reported that hypertriglyceridemia increased CVD risk by 32% and 76% in men and women, respectively. After adjusting for HDL concentrations and other risk factors, the increase in disease risk dropped to 14% and 37% for men and women, respectively, but still remained statistically significant (Austin et al., 1998; Austin, 1998; Hokanson & Austin, 1996). Though many epidemiological studies conclude that elevated TG concentration is an independent CVD risk factor, debate still exists concerning the degree to which hypertriglyceridemia contributes to CVD (Miller et al., 2011). The study by Gotto (1998) is one such study that reported TG concentrations are not independently linked to CHD. In this study, the association between TG and CHD weakened or disappeared when adjusted for HDL-C levels. This association is likely due to the inverse metabolic relationship between HDL-C and triglyceride-rich lipoproteins (Gotto, 1998).
Though the controversy surrounding plasma TG concentration as an independent risk factor of CVD continues, the following three studies provide compelling data from three different populations demonstrating the association between TG and CVD risk. A 14-year follow-up of the Framingham Heart Study concluded that in women 50-69 years of age, blood TG concentration was an independent risk factor (Castelli, 1992). An 8-year follow-up to the Copenhagen Male Study was conducted with male participants 53-74 years of age. Those males in the middle and highest tertiles of baseline TG concentration were at greater risk of ischemic heart disease compared to those in the lowest tertile. The results remained significant after adjusting for age, body mass index (BMI), alcohol, smoking, physical activity, hypertension, noninsulin-dependent diabetes mellitus, social class, LDL-C level, and HDL-C level (Jeppesen, Hein, Suadicani, & Gyntelberg, 1998). An 11-year follow-up of the Paris Prospective Study also suggests that elevated plasma TG concentrations are worth investigating as a potential CVD risk factor. (Fontbonne et al., 1989). An analysis of 943 men, 43 – 54 years of age, with impaired glucose tolerance or diabetes determined that 26 of the 943 died from CHD. Plasma TG levels, among other CVD risk factors (plasma TC and insulin levels), were significantly greater in those individuals that died from CHD compared to those who did not.

TG concentrations have also been considered as a risk factor in conjunction with the ratio of high LDL-C to low HDL-C levels. Considering all three lipids as one risk factor is known as the “lipid triad.” In the Helsinki Heart Study, patients with elevated levels of all three lipid triad components had high relative risk (RR) of CHD (RR = 3.8) as did the patients with both elevated LDL-C and TG concentrations (RR = 2.37) after adjusting for age, smoking and hypertension (Manninen et al., 1992). Similar results were
obtained when analyzing RR for patients with both elevated TC and TG concentrations (RR = 2.37).

In addition to the previous study, a 6-year follow-up of the Prospective Cardiovascular Muenster (PROCAM) study determined that nearly 25% of the CHD events during follow-up were from the small subgroup (4.3% of the 4,559 participants) of participants characterized by the lipid triad (Assmann & Schulte, 1992). The 8-year follow-up of the same study identified plasma TG concentrations as an independent predictor of CHD, even after adjusting for HDL-C level (Assmann, Schulte, & von Eckardstein, 1996).

**Total Cholesterol**

Cholesterol is a lipid molecule classified as a sterol, or modified steroid. A 2016 update of heart disease and stroke statistics published by the American Heart Association (AHA) reports that almost 31 million adults ≥ 20 years of age have TC levels ≥ 240 mg/dl (Mozaffarian et al., 2016). The approximate 31 million cases reported in 2016 is a decrease from the estimated 31.9 million cases reported in the 2014 report (Go et al., 2014).

Originally, TC was identified as a risk factor for CHD (Anderson et al., 1987). In a 30-year follow-up study of 1959 men and 2415 women, age 31 to 65 years, Anderson et al. (1987) reported that all-cause and CVD mortality were associated with TC levels in men and women younger than 48 years of age. When adjusted for smoking, blood pressure, relative weight, and diabetes, the association remains significant only in men. When stratified by age and sex, a 5% increase in all-cause mortality and a 9% increase in CVD mortality for every 10 mg/dL increase in TC concentration remained.
Since lipoprotein analysis has become more precise, and because lipoprotein panel results are better understood, TC values are less utilized for the assessment of CVD risk. Specific lipid and lipoprotein results such as LDL-C, HDL-C, total LDL-P concentration, LDL size, and HDL size are more highly associated with CVD and CVD risk and are therefore more commonly utilized for such purposes.

**Lipoprotein Cholesterol, Particle Size, and Concentration**

Traditionally, LDL and HDL have been quantified by measuring LDL-C and HDL-C which refers to the amount of cholesterol associated with lipoprotein. Well established is the notion that elevated levels of LDL-C are associated with CVD risk. This has been demonstrated in cross-sectional and clinical trials alike. HDL-C levels are inversely associated with CVD risk. This too has been established in both cross-sectional and clinical trials. While LDL-C and HDL-C measurements are well established measurements, they are not without limitation. One limitation to using cholesterol levels as predictors is inter-individual variation in the cholesterol content of LDL and HDL particles (El Harchaoui et al., 2007; Mora et al., 2009; Otvos et al., 2011).

As a solution, NMR spectroscopy quantifies blood lipoprotein concentrations. While lipoproteins are categorized into four relative density classifications (VLDL, IDL, LDL, and HDL), lipoproteins are very heterogeneous in size. Though a complete understanding of all lipoproteins has not been achieved, one well established point is that particle size plays a large role in the functionality of each lipoprotein. Since lipoproteins of different sizes have different functions, a logical conclusion is that the concentration of a lipoprotein subfraction is also important to consider. Due to the differing functions, elevated small LDL-P concentration is associated with increased CHD risk and is
therefore considered an atherogenic lipoprotein (Després, 2007; Krauss, 2010), and even considered a better predictor of CVD compared to LDL-C (Blake, Otvos, Rifai, & Ridker, 2002; El Harchaoui et al., 2009; Kuller et al., 2002; Mora et al., 2007; Otvos et al., 2006; Rosenson, Otvos, & Freedman, 2002). Small LDL-P could potentially contribute to increased CHD risk because this particle can easily penetrate the endothelial tissue of the vasculature (Proctor, Vine, & Mamo, 2004) or has greater potential for oxidization (Liu et al., 2002). Individuals having an increased proportion of small LDL-P have a 2- to 5-fold increase in CHD risk compared to individuals with increased proportion of large LDL-P (Austin et al., 1988; Tornvall, Karpe, Carlson, & Hamsten, 1991).

Even though higher concentrations of small LDL-P are associated with elevated TG concentrations and low HDL-C levels, multiple studies have demonstrated that elevated small LDL-P concentration results in greater CHD risk, independent of other lipid parameters (Griffin et al., 1994; Lamarche et al., 1997; Rajman et al., 1996). Griffin et al. (1994) was the first to demonstrate elevated small LDL-P concentration in CHD patients independent of TG concentration. Though this conclusion was most significant, he also reported that CHD patients had significantly higher TG concentrations compared to the control group (Griffin et al., 1994). In a cohort of normotriglyceridemic men with CAD, Rajman et al. (1996) reported that independent of blood TG and HDL-C levels, mean LDL size was significantly smaller in those individuals with CHD than controls. Also noted was that the LDL subfractions profile was a stronger predictor of CHD (small LDL-P being more abundant in participants with CHD) compared to other lipid parameters (Rajman et al., 1996). Similar results were obtained in a prospective study of
2103 men free of ischemic heart disease at baseline (Lamarche et al., 1997). In a 5-year follow-up period, 114 participants developed ischemic heart disease. Small LDL size was predictive of CHD independently of TG, LDL-C, HDL-C, and other lipid parameters (Lamarche et al., 1997).

Since the publishing of the above studies, many subsequent studies have reported similar results across many populations. Multiple studies have investigated the relationship between mean HDL size and subclass (small HDL-P, medium HDL-P, and large HDL-P) concentrations as related to CVD risk. Like small LDL-P concentration, small HDL-P concentration is also associated with higher CVD risk (Asztalos et al., 2004; Cheung et al., 1991; Krauss, 2010). Unlike small LDL-P, the potential mechanism explaining the association of small HDL-P and CVD is not well established. One possible explanation is the altered activity of lipases involved with HDL maturation (Long et al., 2006).

In a nested, case-control, secondary prevention study termed the Veterans Affairs High-Density Lipoprotein Intervention Trial (VA-HIT), elevated total HDL-P concentration was a significant predictor of reduced CHD risk. During a 5-year follow-up, for every 1 standard deviation (SD) increase in total HDL-P concentration, CHD risk decreased by 29%. Total HDL-P and LDL-P concentrations were not significantly associated with CHD risk (Otvos et al., 2006).

The primary prevention study Multiple Risk Factor Intervention Trial (MRFIT) conducted an 18-year follow-up with men diagnosed with metabolic syndrome. Elevated HDL-P concentration, especially medium HDL-P, was associated with decreased risk of CHD death [hazard ratio (95% confidence interval) 0.45 (0.25-0.83), in quartile 1
compared to quartile 4). Total LDL-P concentration was not associated with risk of CHD, while elevated LDL-C levels, smoking, and white blood cell count were associated with an increased risk (Kuller, Grandits, Cohen, Neaton, & Prineas, 2007).

European Prospective Investigation into Cancer and Nutrition (EPIC)-Norfolk was a case-control study of apparently healthy men and women that determined that at baseline, LDL-C levels were significantly higher and HDL-C levels were significantly lower in CHD participants compared to controls. After adjusting for plasma TG and apoB concentrations, HDL-C levels in the top quartile had decreased CHD risk by 50% compared to the bottom quartile. Also reported was control participants had greater concentration of large HDL-P compared to CHD participants (Harchaoui et al., 2009).

In a randomized trial of statin and antioxidant therapy in high-risk men and women, Parish et al. (2012) using data from Heart Protection Study determined that increments in total HDL-P concentration of 1 SD was associated with a statistically significant risk reduction of 11% for major occlusive coronary events after adjustment for total LDL-P concentration. The association between CVD and HDL-C level was also significant (risk reduction of 9%). Inverse associations of other cardiac events assessed in this study with biomarkers of HDL metabolism were only significant for total HDL-P concentration (risk reduction of 16%) but not for HDL-C level (Parish et al., 2012).

The Multi-Ethnic Study of Atherosclerosis (MESA) evaluated men and women whom were free of CHD and were not utilizing lipid altering therapy. Similar to the Heart Protection Study, total HDL-P concentration was inversely associated with incident of CHD events (30% risk reduction per 1 SD increment in total HDL-P concentration). This association was not significantly affected when adjusted for total LDL-P concentration.
Unlike the Heart Protection Study, HDL-C level was also inversely associated with CHD event (26% risk reduction per 1 SD) (Mackey et al., 2012).

Kontush et al. (2015) concluded that while large-scale clinical studies have demonstrated that a decrease in mean HDL size is associated with CVD, this relationship is secondary to other biomarkers such as plasma levels of total HDL-P, HDL-C and large HDL-P (Kontush, 2015). Despite Kontush’s conclusion, others are of the opinion that a more in-depth HDL-P profile assessment is clinically valuable in providing an improved CVD risk analysis (Mallol et al., 2015; Matyus et al., 2015). While LDL and HDL components are the most common lipid and lipoproteins utilized in CVD risk assessment, VLDL-C is also associated with CVD risk (Koba et al., 2002; J. Liu et al., 2006; Ren et al., 2010). At least one study has reported the association of large VLDL size and atherosclerosis (Colhoun et al., 2002).

In conclusion, elevated levels of LDL-C, and VLDL-C, and decreased levels of HDL-C are associated with higher CVD risk. Analyzing lipoproteins by size, elevated concentrations of small LDL-P and small HDL-P are associated with elevated CVD risk, while elevated levels of large LDL-P and large HDL-P do not increase CVD risk. Elevated mean particle size (LDL and HDL size) is associated with decreased CVD risk.

**LIFESTYLE EFFECTS ON LIPID AND LIPOPROTEIN PROFILES**

Most individuals can decrease dyslipidemia associated CVD risk simply by incorporating lifestyle change. Unfortunately, no one singular lifestyle modification exists that will improve all components of a blood lipoprotein profile associated with CVD. Different lifestyle interventions are likely required to keep all lipoprotein concentrations within a healthy range (Varady, Bhutani, Klempel, & Kroeger, 2011).
According to the Mayo Clinic, lifestyle factors that help an individual maintain healthy TG concentrations include maintaining a healthy weight, eating a healthy well-balanced micronutrient and macronutrient diet, and exercising regularly (Mayo Clinic, 2015). As will be demonstrated, these lifestyle patterns will also aid in obtaining or maintaining other optimal lipid and lipoprotein outcomes. Optimal sleep time will also be discussed as a lifestyle behavior that can be utilized in maintaining an optimal lipid and lipoprotein profile.

**Physical Activity**

Over the past several decades, the effect of physical activity on lipid and lipoprotein profiles has become better understood. As is evident from early observational studies, athletes and more physically active individuals have what is considered a more atheroprotective lipid profile compared to less active counterparts. Specifically, more physically active populations have lower TG concentrations and higher HDL-C levels compared to their less physically active counterparts (Durstine et al., 2001). However, many observational studies reported no significant differences in TC and LDL-C levels comparing physically active to less physically active populations (Kokkinos et al., 1995; Lakka & Salonen, 1992).

Beyond observational evidence, results from clinical trials suggest that endurance training favorably affects lipoprotein subfraction traits that are associated with CVD risk (Brown et al., 2009; Halverstadt et al., 2007; Kraus et al., 2002; Seip et al., 2006; Shadid, LaForge, Otvos, & Jensen, 2006). Varady et al. (2011) completed a 12-week trial of overweight and obese participants that were randomized into alternate day fasting (ADF), calorie restriction (CR), exercise, or control groups. The exercise protocol was moderate
intensity (60% of age predicted maximal heart rate [HRmax]) for 45 minutes, three times per week. All exercise was completed under supervised conditions on a treadmill or elliptical machine. The exercise group was the only intervention that resulted in HDL-P proportion changes, with an increase in large HDL-P, and a decrease in small HDL-P. HDL-C also increased in the exercise group (Varady et al., 2011). Results from the ADF and CR intervention groups are reported below.

To further analyze the effect regular exercise on lipoprotein concentrations, Sarzynski et al. (2015) conducted a meta-analysis that included 10 interventions from six well established endurance training studies. While the exercise protocols were different, each study utilized NMR-based lipoprotein results to analyze lipoprotein subclasses. A brief description of each study will aid in understanding the meta-analysis results.

