LIPOPROTEIN DENSITY DISTRIBUTIONS FOLLOWING DIET AND EXERCISE INTERVENTIONS

A Dissertation

by

ADAM JOHN KIEFFER

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Chair of Committee,	Rosemary L. Walzem
Committee Members,	Stephen F. Crouse
	Steven E. Riechman
	Stephen B. Smith
Head of Department,	Boon Chew

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ABSTRACT

Atherosclerotic-related cardiovascular disease (ASCVD) claims the lives of over 600,000 Americans yearly. Current methodologies of assessment do not distinguish lipoprotein density distributions and instead measure lipoprotein cholesterol, with low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) of primary clinical relevance.

Furthermore, the distinction between the effects of diet and exercise interventions on lipoproteins is frequently indiscernible due to the effects of energy deficit induced by interventions. High-performance lipoprotein density profiling (HPLDP) provides a cost-effective way to rapidly assess the efficacy of diet or exercise interventions. The purpose of this study is to characterize density distribution of HDL subclasses in response to diet or exercise using HPLDP.

Eighty-eight untrained subjects (74% male, average age of 54 years) were pooled from two lifestyle intervention studies that met the inclusion criteria. Following 12 weeks of diet or exercise interventions (750 kcal expenditure or 750 kcal deficit), subjects lost an average of 3.72 kg weight (-3.9%), lost 3.9 kg of body fat (-11.6%), increased lean mass 0.62 kg (+1.1%), and reduced body fat percentage by 3.41% (-9.1%). Average absolute VO_2 max increased 0.16 liters O_2/min (+7.1%) ($p<0.05$). Several lipoprotein density distributions were significantly different ($p<0.05$) between diet and exercise interventions (expressed as percent change from baseline): triglyceride-rich lipoproteins (TRL) (-17.14 vs 19.65), low-density lipoprotein subfraction 5 (LDL5)

(-26.06 vs 8.14), high-density lipoprotein subfraction 3b (HDL3b) (-21.24 vs -0.71), and high-density lipoprotein subfraction 3c (HDL3c) (-17.88 vs 7.94). To elucidate the effects of cardiorespiratory fitness, subjects were further divided into categories of increased absolute VO_2 max and decreased absolute VO_2 max, with no changes between groups at baseline. Associations between absolute VO_2 max percent change on TRL, LDL-5, HDL-3b, and HDL-3c percent change remained significant after controlling for age, gender, and fat mass percent change. A regression equation was constructed from significant correlations and effectively predicted HDL-3c changes using absolute VO_2 max measurements.

A significant linear relationship between improved absolute VO_2 max and increased HDL-3c subfraction AUC exists; lipoprotein subfraction quantification may reveal positive effects of exercise overlooked using traditional clinical cholesterol assessment techniques.

DEDICATION

This dissertation is dedicated to my parents. Without your unwavering support and guidance, I would not be where I am today. I am forever in your debt.

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"*The impediment to action advances action. What stands in the way becomes the way.*"

- Marcus Aurelius

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Contributors

Part 1, faculty committee recognition

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INTRODUCTION AND LITERATURE REVIEW

Introduction

Approximately 610,000 Americans die annually from cardiovascular disease (CVD), which is responsible for one in every four deaths and the leading cause of death in the United States (168). Over half of those deaths are due to atherosclerotic cardiovascular disease (ASCVD). An insidious, multi-factorial disease process, ASCVD has a strong positive correlation with serum cholesterol levels (176). First described by Nikolai Anitschkow in 1913 and further elucidated by the Multiple Risk Factor Intervention Trial (MRFIT) in the 1970s, the "cholesterol hypothesis" describes the exponential increase in ASCVD-related mortality correlated to linear increases in serum cholesterol ASCVD (151, 200, 201). Advancements in cholesterol assessment methods has allowed for the characterization of distinct lipoprotein classes, originally named for their floatation densities within an ultracentrifuge field (5, 152). As such, epidemiological studies on lipoproteins separated by ultracentrifugation found that low-density lipoprotein cholesterol (LDL-C) was positively correlated with the incidence of ASCVD, while high-density lipoprotein cholesterol (HDL-C) was negatively correlated with ASCVD (28, 45, 166, 193).

In a coordinated effort to reduce the prevalence of ASCVD, public health entities such as the National Heart, Lung, and Blood Institute (NHLBI) and the American Heart Association (AHA) developed nutrition intervention policies with guidance to reduce

dietary fat and cholesterol intake, while increasing physical activity (30, 134, 156, 157). Americans have generally adhered to these recommendations, resulting in a 10% reduction in LDL-C and an increase of 4% in HDL-C since 1988 (28, 150). Complicating clinical guidelines are the variable responses observed with exercise on key ASCVD biomarkers, including lipoprotein and cholesterol measurements (123).

Interestingly, 50% of people who die from ASCVD have LDL-C levels at or below therapeutic target concentrations, indicating the presence of residual ASCVD risk due to non LDL-C factors (187). Pharmacological interventions that increase HDL-C, such as cholesterol:ester transfer protein (CETP) antagonists, are able to increase HDL-C up to 80%, but to date have off-target effects and do not reduce CVD risk (11, 147, 187). This unexpected outcome underscores a lack of understanding regarding HDL biology and specifically how it produces anti-ASCVD effects.

A source of confusion regarding the pharmacological modulation of LDL-C and HDL-C is the measurement itself; the current cholesterol measurements included in a standard lipid panel do not capture the variability due to efficacy of lifestyle interventions and as a result are unable to provide for sensitive assessment of changes in ASCVD risk. As will be reviewed in subsequent sections, improved understanding of ASCVD disease processes and lipoprotein biology as well as advances in technologies to characterize lipoprotein particle populations present in circulation can provide for additional insight into the effects of lifestyle intervention and potentially serve as sensitive assessors of intervention efficacy.

Cardiovascular disease and lipoprotein metabolism

Cardiovascular disease is a general term encompassing a plethora of health complications many of which originate from atherosclerosis, an anglicized German term ("atherosklerose") whose origins reside in the conjugation of the Greek words *athērē* ("groats/mealy") and *sklērōsis* ("hardening") (1). Thus, etymologically, atherosclerosis refers to the accumulation of foreign material (namely lipid constituents) within a blood vessel's vasculature that hardens and form plaque deposits within the sub-endothelial space. Plaque accumulation decreases blood flow and increases systemic vascular resistance, increasing sheer stress on the endothelium and in accord with Poiseuille's Law exponentially increases the amount of pressure needed to maintain blood flow. Additionally, the increased force required to produce blood flow through a plaque-laden vessel can lead to plaque rupture and clot release, causing a heart attack or stroke. The formation of atherosclerotic plaque is a complex phenomenon involving local, myocardial, and systemic inflammatory processes, and lipoproteins play a crucial, albeit somewhat paradoxical role in ASCVD (130).

Canonical lipoprotein metabolism and ASCVD development

Understanding the dynamics of canonical lipoprotein metabolism is important for understanding lipoproteins and ASCVD. The term "lipoproteins" refers to a diverse group of spherical and discoid particles composed of cholesterol, cholesterol esters (CE), triacylglycerides (TAG), phospholipids (PL), and apoproteins (**Figure 1**). Given that

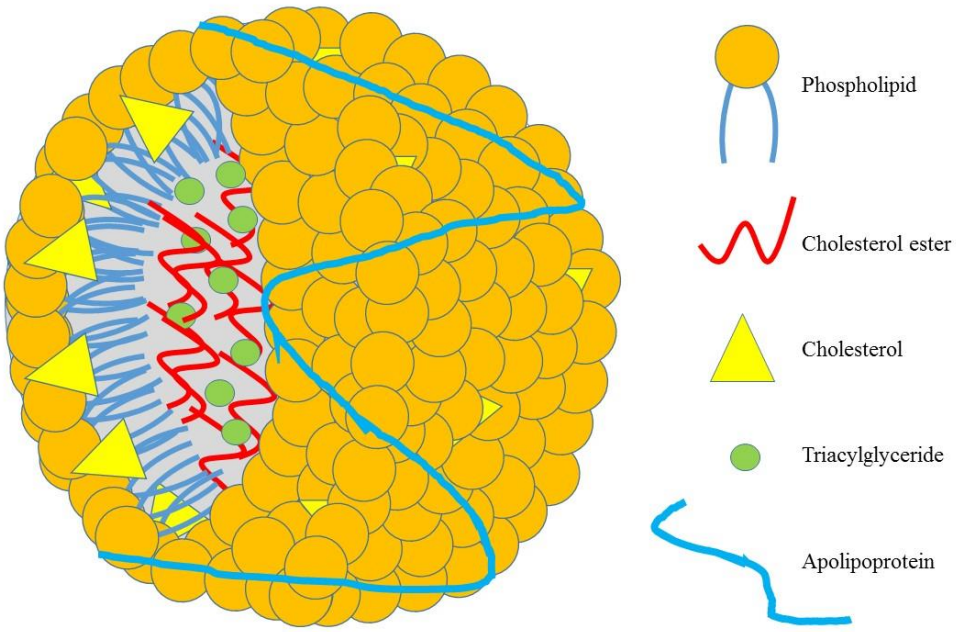

Figure 1. General structure of lipoproteins. Note the lipoprotein core containing cholesterol ester and triacylglycerides, with the outer membrane composed of phospholipid, cholesterol, and apolipoproteins. Adapted from (100).

polar and nonpolar solutions remain relatively immiscible when mixed, lipoproteins provide lipid a means of conveyance through an aqueous environment (214). The amphipathic properties of phospholipids form a hydrophilic outer shell in combination of PL, unesterified cholesterol, and apoproteins surrounding a hydrophobic core of hydrophobic CE and TAG. Apolipoproteins assist in structural stabilization, lipid transfer, and enzymatic activities though specificity with receptors (80). The nomenclature for lipoproteins originates from studies that defined their densities through separation by isopycnic ultracentrifugation, leading to the eponymous categories of the TAG-rich very-low-density lipoproteins (VLDL) with a density of 0.96 to 1.006 g/ml, the intermediate-density lipoproteins (IDL) with a density of >1.006 to 1.019 g/ml, the relatively cholesterol-rich low-density lipoproteins (LDL) with a density range of >1.019 to 1.063 g/ml, and protein-rich high-density lipoproteins (HDL) with a density range of >1.063 to 1.21 g/ml (**Table 1**).

Human lipoprotein metabolism can be divided into forward lipid transport and reverse lipid transport, though this classification minimizes the diverse idiosyncrasies of lipoproteins. Nevertheless, cholesterol transported forward stems from two sources. The process of exogenous lipid transportation is initiated by chylomicron formation. The largest and most TAG-rich lipoprotein are chylomicrons which are formed in response to a fatty meal. Assembled by enterocytes bathed with digested nutrients from the diet, chylomicrons contain both diet-derived and lipid droplet-derived TAG and cholesterol, which are packaged in combination with an apolipoprotein B_{48} (apoB_{48}) protein and

Class	*Origin*	*Major function*	*Diameter (nm)*	*Density (g/ml)*	*Major protein*	*Concentration in interstitial fluid*	*Average composition by weight (%)*				
							TAG	CE	PL	Chol	Protein
Chylomicrons	Ileum	Transport ingested fat and fat-soluble vitamins	200 - 1000	<0.95	$apoB_{48}$	absent	85	3	8	2	1
VLDL	Liver	Transport synthesized TAG	30 - 90	0.95 - 1.006	$apoB_{100}$	absent	55	18	20	5	9
LDL	Lipolysis of VLDL	Deliver cholesterol to cells	22 - 28	1.006 - 1.063	$apoB_{100}$	~9% that in plasma	10	50	29	11	20
HDL	Liver and Ileum	Reverse cholesterol transport	7 - 11	1.063 - 1.21	$apoA_1$	~20% that in plasma	6	40	46	7	50

Table 1. Major classes of lipoproteins. Triacylglycerides, TAG; cholesterol esters, CE; phospholipids, PL; cholesterol, Chol. Adapted from (171).

undergo exocytosis into the lymphatic lacteals adjacent to the enterocytes, entering lymphatic circulation and bypassing the liver. Chylomicrons ultimately enter vascular circulation at the thoracic duct. In general circulation chylomicrons acquire apoC_{II}, apoC_{III}, and apoE from interactions with other lipoproteins. ApoCII is crucial for the activation of lipoprotein lipase (LPL), while apoCIII may delay the action of LPL and apoE is required for receptor-mediated hepatic uptake of chylomicron (39, 64, 98). Extrahepatic tissues expressing LPL, anchored by heparin sulfate proteoglycan (HSPG) chains, hydrolyze TAG on the surface of chylomicrons in a process critically-dependent on the presence of apoC_{II} and glycosylphosphatidylinositol-anchored high-density lipoprotein binding protein 1 (GIPHBP-1) (172, 228). The liberated fatty acids and monoacylglycerol passes through the cell membrane through diffusion or though fatty acid binding protein (FABP)-mediated transport or fatty acid translocase/cluster of determination 36 (FAT/CD36) where they are further metabolized for cellular energy or stored within lipid droplets following re-esterification into TAG (39, 80). As the chylomicron is depleted of its cargo the amphipathic monolayer becomes redundant. The surface PL and unesterified cholesterol become susceptible to dissociation and acquisition by HDL though the enzyme phospholipid transfer protein (PLTP). Eventually the chylomicron is reduced to a chylomicron remnant (CR) containing PL and apoB_{48} (172).

The CR binds to hepatic sinusoids and is internalized by LDL receptors; if apoE is present the CR can also be internalized by the remnant receptor (LRP), where it is hydrolyzed into fatty acids and cholesterol. The liberated fatty acids and cholesterol are

stored in the liver but can be repackaged by the liver into a VLDL particle, assembled within a single apolipoprotein B_{100} ($apoB_{100}$) scaffold. This process occurs within the hepatocytes' endoplasmic reticulum. Upon assembly, VLDL is transported intracellular to the Golgi apparatus, which forms a Golgi vesicle that migrates to the cell membrane and undergoes exocytosis into general circulation. VLDL then travels through the circulation and is hydrolyzed by tissues expressing LPL in a process identical to the hydrolysis of chylomicrons. Exchange of lipid cargos with tissues and the exchange of apolipoproteins and redundant surfaces with other lipoproteins converts VLDL to IDL and eventually to LDL. In addition to density differences, VLDL and IDL retain apoE while LDL lacks apoE. LDL can continue to acquire and exchange lipids in the vascular compartment until $apoB_{100}$ undergoes a conformational change in reference to the particle size, which is the impetus for recognition by ligand receptors in the liver (135). LDL is removed by LDL-receptor mediated endocytosis in the liver, a process that requires proprotein convertase subtilisin/kexin 9 (PCSK9) in order to mark the LDL receptor for degradation or recycling (230). In summary, forward cholesterol transport involves chylomicrons, VLDL, IDL, and LDL, and refers to the packaging and delivery of lipid cargo to muscle and adipose tissue.

As previously mentioned, VLDL particles deliver their lipid cargo to tissues expressing LPL, a crucial function that allows the transportation of cholesterol and TAG to cells throughout the body. It is also well-established that LDL can transverse artery walls and enter the sub-endothelial space, where it can exchange lipid and, ideally, leaves the artery. Small LDL <25.5 nm in diameter may become trapped within the sub-

endothelial space, increasing their likelihood for oxidation (5). This point is crucial because the retention and oxidation of LDL (oxLDL) is considered the progenitor of atherosclerosis (203). The presence of oxLDL triggers a cascade of inflammatory processes including monocyte infiltration into the sub-endothelial space and differentiation into macrophages. Upon engulfing multiple oxLDL, macrophages are transformed into foam cells. Foam cells and inflammatory cytokines trigger smooth muscle cell (SMC) migration into the sub-endothelial space, where they accumulate and form a fibrous cap. Interestingly, the SMC that populate or comprise the fibrous cap appear to be dysfunctional when compared to normal-phenotypic SMC as they lack functional ABCA1 and show uncharacteristic responses to normal stimuli such as vascular endothelial growth factor (VEGF) and nitric oxide (NO) (49). Macrophages can remain trapped within the fibrous cap die via necrosis rather than apoptosis with resultant uncontrolled release of cellular contents to form a necrotic core of lipid and cholesterol within the fibrous cap. Over time, the fibrous cap thins and may rupture, leading to a thrombus that can results in a heart attack or stroke (128-130).

Reverse cholesterol transport (RCT) refers to the removal of lipid from both tissues and other lipoproteins and its transport back to the liver; this activity is performed exclusively by HDL. The act of sequestration by HDL on redundant surface lipids from hydrolyzed lipoproteins occurs through the enzymatic mechanisms of PLTP and lecithin:cholesterol acyltransferase (LCAT). The primary apolipoprotein on HDL, apolipoprotein A1 (apoA1) activates LCAT and is imperative for proper HDL function, to include RCT. ATP-binding cassette transporter member A1 (ABCA1) is also crucial

for cholesterol and phospholipid homeostasis; ABCA1 is expressed ubiquitously in human tissues and aids in the regulation of cellular cholesterol via its exchange activity with HDL (205). HDL may also remove cholesterol and TAG found within tissues such as capillaries and macrophages through the action of LCAT and the transporter ABCA1 (46, 48). Additionally, CE-rich HDL can exchange cholesterol for TAG from apoB-containing lipoproteins through the action of at liver, HDL are internalized by scavenger receptor, type B, class 1 (SR-B1) for hepatic hydrolysis of CE. Smaller HDL, for example an HDL containing a single apoA_1 moiety or delipidated HDL, are not internalized by SR-B1 and re-enter circulation (116, 127, 212). Hepatic lipase (HL) may also hydrolyze surface lipids of HDL, resulting in smaller HDL particles that re-enter circulation.

In summary, lipoproteins such as chylomicrons, VLDL, and LDL deliver cargo from the diet and from storage to peripheral tissues while other lipoproteins, namely HDL, exchange lipid and cholesterol from forward-delivering apoB-containing lipoproteins in order to return these materials to the liver (**Figure 2**). In the classical model of ASCVD, trapped lipoprotein and their cargo become oxidized, triggering a cascade of inflammatory processes that lead to an accumulation of lipid and SMC, which can develop into atherosclerosis.

Classical assessment of lipoproteins and their relationship to ASCVD

The classical assessment of lipoproteins involves quantification of TC, TAG, LDL-C, and HDL-C. Total cholesterol is measured enzymatically by hydrolyzing cholesterol esters and then oxidizing the freed cholesterol, forming the by-product

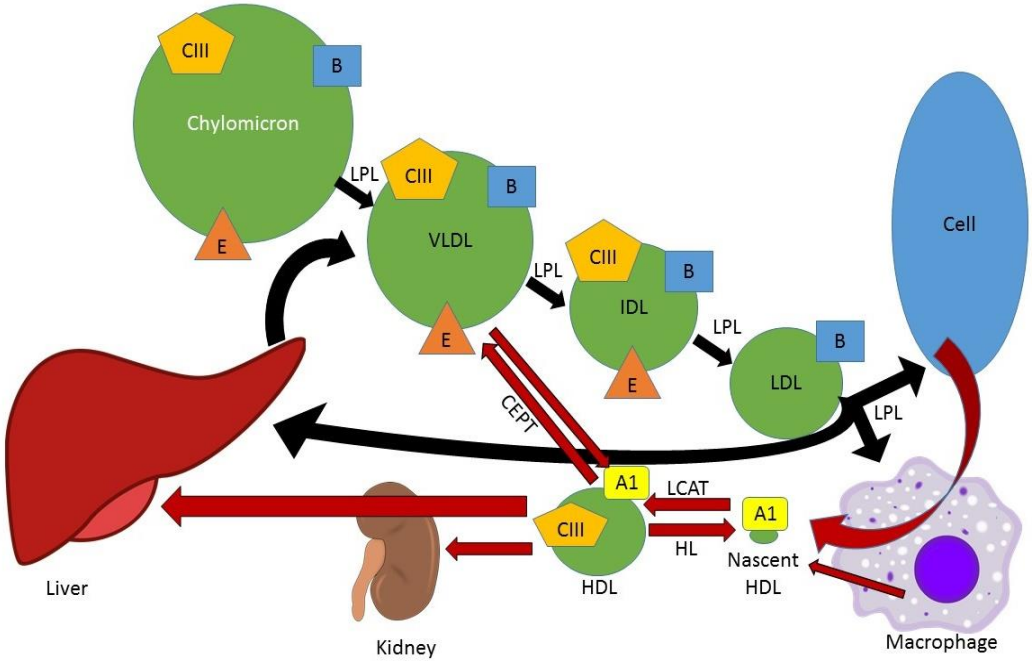

Figure 2. Summary of lipoprotein metabolism. Adapted from (224).

hydrogen peroxide (H_2O_2), which is proportional to the amount of cholesterol oxidized (32, 177, 179). The peroxidase reaction is coupled with a color-producing chromophore; absorbance of this color is measured at 500 nm which determines the quantity of H_2O_2 present, thus the amount of cholesterol present. A similar enzymatic method is used to quantify TAG, hydrolyzing TAG to produce glycerol, then oxidizing the free glycerol to form a proportional amount of H_2O_2, which is coupled to the same chromophore previously mentioned and absorbance is measured at an identical wavelength to determine quantity. Finally, HDL is measured using a direct method, with apoB-containing lipoproteins precipitated from the sample using sulfated alpha-cyclodextrin and magnesium, forming a complex with apoB-containing lipoproteins. The lipoproteins remaining free in solution are non-apoB-containing lipoproteins and presumed to be exclusively HDL. Cholesterol in the fraction is measured enzymatically (32).