The HERITAGE Family Study collected blood samples of 834 participants (17-65 years of age) before and after undergoing a 20-week cycle ergometer training protocol. All participants were sedentary at baseline and gradually increased exercise duration and intensity until able to maintain 75% of the HR associated with baseline maximal oxygen consumption (VO2max) for 50 minutes. A total of 60 training sessions were completed within a 21-week period (3 days/week). The target intensity was maintained for the last 6 weeks of training (Bouchard et al., 1995). The HERITAGE study results provide strong evidence that VLDL-TG significantly decreases, while HDL-C and HDL2-C concentrations significantly increase in response to exercise training in women. Males displayed a significant decrease in plasma TG and VLDL-TG with a significant increase in HDL-C, HDL2-C, and HDL3-C concentrations in response to exercise training (Leon et al., 2000).
The Dose-Response to Exercise in Women (DREW) study included analyzed blood from 464 healthy, postmenopausal, overweight and obese women (45-75 years of age) before and after a 6-month exercise cycle and treadmill training protocols. Participants were randomized to one of three exercise training doses (4, 8, or 12 kilocalorie/kilogram/week [KKW]) or control group. The 8 KKW was calculated as the appropriate dose required for the women to meet the weekly physical activity guidelines (American College of Sports Medicine, 1995). The 4 and 12 KKW exercise doses were chosen to determine if 50% above or below the physical activity guidelines resulted in different physiological outcomes. The control group was asked to maintain a normal routine during the 6-month testing period. All participants were sedentary at baseline. Participants randomized to the exercise group gradually increased exercise duration and intensity until they were able to maintain 50% of the HR associated with baseline peak oxygen consumption (\( \dot{V}O_2 \text{peak} \)) (Morss et al., 2004). Lipoproteins were not affected by exercise in the DREW study (Arsenault et al., 2009).

The University of Maryland Gene Exercise Research Study (GERS) included analyzed blood of 160 men and women (50-75 years of age) before and after 24 weeks of aerobic training. Multiple modes of training were permitted including: cycle, treadmill, elliptical machine, skier machine, stepping machine, and rowers. All participants were sedentary with a BMI \(< 37 \text{ kg/m}^2\) at baseline and gradually increased exercise duration and intensity until able to maintain 70% of the \( \dot{V}O_2 \text{max} \) determined at baseline for 40 minutes 3 times per week. With 12 weeks remaining in the 24-week intervention, a lower intensity, 45-60 minute at home walk was added on the weekends (Halverstadt et al., 2007; Wilund, Colvin, Phares, Goldberg, & Hagberg, 2002). The GERS study reported a
decrease in concentrations of large and small VLDL-P, LDL-C, small, medium small, and very small LDL-P, medium and small HDL-P, and VLDL size. Lipoprotein subfractions that significantly increased included: IDL-P and large LDL-P concentrations, and HDL size (Halverstadt et al., 2007).

Studies of a Targeted Risk Reduction Intervention through Defined Exercise (STRRIDE I) was a randomized controlled trial that included analyzed blood of 240 overweight men and women (40-65 years of age). Blood was collected before and after a 6-month exercise training protocol in which participants were randomized into one of four groups: control; high-amount/vigorous-intensity exercise; low-amount/vigorous-intensity exercise; or low-amount/moderate-intensity exercise. Multiple modes of training were permitted including: cycle, treadmill, and elliptical machine. All participants were sedentary at baseline and gradually increased exercise duration and intensity until they were able to maintain the dose prescribed based on group assignment. Low-amount of exercise was defined as 14 KKW while high-amount was defined as 23 KKW. Moderate-intensity exercise was defined as 40-55% of \( \dot{V}O_2 \)peak while vigorous-intensity exercise was defined as 65-80% of \( \dot{V}O_2 \)peak (Kraus et al., 2002; Slentz et al., 2007). Kraus et al. (2002) concluded that high-amount/vigorous-intensity exercise training decrease of LDL-C and small LDL-P concentration, and an increase of LDL size. Slentz et al. (2009) reported that moderate-intensity training was more effective at decreasing blood TG than vigorous-intensity training. These findings are consistent with previous studies (Berggren, Hulver, Dohm, & Houmard, 2004; Johnson et al., 2007; Kraus & Slentz, 2009).
STRRIDE II – Aerobic Training versus Resistance Training (AT/RT or STRRIDE II) was a randomized control trial that included analyzed blood of 87 overweight men and women (18-70 years of age). Blood was collected before and after an 8-month exercise training protocol in which participants were randomized into one of four groups: control; aerobic training (as defined for STRRIDE I); resistance training; and combine aerobic and resistance training (Bateman et al., 2011).

The apolipoprotein E (APOE) Study included analyzed blood of 106 men and women (18-70 years of age) before and after 6 months of aerobic training. Multiple modes of training were permitted: cycle, treadmill, skier machine, stepping machine, and rowers. All participants were sedentary at baseline and gradually increased exercise duration and intensity until they were able to maintain 60-85% of the HRmax determined at baseline for 40 minutes (Seip et al., 2006; Thompson et al., 2004). Since the primary purpose of this study was to determine how genetics affects the outcome of lipoproteins in response to exercise, the results were reported based on genetic grouping of participants. Despite this grouping, valuable information regarding lipoprotein outcomes independent of genetics was also reported. Exercise training significantly decreased TG and large VLDL-P concentrations as well as mean VLDL size. When analyzed by sex, large VLDL-P concentration was only significantly decreased in males (Seip et al., 2006).

Taking into consideration the above-mentioned studies, the Sarzynski et al. (2015) meta-analysis is better understood. The meta-analysis concluded that exercise training decrease of large VLDL-P, small LDL-P, and medium HDL-P concentrations, as well as mean VLDL size. Significant increases were reported for large LDL-P and HDL-P, as
well as mean LDL size. All values remained significant after adjustment for age, sex, race, and baseline BMI (Sarzynski et al., 2015).

While the mechanism of exercise-induced lipid and lipoprotein changes is not well understood, there is evidence that gives insight into portions of the mechanism that could potentially explain the observed effects of exercise on blood lipid and lipoprotein concentration (Wang & Xu, 2017). Lipoprotein lipase (LPL), which is an enzyme located on the capillary walls that stimulates the release of triglyceride from chylomicron particles and VLDL is upregulated, is upregulated with exercise (Miyashita et al., 2010). Increased LPL activity, in turn, is associated with increased HDL-C (Tsutsumi, 2003). Proprotein convertase subtilisin/kexin type 9 (PCSK9) is an inhibitor of LDL receptor proteins. When PCSK9 binds the LDL receptor protein on the liver, the liver cannot uptake LDL particles, thereby leaving more LDL-P in blood circulation. Increased PA over a 3-month period decrease expression of PCSK9 and lower LDL-C levels (Kamani et al., 2015). Cellular membrane protein ATP-binding cassette transporter A-1 (ABCA1) and the resulting gene expression are upregulated in response to exercise (Tofighi, Rahmani, Jamali Qarakhanlou, & Babaei, 2015). ABCA1 interacts with apoA-1 on an HDL particle thereby preparing the HDL particle to uptake cholesterol. Proliferator-activated receptor gamma (PPARγ) and liver X receptor alpha (LXRα) are two additional receptors involved in RCT. Both PPARγ and LXRα are upregulated in response to exercise training (Butcher et al., 2008).

Some evidence also suggests that cardiorespiratory fitness (CRF) significantly affects lipid and lipoprotein concentration and size of subfractions (Parto, Lavie, Swift, & Sui, 2015). Parto et al.’s (2015) review demonstrates that CRF is associated with an
increase in mean HDL size, and that those with a higher TG concentration at baseline experience a greater reduction in TG concentration in response to increased CRF compared to those with lower TG concentration at baseline. With these results in mind, change in CRF needs to be taken into consideration when analyzing lipid and lipoprotein results with exercise intervention.

In summary, significant evidence exists demonstrating that physical activity and exercise beneficially affect multiple lipids, lipoproteins, and lipoprotein subfractions associated with CVD. What is yet to be reported in the literature is if exercise training of different doses affects lipoprotein subfractions differently. To the best of our knowledge, this dissertation is one of the first to report the effects of exercise dose on NMR lipoprotein subfraction outcomes associated with CVD.

**Diet/Weight Loss**

Vast amounts of research report lipid responses to CR and weight loss. Weight loss and body composition changes via CR generally decrease CVD risk by decreasing TC and LDL-C and/or elevating HDL-C (Fontana et al., 2004; Katzel et al., 1995; Verdery & Walford, 1998). Since the above responses are well established, this review primarily focuses on the limited studies that reported lipoprotein particle responses in these categories.

Being overweight or obese is associated with lipoprotein profiles high in small LDL-P and small HDL-P (Després, 2007; Magkos, Mohammed, & Mittendorfer, 2008). Siri-Tarino et al. (2009) reported that small LDL-P concentration is also reduced in overweight, otherwise healthy, men after CR induced weight loss (Siri-Tarino, Williams, Fernstrom, Rawlings, & Krauss, 2009).
Varady et al. (2011) completed a 12-week trial of overweight and obese participants. Participants were randomized into ADF, CR, exercise, or control groups. Weight decreased by approximately 5% in the three intervention groups while remaining unchanged in the control group. TC remained unchanged in all groups while LDL-C decreased with ADF and CR. TG concentrations were only decreased in the ADF group. In both the ADF and CR interventions, mean LDL-P size increased. Only the ADF group increased proportion of large LDL-P and a decreased proportion of small LD-P (Varady et al., 2011).

In summary, CR induced weight loss significantly decreases CVD risk in terms of multiple lipid and lipoprotein markers. This dissertation significantly contributes to the limited literature by determining if the beneficial effects of CR weight loss on lipoprotein particle concentration and size are negatively affected by other lifestyle behavior, specifically sleep restriction (SR).

Sleep

In recent years, sleep has been recognized as a modifiable behavior associated with morbidity and mortality (Broussard & Van Cauter, 2016; Jean-Louis et al., 2014; Koyanagi et al., 2015; Y. Liu et al., 2013; Luyster et al., 2012; Zawisza et al., 2015). One early study determined that sleep duration had no significant association with serum lipid and lipoproteins, however, the participants from this study were men from a single place of work (Nakanishi, Nakamura, Ichikawa, Suzuki, & Tatara, 1999). Despite this limitation, other studies in the last decade have identified sleep duration as a potential modifier of blood lipid and lipoproteins, which are described below.
Kaneita et al. (2008) analyzed data from Japan’s 2003 National Health and Nutrition Survey. Data from 3995 men and women were utilized for the analysis. For men, LDL-C concentration was higher in those sleeping $\geq$8 hours compared to those sleeping 6-7 hours. For women, sleeping shorter or longer than 6-7 hours was associated with elevated TG, and lower HDL-C concentrations compared to those sleeping 6-7 hours (Kaneita et al., 2008).

Bjorvatn et al. (2007) analyzed data from data from 8860 men and women (40-45 year of age) who participated in the Hordaland Health Study. Those men and women that averaged less than 7-8 hours of sleep per night had significantly higher TC and TG concentrations, and significantly lower total HDL-C concentrations compare to those that averaged 7-8 hours of sleep per night (Bjorvatn et al., 2007).

In a study of 503 men and women (32-51 years of age) longer sleep duration was associated with a significant 10-year increases in TC and LDL-C among both men and women. These associations were attenuated and no longer significant among women once menstrual status was taken into account. Premenopausal women generally report lower TC, LDL-C, and HDL-C concentrations compared to their male counterparts. These differences are potentially due to sex hormones, and their effects on hormone metabolism. These differences were diminished in postmenopausal women (Petrov et al., 2013).

Similar results to the above-mentioned studies have been observed in other studies (Cooper et al., 2015; Doo, Chun, & Doo, 2016; Shin et al., 2016; Zhan, Chen, & Yu, 2014). In general, sleeping less than 6 hours or more than 8 hours is associated with blood lipid and lipoprotein outcomes that are associated with higher CVD risk.
While cross-sectional data demonstrates significant association between sleep time and CVD related lipids and lipoproteins, little experimental research has been published concerning SR and blood lipid profile or NMR-measured lipoprotein subfractions, and the result of SR on lipids in clinical studies is inconsistent. Kerkhofs et al. (2007) reported that 3 nights of sleep restricted to 4 hours resulted in elevated levels of TC and LDL-C (Kerkhofs et al., 2007), while O’Keeffe et al. (2013) reported no significant lipid changes following 5 nights of sleep restricted to 4 hours (O’Keeffe, Roberts, Kelleman, Roychoudhury, & St-Onge, 2013). Differences in results may be due to population differences as Kerkhofs utilized post-menopausal women on hormone replacement therapy, and O’Keeffe utilized young healthy individuals. To the best of my knowledge, only one interventional sleep studied has examined NMR-measured lipoprotein particles in human subjects. Sleep was reduced to 4 hours per night for five nights in 14 young male participants. SR decreased small, medium and large LDL-P, and small VLDL-P. There was no significant change in small HDL-P, medium HDL-P, or large HDL-P (Aho et al., 2016).

Experimental SR and analysis of epidemiological SR data by Aho et al. (2016) reviled that SR affects many of the same portions of the lipoprotein altering mechanism as exercise. While acute sleep restriction appears to alter the LDL profile and associated mechanisms, long term SR decreased expression of LXR and ABCA1. Both LXR and ABC1 results in reduced RCT, lower HDL concentration, and larger VLDL-P size (Aho et al., 2016; Liu, Chung, Shelness, & Parks, 2012). Caveolin-1 (CAV1) is an additional membrane protein thought to play a role in cholesterol traffic and homeostasis (Fielding & Fielding, 2001). Though the effects of CAV1 on RCT are complex and not completely
understood, Aho et al. (2016) reports a significant decrease of CAV1 in response to SR that could potentially play a role in negatively altering RCT with SR.

In mice, many of the receptors and transporters above have been demonstrated to be regulated by circadian rhythm (Gnocchi, Pedrelli, Hurt-Camejo, & Parini, 2015). Though it is yet to be investigated in humans, sleep restriction may affect the circadian regulation of some receptors and transporters involved in lipid and lipoprotein transport, thereby affecting measurable concentrations of blood lipoprotein subfractions.

In summary, though evidence is limited, previous research indicates that an association exists between SR and blood lipid and lipoprotein concentrations. Only one study has reported the effect of short sleep duration on lipoprotein subfractions, and SR was limited to 5 nights in that study. No study has reported the effect of long-term SR on lipoprotein concentration and size.