LDL cholesterol (LDL-C) can be measured directly or indirectly, with indirect methods being the most common (32). Direct methods are not typically utilized in the clinical setting due to cost and specificity of required instrumentation (154). The most frequently used indirect method calculates LDL-C following quantification of TC, TAG, and HDL-C by use of the Freidewald equation (32, 65):

$$[\text{LDL-C}_{\text{calc}}] = [\text{TC}] - [\text{HDL-C}] - [\text{TG}]/5$$

The Freidewald equation estimates LDL-C using the assumption that LDL-C can be calculated by TC minus the presence of HDL-C, minus TAG divided by 5 to account for the presence of VLDL. It has been determined that TAG levels >4000 mg/L (>400 mg/dl) will distort LDL-C measurements estimated from Freidewald equation (154).

The method of enzymatically quantifying TC, TAG and HDL-C, then estimating LDL-C is commonly referred to as a "cholesterol panel" in the healthcare community (73). It is also important to note that this method is the only standardized method for assessing lipoprotein cholesterol concentrations. It does not characterize lipoprotein physical properties including composition other than cholesterol content. This standardized clinical methodology is what is used to assess risk at the population level. Its ability to assess the efficacy of TC and LDL-C lowering is well documented. The efficacy of this technology to assess risk reduction due to artificial HDL-C raising therapies is less certain. The interpretation of a cholesterol panel based on the Adult Treatment Panel III by the National Cholesterol Education Program (NCEP) is provided (**Table 2**).

Other methodologies

Although national public health agencies utilize the aforementioned clinical assessment method for lipoprotein cholesterol, novel and increasingly accurate processes to capture more information than cholesterol content have been developed that range from floatation/density characterization to nuclear magnetic resonance (NMR). Lipoproteins are increasingly resolved into specific subfractions whose components are being quantified via immunoassays, gradient gel electrophoresis, ion mobility, and NMR in research in order to describe the particle itself in addition to its cargo.

Gel electrophoresis utilizes size-exclusion or charge to separate lipoproteins that, typically, have been separated into broad density subfractions, i.e. VLDL, LDL and

Cholesterol Panel Guidelines	
LDL-cholesterol (mg/dl)	
Optimal	< 100
Near optimal	100 to 129
Borderline high	130 to 159
High	160 to 189
Very high	≥ 190
Total cholesterol (mg/dl)	
Desirable	< 200
Borderline high	200 to 239
High	≥ 240
HDL-cholesterol (mg/dl)	
Low	< 40
Optimal	≥ 60

Table 2. Cholesterol panel guidelines. Displays ASCVD-risk using results from a classic cholesterol panel. Adapted from (162).

HDL, by preparatory ultracentrifugation prior to analysis (5). Immunoassays utilizes specific antibodies for various apolipoproteins to differentiate lipoproteins (such as apoB within LDL and $apoA_1$ within HDL). Notably, apoB-containing lipoproteins contain one apoB protein per particle and this allows absolute particle number to be determined by immunoassay. The same situation does not exist in HDL as particles floating in the HDL density range can contain one or several apoA1 proteins as well as containing no apoA1 protein; albeity as we will discover non-apoA1-containing HDL are dysfunctional in RCT and can be proinflamatory (177, 179). Two-dimensional gradient gel electrophoresis separates HDL using electrostatic charges or density gradient centrifugation followed by polyacrylamide gel particle size separation. Visualization of HDL particles is achieved through staining or blotting with antibodies (170, 177). Another separation methodology is ion mobility; this method also requires ultracentrifugation to separate HDL from other lipoproteins and albumin prior to separation by size in a voltage gradient. Particles are condensed and quantified by light scattering (31, 177). Nuclear magnetic resonance spectroscopy relies on magnetic properties inherent in lipoprotein particles that are based on size and which produce distinct signals when exposed to a magnetic field. In this analysis magnetic resonance is proportional to the methyl group protons of lipids that comprise each lipoprotein particle and so allows concentration of subclasses to be determined (160, 177).

In the last 10 years, high-performance lipoprotein density profiling (HPLDP) has gained popularity (119). Based on one of the earliest and most high-resolution methodologies of analytic ultracentrifugation, HPLDP embodies technical changes that

reduce analysis time and simplify subfraction imaging. This method allows for rapid (six hours) separation of lipoprotein subfractions using ultracentrifugation, which have been incubated with a fluorophore (101) . Using a salt solution that forms a density gradient upon centrifugation, the lipoprotein subfractions are visualized by the use of a light source that excites only fluorophore that has partitioned into the lipoprotein surface. Subfraction density distribution is imaged using specific filters that match the excitation and emission paramenters of the fluorophore. The image of the emitted light is captured using a CCD camera. The image is analyzed by first converting the image into a pixel matrix whose values correspond to fluorescent intensity/quantity. This relationship allows for lipoprotein particle mass quantification. This method is rapid (<6 hours for gradient formation) and inexpensive compared to other methods.

Lipoprotein cholesterol, ASCVD risk, and current treatment guidelines

As previously discussed, clinical methods used to assess ASCVD risk reveal only the Chol and TAG content of lipoproteins, revealing little about their structure or function. Nevertheless, the ranges for lipoprotein cholesterol outlined in Table 2 are the primary means of classifying ASCVD-risk in the healthcare setting. These guidelines originated from a plethora of research over the past 75 years regarding a relationship between lipoprotein cholesterol and ASCVD mortality (6, 152, 199).

The most agreed upon relationship between lipoprotein cholesterol and ASCVD risk is that LDL-C is positively-correlated with ASCVD mortality, while HDL-C is inversely-correlated with ASCVD mortality (200, 209). Reducing LDL-C is as simple as taking a medication due to the development of 3-hydroxy-3-methylglutaryl-coenzyme A

(HMG-CoA) reductase inhibitors, generically referred to as statins, which effectively limit cellular cholesterol synthesis while upregulating LDL-receptor expression, resulting in an increase in hepatic clearance of LDL-C (33). Statins also may modestly raise HDL-C in some patients (26). Increasing HDL-C through medication that inhibits the CETP enzyme can increase HDL-C by up to 80% (11).

In the time periods of 1988–1994 and 2007–2010, the use of cholesterol-lowering medication in the United States significantly increased from 5% to 25% in men and 6% to 21% in women (117). In adults aged 40-64, statin use increased from 4% to 19% and adults aged 65–74 statin use increased 10% to 39% (117). Correspondingly, the prevalence of elevated LDL–C decreased significantly from 59% of adults having elevated LDL-C in 1976–1980 to 42% in 1988–1994, which further decreased to only 27% of adults having elevated LDL-C in 2007–2010 (117). Additionally, several HDL-C raising drugs are currently in clinical trial (110).

Despite the improvements in LDL-C and HDL-C, the number one symptom of ASCVD remains sudden death due to cardiac arrest and is responsible for 50% of all ASCVD deaths (182, 231). Furthermore, 50% of those who die from ASCVD have LDL-C equivalent to or below 100 mg/dl, a concentration associated with significant reduction in risk of ASCVD mortality. In addition approximately half of those who suffer a clinical ASCVD event have HDL-C greater than>40 mg/dl, a concentration with reduced ASCVD mortality risk (18, 187). Adding to the confusion, several studies examining the efficacy of CETP-inhibitors to reduce ASCVD risk by increasing HDL-C were ended early due to a statistically significant increase in mortality attributed to off-

target effects (11) or lack of efficacy in risk reduction. This is seems that medical interventions can successfully reduce LDL-C and associated ASCVD risk additional information is needed to understand whether HDL-C can be used to accurately assess residual risk or whether other techniques would better serve this purpose.

Lipoprotein diameter and ASCVD risk

Not assessed by cholesterol panels, the atherogenicity of lipoproteins is directly related to time spent in circulation (218). The longer particles remain in circulation, the greater their susceptibility to oxidization; for example, delayed clearance of LDL results in more metabolic interactions, reducing particle diameter and increasing atherogenicity (217, 218). Similarly, HDL exhibits functions beyond RCT that appear to be significantly related to its diameter. Thus, alternate means of measuring the diameter of lipoproteins have emerged as a prognostic indicator for assessing ASCVD risk (42, 69, 145).

Low-density lipoproteins classified as small and dense (sdLDL) are ~22 nm and d = 1.050-1.063 g/ml, appear to increase ASCVD risk due to the ease at which they enter the sub-endothelial space and their increased susceptability to oxidization (99, 118, 211). This subfraction is reportedly associated with a three-fold increase in risk of myocardial infarction (5). Additionally, patients with coronary artery disease exhibit sdLDL, which is inversely correlated to HDL-C and apoA1 and positively correlated to prevalence of smaller HDL particles (< 10.5 nm)(25). A shift in the diameter of LDL from small (~22 nm) to large (~26 nm) is considered beneficial for ASCVD risk reduction (195). In people free ofASCVD, large LDL are common while sdLDL are not (202). These

studies demonstrate the heterogenity of LDL size and emphasize the need to classify LDL by not only LDL-C but also LDL diameter (71).