Summary

Analysis of lipid and lipoprotein profiles is indicative of our health and health risks yet the relationship between lipoproteins and health implications is quite complex. Many factors influence lipoproteins and make understanding these relationships even more complex. Advancements in technology, including NMR, have provided tools to advance our understanding of these relationships, however we are still left with important unanswered questions. This dissertation focuses on the effects of two specific modifiable lifestyle behaviors (exercise dose and sleep duration) on the concentration and size of lipoprotein subfractions. To date, few studies have explored the link between exercise dose and lipoprotein subfraction concentration and size. To the best of our knowledge, no experimental study has investigated the effect of SR on lipoproteins subfraction
concentration and size beyond 5 days of SR. Therefore, the purpose of this dissertation is to bridge the gap in the literature by analyzing the effects of different exercise doses and SR on NMR-based lipid and lipoprotein phenotypes. Specifically, the purpose of aim 1 is to analyze the exercise dose effect of a 16-week exercise protocol in older women utilizing NMR spectroscopy in determining blood lipid and lipoprotein profiles. In specific aim 2, NMR spectroscopy was utilized to determine the effects of SR compared to normal sleep during 8 weeks of CR in overweight adults on lipid and lipoprotein profiles.
CHAPTER 3

GENERAL METHODOLOGY

INTRODUCTION

The two studies in this dissertation utilize data collected from two separate studies. One study was the Women’s Energy Expenditure in Walking Programs (WEWALK). This study was a randomized clinical exercise trial that investigated the effect of two different moderate-intensity exercise training doses on energy expenditure in sedentary older women (ClinicalTrials.gov identifier: NCT01722136) (Wang, Bowyer, Porter, Breneman, & Custer, 2017). A second study was the Weight Outlooks by Restriction of Diet and Sleep (WORDS) study. The WORDS study was a randomized trial that investigated the effect of sleep restriction (SR) on body composition in individuals undergoing a caloric restriction (CR) dietary weight loss program (ClinicalTrials.gov identifier: NCT02413866) (Wang, Sparks, Bowyer, & Youngstedt, 2018).

OVERALL MEASUREMENTS

Nuclear Magnetic Resonance (NMR) Spectroscopy

For both studies, 12-hour fasted blood samples were obtained before and after intervention and shipped to an offsite facility for NMR analysis (LipoScience, Inc. Raleigh, NC). Utilizing the magnetic resonance signal of the protons associated with each lipoprotein, the NMR technique measures the concentration (nmol/L; µmol/L) and average size (nm) of VLDL, LDL, and HDL particles from a single plasma or serum
sample (Jeyarajah, Cromwell, & Otvos, 2006). Concentration outcomes obtained utilizing NMR spectroscopy include: total VLDL particles (VLDL-P), large, medium and small VLDL-P, total LDL particles (LDL-P), IDL particles (IDL-P), large and small LDL-P, total HDL particles (HDL-P), large, medium and small HDL-P. Mean particle size outcomes obtained utilizing NMR spectroscopy include VLDL size, LDL size, and HDL size.

**Body Mass Index (BMI)**

Height and weight were measured to the nearest 0.1 cm and 0.1 kg, respectively. Body weight was measured on a digital scale (Health O Meter® 10 Professional, Pelstar LLC, McCook, IL) with participants wearing standard scrubs and without shoes or outer garments. The average of two consecutive height and weight measurements were utilized to calculate the BMI (kg/m$^2$) for each individual before and after intervention.

**STUDY 1**

**Purpose**

The purpose of study 1 is to investigate and compare higher-dose and lower-dose exercise training’s effects on blood lipid and lipoprotein particle concentrations and particle size.

**Hypothesis**

We hypothesized that moderate-intensity exercise training in sedentary older women would have positive effects on lipoprotein particle size and lipoprotein subfraction concentrations. Specifically, mean HDL and LDL size would increase, small HDL-P and small LDL-P concentrations would decrease, and large HDL-P and large
LDL-P concentrations would increase. We also hypothesized that these results would be greater in the higher-dose training group compared to the lower-dose training group.

**Participants and Enrollment**

Participants were recruited from the greater Columbia, South Carolina area between October 2012 and December 2014. The WEWALK study protocol was reviewed and approved by the University of South Carolina Institutional Review Board. Prior to beginning the study, all participants signed an informed consent. Participants for this study were female, 60 – 75 years of age, weight stable (± 3% body weight for previous 3 months), sedentary (no more than 20 minutes of structured exercise 3 times per week), non-smoking in the last year, BMI ≥ 18 and ≤ 30 kg/m², and free from CVD, metabolic or respiratory disease, or any other condition that might affect adherence to study protocol. Individuals reporting contraindications to exercise according to the American College of Sports Medicine Guidelines (ACSM, 2010) or use of metabolism affecting medications were excluded from the study, in addition to those self-reporting excessive caffeine use (> 500 mg per day).

Medical conditions that could potentially interfere with participation in the exercise intervention were identified with medical examination, cognitive and depression screening, and fasting blood draw. Exclusionary signs included: hypertension (blood pressure ≥ 160/90 mmHg); diabetes (fasting glucose ≥ 126 mg/dL); liver, renal, hematologic or thyroid disorder; cognitive dysfunction (Mini-Mental State Examination <24); or depression (Center for Epidemiologic Studies Depression [CES-D] Scale >16).
**Study Design**

This study was a randomized clinical exercise trial using a lower-dose exercise group and a higher-dose exercise group.

**Study Intervention**

Prior to commencement of the exercise intervention, participants were randomized to one of two moderate-intensity walking groups that differed by dose, defined by weekly exercise training energy expenditure. The lower-dose group was prescribed 8 kilocalories (kcal) per kilogram (kg) of body weight per week (KKW) and the higher-dose group 14 KKW. Weekly energy expenditure was determined by multiplying the participant’s weight by their assigned dosage. The two different doses were achieved by varying the duration of total weekly exercise. All training sessions were supervised in a clinical exercise setting for a period of 4 months.

Since all participants were considered sedentary upon study entry, the exercise intensity and weekly caloric expenditure was incrementally increased. Training intensity started at 40% of heart rate reserve (HRR) and increased by 5% every two weeks until achieving the target level of 50-55% HRR. The HRR was calculated from the heart rate max achieved during the baseline fitness test. Both exercise groups began at a weekly caloric expenditure of 4 KKW during the first week of the intervention and progressed until reaching the assigned exercise dosage. The target exercise intensity and dose were reached by week five in the lower-dose group and week eight in the higher-dose group. A 3-minute warm-up and cool-down was conducted for each exercise session. Heart rate (HR) monitors (FT1; Polar, Lake Success, NY, USA) were utilized to continuously monitor training intensity throughout each exercise session. The HR was recorded every
five minutes. Blood pressure was measured before, at the mid-point, and after each
exercise session.

Compliance to the exercise protocol (frequency, intensity, and duration) was
reviewed weekly for each participant. Any participant missing an exercise session
without notifying study personnel was contacted via phone to encourage further
attendance.

Measurements

Graded Exercise Test (GXT)

Graded exercise testing is a method utilized to determine cardiorespiratory fitness
(CRF). A graded treadmill test was utilized to determine the CRF of all participants
before and after exercise training intervention. The protocol began at 0% grade, and the
participants self-selected pace defined as, “comfortable but challenging.” Every two
minutes the incline was increased by 2%. Oxygen consumption (V̇O₂) was measured
utilizing a metabolic cart (TrueOne 2400; ParvoMedics, Sandy, UT, USA). Blood
pressure was measured at rest and in the last 30 seconds of every exercise stage utilizing
a stethoscope and sphygmomanometer. Participants were encouraged to continue to
volitional fatigue. During the GXT, heart rhythm and HR were monitored utilizing a
standard 12-lead electrocardiogram (ECG) (Q-Stress ®; Cardiac Science, Bothell, WA,
USA). The ECG and HR were continuously monitored for the entirety of the GXT and
for 10 minutes following the test by a trained medical professional. Test results were
considered satisfactory if at least two of the following four criteria were met: a plateau in
HR or V̇O₂, achieving a maximal HR greater than 90% of age predicted maximal HR
(HRmax) (220 – age), a self-reported rating of perceived exertion (RPE) greater than 17
on the 6 – 20 Borg RPE scale, and/or a respiratory exchange ratio greater than or equal to 1.10. Peak oxygen consumption (\(\dot{V}O_2\)peak) was determined by the highest 30-second \(\dot{V}O_2\) average recorded during the test.

**Blood Collection**

For this study, 12-hour fasted blood samples were collected before and after exercise training intervention. The pre-intervention blood sample was collected at least 24 hours before the commencement of exercise training intervention and the post-intervention blood sample was collected at least 24 hours after the last bout of exercise training. All blood samples were collected by a trained phlebotomist. Blood was collected from the median cubital or cephalic vein in the cubital fossa of the elbow unless those veins were compromised. Blood was collected in a 7 mL EDTA plasma vial and centrifuged at 3000 rotations per minute (rpm) to separate the red blood cells from plasma. Plasma was then aliquoted and stored at -80°C until being shipped to an offsite facility for NMR analysis (LipoScience, Inc. Raleigh, NC).

**Statistical Analysis**

Analysis was done for all participants who successfully completed the exercise intervention and had complete NMR blood results (n=65). Baseline descriptive statistics were calculated and reported as means and standard deviations (SD). Independent sample t-tests tests were utilized to determine differences in baseline characteristics (age, BMI, and \(\dot{V}O_2\)peak) between participants randomized into higher-dose versus lower-dose exercise training.

A repeated measure analysis of variance (ANOVA) was utilized to compare pre-versus post-intervention NMR results (total VLDL-P, large, medium and small VLDL-P,
LDL-P, IDL-P, large and small LDL-P, total HDL-P, large, medium and small HDL-P, VLDL size, LDL size, and HDL size) between the two intervention groups. Separate models were run for each of the NMR variables. The model adjusted for age, BMI, and \( \dot{V}O_2 \text{peak} \) as covariates. The independent variables of interest were exercise dose (lower versus higher), time (pre- versus post-), and interaction between group and time. Data were analyzed using PROC MIXED and statistical significance was set at \( P < 0.05 \). All analyses were performed utilizing SAS 9.4 (SAS Institute Inc., Cary, NC).

**Strengths and Limitations of Study 1**

The strengths of this study included the use of NMR spectroscopy to analyze lipoprotein particle concentration and size, and each exercise session was supervised in a clinical exercise setting to assure protocol compliance. Also, because all participants in this study were post-menopausal women, menses did not affect blood lipoprotein variables. Since older women have unique physiological characteristics, the generalizability of this study is limited to generally healthy, postmenopausal women. The participants in this study did not have a high risk of CVD, therefore, the exercise training effect may be limited. The lack of a control group for comparison is also a significant limitation.

**STUDY 2**

**Purpose**

The purpose of study 2 was to investigate the effect of SR on blood lipid and lipoprotein particle concentrations and particle size in individuals undergoing a CR weight loss program. This study also determined the effect of caloric restriction
combined with sleep restriction (CR+SR) on blood lipid and lipoprotein particle concentrations and particle size compared to CR alone.

**Hypothesis**

We hypothesized that CR alone would result in an increase in HDL and LDL size, and an increase in large HDL-P and large LDL-P concentration. We hypothesized that SR in individuals undergoing a CR weight loss program would reduce the beneficial effects on lipoprotein particle size and lipoprotein subfraction concentrations compared to individuals without SR intervention, undergoing a CR weight loss program. Specifically, mean HDL and LDL size would decrease, small HDL-P and small LDL-P concentrations would increase, and large HDL-P and large LDL-P concentrations would decrease in the sleep-restriction compared to non-sleep-restriction group.

**Study Design**

This study was a randomized clinical trial including two groups - CR and CR+SR.

**Participants and Enrollment**

Participants who completed the intervention including pre- and post-intervention blood collection (n = 28) were recruited from the greater Columbia, South Carolina area between January 2015 and May 2016. The WORDS study protocol was reviewed and approved by the University of South Carolina Institutional Review Board. Prior to beginning the study, all participants signed an informed consent. Male and female participants were recruited for this study, and were 35 – 55 years of age, weight stable (± 3% body weight for previous 3 months), self-reported sleeping between 6.5 – 8 hours per day with <90 minutes per day of daytime napping, have <120 minutes of exercise training per week during the previous 3 months, BMI ≥ 25 and ≤ 40 kg/m², and free from
CVD, diabetes, chronic respiratory disease, active cancer, sleep or eating disorders, or any other condition that would prevent adherence to study protocol. Individuals reporting shift work, psychological disorders, smoking within the past year, use of metabolism affecting medications, lactating or pregnant females, or peri-menopausal females with irregular menses were excluded from the study. Individuals self-reporting excessive caffeine use (> 500 mg per day) were also excluded from study participation.

Medical conditions and sleeping patterns that could potentially interfere with participation in the study were identified with medical history questionnaire, cognitive and depression screening, and fasting blood draw. Exclusionary signs included: elevated fasting blood glucose (≥126 mg/dL), significant anemia (hemoglobin <10 g/dL), depression (CES-D ≥ 16), and Epworth Sleepiness Scale (ESS) to exclude those with high daytime sleepiness (≥12).

Prior to study intervention, all participants completed a 1-week run-in phase to exclude individuals unlikely to comply with intervention guidelines. Sleep duration was recorded subjectively and objectively during the run-in phase to assure participants met the inclusion criteria. Sleep was recorded subjectively utilizing a time in bed/time out of bed sleep record, and objectively utilizing the Actigraph monitor (GT3X+; Actigraph, Pensacola, FL, USA) that was worn on the wrist at all times during the run-in phase. If determined that average time in bed (TIB) was <6 hours or >9 hours, participants were excluded from the study. Participants were instructed how to record all food and drink intake for the entirety of the 1-week run-in phase. The Pittsburgh Sleep Quality Index (PSQI) was utilized to assess sleep quality. On the last day of the run-in phase, participants completed a series of questionnaires including the ESS and PSQI in addition
to health-related quality of life (SF-36) (Ware, 2000), Functional Outcomes of Sleepiness (FOSQ) (Weaver et al., 1997), mood (POMS), and a Psychomotor Vigilance test (PVT) (Dinges et al., 1997).

All measurements completed prior to intervention were repeated after intervention in order to analyze intervention associated change.

Study Intervention

Caloric Restriction

Prior to the 8-week dietary intervention, participants were randomized into one of two groups [CR and CR+SR]. The dietary intervention was implemented as an 8-week CR diet plan. Caloric intake was reduced to 95% of each individual’s resting metabolic rate (RMR) which was measured utilizing indirect calorimetry. Prepackaged frozen meals were provided for lunch and dinner on 4 days each week. In order to assess total caloric consumption and caloric breakdown (fats, carbohydrate, and protein), all food and beverage intake was recorded for the entirety of the intervention period utilizing the “My Fitness Pal” phone application. Participants returned the Clinical Research Center on a weekly basis to record weight, report diet, and obtain meals for the upcoming week. Any compliance issues were addressed at these visits.

Sleep Restriction

The individuals assigned to CR group were asked to maintain their normal sleep and napping habits for the 8-week intervention period. The individuals assigned to CR+SR group were asked to decrease total TIB by 90 minutes for 5 days each week. Participants were asked to maintain similar sleep-wake timing during the 5 sleep restricted days. The other 2 days, participants were allowed to sleep ad libitum. This
schedule may reflect a sleep pattern where individuals sleep less during the work week, and more on non-work days, or weekends. Sleep duration was recorded subjectively utilizing a time in bed/time out of bed sleep record, and objectively utilizing the Actigraph monitor that was worn on the wrist at all times during intervention. Quality of life, depression, sleepiness, mood, and alertness were assessed every other clinical visit (every 2 weeks) utilizing CES-D, ESS, PSQI, SF-36, FOSQ, POMS, and PVT. If there were indication of clinical depression (CES-D > 16), excessive sleepiness (ESS > 12), large changes of the FOSQ (>10%), or an increase in PVT response time to >500 milliseconds, participants discontinued participation in the study and were referred to appropriate care.

Measurements

Sleep

Sleep duration was measured for all participants. Sleep quantity was objectively assessed by actigraphy utilizing Actigraph accelerometers (GT3X+; Actigraph, Pensacola, FL, USA). The accelerometer was worn on their non-dominant wrist for the duration of the 8-week intervention period. All participants kept a daily sleep log that recorded the time they laid down with light out trying to sleep (regardless of when they actually fell asleep), and the time they moved to a sitting position or got out of bed.

Accelerometer data was analyzed utilizing the manufacture provided software (ActiLife version 6.11.2). Data from the monitor were assessed in 60-second epochs, and time in bed/wake time recorded on the sleep log was manually input to allow an algorithmic calculation of total sleep time. Missing sleep log entries were estimated or adjusted for based on hierarchical ranking of inputs (i.e., sleep diary, light intensity and
activity counts). The Cole-Kripke sleep scoring algorithm was used to determine when each participant was asleep or awake (Cole, Kripke, Gruen, Mullaney, & Gillin, 1992). Application of this algorithm provides information on SOL (duration from specified bedtime to when the algorithm scored sleeping), TST (total length of time specified by the algorithm as being “asleep”), WASO (number of minutes the algorithm scored as being “awake” after sleep onset), and frequency of awakenings (number of awakenings occurring during the sleep period). While measuring sleep time with actigraphy based algorithm is a limitation, epoch-by-epoch analysis of wrist actigraphy is a feasible method of gathering objective sleep data measurements that are valid and in accordance with the gold standard of polysomnography recordings (de Souza et al., 2003; Taibi, Landis, & Vitiello, 2013).

Resting Metabolic Rate

RMR was measured for each participant in order to calculate the caloric intake required for the CR portion of this study. After an overnight fast, indirect calorimetry with a ventilated hood was utilized to determine the RMR of each participant. Following 15 minutes of rest, data were collected for a period of 30 minutes. Expired air was collected and analyzed with a metabolic cart (TrueOne 2400; ParvoMedics, Sandy, UT, USA). The \( \dot{V}O_2 \) and \( \dot{V}CO_2 \) data collected from the metabolic cart were utilized to calculate RMR with Weir’s equation (Weir, 1949). All measurements were completed in the morning.

Blood Collection

For this study, 12-hour fasted blood samples were collected before and after intervention. All blood samples were collected by a trained phlebotomist. Blood was
collected from the median cubital or cephalic vein in the cubital fossa of the elbow unless those veins were compromised. Blood was collected in a 7 mL serum vial and centrifuged at 3000 rpm to separate the red blood cells from serum. Serum was then aliquoted and stored at -80°C until being shipped to an offsite facility for NMR analysis.

**Statistical Analysis**

Analysis was done for all participants who successfully completed the diet intervention and had had NMR blood results (n= 28). Baseline descriptive statistics were calculated and reported as means and SD. Independent sample t-tests were utilized to determine differences in baseline characteristics (age, BMI, caloric intake, and sleep time) between participants randomized into CR versus CR+SR.

A repeated measure analysis of variance (ANOVA) was utilized to compare pre-versus post-intervention NMR results (total VLDL-P, large, medium and small VLDL-P, LDL-P, IDL-P, large and small LDL-P, total HDL-P, large, medium and small HDL-P, VLDL size, LDL size, and HDL size) between the two intervention groups. Separate models were run for each of the NMR variables. The model adjusted for age, BMI, and sex as covariates. The independent variable of interest were sleep (SR versus non-SR), time (pre- versus post-), and interaction between sleep and time. Data were analyzed using PROC MIXED and statistical significance was set at P < 0.05. All analysis were performed utilizing SAS 9.4 (SAS Institute Inc., Cary, NC).

**Strengths and Limitations of Study 2**

The strengths of this study include the use of NMR spectroscopy to analyze lipoprotein particle concentration and size, and the utilization of the actigraphy to obtain objective sleep time measurements. The watch-like monitor allowed objective sleep
measurement throughout the entirety of the intervention in addition to allowing participants to undergo a long-term sleep restriction protocol with the comfort of sleeping in their own home. The 12-week experimental SR protocol is one of the greatest strengths of this study. The effect of SR on lipoprotein particles has not been examined beyond five days of SR. Though self-report caloric intake is one of the limitations of this study due to the bias of self-report dietary intake, lunch and dinner being provided to participants 4 days a week provided meal samples and helped to reduce the potential effects of SR on modifying diet. Additional limitations of this study include the small sample size, and the lack of information collected concerning the menstrual cycle of female participants in relationship to the timing of blood collection. Menstrual cycle phase was not taken into account; therefore, pre- and post-intervention blood collection of female participants may have been completed in different phases of the menstrual cycle, which could affect blood lipoprotein results (Vashishta, Gahlot, & Goyal, 2017).
CHAPTER 4

MANUSCRIPT 1 – THE EFFECT OF DIFFERENT DOSES OF EXERCISE TRAINING ON LIPOPROTEIN PARTICLES IN OLDER WOMEN

\[\text{Porter, RR, Durstine, JL, Breneman, CB, Thompson, RW, Sarzynski, MA, Hussey, JR, & Wang, X. To be submitted to Medicine & Science in Sports & Exercise.}\]
ABSTRACT

INTRODUCTION: Lipoprotein concentrations are well established biological markers associated with cardiovascular disease (CVD) risk. Recent research has placed great importance on the various subfractions of lipoprotein particles. Current literature supports exercise as having a positive effect on lipoprotein profiles, however little research has been conducted to determine the effects of exercise dose on these lipoprotein subfraction concentration and size outcomes. PURPOSE: To investigate the effects of 16-weeks of exercise training at higher-dose (14 kilocalories per kilogram body weight per week [KKW]) or lower-dose (8 KKW) on the concentration and size of blood lipoprotein particles. METHODS: Sixty-five women (age = 64.7 ± 4.2 years) were randomized into higher-dose (n = 30) and lower-dose (n = 35) exercise groups. Exercise training consisted of supervised treadmill walking sessions, 3 times per week. All exercise training was completed at an intensity of 50-55% of heart rate reserve, therefore, dose primarily differed due to exercise amount. Fasting plasma samples were collected before and after exercise intervention. Plasma lipoprotein particle concentrations and average sizes were determined by nuclear magnetic resonance (NMR) spectroscopy. Statistical analyses were performed to compare within and between exercise group differences for all outcomes. A collapsed group analysis was completed to determine whether there was a significant effect of exercise training regardless of exercise dose. RESULTS: In the collapsed exercise group analysis (n = 65), exercise training reduced total high-density lipoprotein particle (HDL-P) concentration (p = 0.002) and increased mean HDL-P size (p = 0.002). When analyzed by exercise groups, the lower-dose group displayed a decrease in total HDL-P concentration (p = 0.001), while the higher-dose group displayed
an increase in mean low-density lipoprotein particle (LDL-P) size (p = 0.007). Both exercise lower- and higher-doses groups significantly increase mean HDL-P size (p = 0.03 and p = 0.02, respectively) with no significant difference between groups.

CONCLUSION: The results from this study support that exercise in sedentary older women leads to changes in the lipoprotein profile. Though the total HDL-P concentration decreased in the lower-dose group, HDL-P size increased. Maintenance of total HDL-P concentration in the higher-dose group along with the increase in mean HDL-P and LDL-P size are characteristics associated with lower CVD risk.

INTRODUCTION

Dyslipidemia is a lipoprotein metabolism disorder that results in abnormal plasma concentrations of lipids and lipoproteins. Data from the National Health and Nutrition Examination Survey 2003 to 2006 determined that approximately half the United States (US) population had at least one lipid abnormality (Parto, Lavie, Swift, & Sui, 2015). The association between dyslipidemia and cardiovascular disease (CVD) risk and mortality is well-established (Anderson, Castelli, & Levy, 1987; Cui et al., 2001; Gordon et al., 1989; Law, Wald, & Rudnicka, 2003; Park et al., 2015). Disease risk is important, as CVD is the leading cause of death in the US and globally (Centers for Disease Control [CDC], 2015; World Health Organization, 2017). Fortunately, treating dyslipidemia dose reduce the risk of coronary heart disease (CHD) (a specific type of CVD) and coronary events by approximately 30% (Goff et al., 2006; Grundy et al., 2004).

Results from observational and clinical trials demonstrate that increased exercise contribute to a more favorable traditional blood lipid and lipoprotein profile which includes: triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and the ratio of TC to HDL-C.
Specifically, populations that exercise more have lower TG and higher HDL-C concentrations compared to their counterparts that exercise less (Durstine, Grandjean, Cox, & Thompson, 2002). The beneficial effects of exercise on lipoprotein metabolism are not limited to the traditional lipid profile. Recent studies have shown lipoprotein subfraction traits are better markers of CVD risk compared to the traditional lipid profile. For example, studies have shown LDL particles, small LDL particles, large HDL particles, and large VLDL particles are interpedently associated with CVD risk (El Harchaoui et al., 2009; Krauss, 2010; Parish et al., 2012; Rosenson, Otvos, & Freedman, 2002). Several studies have demonstrated that exercise can impact the lipoprotein subfraction profile (Brown et al., 2009; Halverstadt, Phares, Wilund, Goldberg, & Hagberg, 2007; Kraus et al., 2002; Seip et al., 2006; Shadid, LaForge, Otvos, & Jensen, 2006). A meta-analysis by Sarzynski et al. (2015) found that exercise training decreased the concentrations of large VLDL-P, small LDL-P, and medium HDL-P, as well as decreased mean VLDL-P size. Exercise training also increased large LDL-P and large HDL-P concentrations and mean LDL-P size (Sarzynski et al., 2015).

Clearly, PA and exercise influences the lipoprotein profile, but the lipoprotein subfractions being significantly affected varies from study to study. One possible explanation for these inconsistent results is the wide variety of exercise training protocols utilized across studies. Few studies have thoroughly investigated whether the dose or amount of exercise influences lipoprotein subfractions. Kraus et al. (2002) demonstrated that higher exercise training amount had greater effect on 10 of 11 lipoprotein outcomes compared to lower amount of exercise of similar intensity. Multiple cross-sectional studies demonstrated increasing exercise dose was inversely associated with TG and
TC:HDL-C ratio, and positively associated with HDL-C concentration (Drygas et al., 1988, 2000; Durstine et al., 2001; Kokkinos et al., 1995; Lakka & Salonen, 1992; Williams, 1996, 1997, 1998). Though these studies demonstrate a dose-response effect of exercise on the traditional lipid and lipoprotein profiles, few studies have examined the effects of exercise dose on lipoprotein subfractions. Therefore, the purpose of this study was to investigate the effects of 16-weeks of exercise training at a higher-dose or lower-dose on blood lipoprotein particles concentrations and size.

METHODS

Study Population

The current study utilized data from the Women’s Energy Expenditure in Walking Programs (WEWALK) study. The WEWALK study was a 16-week randomized clinical exercise trial that investigated the effects of two different moderate-intensity exercise training doses on changes in energy expenditure and physical activity in sedentary older women (ClinicalTrials.gov identifier: NCT01722136) (Wang et al., 2017). The WEWALK study protocol was reviewed and approved by the University of South Carolina Institutional Review Board. Prior to beginning the study, all participants signed an informed consent. All participants recruited for this study were female, 60 – 75 years of age, weight stable (± 3% body weight for previous 3 months), sedentary (no more than 20 minutes of structured exercise 3 times per week for the past 3 months), non-smoking in the last year, BMI ≥ 18 and ≤ 30 kg/m², and free from CVD, metabolic or respiratory disease, or any other condition that would affect adherence to the study protocol, and were post-menopausal. Individuals reporting contraindications to exercise developed by the American College of Sports Medicine Guidelines (ACSM, 2010) or use
of metabolism affecting medications were excluded from the study. In addition, those self-reporting excessive caffeine use (> 500 mg per day) were also excluded. Medical conditions that could potentially interfere with participation in the exercise intervention were identified with a medical examination, and cognitive and depression screenings were completed. Exclusionary signs included: hypertension (blood pressure ≥ 160/90 mmHg); diabetes (fasting glucose ≥ 126 mg/dL); liver, renal, hematologic or thyroid disorder; cognitive dysfunction (Mini-Mental State Examination <24); or depression (Center for Epidemiologic Studies Depression [CES-D] Scale >16).

**Study Intervention**

Prior to commencing the exercise intervention, participants were randomized to one of two moderate-intensity treadmill-walking groups that differed by exercise dose, defined by weekly exercise energy expenditure. The lower-dose group was prescribed an exercise dose of 8 kilocalories per kilogram of body weight per week (KKW) and the higher-dose group was prescribed 14 KKW. Weekly energy expenditure was determined by multiplying the participant’s weight by their assigned dosage. The two different doses were achieved by varying the amount (duration) of total weekly exercise. All training sessions were supervised in a clinical exercise setting for a 16-week period.

Since all participants were considered sedentary upon study entry, the exercise intensity and weekly caloric expenditure were incrementally increased. Exercise training intensity started at 40% of heart rate reserve (HRR) and increased by 5% every two weeks until achieving the HRR target level of 50-55%. HRR was calculated from the maximum heart rate achieved during the baseline graded exercise test (GXT). Both exercise groups began at a weekly caloric expenditure of 4 KKW during the first week of
the intervention and progressed until reaching the assigned exercise dosage. The target intensity and amount were reached by week five in the lower-dose group (8 KKW) and week eight in the higher-dose group (14 KKW). A 3-minute warm-up and cool-down was conducted for each exercise session. Heart rate monitors (FT1; Polar, Lake Success, NY, USA) were utilized to continuously monitor exercise training intensity throughout each exercise session. During each exercise training session HR was recorded every five minutes. If HR was out of range, exercise intensity was adjusted in the following training session. Blood pressure was measured before, at the mid-point, and after each exercise session.

Compliance to the exercise protocol (frequency, intensity, and duration) was reviewed weekly for each participant. Participants missing an exercise session without notifying study personnel were contacted via phone and encouraged to attend. Refer to Table 4.1 for adherence to exercise parameters. Adherence was calculated by dividing actual HR (bpm) and dose (KJ) by prescribed HR and dose.