Equally significant, HDL particles of different diameters are associated with varying ASCVD risk (57). In general, larger HDL-2 particles, 10.5 to 14.5 nm, are associated with a decrease in ASCVD risk, while smaller HDL-3 particles , 7.7 to 10.5 nm, are associated with increased ASCVD-risk (93, 99). Using NMR and gel electrophoresis, Harchaoui et al. (57) found a strong association with small HDL particles and metabolic syndrome. Kontush et al. (177) have reported that large diameter HDL (> 10.5 nm) are strongly associated with HDL-C and are thus indicative of reduced ASCVD risk. Furthermore, a recent study by Hussein et al. (93) demonstrated a defect in the antioxidant capacity of HDL-3 in patients exhibiting familial hypercholesterolemia. Taken together, these studies demonstrate a similar association between larger diameter HDL and reduced ASCVD risk.

High-density lipoproteins: functions beyond reverse cholesterol transport

An emerging charateristic of HDL not assessed in a cholesterol panel is its anti-inflammatory, anti-oxidant, anti-apoptotic, anti-glycation, and anti-thrombotic capacity (196). As previously stated, HDL can attenuate the effects of oxLDL through RCT, mediated by ABCA1 translocation of internal cholesterol stores, or from oxLDL from within the sub-endothelial space, to the outer cellular membrane for acquisition by HDL (178).

Another action of HDL in the reduction of oxLDL is through the action of paraoxonase-1 (PON1) (53). Synthesized by the liver, PON1 activity has been shown to

reduce lipid peroxide accumulation and low PON1 activity despite normal HDL-C was shown in ASCVD patients (192, 197). Interestingly, the anti-oxidant characteristics of HDL mediated by PON1 appear to reduce LDL glycosylation, an independent risk factor for developing ASCVD; this phenomena is frequently observed in patients with type 2 diabetes and metabolic syndrome (MetS) (47, 225). High-density lipoproteins can also reduce tumor necrosis factor-α mediated expression of adhesion molecules commonly associated with aortic endothelial cells such as vascular cell adhesion protein-1 (VCAM-1) and intracellular adhesion molecule-1 (ICAM-1) (178). These adhesion proteins are strongly associated with hyperlipidemia and acute coronary syndrome (23).

Paradoxically, HDL may exhibit pro-inflammatory characteristics, including reduced RCT from macrophages and the production of pro-oxidants, especially in patients with MetS and diabetes (43, 136, 178, 190). In subjects who have ST segment elevation myocardial infarction (STEMI), HDL appears to be saturated with serum amyloid A (SAA), an acute phase reactant and marker of inflammation, and exhibits attenuated antioxidant capabilities compared to subjects who have not had STEMI (169). This supports the hypothesis that HDL may be rendered dysfunctional due to chronic inflammation that may damage or impair the action of $apoA_1$ (178). As demonstrated in the pharmokinteics of CETP-inhibitors, HDL may also lack the ability to transfer CE due to inhibition of CETP, leading to a reduced capacity to interact with SR-B1 and reduced RCT, which may result in elevated HDL-C (178). Unfortunately, functional HDL is indistinguishable from dysfunctional HDL when measuring HDL-C.

These anti- and pro-atherogenic characteristics of HDL underline the diverse nature of the particle depending on the influence of the environment, which are not assessed in a cholesterol panel.

Modifiable lifestyle factors and ASCVD risk

It has been estimated that 90% of ASCVD is preventable and related directly to lifestyle factors, primarily through poor dietary and physical activity habits (139, 143). In an effort to reduce ASCVD risk by capitalizing on these modifiable risk factors, guidance by international organizations has focused on modifying diet and physical activity. It is important to note that these intervention strategies are evaluated using changes in ASCVD rates and cholesterol panel outcomes.

Dietary recommendations to reduce ASCVD risk

Traditional dietary guidelines for the prevention of ASCVD are focused on reducing total fat intake and increasing carbohydrate intake, and were established from strong correlations observed in epidemiological studies to relate diet composition to ASCVD mortality rates (59, 96, 120, 200). Nutrient targets to reduce ASCVD are commonly presented as food-based guidelines as opposed to individualized nutrition strategies; these guidelines include replacing saturated fats with unsaturated fats, increasing consumption of low-fat dairy products, whole-grain cereals, fruits, vegetables, and fish, while avoiding fatty meat, red meat products, added salt, and added sugar (173).

Fat intake

The findings of the Seven Countries Study by Ancel Keys, in addition to other ASCVD epidemiological studies, revealed strong-correlations between saturated fat intake (SFA) and ASCVD (58, 108, 133, 144, 200, 220). Reducing fat intake is a primary recommendation for ASCVD risk reduction (61, 149, 194, 213). The central hypothesis regarding fat intake reduction to reduce serum cholesterol has been challenged as more studies fail to reaffirm the association with fat intake and ASCVD risk (117, 183). The biochemical processes that related to the effect of SFA and ASCVD are more complex than previously thought; for example, SFA stimulates ABCA1 expression which increases HDL-C and TC, while polyunsaturated fatty acid (PUFA) appears to diminish ABCA1 expression (137). Vafeiadou et al. found that replacing SFA with monounsaturated fatty acids or PUFA increased HDL-C and TC but did not impact vascular function (213). The central hypothesis regarding fat intake reduction to reduce serum cholesterol continues to be challenged as more studies fail to reaffirm the association with fat intake and ASCVD risk (117, 183).

A seminal analysis by Hu et al. using the Nurses' Health Study found the risk for coronary heart disease is directly related with total fat intake (89). However, upon controlling for smoking, alcohol use, and exercise, these associations no longer were significant. Another study which revisited subjects who participated in the 1995 Nurses' Health Study found only PUFA consumption remained directly correlated with an increased risk for coronary heart disease, not total fat intake (159). Further complicating matters, the Woman's Health Initiative Randomized Controlled Dietary Modification

Trial found that reducing fat intake from 37.0% of total energy intake to 28.8% of total energy intake over the course of eight years did not reduce ASCVD risk compared to controls (88). Another study found a reduction in ASCVD risk with higher fat intakes of PUFA and MUFA, while ASCVD risk increased with SFA intake associated with pastries and processed goods (79). Finally, two meta-analysis found no evidence to continue to recommend reduced fat intake or reduced SAT fat intake for the prevention of ASCVD (36, 194). In the first, Chowdhury et al. (35) reviewed 32 studies on fatty acid consumption and ASCVD risk and determined that no evidence exists to support the consumption of PUFA in place SFA to reduce ASCVD risk, though total fat intake was not assessed (35). In the second, Siri-Tarino et al. looked at 21 studies that included ranges from 5 to 23 years to determine rates of ASCVD and diet composition; the authors concluded that no association existed between dietary SFA intake and ASCVD risk (194). Together, these studies reveal a conflicted scientific consensus on the impact of total and SFA on ASCVD risk.

Calorie restriction

Although a myriad of dietary modifications exists to reduce ASCVD risk in both the scientific community and popular press, the common denominator is unquestionably calorie restriction (CalR). When basal dietary caloric intake is reduced by 25%, ASCVD risk is reduced by up to 20% (122). In support of this finding, isocaloric diets with differing macronutrient ratios have been found to produce identical body fat reduction, reduced ASCVD risk, and improved markers of inflammation (50, 185). Due et al. compared the effectiveness of three diets with varying percentages of fat and

carbohydrates (51). Although fat composition varied by 15% of total calories, total calories were identical and no markers for ASCVD varied between groups. Other physiological processes may be influenced by CalR that contribute to the effectiveness of CalR for ASCVD risk reduction. The reader is directed to a phenomenal review on the topic by Weiss and Fontana (221).

Adherence to dietary recommendations

Adherence to food-based dietary recommendations such the Healthy Eating Index is associated with reduced ASCVD-risk (173). "Healthy Eating Indexes" are commonly used to assess the compliance of a specific population to dietary guidelines, which can be compared to rates of ASCVD. As such, increasing compliance with a number of healthy eating indexes (Alternative Healthy Eating Index (AHEI-2000), Alternative Mediterranean Diet (AMED), or Dietary Approaches to Stop Hypertension (DASH)) can reduce ASCVD risk by up to 20% (198). These indexes follow similar guidance, advocating consuming less animal product while increasing consumption of nuts, seeds, fruits, vegetables, vegetable oils, and whole grains (34, 67, 68). Interestingly, Americans have generally met these dietary recommendations, improving their healthy eating index scores (assessed using the HEI-2010) by more than 20% since 1999 (**Figure 3**) (144, 219). The upward trend in healthy diet scores is attributed to the decline in total fat intake from 36.9% to 33.4% in men and 36.1% to 33.8% in women; not surprising, an 80% increase in the consumption of carbohydrates accompanied the reduction in total fat intake (19, 86). The percentage of adults meeting reduced saturated-fat intake guidelines also increased significantly from 1976–1980 to 1988–1994 (from

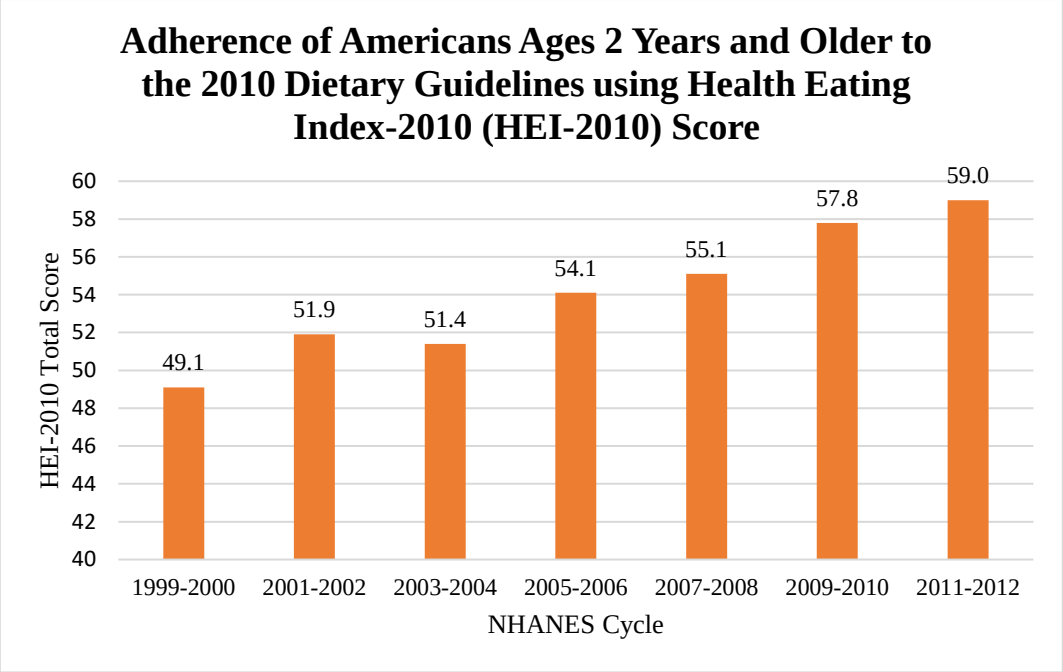

Figure 3. Healthy Eating Index 2010. Adherence of US population ≥ 2 years of age to the 2010 Healthy Eating Index. Adapted from (219).

25% to 41%), which has held constant up to 2010. Additionally, since 1979 total saturated fat intake has steadily decreased (19, 117). Despite improving diet scores and meeting macronutrient guidelines, energy intake (kcal/d) has increased significantly in the US; since 1971 energy intake has increased 22% in women (from 1542 to 1886 kcals/d) and 10% in US men (from 2450 to 2693 kcal/d) (150).

Assessment of body composition

The gold standard for body composition measurements is dual energy x-ray absorptiometry (DEXA) (91, 180). Using two distinct energy levels, 40 and 70 keV, photons pass through subjects' body and can distinguish fat and non-fat tissue (2). Compute models interpret this data and quantify not only body fat percentage but also the location and quantity of body fat in various anatomical regions. Advantages of using DEXA as opposed to classic body composition measurements such as eight for height or skinfold thickness measurement, is the precision and repeatability of these measurements and the ability to combine DEXA information recorded indifferent locations or using different machines when proper validation of measurements are made (2, 91).

Exercise prescription and assessment techniques

Multiple means of assessing the efficacy of exercise interventions exist, but maximal aerobic power or $\dot{V}O_2$ max (VO_2 max) is "accepted as the criterion measurement of cardiorespiratory function" (140). Maximal aerobic power is considered the best measurement of cardiovascular functional limits and predicts mortality from all causes in health and unhealthy individuals (17, 82, 153, 181).

The Fick Equation outlines the primary determinants of VO_2 max:

$$\dot{V}O_2\ max = \dot{Q}\ x\ (C_aO_2 - C_vO_2)$$

Where $\dot{Q}$ is cardiac output (CO), expressed as liters of blood per minute (L blood x min^{-1}) and $C_aO_2 - C_vO_2$ is arterial-venous oxygen difference (A-V O_2 diff), expressed as milliliters of oxygen per liter of blood (ml O_2 x L $blood^{-1}$) (140). This measurement is specifically referred to as "absolute VO_2 max" and can be adjusted to include body weight of the subject as means to compare VO_2 max measurements.

One such modification to absolute maximal aerobic power is "*relative VO_2 max"* which is expressed as milliliters of oxygen consumed per minute per kilogram body weight (ml O_2 x min^{-1} x kg^{-1}). This measurement is most frequently used in literature to compare VO_2 max measurements between subjects. A more accurate modification is "*relative lean body mass VO_2 max*" which is expressed as milliliters oxygen consumed per minute per kilogram lean body mass (LBM) or non-fat mass (ml O_2 x min^{-1} x kg LBM^{-1}). This modification less variable because it is not influenced by fat or weight reduction commonly associated with diet and exercise. Albeit changes in LBM can occur. Nevertheless, it allows for an accurate assessment of absolute VO_2 expressed as a ratio to LBM. At the time of this writing, definitive norms for relative LBM VO_2 max are virtually nonexistent; most research continues to report VO_2 max expressed as a percent change from baseline using relative VO_2 max (7). Note that using relative VO_2 max can be misleading. For example, subjects may lose mass and not improve their absolute VO_2 max, but will improve their relative VO_2 max since it is expressed per kilogram body weight, misleading the researcher into reporting improvements in

cardiorespiratory fitness despite there being none. Since relative VO_2 max is inextricably influenced by body composition changes, efforts to use relative LBM VO_2 max in place of relative VO_2 max have begun. Relative LBM VO_2 max may change in response to changes in LBM and/or VO_2 max and is unbiased by changes in non-oxygen consuming tissues. Alternately, absolute VO_2 max indicates true changes to cardiorespiratory function albeit without the influence of body composition. The problem is absolute VO_2 max doesn't account for body composition differences between subjects: two people may have identical absolute VO_2 max assessments but be at much different levels of fitness if one weighs 50 kg and the other weighs 100 kg. Regardless, both relative LBM VO_2 max and absolute VO_2 max are more accurate than relative VO_2 max in regards to assessing the O_2 consumption of working tissues, especially when used for comparing baseline and final measurements of lifestyle interventions that are known to impact body weight and composition.

Differences in VO_2 max between male and female genders is well-documented (20, 112). Men typically have a 40% greater absolute VO_2 max than women, which is reduced to a 10% difference when relative LBM VO_2 max is used (20). In addition to difference with lean body mass, the influence of androgens on hemoglobin production also is responsible for the disparity of oxygen-carrying capacity between sexes (20). Interestingly, the ability to improve VO_2 max is virtually identical between males and females; training adaptations can result in a 20% increase in VO_2 max capacity (20).

Age also influences VO_2 max. Between ages 20 and 65, VO_2 max declines approximately 30% (20). Referring to the Fick Equation, the decrease in VO_2 max is

attributable to reduced maximal heart rate and reduced stroke volume, as well as reduced power output caused by a decline in muscle mass, especially adults 65 years of age or older (20).

Traditional, individualized exercise prescription begins with an absolute VO_2 max measurement converted into a relative VO_2 max. With this value established, target heart rates or VO_2 max goals are prescribed, the latter as a percentage of baseline relative VO_2 max. Although a maximal VO_2 max test can be conducted where the subject exercises to failure, various health and safety concerns make sub-maximal testing an attractive alternative, especially in at-risk population such as subjects with ASCVD (140). Common sub-maximal VO_2 tests include the Bruce Protocol, which is covered in extensive detail elsewhere (21). These sub-maximal tests utilize algorithms to predict absolute VO_2 max with acceptable results (76, 140). Once a baseline VO_2 max is established, exercise prescription as a percentage of relative VO_2 max can be determined, using the subject's heart rate as a means of self-assessing exercise intensity. In a laboratory, exercising at a percent of VO_2 max can be monitored using O_2 consumption or other means.

Exercise intensity is commonly expressed as a percentage of relative VO_2 max or as multiples of resting metabolic rate or metabolic equivalents (METs), which at rest is expressed as 3.5 ml O_2/kg/min (97). Both are compared for reference in **Table 3**. Exercise intensity is crucial for effective exercise intervention and the subsequent reduction in CVD risk. In fact, the need to engage in high-intensity, peak or near peak VO_2 max (at or above 85% relative VO_2 max) activities to reduce ASCVD risk rather

Five-Level Classification of Physical Activity Based on Energy Intensity			
	Energy Expenditure[a]		
	L x min^{-1}	ml x kg^{-1} x min^{-1}	METs
Men			
Light	0.40-0.99	6.1-15.2	1.6-3.9
Moderate	1.00-1.49	15.3-22.9	4.0-5.9
Heavy	1.50-1.99	23.0-30.6	6.0-7.9
Very Heavy	2.00-2.49	30.7-38.3	8.0-9.9
Unduly Heavy	≥2.50	≥38.4	≥10.0
Women			
Light	0.30-0.69	5.4-12.5	1.2-2.7
Moderate	0.70-1.09	12.6-19.8	2.8-4.3
Heavy	1.10-1.49	19.9-27.1	4.4-5.9
Very Heavy	1.50-1.89	27.2-34.4	6.0-7.5
Unduly Heavy	≥38.4	≥38.4	≥38.4
[a]L x min^{-1} based on 5 kcal per liter of O_2; ml x kg^{-1}x min^{-1} based on 65 kg man and 55 kg woman; one MET equals the average resting oxygen consumption (250 ml x min^{-1} for men, 200 ml x min^{-1} for women)			

Table 3. Five-level classification of physical activity based on exercise intensity. Adapted from (138).

than simply engaging in low to moderate intensity physical activity has recently been discussed (188, 223). Sassen et al. (188) found an inverse relationship between engaging in peak relative VO_2 max exercise (assessed as METs) and ASCVD risk factors, concluding that engaging in high-intensity exercise (>6 METs), not low-intensity physical activity (<4 METs), is primarily responsible for ASCVD risk reduction. Additionally, engaging in activity that produced peak oxygen uptake was attributed to approximately 78% of ASCVD risk reduction derived from exercise (188). Supporting this finding, a meta-analysis by Williams et al. demonstrated a large statistical difference in the relative risk reduction for ASCVD when subjects engage in physical activity versus engaging in physical fitness, expressed as person-years engaged in physical activity (223). The authors determined that relative to the least fit or active percentiles, the relative risk reduction for CVD is significantly greater for fitness than physical activity and is precipitous up until the 25^{th} percentile for fitness.

Exercise recommendations for ASCVD risk reduction

Exercise in almost any capacity and frequency reduces the risk of mortality across most ages, diseases, and medical conditions (12). Accordingly, sedentary behavior is directly related to increased mortality (52, 102). However, evaluation of the effectiveness for exercise on ASCVD risk has been limited to studies assessing changes in LDL-C and HDL-C (41, 95, 113). Nonetheless, the effect of exercise on lipoprotein cholesterol is variable and appears highly individualized depending on, modality, intensity, duration, and frequency of exercise as well as the subject's anthropometrics.

The effect of exercise on lipoprotein cholesterol measured by lipid panel

It is widely accepted that regular exercise at a moderate-intensity reduces LDL-C and raises HDL-C compared to sedentary controls (3, 27, 124, 125, 146). Exercise is recommended by the National Cholesterol Education Program to reduce ASCVD risk (156). Mechanistically, an increased turnover of lipid substrates through increased LPL activity in adipose and skeletal muscle is thought to reduce ASCVD risk (204). Increased LPL activity may also reduce the total amount of chylomicrons and VLDL in cirulation, reducing TAG concentration (204). Exercise may also increase HDL-C through decreased HL activity, reducing HDL clearance (204).