**Measurements**

*Body Mass Index (BMI)*

Height and weight were measured to the nearest 0.1 cm and 0.1 kg, respectively. Body weight was measured on a digital scale (Health O Meter® 10 Professional, Pelstar LLC, McCook, IL) with participants wearing standard scrubs, without shoes, or outer garments. The average of two consecutive height and weight measurements were averaged and utilized to calculate the BMI (kg/m²) for each individual before and after completion of the intervention.
**Graded Exercise Test (GXT)**

Graded treadmill exercise testing was completed to determine cardiorespiratory fitness (CRF) of all participants before and after exercise training. The protocol began at 0% grade and the participants’ self-selected pace defined as, “comfortable but challenging.” Every two minutes the incline was increased by 2%. Oxygen consumption ($\dot{V}O_2$) was continuously measured utilizing a metabolic cart (TrueOne 2400; ParvoMedics, Sandy, UT, USA). Blood pressure was measured at rest and in the last 30 seconds of every exercise stage utilizing a stethoscope and sphygmomanometer. Participants were encouraged to continue exercising to volitional fatigue. During the GXT, heart rhythm and HR were monitored utilizing a standard 12-lead electrocardiogram (ECG) (Q-Stress ®; Cardiac Science, Bothell, WA, USA). The ECG and HR were continuously monitored for the entirety of the GXT and for 10 minutes following the test. Test results were considered maximal if at least two of the four criteria were met, and peak if two of the following four criteria were not met: a plateau of $\dot{V}O_2$ (within 2 ml/kg/min in last 2 min), achieving a maximal HR greater than 90% of age predicted maximal HR (HRmax) (220 – age), a self-reported rating of perceived exertion (RPE) greater than 17 on the 6 – 20 Borg RPE scale, and/or a respiratory exchange ratio greater than or equal to 1.10. Peak oxygen consumption ($\dot{V}O_2$peak) was determined by the highest 30-second $\dot{V}O_2$ average recorded during the test.

**Blood Collection**

Twelve-hour fasted blood samples were collected before and after the 16-week exercise training intervention. The pre-intervention blood sample was collected within 7 day the first exercise session but at least 24 hours before the commencement of the
exercise training intervention. The post-intervention blood sample was collected at least 24 hours after but within 7 days of the last exercise training session. The median cubital or cephalic vein in the cubital fossa of the elbow was used to collect blood unless these veins were compromised. Blood was collected into an EDTA plasma vial and centrifuged at 3000 rpm to separate the red blood cells from plasma. Plasma was then aliquoted and stored at -80°C until NMR analysis.

**Lipoprotein Subfraction and Particle Size Measurements**

Comprehensive lipoprotein analysis was performed on fasting plasma samples collected before and after completion of exercise training by NMR spectroscopy at LipoScience, Inc (Raleigh, N.C.) using the LipoProfile-3 algorithm (Jeyarajah, Cromwell, & Otvos, 2006). Each measurement provides concentrations of large, medium, and small VLDL-P and HDL-P, large and small LDL-P, and intermediate-density lipoprotein particles (IDL-P), as well as weighted-average VLDL-P, LDL-P, and HDL-P sizes. The weighted average particle diameter for each lipoprotein is calculated as the sum of the lipoprotein subclass diameters multiplied by its relative mass percentage as estimated from the amplitude of its methyl NMR signal. Total VLDL-P, LDL-P, and HDL-P concentrations were calculated as the sum of their respective subclass concentrations.

**Statistical Analyses**

Statistical analysis only included participants who successfully completed the exercise intervention and had complete NMR blood analysis (n=65). Baseline descriptive statistics were calculated and reported as means and standard deviations (SD) for each exercise intervention group. In addition, both intervention groups were collapsed into one
group to determine whether there was a significant effect of exercise training regardless of exercise dose. Independent sample t-tests tests were utilized to determine differences in baseline characteristics (age, BMI, and V̇O₂peak) between exercise groups.

Repeated measure analysis of variance (ANOVA) was utilized to compare pre-versus post-intervention NMR traits between the two intervention groups. Separate models were run for each of the NMR variables. The model included exercise dose (lower versus higher), time (pre- versus post-intervention), and interaction between group and time. The model adjusted for age, BMI, and V̇O₂peak as covariates. Data was analyzed using PROC MIXED and statistical significance was set at P < 0.05. All analysis was performed utilizing SAS 9.4 (SAS Institute Inc., Cary, NC).

RESULTS

Participant Characteristics

A total of 87 participants enrolled into the study and 72 participants completed the intervention. Of those that completed the intervention, 65 subjects also had viable pre- and post-intervention blood samples. While blood collection was attempted for all participants, we were unable to collect blood for seven participants (lower-dose, n=2; higher-dose, n=5) at one or both time points. The energy prescription and intervention adherence of those that completed the study are reported in Table 4.1. Baseline and post-training characteristics for the 65 participants are found in Table 4.2 for the total population and by exercise group. No significant baseline differences were found between groups for age, body weight, BMI, V̇O₂peak, or NMR lipoprotein traits. No differences in baseline characteristics were observed between participants that completed
the exercise training program and had full data (n = 65) and those missing blood data (n = 7).

**Effects of Exercise Training on Lipoprotein Subfractions**

No significant difference between the two groups for changes of all lipoprotein subfractions was found (all p values for group x time interactions > 0.05). Therefore, data were analyzed first with the two groups collapsed, then separately in order to determine changes within each group. Total HDL-P concentration significantly (p = 0.002) decreased in the collapsed group (1.4 µmol/L), which appeared to be driven by significant (p = 0.001) decreases in the lower-dose group only (1.8 µmol/L). Mean HDL-P size significantly increased in the collapsed, lower-dose, and higher-dose groups (Table 4.3). LDL-P size significantly (p = 0.007) increased only in the higher-dose exercise group by 0.3 nm. However, overall, we found that the effects of exercise training on the concentration and size of lipoproteins did not differ between exercise dose groups (data not shown) as we did not find any significant interaction terms between time and exercise group.

**Effects of Exercise Training on Cardiometabolic Traits**

BMI significantly decreased by 0.4 kg/m² for both the higher-dose (p = 0.005) and lower-dose (p = 0.02) exercise groups, and by 0.3 kg/m² (p < 0.001) in the collapsed group after 16-weeks of intervention compared to baseline. Weight significantly decreased by 0.8 kg (p = 0.007) only in the collapsed group analysis. \( \dot{V}O_2\text{peak} \) significantly increased in the higher-dose group by 4.7 ml/kg/min (p < 0.001) and collapsed group analysis by 4.9 ml/kg/min (p < 0.001) but remained unchanged in the lower-dose exercise group after intervention.
Lipoprotein Associations with Cardiometabolic Traits

Statistical analysis resulted in noted associations between multiple lipoprotein variables and age, BMI, and \( \dot{V}O_2 \)peak. Large and medium VLDL-P (\( r = 0.229, p = 0.009; r = 0.197, p = 0.025 \), respectively) and small LDL-P concentration (\( r = 0.207, p = 0.018 \)), and mean VLDL-P size (\( r = 0.201, p = 0.022 \)) were all positively associated with age. Lipoprotein variables positively associated with BMI included large VLDL-P (\( r = 0.248, p = 0.004 \)), and small LDL-P concentration (\( r = 0.375, p < 0.001 \)). Large LDL-P and HDL-P concentrations (\( r = -0.207, p = 0.002; r = -0.431, p < 0.001 \), respectively) and mean LDL-P and HDL-P size (\( r = -0.322, p < 0.001; r = -0.476, p < 0.001 \), respectively) were negatively associated with BMI (\( p < 0.05 \)). Medium HDL-P concentration (\( r = 0.202, p = 0.022 \)) was positively associated with \( \dot{V}O_2 \)peak, while small HDL-P concentration (\( r = -0.292, p = 0.001 \)) was negatively associated with \( \dot{V}O_2 \)peak (\( p < 0.05 \)).

**DISCUSSION**

We investigated the effect of exercise dose on lipoprotein particle concentration and size. Our study is one of the first investigations designed to analyze the differences between higher-dose and lower-dose exercise on the lipoprotein particle concentration and size. Though we hypothesized the higher-dose exercise group would have more beneficial changes in lipoprotein particle subclass outcomes compared to the lower-dose group, no between-group differences were observed for any lipoprotein subclass trait. These findings are inconsistent with the findings of Kraus et al. (2002), who found that higher-dose exercise did elicit greater change in some lipoprotein subfractions (large VLDL-P, IDL-P, total and small LDL-P, and large HDL-P concentration; mean LDL-P and HDL-P size) compared to lower-dose exercise. This inconstancy could be due, in
part, to study design differences. First, our study was comprised of normal weight and overweight women, 60-75 years of age, while Kraus et al. (2002) included overweight and obese men and women, 40-65 years of age. Because of the body composition differences alone, baseline lipoprotein profiles may have been significantly different as small LDL-P and small HDL-P concentrations are associated with overweight and obesity (Després, 2007). Second, our study did not include a control group for comparison which likely impacted the statistical analysis and the potential for group differences. Third, Kraus et al. (2002) analyzed exercise intensity and amount separately, whereas our study fixed exercise intensity and prescribed different exercise amounts. Their study design included four groups; a control group, a low-amount of work (14 KKW) completed at a moderate intensity (40% to 55% \( \dot{V}O_2 \)peak), a low-amount of work completed at a high intensity (65% to 80% \( \dot{V}O_2 \)peak), and a high-amount of work (23 KKW) completed at a high intensity. Our study design incorporated a lower-dose group at 8 KKW and a higher-dose group at 14 KKW, both at intensities of 50-55% HRR. Thus, our higher-dose group was only equivalent to the low-amount of work at a moderate intensity group of Kraus et al. (2002). Since Kraus et al. (2002) utilized higher doses of exercise than our study; further investigation is needed to determine if our lack of observable difference between doses is due to insufficient intensity or volume of exercise.

Though our study found no significant differences between groups after the exercise intervention, multiple variables did significantly change within exercise groups and/or the collapsed group indicating that exercise did have an effect on the lipoprotein profile. In our study, weight only decreased in the collapsed group analysis, but the body
weight change was large enough in both higher-dose and lower-dose exercise groups to cause significant change in BMI outcomes. BMI was statistically controlled for in the lipoprotein analysis as BMI was positively associated with change in large VLDL-P and small LDL-P concentration, and VLDL-P size. BMI was also negatively associated with change in total LDL-P and large HDL-P concentration, and LDL-P and HDL-P size. Cardiorespiratory fitness significantly increased in the collapsed and higher-dose exercise group but remained unchanged in the lower-dose group. This change in cardiorespiratory fitness is consistent with the work of Church et al. (2007) who completed a randomized control trial in postmenopausal women that compared the dose effect of moderate-intensity aerobic exercise at 4, 8 and 12 KKW. Church et al. (2007) reported no difference in cardiorespiratory fitness after 6-months of exercise between the 8 KKW and 12 KKW exercise groups.

The decrease of total HDL-P concentration in the lower-dose and collapsed exercise groups could be viewed as a less favorable outcome because HDL-P is inversely associated with CHD risk (Mackey et al., 2012). However, one must consider the entire HDL-P profile. While total HDL-P concentration decreased, mean HDL-P size increased in both training groups and the collapsed group. This increase in HDL-P size is viewed as favorable and is indicative of greater cholesterol carrying capacity of the circulating HDL particles and may, in part, explain the decreased HDL-P concentrations (Kontush, 2015). The same rationale can also apply to the increase in LDL-P size observed only in the higher-dose exercise group (Mora et al., 2007). Since the increase was only observed in the higher-dose group, these data provide preliminary evidence that a higher dose of
aerobic exercise training potentially provides greater benefit to the lipoprotein profile compared to lower dose aerobic exercise training doses.

In addition to the above observations, associations were noted between select lipoprotein variables, age, BMI, and \( \dot{V}O_2 \text{peak} \). Among this population of older women, large and medium VLDL-P concentration, small LDL-P concentration, and mean VLDL-P size were all positively associated with age. Evidence from our study combined with Kraus et al. (2002) and Sarzynski et al. (2015) suggests that individuals may improve CVD associated lipoprotein particle subfractions such as large VLDL-P concentration and size, and small LDL-P concentration with exercise. Exercise of greater amount may result in even greater improvements in older individuals (Kraus et al., 2002). Exercise in older individuals Lipoprotein variables that positively associated with BMI included large VLDL-P, small LDL-P concentration, and VLDL-P size. Total LDL-P and large HDL-P concentrations, and mean LDL-P and HDL-P size were negatively associated with BMI. Because BMI significantly decreased with exercise in our study, the BMI associated lipoprotein subfractions may potentially result in decreased CVD risk. A longitudinal exercise training study is needed to determine the above mentioned potential of improving CVD risk. The associations observed with increasing BMI and age aligned with the associations between lipoprotein profile and increased CVD risk. Medium HDL-P concentration was positively associated with \( \dot{V}O_2 \text{peak} \), while small HDL-P concentration was negatively associated with \( \dot{V}O_2 \text{peak} \). These associations also aligned with reduced CVD risk associations.

While the mechanisms for exercise-induced lipid and lipoprotein changes is not well understood, existing studies provide insight into potential mechanisms (Wang & Xu,
Lipoprotein lipase (LPL), the enzyme located on the capillary walls that stimulates the release of triglyceride from chylomicron particles and VLDL, is known to be acutely (Ferguson et al., 1998; Kantor et al., 1984; Seip & Semenkovich, 1998) and chronically (Bergeron et al., 2001; Miyashita et al., 2010; Thompson et al., 1997) affected by exercise. Increased LPL activity, in turn, is associated with increased HDL-C (Durstine & Haskell, 1994; Tsutsumi, 2003). Cellular membrane protein levels of ATP-binding cassette transporter A-1 (ABCA1) and ABCA1 gene expression are upregulated as an adaptation to exercise training (Tofighi, Rahmani, Jamali Qarakhanlou, & Babaei, 2015). ABCA1 interacts with apoA-1 on an HDL particle and thereby preparing the HDL particle to uptake cholesterol in the process of reverse cholesterol transport. Proliferator-activated receptor gamma (PPARγ) and liver X receptor alpha (LXRα) are two additional receptors involved in reverse cholesterol transport. Both PPARγ and LXRα are upregulated as an adaptation to exercise training (Butcher et al., 2008). While LPL, ABCA1, PPARγ, and LXRα were not measure in our study, the effects of exercise training on these receptors and enzymes may be potential mechanisms that led to significant changes in HDL-P concentration and size. Proprotein convertase subtilisin/kexin type 9 (PCSK9) is an inhibitor of LDL receptor proteins. When PCSK9 binds the LDL receptor protein in the liver, LDL cannot be taken up by liver cells, thereby leaving more LDL in blood circulation. Increased PA over a three-month period decreased expression of PCSK9 and lower LDL-C levels (Kamani et al., 2015). Further investigation is needed to determine if this mechanism is involved in alteration of LDL-P concentration composition with exercise reported by Sarzynski et al. (2015).
The strengths of this study include the use of NMR spectroscopy to analyze lipoprotein particle concentration and size, and the exercise intervention sessions were supervised in a clinical exercise setting to assure protocol compliance. The generalizability of this study is limited to generally healthy, postmenopausal women. The participants in this study were not at high CVD risk. Therefore, the impact of exercise training on altering CVD risk is potentially limited. The absence of a control group (due to lack of funding) for comparison is also a significant limitation. Significant results may also be due to the lower volume and intensity of our exercise protocol compared to that of other studies (Kraus et al., 2002).