Complicating the effects of exercise on lipoproteins is the inherent variability of time, duration, and type of activity that is possible. Exercise adaptations depend greatly on 1) the subject's previous exposure to exercise, and 2) the modality, intensity, duration, and frequency of training (103, 104, 106, 164, 210). Finally, cessation/non-adherence to physical exercise and the confounding variables of anabolic activities such as strength training versus catabolic activities such as cardiovascular activities, affect ASCVD risk in a myriad of different ways (14, 15). Nevertheless, the following sections summarize the most current research and national guidelines regarding exercise and its effects on lipoproteins.

Acute endurance exercise

Acute endurance exercise refers to a single bout of aerobic exercise. A single bout of endurance exercise has been shown to improve blood lipids (54). Yu et al. used NMR and a cholesterol panel to measure acute changes in lipoprotein cholesterol

concentrations and subclass distribution in elite athletes who participated in a triathlon (229). After the triathlon, sdLDL decreased by 62% compared to baseline, while small HDL decreased by 16%. The authors also noted a 7% decrease in TC, 23% decrease in LDL-C, and 30% increase in HDL-C (229). In untrained individuals, acute exercise of high-intensity produces identical changes in LDL-C observed in elite athletes (115, 155). Kraus et al. found that acute, high-intensity exercise (>85% relative VO_2 max) can slightly increase HDL-C, but dramatically increased LDL particle size (115).

Alternately, Nelson and Horowitz (155) found that acute exercise in sedentary obese and mildly dyslipidemic individuals had no effect on TC, LDL-C and HDL-C. Despite the lack of change in lipid panel cholesterol values with single bout of exercise, the authors found that for three days following the exercise session, insulin sensitivity was identical to trained exercisers, dramatically reducing ASCVD risk. Crouse et al. (40) on the other hand found that HDL-2 cholesterol rose with training, while HDL-3 cholesterol was lower. Finally, Greene et al. (78) observed increases in HDL subfractions without changes in HDL-C following 12 weeks of progressive endurance training.

Summarizing the effects of acute exercise on lipoprotein cholesterol, despite individual variability in response to an exercise training session and the subsequent heterogeneity observed with lipoprotein cholesterol, indicators of ASCVD risk are improved with participation in acute exercise programs.

Chronic endurance exercise

Chronic endurance exercise in this document refers to habitual exercise occurring over the course of a minimum of eight weeks. Chronic endurance exercise, regardless of intensity, increases HDL-C and reduces LDL-C (72). Typically, studies demonstrate an increase in the more cardio-protective HDL_2 subfraction, while sdLDL concentrations decrease in conjunction with increased concentrations of larger diameter LDL (85). Additionally, chronic exercise reduces plasma TG concentration in a dose-dependent manner, with changes that can vary from immediately post-exercise up to a few days later (60). These repeated bouts of exercise alter adipose and muscle tissue blood flow and improve utilization of fatty acids for fuel (87). As demonstrated by Horowitz and Klein (87), intramuscular TG increase as plasma TG decrease, distinguishing active individuals from sedentary overweight individuals with increased intramuscular TG. The adaptations associated with chronic endurance training may explain the changes seen in cholesterol panels. In elderly subjects, although exercise may not produce marked statistical changes in lipoprotein profiles, it significantly reduces the susceptibility of LDL subfractions to oxidation, which is a risk for developing atherosclerosis (142). A recent meta-analysis of nine randomized controlled trials on obese and overweight adults seemed to concur with the previous study by demonstrating that aerobic exercise had little effect on TAG, TC, LDL-C and HDL-C (22).

Summarizing the available research, any endurance training improves blood lipids, with an initial reduction in TAG and sdLDL, while increasing TAG-rich LDL.

These changes may occur in the absence of changes to LDL-C concentrations and would be overlooked by standard clinical blood lipid measurements.

Acute and chronic strength training

Strength training involves progressive resistance against a muscle to induce muscular contraction, which is beneficial for muscular endurance, strength, and injury prevention (63) There is contradicting evidence thought regarding the effect of strength training, either acute or chronic, on lipoprotein cholesterol. Historically, strength-based exercise was deemed to be ineffective in blood lipid modulation, and has even been suggested as deleterious to ASCVD risk, however no deleterious effects were found in a small study of 27 previously untrained men that directly compared strength, endurance, and combined training (72). Studies using animal models found no untoward effects of strength training alone on lipoproteins, but found that testosterone injections combined with strength training lowered HDL-C (92). Several studies conducted in the mid-1980s and 1990s used lipid panel outcomes to demonstrate that chronic resistance training in the absence of diet modification could improve blood lipids (66, 75, 92). A meta-analysis on 29 studies conducted by Kelly and Kelly (104) found reductions of 2.7% for TC, 11.6% for TC/HDL-C, and 6.4% for TAG when subjects performed strength-based exercise. When combined with strength training, diet modification does not appear to enhance the effects of strength training alone on lipoprotein cholesterol levels (148). Interestingly, a 2013 report noted that strength training improved HDL functionality in regards to its antioxidant abilities compared to sedentary controls (175).

In summary, strength training appears to have a favorable impact on reducing ASCVD risk, either through reducing LDL-C and increasing HDL antioxidant capacity, or through other means not assessed via lipoprotein cholesterol panels.

Concurrent training

It should be noted that although aerobic and strength training activities performed individually produce an improved lipid profile, a combination of aerobic and strength training activities achieves the same results but more rapidly (146). A meta-analysis of six studies at least four weeks in duration found that diet and exercise combined produced greater reductions in LDL-C while increasing HDL-C, compared to either diet or exercise alone (107). It should be also be noted that studies that include diet and exercise typically result in weight loss; body fat reduction results in reduced LDL-C and may also increase HDL-C if exercise is included in the intervention (216). Alternately, Varady et al. found that combined diet and endurance exercise had no effect on LDL-C and LDL particle size in obese, untrained women (215). Finally, a seminal study by Lefvere et al. (122) found that when non-obese healthy individuals partake in calorie restriction of 12.5% from baseline and expended an additional 12.5% of baseline energy intake (25% total caloric reduction), ASCVD risk is reduced by 39% compared to 29% with CalR alone. Although the combination of diet and exercise is ubiquitously suggested as a means to reduce ASCVD, the effects of these interventions alone or combined and assessed by lipoprotein density distribution remains to be exhaustively characterized.

Exercise recommendations for reducing ASCVD risk

Despite the variable response of lipoproteins to exercise, exercise is nonetheless associated with reduced ASCVD risk. Multiple organizations issued recommendations to increase physical activity to reduce both the risk of developing and for treating ASCVD (44, 59, 81, 105, 184). The AHA recommends 150 minutes per week of moderate exercise or 75 minutes per week of vigorous exercise (or a combination of moderate and vigorous activity) for general cardiovascular health, while those with high blood pressure or high cholesterol are encouraged to engage in 40 minutes of moderate intensity exercise three or four times weekly (55). Other organizations such as the ACSM and NHLBI echo these recommedations (70, 94, 141, 206). These exercise duration thresholds are based on combined epidemeological data that summarily show a dramatic reduction in ASCVD risk at or near durations of 90 minutes of physical activity weekly.

As outlined above, assessing the efficacy of diet and exercise interventions on ASCVD risk relies heavily on the individual's baseline anthropometrics, degree of previous exercise training, a multitude of exercise-related factors and conditions, and pre-existing medical conditions that may influence lipoproteins. It seems that current lipid panel is best used in the assessment of changes in apoB-containing lipoprotein parameters. However, this technique appears inadequate to characterize the functionally and physicochemically diverse particles that can be isolated within the nominal HDL fraction of plasma. For these reasons assessment techniques that go beyond measurement of lipoprotein cholesterol content are necessary to adequately describe risk

and/or benefit associated with particles recoverable within the HDL density fraction of plasma.

Statistical methods to assess high dimensional and interdependent data

Given the high probability of confounding variables in lifestyle modification intervention studies, a statistical test to control for these dimensions is desired. Although a one-way or two-way analysis of variance (ANOVA) can be conducted, the statistical power of such repeated measures is inherently biased due to the increased error rate when ANOVA is conducted in series (74). Although a number of post hoc tests could theoretically account for this bias, the risk of neglecting the influence of continuous and binary variables when assessed in unison on the dependent variables still remains. Furthermore, a T-Test cannot distringuish the effects of a binary variable such as gender *and* multiple dependent variables or responses without neglecting to assess the influence of the covariates (90). Thus, the use of a multivariate analysis of variance (MANOVA) or multivariate analysis of covariance (MANCOVA) is warranted (62).

Plasma transport of sphingosine 1-phosphate (S1P)

Upon secretion into plasma, S1P is bound to either albumin (20%) or lipoproteins (80%), with HDL being the primarily lipoprotein (>85%) (126, 132, 190, 222). More recent studies indicate considerable heterogeniety with the contribution of albumin and HDL to S1P transport (83, 109, 132, 189). Egress of S1P from erythrocytes is critically-dependent on the presence of albumin or HDL (207). Albumin-bound S1P signaling is neither enhanced nor degraded, but HDL-bound S1P (HDL-S1P) exhibits enhanced

signaling and can indirectly increase total plasma S1P by increasing transport capacity and S1P production (24, 38).

The transport of S1P on albumin is not fully understood. Modeling has revealed that S1P is not bound to albumin but rather co-localized in close proximity to albumin, perhaps to increase S1P solubility (4). On the other hand, S1P is bound to HDL by apolipoprotein M (apoM). S1P is transported by HDL only if the HDL particle contains apoM; increasing apoM expression in mice (fold or % change) increases plasma S1P levels by 267% (38). A member of the lipocalin family, apoM displays anti-parallel β-sheets that form a hydrophobic binding pocket (37). This pocket allows for the transport of S1P by HDL. Plasma HDL-C and apoM expression is strongly correlated; apoM-knockout mice have a 17% to 21% reduction in HDL-C (226). Recently it was shown that patients with CAD have decreased HDL-S1P compared to healthy controls, though albumin-bound and free S1P is typically increased (191). Loading HDL with S1P from healthy control erythrocytes or C17-sphingosine 1-phosphate can increase the amount of HDL-S1P in plasma and improve HDL function in HDL dervived from CAD patients (190). Not all HDL carries S1P. The most dense HDL_3 subfraction carries approximately two to three times the quantity of S1P as the less dense HDL_2 subfraction. The difference in S1P content appears attributable to increased apoM content of HDL_3 compared to HDL_2 (114).