In conclusion, we did not find a dose effect of exercise training on lipoprotein concentrations and size, as training effects were observed with both lower- and higher-dose exercise training. A potential reason for not finding a difference is that greater exercise volume at higher exercise intensity is likely required for blood lipid and lipoprotein change (Davis et al., 1992; Ferguson et al., 1998; Kraus et al., 2002). However, the lipoprotein variables that changed significantly from baseline are indicative of decreased CVD risk. Because total HDL-P concentration and LDL-P size only changed from baseline in the lower-dose and higher-dose exercise groups respectively, further clinical research including a control group is needed to better determine if exercise dose plays a role in the effect of exercise training on lipoprotein particle concentration and size.
### Table 4.1 Exercise prescription and intervention adherence

<table>
<thead>
<tr>
<th></th>
<th>Lower-dose (n = 37)</th>
<th>Higher-dose (n = 35)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Exercise intensity</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prescribed HR (contractions/min)</td>
<td>118 ± 11</td>
<td>119 ± 7</td>
</tr>
<tr>
<td>Actual HR at 8 weeks (beats/min)</td>
<td>114 ± 11</td>
<td>116 ± 10</td>
</tr>
<tr>
<td>Adherence to intensity (actual/prescribed HR), %</td>
<td>97 ± 7.6</td>
<td>98 ± 8.4</td>
</tr>
<tr>
<td><strong>Exercise dose</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prescribed weekly exercise dose (KJ)</td>
<td>2268 ± 328</td>
<td>3948 ± 516</td>
</tr>
<tr>
<td>Actual weekly exercise dose (KJ)</td>
<td>2365 ± 386</td>
<td>3902 ± 643</td>
</tr>
<tr>
<td>Adherence to exercise dose (actual/prescribed dose), %</td>
<td>104 ± 8.5</td>
<td>99 ± 7.5</td>
</tr>
</tbody>
</table>

HR – heart rate; KJ – kilojoule

Actual weekly exercise dose calculated using American College of Sports Medicine formula (American College of Sports Medicine, 2010)
<table>
<thead>
<tr>
<th></th>
<th>Collapsed (Baseline)</th>
<th>Collapsed (Post-training)</th>
<th>Lower-dose (Baseline)</th>
<th>Lower-dose (Post-training)</th>
<th>Higher-dose (Baseline)</th>
<th>Higher-dose (Post-training)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body weight, kg</strong></td>
<td>67.5 ± 9.7</td>
<td>66.7 ± 9.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>67.4 ± 10.1</td>
<td>66.7 ± 10.0</td>
<td>67.6 ± 9.3</td>
<td>66.8 ± 9.2</td>
</tr>
<tr>
<td><strong>BMI, kg/m&lt;sup&gt;2&lt;/sup&gt;</strong></td>
<td>25.6 ± 3.6</td>
<td>25.3 ± 3.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>25.8 ± 4.0</td>
<td>25.4 ± 4.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>25.5 ± 3.1</td>
<td>25.1 ± 3.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>(\dot{V}O_2)peak, ml/kg/min</strong></td>
<td>20 ± 3.7</td>
<td>22 ± 4.7&lt;sup&gt;c&lt;/sup&gt;</td>
<td>20 ± 3.7</td>
<td>21 ± 4.3</td>
<td>20 ± 3.7</td>
<td>23 ± 5.1&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> – statistically different from baseline (p < 0.05); <sup>b</sup> – statistically different from baseline (p < 0.01); <sup>c</sup> – statistically different from baseline (p < 0.001)
Table 4.3 Lipoprotein Outcomes by Exercise Dose at Baseline and Post-training

<table>
<thead>
<tr>
<th>Lipoprotein Particle</th>
<th>Collapsed (Baseline)</th>
<th>Collapsed (Post-training)</th>
<th>Lower-dose (Baseline)</th>
<th>Lower-dose (Post-training)</th>
<th>Higher-dose (Baseline)</th>
<th>Higher-dose (Post-training)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N = 65</td>
<td>N = 35</td>
<td>N = 30</td>
<td>N = 30</td>
<td>N = 30</td>
<td>N = 30</td>
</tr>
<tr>
<td>VLDL Particle (nmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total VLDL-P</td>
<td>49.1 ± 20.0</td>
<td>45.3 ± 17.7</td>
<td>48.8 ± 18.2</td>
<td>42.6 ± 15.0</td>
<td>49.5 ± 22.3</td>
<td>48.5 ± 20.2</td>
</tr>
<tr>
<td>Large VLDL-P</td>
<td>3.8 ± 2.9</td>
<td>3.6 ± 3.6</td>
<td>4.1 ± 3.0</td>
<td>3.2 ± 3.2</td>
<td>3.6 ± 2.8</td>
<td>4.0 ± 3.9</td>
</tr>
<tr>
<td>Medium VLDL-P</td>
<td>14.7 ± 9.7</td>
<td>15.3 ± 9.8</td>
<td>14.6 ± 9.8</td>
<td>14.4 ± 8.8</td>
<td>14.8 ± 9.8</td>
<td>16.4 ± 10.9</td>
</tr>
<tr>
<td>Small VLDL-P</td>
<td>31.0 ± 14.4</td>
<td>27.2 ± 12.6</td>
<td>31.0 ± 12.7</td>
<td>26.4 ± 14.0</td>
<td>31.1 ± 16.4</td>
<td>28.1 ± 11.1</td>
</tr>
<tr>
<td>LDL Particle (nmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total LDL-P</td>
<td>1034.4 ± 290.5</td>
<td>1020.8 ± 318.5</td>
<td>1016.0 ± 325.5</td>
<td>1015.1 ± 355.3</td>
<td>1055.9 ± 247.2</td>
<td>1046.9 ± 274.3</td>
</tr>
<tr>
<td>IDL-P</td>
<td>274.7 ± 147.9</td>
<td>282.6 ± 133.0</td>
<td>270.7 ± 143.3</td>
<td>303.7 ± 122.8</td>
<td>279.4 ± 155.5</td>
<td>258.0 ± 142.1</td>
</tr>
<tr>
<td>Large LDL-P</td>
<td>281.8 ± 195.2</td>
<td>303.4 ± 196.0</td>
<td>307.4 ± 204.7</td>
<td>315.0 ± 215.3</td>
<td>252.8 ± 183.0</td>
<td>290.2 ± 174.4</td>
</tr>
<tr>
<td>Small LDL-P</td>
<td>490.8 ± 300.4</td>
<td>448.6 ± 371.1</td>
<td>455.5 ± 320.5</td>
<td>405.5 ± 405.7</td>
<td>532.0 ± 274.8</td>
<td>495.8 ± 325.8</td>
</tr>
<tr>
<td>HDL Particle (µmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total HDL-P</td>
<td>38.8 ± 5.9</td>
<td>37.4 ± 5.5 a</td>
<td>39.4 ± 5.6</td>
<td>37.6 ± 5.6 b</td>
<td>38.1 ± 6.3</td>
<td>37.2 ± 5.5</td>
</tr>
<tr>
<td>Large HDL-P</td>
<td>9.9 ± 3.2</td>
<td>10.2 ± 3.4</td>
<td>10.2 ± 3.7</td>
<td>10.5 ± 3.7</td>
<td>9.7 ± 2.4</td>
<td>9.8 ± 3.0</td>
</tr>
<tr>
<td>Medium HDL-P</td>
<td>13.7 ± 8.0</td>
<td>12.8 ± 7.3</td>
<td>14.6 ± 7.5</td>
<td>13.3 ± 7.0</td>
<td>12.8 ± 8.7</td>
<td>12.1 ± 7.7</td>
</tr>
<tr>
<td>Small HDL-P</td>
<td>15.6 ± 7.6</td>
<td>15.2 ± 8.3</td>
<td>15.1 ± 8.1</td>
<td>14.5 ± 9.0</td>
<td>16.2 ± 7.1</td>
<td>16.2 ± 7.4</td>
</tr>
<tr>
<td>Particle Size (nm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VLDL-P size</td>
<td>49.4 ± 7.0</td>
<td>49.2 ± 7.1</td>
<td>50.5 ± 7.6</td>
<td>49.9 ± 7.1</td>
<td>48.0 ± 6.1</td>
<td>48.3 ± 7.1</td>
</tr>
<tr>
<td>LDL-P size</td>
<td>20.7 ± 0.6</td>
<td>20.8 ± 0.7</td>
<td>20.8 ± 0.6</td>
<td>20.6 ± 0.5</td>
<td>20.6 ± 0.5</td>
<td>20.9 ± 0.7 a</td>
</tr>
<tr>
<td>HDL-P size</td>
<td>9.7 ± 0.4</td>
<td>9.8 ± 0.5 a</td>
<td>9.7 ± 0.5</td>
<td>9.8 ± 0.6 a</td>
<td>9.7 ± 0.4</td>
<td>9.8 ± 0.5 a</td>
</tr>
</tbody>
</table>

a – statistically different from baseline (p < 0.01); b – statistically different from baseline (p = 0.001)
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CHAPTER 5

MANUSCRIPT 2 – THE EFFECT OF SLEEP RESTRICTION DURING CALORIC RESTRICTION ON LIPOPROTEIN PARTICLES

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2Porter, RR, Durstine, JL, Youngstedt, SD, Sparks, JR, Thompson, RW, Sarzynski, MA, Hussey, JR, & Wang, X. To be submitted to *Lipids in Health and Disease*. 
ABSTRACT

INTRODUCTION: Caloric restriction (CR) is a dietary technique utilized for weight loss and cardiovascular disease (CVD) risk management. Weight loss decreases CVD risk through various mechanisms, including favorably altering the lipid profile. Short durations of sleep may increase CVD risk partially evident by less favorable lipoprotein profiles. However, few studies have examined the effects of sleep restriction (SR) during CR to determine whether SR limits the beneficial effects of CR on lipoprotein profiles.

PURPOSE: To investigate 8-weeks of SR during CR compared to CR alone on blood lipoprotein particle concentrations and size.

METHODS: Twenty-eight male and female participants (age = 44.0 ± 5.8 years) were randomized into CR (n = 12) and CR+SR (n = 16) groups. CR consisted of caloric intake reduced to 95% of each individual’s baseline resting metabolic rate (RMR). SR consisted of reducing total time in bed (TIB) by 90 minutes, 5 nights per week. Fasting plasma samples were collected before and after the intervention. Plasma lipoprotein particle concentrations and average sizes were determined by nuclear magnetic resonance spectroscopy. Statistical analysis was completed to compare within and between intervention group differences for all outcomes. A collapsed group analysis was completed to determine whether a significant effect of CR exists regardless of sleep duration.

RESULTS: No significant differences between groups were observed at baseline for any lipoprotein variable. After the intervention, weight significantly decreased by an average of 3.0 kg (p = 0.02) in the CR group, 3.2 kg (p < 0.001) in the CR+SR group, and 2.9 kg (p < 0.001) in the collapsed group. Large high-density lipoprotein particle (HDL-P) concentration decreased in the CR group by an average of 0.8 µmol/l (p = 0.004) and in the collapsed group by 0.9
μmol/l (p = 0.006), while mean HDL-P size decreased in the CR+SR group by 0.3 nm (p = 0.02). CONCLUSION: No between-group differences were found for the effects of CR or CR+SR on lipoprotein subclasses. However, several lipoprotein variables significantly changed in intervention groups, with our results suggesting that CR+SR may negatively affect certain lipoproteins that remained unaffected by CR alone.

INTRODUCTION

Weight loss by means of dietary caloric restriction (CR) is commonly utilized as a method to decrease CVD risk (Siri-Tarino & Krauss, 2016). Some mechanisms aiding in CVD risk reduction via CR include: reduced metabolic rate and oxidative damage (Ungvari, Parrado-Fernandez, Csizsar, & de Cabo, 2008), decreasing total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) and elevating high-density lipoprotein cholesterol (HDL-C) concentrations (Fontana, Meyer, Klein, & Holloszy, 2004; Katzel et al., 1995; Verdery & Walford, 1998). Recent studies have also reported the effect of CR on lipoprotein subfractions as determined by nuclear magnetic resonance (NMR) spectroscopy. Higher concentrations of small LDL-P and small HDL-P are associated with overweight and obesity (Després, 2007). Interventions causing weight loss by means of daily CR or alternate day fasting (ADF) report decreased LDL-C and decreased proportion of small LDL-P among total LDL-P, as well as an increase in mean LDL-P size (Bhutani et al., 2013; Tzotzas et al., 2011; Varady et al., 2011; Varady & Hellerstein, 2007).

Sleep as a modifiable lifestyle behavior in relation to lipoprotein particles and CVD is a relatively new area of study, thus our understanding of this relationship is limited. Existing evidence also supports sleep duration as being associated with blood lipid and lipoprotein profiles. Population-based studies have concluded that sleep
duration is associated with plasma concentrations of triglyceride (TG), TC, HDL-C, and LDL-C, and consistent patterns of sleeping <6 hours or >8 hours were associated with abnormal blood lipid and lipoprotein concentrations and elevated CVD risk (Bjorvatn et al., 2007; Kaneita, Uchiyama, Yoshiike, & Ohida, 2008). Only one human study has utilized NMR to analyze the effect of short term (5 days) sleep deprivation on lipoprotein subfractions (Aho et al., 2016). The study found a decreased concentration of small, medium and large LDL-P, and small VLDL-P, but no change in concentration of small, medium, or large HDL-P after 5 days of sleep deprivation (Aho et al., 2016). To our knowledge, no previous study has examined the effects of experimental chronic sleep restriction (SR) on lipoprotein subfractions. More specifically, no study has investigated the effects of SR in individuals undergoing CR. The literature containing information showing that a dose-response relationship between short sleep, and high BMI and weight gain are due to increased caloric intake (Calvin et al., 2013). Therefore, the purpose of this study was to determine the effects of an 8-week CR on blood lipid and lipoprotein particle concentrations and particle size, and to determine the effect of CR combined with SR (CR+SR) on blood lipoprotein particle concentrations and particle size compared to CR alone.

METHODS

Study Population

Data used in this study were part of the Weight Outlooks by Restriction of Diet and Sleep (WORDS) study. The WORDS study was a randomized trial that investigated the effect of SR on body composition in individuals undergoing a CR dietary weight loss program (ClinicalTrials.gov identifier: NCT02413866) (Wang, Sparks, Bowyer, &
Youngstedt, 2018). The WORDS study protocol was reviewed and approved by the University of South Carolina Institutional Review Board. Prior to beginning the study, all participants signed an informed consent. Male and female participants recruited for this study were 35 to 55 years of age, weight stable (± 3% body weight for the previous 3 months), self-reported sleeping between 6.5 to 8 hours per day with <90 minutes per day of daytime napping for the previous 3 months, had < 120 minutes of exercise training per week during the previous 3 months, BMI ≥ 25 and ≤ 40 kg/m², and were free from CVD, diabetes, chronic respiratory disease, active cancer, sleep or eating disorders, or any other condition that would prevent adherence to study protocol. Individuals reporting shift work, psychological disorders, smoked within the past year, use of metabolism affecting medications, lactating or pregnant females, or peri-menopausal females with irregular menses were excluded from the study. Individuals self-reporting excessive caffeine use (> 500 mg per day) were also excluded from study participation.

Prior to the study intervention, all participants completed a 1-week run-in phase to familiarize them to the measurements. Participants were instructed to record the time in and out of bed and to wear an Actigraph monitor (GT3X+; Actigraph, Pensacola, FL, USA) on the wrist at all times during the run-in phase. Participants were instructed how to record all food and drink intake for the entirety of the 1-week run-in phase.

**Study Intervention**

**Caloric Restriction**

Prior to the 8-week dietary intervention, participants were randomized into one of two groups [CR and CR+SR]. The dietary intervention was implemented as an 8-week CR diet plan. Caloric intake was reduced to 95% of each individual’s resting metabolic
rate (RMR) which was measured utilizing indirect calorimetry, as previously described by Wang et al. (2018). Prepackaged frozen meals were provided for lunch and dinner on 4 of the CR days each week. Participants were instructed to record all food and beverage intake for the entirety of the intervention period utilizing the “My Fitness Pal” internet-based website, or phone application. Participants visited the Clinical Research Center on a weekly basis to record weight, report diet, and obtain meals for the upcoming week. Any compliance issues were addressed at these visits.

Sleep Restriction

The individuals assigned to the CR group were asked to maintain their normal sleep and napping habits for the 8-week intervention period. Individuals assigned to the CR+SR group were asked to decrease total TIB by 90 minutes over 5 days each week. The 5 days did not have to be concurrent but could be if the participant so desired. The other 2 days, participants were allowed to sleep ad libitum. Participants were asked to maintain similar sleep-wake timing during the intervention. Participants were instructed to record the time in and out of bed, and to wear an Actigraph monitor on the wrist at all times during the 8-week intervention period. On the last day of the run-in phase, participants completed a series of questionnaires including the Center for Epidemiologic Studies Depression Scale (CES-D), the Epworth Sleepiness Scale (ESS), the Functional Outcomes of Sleepiness (FOSQ) (Weaver et al., 1997), and a Psychomotor Vigilance test (PVT) (Dinges et al., 1997). Depression, sleepiness, mood, and alertness were also assessed every other clinical visit (every 2 weeks) utilizing CES-D, ESS, FOSQ, and PVT to determine whether adverse changes occurred.
Sleep

Accelerometer data was analyzed utilizing the manufacture provided software (ActiLife version 6.11.2). Data from the monitor were assessed in 60-second epochs and time in bed/wake time recorded on the sleep log was manually inputted to allow an algorithmic calculation of total sleep time (TST). Missing sleep log entries were estimated or adjusted for based on hierarchical ranking of inputs (i.e., sleep diary, light intensity and activity counts). The Cole-Kripke sleep scoring algorithm was used to determine when each participant was asleep or awake (Cole, Kripke, Gruen, Mullaney, & Gillin, 1992). TST was defined as the total length of time specified by the algorithm as being “asleep”. TIB was determined utilizing self-report sleeping logs in which participants recorded the time they got in and out of bed, regardless of actual sleep time.

Measurements

Body Mass Index (BMI)

Height and weight were measured to the nearest 0.1 cm and 0.1 kg, respectively. Body weight was measured on a digital scale (Health O Meter® 10 Professional, Pelstar LLC, McCook, IL) with participants wearing standard scrubs and without shoes or outer garments. Height and weight measurements taken on two consecutive days were averaged and utilized to calculate the BMI (kg/m²) of each individual before and after intervention.

Blood Collection and Analysis

Fasting blood samples were collected before and after the intervention following an overnight fast (12 hours). Blood was taken from the median cubital or cephalic vein in the cubital fossa of the elbow (unless those veins were compromised) and collected into a
serum vial, allowed to set at room temperature for 30 minutes, and centrifuged at 3000 rpm to separate the clotted cells from serum.

**Lipoprotein Subfraction and Particle Size Measurements**

Fasting blood samples were utilized for a comprehensive lipoprotein analysis before and after completion of the intervention by NMR spectroscopy at LipoScience, Inc (Raleigh, N.C.) using the LipoProfile-3 algorithm (Jeyarajah, Cromwell, & Otvos, 2006). Concentrations of large, medium, and small VLDL-P and HDL-P, large and small LDL-P, and intermediate-density lipoprotein particles (IDL-P), as well as weighted-average VLDL-P, LDL-P, and HDL-P sizes are provided for each blood sample. The weighted average particle diameter for each lipoprotein is calculated as the sum of the lipoprotein subclass diameters multiplied by its relative mass percentage as estimated from the amplitude of its methyl NMR signal. Total VLDL-P, LDL-P, and HDL-P concentrations were calculated as the sum of their respective subclass concentrations.

**Statistical Analyses**

Analysis was completed on all participants who successfully completed the diet intervention and had NMR results at both pre- and post-intervention (n=28). Baseline descriptive statistics were calculated and reported as means and standard deviation (SD) for each study group in addition to the whole study population (referred to as “collapsed group”). Independent sample t-tests were utilized to determine differences in baseline characteristics (age, BMI, caloric intake, and sleep time) between participants randomized into CR (n = 12) versus CR+SR (n = 16).

Repeated measure analysis of variance (ANOVA) was utilized to compare pre-versus post-intervention NMR results (total VLDL-P, large, medium and small VLDL-P,
LDL-P, IDL-P, large and small LDL-P, total HDL-P, large, medium and small HDL-P, VLDL size, LDL size, and HDL size) and between the two intervention groups. Separate models were run for each of the NMR variables. The model adjusted for age, BMI, and sex as covariates. The independent variable of interest was sleep (SR versus non-SR). Data was analyzed using PROC MIXED and statistical significance was set at P < 0.05. All analysis was performed utilizing SAS 9.4 (SAS Institute Inc., Cary, NC).

RESULTS

Participant Characteristics

Of the 36 participants that completed the study, 28 completed the intervention with viable pre- and post-intervention blood samples. While blood collection was attempted for all participants, blood was not collected for eight participants (CR, n=3; CR+SR, n=5) at one or both time points due to unsuitable veins. Baseline characteristics for the 28 participants are found in Table 5.1 for the collapsed group and each intervention group. No significant difference existed between intervention groups for baseline values of age, sleep time, or any lipoprotein variable. However, mean body weight and BMI of the CR+SR group was significantly (p < 0.01 and p < 0.05, respectively) greater compared to the CR group at baseline.

Effects of Sleep Restriction on Lipoprotein Profile

The non-sleep restriction group (CR only) had no change in TST during the intervention, whereas the CR+SR group significantly decreased average sleep time by 53 minutes per night (p < 0.001). On nights of SR, participants averaged 342 ± 45 minutes (an average of 69 minutes less per night compared to baseline), compared to 453 ± 47
minutes (an average of 42 minutes more per night compared to baseline) per night on *ad libitum* sleep nights.

There were no differences between groups on the effects of the intervention on changes in lipoprotein particle traits. Within-group and/or collapsed group outcomes of several variables significantly changed with the intervention (Table 5.2). BMI significantly decreased by an average of 1.0 kg/m$^2$ (p = 0.002) in the CR group, 1.1 kg/m$^2$ (p < 0.001) in the CR+SR group, and 1.1 kg/m$^2$ (p < 0.001) in the collapsed group. Weight significantly decreased by 3.0 kg (p = 0.002) in the CR group, 3.2 kg (p < 0.001) in the CR+SR group, and 2.9 kg (p < 0.001) in the collapsed group. Large HDL-P concentration decreased in the CR group by 0.8 µmol/l (p = 0.004) and by 0.9 µmol/l (p = 0.006) in the collapsed group, while mean HDL-P size decreased in the CR+SR group by 0.3 nm (p = 0.02).

**DISCUSSION**

The current study investigated the effect of SR compared to normal sleep during CR diet on CVD associated lipoprotein particle concentration and size. The present study is one of the first designed to analyze the effect of SR on lipoprotein particle concentration and size. Though we expected the CR+SR group to have less beneficial change in certain lipoprotein particle subclass outcomes compared to the CR group, no differences were observed in any lipoprotein subclass between the two intervention groups. In addition, no significant changes of lipoproteins were observed in the CR group.

In our study, within-group differences do provide evidence that CR+SR may negatively affect certain lipoproteins that remained unaffected by CR alone. While body
weight and BMI changes within groups were similar, sleep was significantly reduced in
the CR+SR group but not in the CR group. These results allow us to conclude that the
differences in lipoprotein outcomes are more likely due to differences in sleep patterns as
opposed to differences in body composition. Observed changes included, decreased large
HDL-P concentration in the CR group and collapsed group, and decreased HDL-P size in
the CR+SR group. These changes are not consistent with the only other study that has
investigated the lipoprotein outcomes in response to a SR intervention. Aho et al. (2016)
reported a decrease of small, medium, and large LDL-P, and small VLDL-P. No
significant change in small, medium, or large HDL-P were found. These inconsistencies
are likely due to various differences in experimental design. First, our participants were
undergoing CR and SR simultaneously. The CR produced significant weight loss and
decreased BMI. In obese men with metabolic syndrome, weight loss did lead to
significant changes in LDL and HDL kinetics (Ng, Watts, Barrett, Rye, & Chan, 2007).
Though no change in LDL or HDL production was reported in the study by Ng et al.
(2007), weight loss increased catabolism of LDL apolipoprotein B-100 (apoB-100) and
delayed catabolism of HDL apolipoprotein A-1 (apoA-1). Second, the participants in the
Aho et al. (2016) study only underwent SR for 1 period of 5-nights compared to the 5-
nights per week for 8 weeks that our study participants completed. The epidemiological
data developed by Aho et al. (2016) may provide, in part, an explanation for the
lipoprotein changes and insight into the mechanisms underlying our 8-week chronic SR
intervention. Examination of the epidemiological SR data by Aho et al. (2016)
demonstrated the while acute SR appears to alter the LDL profile, long term SR results in
a decreased expression of liver X receptor (LXR) and ATP-binding cassette transporters
A1 (ABCA1), which are key components of cholesterol delivery by HDL to the liver. The delivery of cholesterol to the liver by HDL is known as reversed cholesterol transport (RCT). Chronic SR induces decreased expression of LXR and ABCA1, which resulted in reduced RCT, lower HDL-C concentration, and larger VLDL-P size (Aho et al., 2016; Liu, Chung, Shelness, & Parks, 2012).

The strengths of this study include the use of NMR spectroscopy to analyze lipoprotein particle concentration and size, and the utilization of actigraphy to obtain objective sleep time measurements. The watch-like monitor allowed objective sleep measurement throughout the entirety of the intervention in addition to allowing participants to undergo a long-term sleep restriction protocol with the comfort of sleeping in their own home. The 12-week experimental SR protocol is one of the greatest strengths of this study. The effect of SR on lipoprotein particles has not been examined beyond five days of SR. Though self-reported caloric intake is one of the limitations of this study due to the bias of self-reported dietary intake, lunch and dinner being provided to participants 4 days a week helped to reduce the potential effects of SR on modifying diet. Additional limitations of this study include the small sample size and the lack of information collected concerning the menstrual cycle of female participants in relationship to the timing of blood collection. Menstrual cycle phase was not concerned; therefore, pre- and post-intervention blood collection of female participants may have been completed in different phases of the menstrual cycle, which could affect blood lipoprotein results (Vashishta, Gahlot, & Goyal, 2017).

In conclusion, we did not observe any between-group differences comparing lipoprotein subclasses of CR and CR+SR intervention groups. However, within-group
lipoprotein variables that differed significantly post-intervention compared to baseline suggest that CR+SR may negatively impact the lipoprotein profile that was unchanged by CR alone. Since this study is the first to report the effect of long term SR on lipoprotein particle concentration and size and because this study was completed in conjunction with a CR weight loss intervention, further studies are needed to determine the effect of SR on lipoprotein particle outcomes, and to determine whether SR limits the effects of weight loss on CVD associated lipoprotein particle concentrations.
<table>
<thead>
<tr>
<th></th>
<th>Collapsed (Baseline) N = 28</th>
<th>Collapsed (Post-intervention) N = 12</th>
<th>CR (Baseline) N = 16</th>
<th>CR (Post-intervention) N = 16</th>
<th>CR+SR (Baseline) N = 16</th>
<th>CR+SR (Post-intervention)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, kg</td>
<td>96.1 ± 10.9</td>
<td>93.0 ± 11.0c</td>
<td>89.4 ± 9.0</td>
<td>86.4 ± 8.8b</td>
<td>101.1 ± 9.7</td>
<td>97.9 ± 10.0c</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>33.8 ± 4.4</td>
<td>32.7 ± 4.3c</td>
<td>31.5 ± 3.5</td>
<td>30.5 ± 3.4b</td>
<td>35.5 ± 4.2</td>
<td>34.4 ± 4.3c</td>
</tr>
<tr>
<td>Sleep, min/week</td>
<td>399 ± 53</td>
<td>363 ± 48</td>
<td>384 ± 40</td>
<td>370 ± 38</td>
<td>411 ± 60</td>
<td>358 ± 55a</td>
</tr>
</tbody>
</table>

a – statistically different from baseline (p < 0.05); b – statistically different from baseline (p < 0.01); c – statistically different from baseline (p < 0.001)
Table 5.2 Lipoprotein Outcomes by Intervention Group and Time Point