HDL functionality attributed to S1P

As previously mentioned, the function of HDL goes far beyond RCT. In fact, evidence suggests that HDL play a pivotal role in immunology and vascular health

(158). Furthermore, diseases states such as atherosclerosis regularly exhibit HDL with less functional or even dysfunctional phenotypes (178). Many of the advantageous and positive attributes associated with HDL can be attributed to S1P (**Figure 4**).

Stimulating endothelial cell proliferation and mobility, inhibiting leukocyte adhesion and promoting extravasation of oxidized lipoproteins, stimulating prostacyclin and NO production, and inhibit matrix metalloproteinases are all processes influenced by HDL-S1P (16, 37, 38, 56, 126, 131, 158, 163, 167, 222).

The effect of diet and exercise on S1P

No research currently exists on changes in HDL-S1P quantity following a diet and/or exercise intervention. Studies have focused on fluctuations in plasma S1P quantity and have mixed results. Acute exercise bouts of one session of cycling at 70% VO_2 max to exhaustion produces increased plasma S1P release by erythrocytes and trained individuals had S1P levels 37% higher than untrained subjects (9). Another study found that high-intensity exercise to exhaustion significantly increased plasma S1P, but resistance training did not (8). In trained ultramarathon runners, plasma S1P drops during exercise and remains reduced until 24 hours following rest from exercise (10). Interestingly, this effect is also observed in acute myocardial infarction, with sustained reductions in plasma S1P following cardio-injury (111).

Although the effects of exercise on HDL-S1P is not currently known, the effect of exercise on atherosclerosis has been attributed to many of the same processes that are influenced by S1P, and therefore could plausibly be attributed to increased HDL-S1P

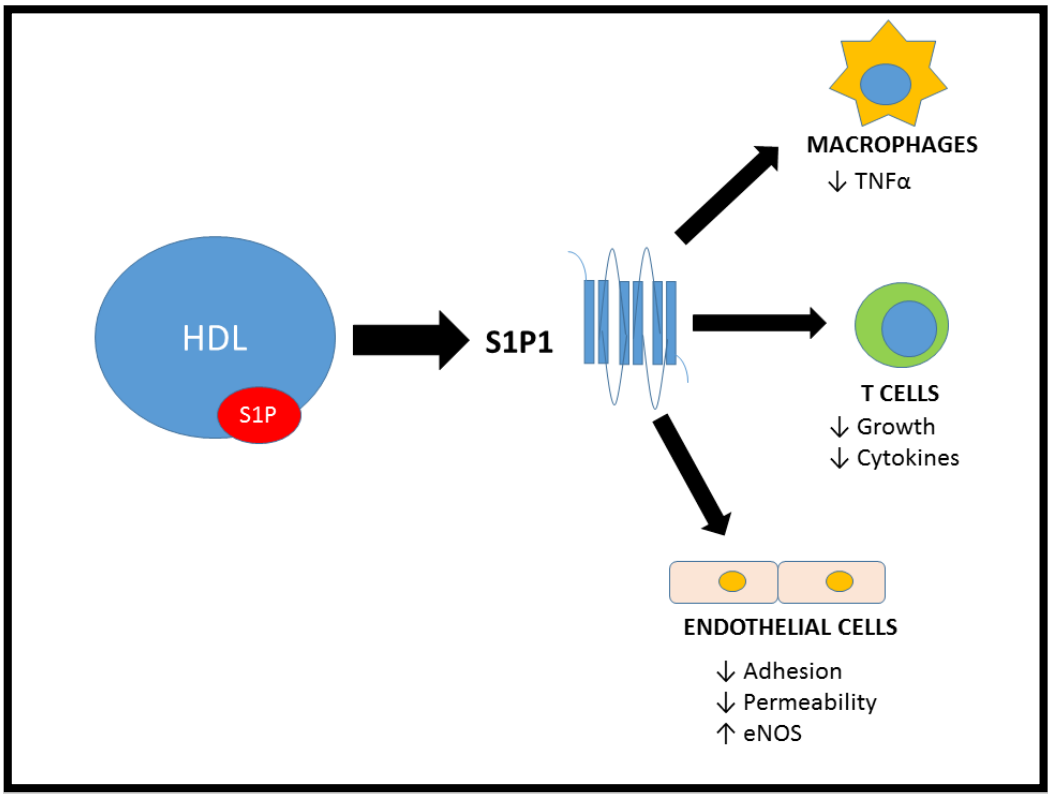

Figure 4. Proposed functions of HDL-S1P. Adapted from (167).

following chronic exercise (161). An extensive review by Palmefors et al. (161) attributes reduced IL-6, decreased VCAM-1, and reduced ICAM-1 as an effect of exercise, though the authors do not give specific mechanisms as to why exercise produces such an effect. Coincidentally or not, the benefits of exercise can be mimicked by S1P in vivo, which may be the reason exercise has such a dramatic effect on atherosclerosis reduction.

Hypothesis

Exercise produces effects on lipoprotein particle populations that are independent of changes in dietary intake. These effects are most likely to manifest within the HDL fraction of plasma and be unrelated to changes in particle cholesterol content *per se*. Changes in VO_{2max} can be used as an index of exercise impact and can be statistically related to changes in HDL particle properties.

This study makes an initial assessment of this hypothesis through the use of an isopycnic ultracentrifugal separation methodology to determine whether changes in VO_2 max (absolute and relative LBM VO_2 max) can be specifically linked to changes in density distributions of lipoprotein subclasses.

CHAPTER II

METHODS

Study inclusion criteria

To evaluate the effect of diet and exercise on lipoprotein density profiles, and to reduce bias associated with body composition and VO_2 max changes, only studies meeting the following inclusion criteria were considered: study duration of a minimum of eight weeks, a negative daily energy balance achieved through either supervised exercise intervention or controlled diet restriction of approximately 750 kcals per week with outcomes assessed of body composition by DEXA, VO_2 max measurements utilized standardized protocols, and serum samples properly stored and available for analysis. Samples for each subject had to include both pre-intervention baselines and post-intervention endpoints, in order to ensure that each subject was compared to their own data. Two studies met the required criteria and the details of their design and execution are published elsewhere for reference (77, 165).

Subject characteristics

The final data set comprised 90 individuals aged 20 – 74 years of age. Gender, baseline body composition and VO_2 max details of the combined study population are shown in (**Table 4**).

Descriptive Statistics		
Gender	74.2% male	25.8% female
Race		97.8% Caucasian
	Mean	*Std deviation*
Age (years)	54.06	14.95
Height (cm)	173.62	8.36
Weight (kg)	94.54	15.53
BMI (kg/m^2)	31.23	3.81
DXA (% body fat)	37.51	8.17
Fat Mass (kg)	33.74	9.53
Lean Mass (kg)	55.93	10.55
Relative VO_2 max (ml/kg/min)	24.17	7.06
Relative LBM VO_2 max (ml/kg LBM/min)	40.89	11.21
Absolute VO_2 max (L/min)	2.26	0.67

Table 4. Comparison of merged subject characteristics.

High performance lipoprotein density profiling

High performance lipoprotein density profiling (HPLDP) is a novel laboratory technique utilizing NBD-ceramide to pre-stain lipoproteins prior to isopycnic separation by ultracentrifugation using a metal ion EDTA salt mixture that self-forms a density gradient. This allows for sensitive separation of lipoprotein subclasses that can then be quantified by documentation of the fluorescent dye distribution along the density gradient (119).

The staining and separation of lipoproteins was achieved by mixing 6.0 µl of subject serum or plasma that were then mixed with 0.18 M NaBiEDTA. Ten microliters of NBD-ceramide (Cayman Chemical, Ann Arbor, Michigan) was reconstituted at a 1 mg to 1 ml ratio using dimethyl sulfide (Sigma-Aldrich Corp, St. Louis, Missouri) was added to the mixture within a 11 mm x 34 mm polycarbonate tube (Beckman-Coulter, Palo Alto, California) and incubated at four degrees Celsius for 30 minutes. The mixture was then centrifuged using a MLA-130 fixed angle rotor (Beckman-Coulter, Palo Alto, California) and an Optima MAX-LP Ultracentrifuge (Beckman-Coulter, Palo Alto, California) at 120,000 rpm for six hours at four degrees Celsius with the acceleration and deceleration set at “five” and the vacuum at a maximum of 20 microns. Once centrifugation was completed, tubes were then transferred to a custom-built enclosure which housed an Optical Breadboard (Thorlabs Inc, Newton, New Jersey) with a custom-built tube holder and Quantifire CCD camera (Optronics, Muskogee, Oklahoma). Flotation of 270 µl of high-performance liquid chromatography-grade hexane (Sigma-Aldrich Corp, St. Louis, Missouri) aided in the suppression of the

meniscus without disturbing the triglyceride-rich lipoprotein layer (119). The room lights were turned off and the tube was illuminated using a metal halide Fiber-Lite Illuminator (MH100A, Edmund Industrial Optics, Barrington, New Jersey). Two filters (Edmund Industrial Optics, Barrington, New Jersey) matching NBD excitation and emission wavelengths were placed in front of the light source and CCD camera respectively. The filters chosen to match NBD excitation and emission were a blue-violet band-pass filter (BG-12) with a wavelength centered at 407 nm and a yellow long-pass filter (OG-515) with a cut-on wavelength of 515 nm.

Settings for the Picture Frame camera software (Optronics, Muskogee, Oklahoma) were at an exposure time of "3 seconds", gain of 1.000, and a target intensity of 30%. Pixel values of the center of the tube (11-pixel wide) were converted into fluorescent intensity using Origin 8.0 software (OriginLab, Northampton, Massachusetts) and Origin 2015 (OriginLab, Northampton, Massachusetts) and plotted as a function of the tube coordinates (**Table 4, Figure 4**). Pixel values were converted to area under the curve (AUC and %AUC) for each lipoprotein subclass using Microsoft Excel 2010 (Microsoft, Redmond, Washington). Image processing included data adjustment for 16 pixels divided by 16 bits per pixel (BPP) to ensure accuracy of pixel data.