<table>
<thead>
<tr>
<th></th>
<th>Collapsed (Baseline) N = 28</th>
<th>Collapsed (Post-intervention)</th>
<th>CR (Baseline) N = 12</th>
<th>CR (Post-intervention)</th>
<th>CR+SR (Baseline) N = 16</th>
<th>CR+SR (Post-intervention)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total VLDL-P</strong></td>
<td>44.1 ± 17.6</td>
<td>46.4 ± 21.3</td>
<td>47.2 ± 19.1</td>
<td>43.2 ± 22.6</td>
<td>41.8 ± 16.7</td>
<td>48.8 ± 20.8</td>
</tr>
<tr>
<td><strong>Large VLDL-P</strong></td>
<td>4.0 ± 3.1</td>
<td>4.0 ± 2.6</td>
<td>4.4 ± 3.9</td>
<td>4.3 ± 3.5</td>
<td>3.6 ± 2.4</td>
<td>3.8 ± 1.7</td>
</tr>
<tr>
<td><strong>Medium VLDL-P</strong></td>
<td>8.7 ± 6.4</td>
<td>8.5 ± 7.1</td>
<td>10.6 ± 7.5</td>
<td>8.8 ± 6.9</td>
<td>7.4 ± 5.1</td>
<td>8.3 ± 7.4</td>
</tr>
<tr>
<td><strong>Small VLDL-P</strong></td>
<td>31.4 ± 15.6</td>
<td>30.4 ± 18.2</td>
<td>32.3 ± 18.9</td>
<td>30.8 ± 18.9</td>
<td>30.8 ± 13.2</td>
<td>36.8 ± 17.8</td>
</tr>
<tr>
<td><strong>LDL-P</strong></td>
<td>936.8 ± 247.9</td>
<td>947.2 ± 267.7</td>
<td>1008.1 ± 249.3</td>
<td>976.5 ± 284.9</td>
<td>881.6 ± 240.2</td>
<td>925.2 ± 261.2</td>
</tr>
<tr>
<td><strong>IDL-P</strong></td>
<td>208.3 ± 96.6</td>
<td>183.6 ± 113.0</td>
<td>216.7 ± 113.7</td>
<td>183.8 ± 130.0</td>
<td>202.0 ± 85.0</td>
<td>183.6 ± 102.9</td>
</tr>
<tr>
<td><strong>Large LDL-P</strong></td>
<td>364.2 ± 273.6</td>
<td>426.3 ± 218.6</td>
<td>353.6 ± 229.1</td>
<td>433.7 ± 214.1</td>
<td>373.8 ± 255.0</td>
<td>419.6 ± 232.8</td>
</tr>
<tr>
<td><strong>Small LDL-P</strong></td>
<td>428.5 ± 217.5</td>
<td>443.8 ± 241.0</td>
<td>467.4 ± 275.8</td>
<td>431.3 ± 279.7</td>
<td>399.3 ± 165.0</td>
<td>453.2 ± 216.7</td>
</tr>
<tr>
<td><strong>HDL-Particles (µmol/L)</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total HDL-P</strong></td>
<td>27.9 ± 9.8</td>
<td>26.7 ± 9.7</td>
<td>28.5 ± 7.5</td>
<td>27.1 ± 8.1</td>
<td>27.4 ± 11.5</td>
<td>26.5 ± 10.9</td>
</tr>
<tr>
<td><strong>Large HDL-P</strong></td>
<td>7.5 ± 3.4</td>
<td>6.6 ± 3.4b</td>
<td>7.6 ± 3.9</td>
<td>6.8 ± 3.9b</td>
<td>7.4 ± 3.2</td>
<td>6.4 ± 3.0</td>
</tr>
<tr>
<td><strong>Medium HDL-P</strong></td>
<td>8.6 ± 4.5</td>
<td>9.0 ± 4.5</td>
<td>7.8 ± 4.2</td>
<td>8.8 ± 4.9</td>
<td>9.2 ± 4.8</td>
<td>9.1 ± 4.4</td>
</tr>
<tr>
<td><strong>Small HDL-P</strong></td>
<td>12.1 ± 8.2</td>
<td>12.0 ± 8.5</td>
<td>13.8 ± 8.6</td>
<td>13.3 ± 8.0</td>
<td>10.8 ± 7.9</td>
<td>11.0 ± 9.0</td>
</tr>
<tr>
<td><strong>Particle Size (nm)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>VLDL-P size</strong></td>
<td>50.2 ± 6.3</td>
<td>50.0 ± 6.8</td>
<td>50.7 ± 7.0</td>
<td>49.5 ± 6.5</td>
<td>49.8 ± 5.9</td>
<td>50.4 ± 7.2</td>
</tr>
<tr>
<td><strong>LDL-P size</strong></td>
<td>20.7 ± 0.7</td>
<td>20.7 ± 0.8</td>
<td>20.8 ± 0.8</td>
<td>20.7 ± 0.6</td>
<td>20.7 ± 0.8</td>
<td>20.7 ± 0.9</td>
</tr>
<tr>
<td><strong>HDL-P size</strong></td>
<td>9.8 ± 0.7</td>
<td>9.7 ± 0.6</td>
<td>9.7 ± 0.8</td>
<td>9.7 ± 0.7</td>
<td>9.9 ± 0.6</td>
<td>9.6 ± 0.6a</td>
</tr>
</tbody>
</table>

a – statistically different from baseline (p < 0.05); b – statistically different from baseline (p < 0.01)
REFERENCES


CHAPTER 6

OVERALL DISCUSSION

Many modifiable lifestyle behaviors have been demonstrated to have direct effects on our health, and many of these behaviors affect multiple health outcomes. According Byrne et al. (2016), three modifiable lifestyle behaviors highly associated with health outcomes are exercise, diet, and sleep. Since there are many modifiable lifestyle behaviors, and numerous health outcomes associated with such behaviors, this dissertation focused on the effects of exercise, caloric restriction (CR), and sleep restriction (SR) on blood lipoprotein particle concentrations and size. Lipoprotein profiles were the chosen outcome to analyze because lipoproteins are well established biological markers associated with cardiovascular disease (CVD) risk irrespective of age, race, nationality, or sex (Austin, Hokanson, & Edwards, 1998; Austin & Hokanson, 1994; Benjamin et al., 2017; Cullen, 2000; Durstine, 2004; Mykkänen, Kuusisto, Haffner, Laakso, & Austin, 1999). More specifically, recent research has placed great importance on the various functions of the different lipoprotein subfractions (small and large LDL; small, medium and large HDL). Treating abnormal blood lipid levels (dyslipidemia) can reduce the risk of coronary heart disease (CHD) (a specific type of CVD) and coronary events by about 30% over a 5-year period (Goff et al., 2006; Grundy et al., 2004).

An increase in physical activity and exercise, a decrease in caloric intake for overweight and obese individuals, and meeting recommended sleep patterns have all been shown in the literature to result in blood lipid and lipoprotein profiles associated with
lower CVD risk (Durstine et al., 2001; Fontana, Meyer, Klein, & Holloszy, 2004; Katzel et al., 1995; Verder & Walford, 1998; Bhutani et al., 2013; Tzotzas et al., 2011; Varady et al., 2011; Varady & Hellerstein, 2007; Bjorvatn et al., 2007; Kaneita, Uchiyama, Yoshiike, & Ohida, 2008; Aho et al., 2016). However, less is understood about how alterations within modifiable lifestyle behaviors may affect the degree to which behavior change effects CVD risk in terms of lipoprotein subfractions concentration and size.

Therefore, the general purpose of this dissertation was to determine if specific modifiable lifestyle behaviors affected lipoprotein subfractions concentration and size.

The specific goals of this dissertation were to 1) investigate the effects of 16-weeks of higher-dose (14 kilocalories per kilogram body weight per week [KKW]) compared to lower-dose (8 KKW) exercise training on blood lipid and lipoprotein particle concentrations and size and 2) investigate the effects sleep restriction (SR) during 8-weeks of a caloric restriction (CR) diet compared to CR alone on blood lipid and lipoprotein particle concentrations and size. Data for the first study were collected from the WEWALK study, a clinical exercise trial involving older women. Data for the second study were collected from the WORDS study, a clinical diet and sleep trial involving overweight men and women. For both studies, blood lipoprotein profiles were analyzed pre- and post-intervention utilizing nuclear magnetic resonance (NMR) spectroscopy. The primary statistical analysis compared the change in lipoprotein subfractions concentration and size between and within intervention groups. For each study, we also analyzed the whole study population (reported as “collapsed group”) pre- and post-intervention lipoprotein concentration and size to determine if the lifestyle behavior
resulted in lipoprotein profile changes regardless of specific alterations within each behavior change.

The hypothesis for the first study was that higher-dose exercise training would result in greater reduction of CVD risk than lower-dose exercise training based on lipoprotein subfraction results. Specifically, mean HDL and LDL size will increase, small HDL-P and LDL-P concentrations will decrease, and large HDL-P and LDL-P concentrations will increase more in the higher-dose group compared to the lower dose group.

Our first study found no significant changes between groups after exercise intervention, but several lipoprotein variables did significantly change within exercise groups and/or the collapsed group when comparing baseline to post-intervention outcomes. Lower-dose exercise group displayed a decrease in total HDL-P concentration (p=0.001), while the higher-dose group displayed an increase in mean LDL-P size (p<0.05). Both exercise dose treatments were found to significantly increase mean HDL particle size (p<0.05). Changes in variables as an adaptation to exercise training observed in our study are supported by previous findings in the literature (Halverstadt et al., 2007; Kraus et al., 2002; Ross et al., 2000; Slentz et al., 2007).

The decrease of HDL-P concentration in the lower-dose and collapsed exercise group analysis could be viewed as a less favorable outcome, because the function of HDL particles is to remove cholesterol from the circulatory system. However, to better understand what is actually happening, one must consider the entire HDL-P profile. While HDL-P concentration decreased, mean HDL-P size increased in both training groups and the collapsed group. This increase in HDL-P size is viewed as favorable and
is indicative of greater cholesterol carrying capacity of the circulating HDL particles and in part explains the decreased HDL-P concentrations. The same rationale can also apply to the increase in LDL-P size observed only in the higher-dose exercise group. Since the increase was only observed in the higher-dose group, these data provide preliminary evidence that higher aerobic exercise training dose does potentially provide greater benefit to CVD risk when compared to lower aerobic exercise training doses.

The hypothesis for the second study was that CR+SR would result in reducing the beneficial effects on lipoprotein particle size and lipoprotein subfraction concentrations compared to individuals without SR intervention, undergoing a CR weight loss program. Our second study found no significant changes between CR and CR+SR intervention groups, but lipoprotein variables did significantly change within intervention groups and/or the collapsed group when comparing baseline to post-intervention outcomes. Large HDL-P concentration decreased in the CR and collapsed group while mean HDL-P size decreased in the CR+SR group.

The changes observed in this study are not consistent with the only other study that has investigated the lipoprotein outcomes in response to a SR intervention. Aho et al. (2016) reported a decrease of small, medium, and large LDL-P, and small VLDL-P. No significant change in small HDL-P, medium HDL-P, or large HDL-P were found. These inconsistencies are likely due to various differences in experimental design. First, our participants were undergoing CR and SR simultaneously. The CR produced significant weight loss and decreased BMI. In obese men with metabolic syndrome, weight loss did led to significant changes in LDL and HDL kinetics (Ng, Watts, Barrett, Rye, & Chan, 2007). Though no change in LDL or HDL production was reported in the study by Ng et
al. (2007), weight loss resulted in increased catabolism of LDL apoB-100 and delayed catabolism of HDL apoA-1. Second, the participants in the Aho et al. (2016) study only underwent SR for 1 period of 5-nights compared to the 5-nights per week for 8 weeks that our study participants completed.

Considering the outcomes of both the exercise and SR studies together, there are interesting findings to deliberate. Primarily, neither investigation resulted in between-group differences, yet both resulted in significant changes of different lipoprotein subfractions within intervention groups. While there is still much to be learned concerning the underlying mechanisms responsible for the lipoprotein changes resulting from lifestyle behavior change, our current understanding supports the differences we observed between the two studied.

Lipoprotein lipase (LPL), which is an enzyme located on the capillary walls that stimulates the release of triglyceride from chylomicron particles and VLDL, is upregulated with exercise (Miyashita et al., 2010). Increased LPL activity, in turn, is associated with increased HDL-C (Tsutsumi, 2003). Proprotein convertase subtilisin/kexin type 9 (PCSK9) is an inhibitor of LDL receptor proteins. When PCSK9 binds the LDL receptor protein on the liver, the liver cannot uptake LDL particles, thereby leaving more LDL-P in blood circulation. Increased PA over a 3 month period resulted in a decrease expression of PCSK9 and lower LDL-C levels (Kamani et al., 2015). Cellular membrane protein ATP-binding cassette transporter A-1 (ABCA1) and the resulting gene expression are upregulated in response to exercise (Tofighi, Rahmani, Jamali Qarakhanlou, & Babaei, 2015). ABCA1 interacts with apoA-1 on an HDL particle thereby preparing the HDL particle to uptake cholesterol. Proliferator-activated receptor
gamma (PPARγ) and liver X receptor alpha (LXRα) are two additional receptors involved in RCT. Both PPARγ and LXRα are upregulated in response to exercise training (Butcher et al., 2008).

Experimental SR and analysis of epidemiological SR data by Aho et al. (2016) reviled that SR affects some of the same portions of the lipoprotein altering mechanism as exercise. However, SR also effects proteins and receptors that do not appear to be affected by exercise. While acute sleep restriction appears to alter the LDL profile and associated mechanisms, long term sleep restriction resulted in a decreased expression of LXR and ABCA1. Both LXR and ABC1 results in reduced RCT, lower HDL concentration, and larger VLDL-P size (Aho et al., 2016; Liu, Chung, Shelness, & Parks, 2012).

The effect of SR on circadian rhythms must also be considered when discussing potential mechanisms that affect blood lipoprotein profiles. In mice, many of the receptors and transporters above have been demonstrated to be regulated by circadian rhythm (Gnocchi, Pedrelli, Hurt-Camejo, & Parini, 2015). Though not yet investigated in humans, sleep restriction may affect the circadian regulation of some receptors and transporters involved in lipid and lipoprotein transport, thereby affecting measurable concentrations of blood lipoprotein subfractions.

Despite the ambiguity in the mechanisms of change, this dissertation found that alterations within modifiable lifestyle behaviors may affect the degree to which behavior change effects CVD risk in terms of lipoprotein subfractions concentration and size. Though these two study protocols were not specifically designed to investigate the effect of modified lifestyle behavior on blood lipoprotein profiles, the reported outcomes will
contribute greatly to the literature because little is understood about the alteration of lifestyle behavior change on lipoprotein outcomes. While we were limited to the analysis of two exercise doses in postmenopausal women, we conclude that higher-dose exercise may be more beneficial than lower-dose exercise in older women. Future research on the topic should include a wider range of exercise doses, and male and female participants with a wider age range. From the second study, we conclude that CR may be more beneficial for overweight individuals if sleep is not restricted during CR. Since this is the first longitudinal study to report the effect of long term sleep restriction on blood lipoprotein profiles, further investigations utilizing larger subject numbers are needed. Eventually, future research needs to be completed to understand how simultaneous change of multiple lifestyle behaviors effects CVD risk in terms of blood lipoproteins concentration and size.
BIBLIOGRAPHY