Lipoprotein Subclass	Density Range (g/ml)
TRL	<1.019
LDL-1	1.019-1.023
LDL-2	1.023-1.029
LDL-3	1.029-1.039
LDL-4	1.039-1.050
LDL-5	1.050-1.063
HDL-2b	1.063-1.091
HDL-2a	1.091-1.110
HDL-3a	1.110-1.133
HDL-3b	1.133-1.156
HDL-3c	1.156-1.179

Table 5. Lipoprotein subclasses and their density ranges using HPLDP.

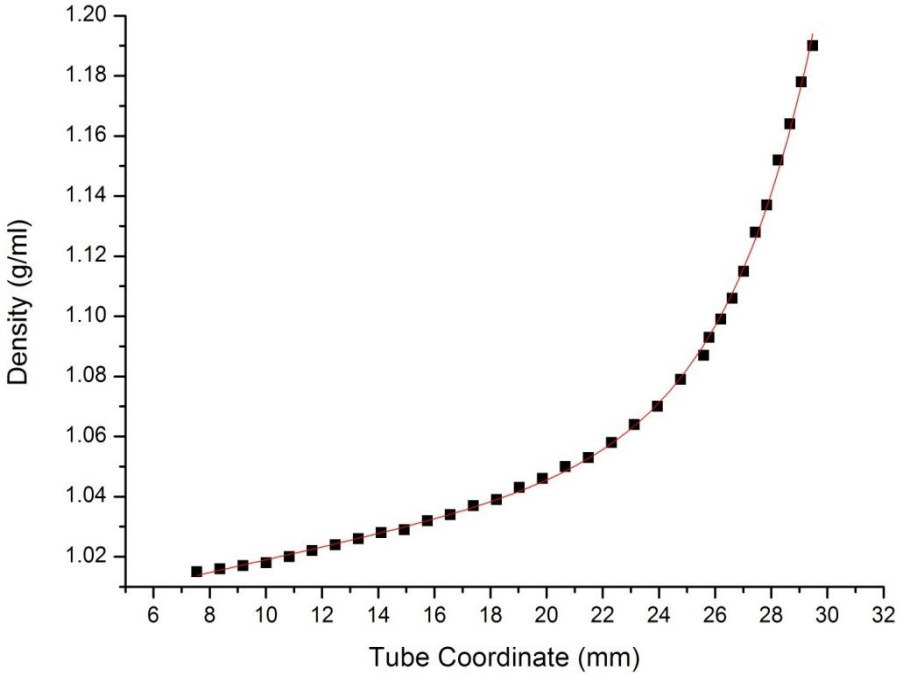

Figure 5. Graphical representation of density versus tube coordinates. of y = A1* exp (-x/t1) + A2 * exp (-x/t2) + y0, where y is the density value, x is the tube coordinate and A1, A2, t1, t2 and y0 are constants. Values were y = -872.17113 + 873.17712 * exp (A2 / 10843400) + 0.0000389069 * exp (A2 / 80.00782).

Data transformation and analysis

Sample data was analyzed using SPSS (SPSS Statistics for Windows, Version 24.0, Armonk, NY). Graphs and tables were either generated within SPSS or using Microsoft Excel 2010 (Microsoft, Redmond, Washington).

CHAPTER III

RESULTS

Eighty-eight subjects were included in the final data set, with two subjects excluded for abnormal data points observed during box plot visualization. Mean age was 54.4 ± 14.6 years. Mean height was 68.4 ± 3.2 inches. Mean weight was 209.2 ± 33.4 lbs. Average BMI was 31.3 ± 3.8 kg/m^2. Average DEXA was 37.6 ± 8.2 percent body fat. Average fat mass was 33.9 ± 9.4 kg. Average lean mass was 56.1 ± 10.4 kg. Average absolute VO_2 max was 2.3 ± 0.7 liters O_2/min. Average relative VO_2 max was 24.0 ± 6.9 ml O_2/kg/min. Average relative LBM VO_2 max was 40.7 ± 11.1 ml O_2/kg LBM/min.

After 12 weeks of participating in a diet or exercise intervention, subjects lost an average of 3.72 kg weight (-3.9%), lost 3.9 kg of body fat (-11.6%), increased lean mass 0.62 kg (+1.1%), and reduced body fat percentage by 3.41% (-9.1%). Average absolute VO_2 max increased 0.16 liters O_2/min (+7.1%). Average relative VO_2 max increased 2.65 ml O_2/kg/min (+11.0%). Average relative LBM VO_2 max increased 2.36 ml O_2/kg LBM/min (+5.8%). A paired-samples T-Test indicated that all metrics were significantly changed from baseline ($p<0.05$). Lipid profiles are summarized in **Table 6**. Statistically

Density Fraction	Baseline		Final		Change	
	mean	*SD*	*mean*	*SD*	*Gross*	*%Δ*
TRL	452.60	269.78	402.61	325.50	-49.99	5.46
LDL-1	70.60	37.58	71.65	53.73	1.04	14.31
LDL-2	108.92	46.74	97.91	49.25	-11.01	-4.20*
LDL-3	308.87	135.57	306.72	166.12	-2.14	4.43
LDL-4	718.16	369.22	677.31	384.78	-40.85	-1.38
LDL-5	471.37	211.91	435.83	248.31	-35.54	-3.63
LDL Total	1677.93	801.01	1589.42	902.19	-88.51	-3.71
HDL-2b	546.05	251.30	524.25	283.48	-21.80	0.56
HDL-2a	516.45	207.57	455.44	201.33	-61.00	-7.23*
HDL-3a	688.29	170.83	593.30	153.26	-94.99	-10.57*
HDL-3b	466.29	116.75	414.97	106.98	-51.32	-6.39*
HDL-3c	178.99	46.94	171.88	53.67	-7.11	0.48
HDL Total	2396.06	793.39	2159.84	798.72	-236.22	-7.43*

Table 6. Baseline and final lipid profiles of all subjects. AUC = area under the curve (arbitrary units), %AUC = percent of area under the curve. * indicates significance ($p<0.05$).

Density Fraction	Baseline		Final		Change	
	mean	*SD*	*Mean*	*SD*	*Gross*	*%Δ*
LDL-1 %AUC	1.53	0.66	1.68	0.90	0.15	--
LDL-2 %AUC	2.38	0.73	2.33	0.83	-0.06	--
LDL-3 %AUC	6.74	2.07	7.19	2.61	0.45	--
LDL-4 %AUC	15.49	5.30	15.76	5.67	0.28	--
LDL-5 %AUC	10.40	3.71	10.18	3.81	-0.22	--
LDL Total %AUC	36.54	12.47	37.13	13.82	0.59	--
HDL-2b %AUC	11.99	4.10	12.72	5.03	0.73	--
HDL-2a %AUC	11.40	3.36	11.13	3.63	-0.26	--
HDL-3a %AUC	15.42	2.64	14.75	2.99	-0.68	--
HDL-3b %AUC	10.54	2.39	10.44	2.79	-0.10	--
HDL-3c %AUC	4.10	1.19	4.33	1.37	0.22	--
HDL Total %AUC	53.45	13.68	53.37	15.80	-0.08	--

Table 6 continued. Baseline and final lipid profiles of all subjects, n = 88. AUC = area under the curve (arbitrary units), %AUC = percent of area under the curve. * indicates significance ($p<0.05$).

significant findings were decreased LDL-2 (-4.20%), HDL-3a (-10.57%), HDL-3b (-6.39%), and Total HDL (-7.43%) ($p<0.05$).

Differences between diet and exercise interventions

Using a priori knowledge, subjects separated into diet and exercise groups to ascertain if differences existed between interventions (**Table 7**). No differences existed at baseline. Using percent change from baseline, an independent T-Test was used to detect differences between interventions at 12 weeks. Significant differences were detected between groups (**Table 7**). Lipoprotein density distributions for diet and exercise were -17.14% vs 19.65% for TRL, -26.06% vs 8.14% for LDL5, -19.12% vs -0.71% for HDL3b, and -17.88% and 7.94% for HDL3c, respectively.

Characterizing anthropometrics and lipid profiles of subjects who changed VO_2 metrics

Since these findings were unexpected, we explored the effects of cardiorespiratory fitness on lipoprotein subfraction density distributions alone by dividing subjects into two groups: those who reduced absolute VO_2 max and those who increased absolute VO_2 max. The intent was to determine if differences exist between each group's anthropometric measurement and lipid profile changes that would be specifically attributable to exercise. At baseline, there were no significant differences between these subjects expect for baseline absolute VO_2 max which differed approximately 13% between subjects; since percent change was used to baseline and final measurements, this variance between groups was accounted for in the normalization process and did not impact results.

%Δ	*Diet (32)*		*Exercise (56)*	
	Mean	*Std. Deviation*	*Mean*	*Std. Deviation*
Weight	-9.35**	4.24	-0.78**	2.66
DXA	-17.50**	11.05	-4.64**	6.65
BMI	-9.35**	4.24	-0.83**	2.62
Lean Mass	-0.44*	3.75	1.97*	3.78
Fat Mass	-24.76**	12.62	-4.70**	7.30
Abs VO_2	-4.10**	8.51	12.87**	12.74
Rel VO_2	5.93**	9.52	13.85**	12.87
Rel LBM VO_2	-3.62**	8.31	10.79**	13.20
Total HDL	-12.25	17.96	-6.26	22.15
Total LDL	-15.37*	27.71	2.60*	29.30
TRL	-17.14*	52.02	19.65*	103.51
LDL1	10.98	58.48	17.74	85.40
LDL2	-11.43	41.31	0.60	40.71
LDL3	-5.77	35.34	9.52	54.46
LDL4	-11.02	32.41	3.78	40.49
LDL5	-26.06**	25.48	8.14**	41.84
HDL2b	0.15	27.04	-0.35	39.87
HDL2a	-6.59	23.45	-9.11	34.89
HDL3a	-15.55	18.40	-9.65	24.83
HDL3b	-19.12*	21.24	-0.71*	31.29
HDL3c	-17.88**	21.75	7.94**	31.38

Table 7. Differences between diet and exercise interventions. Data expressed as percent change from baseline. *indicates significance (p<0.05). **indicates significance (p<0.001)

Approximately 33% of subjects (n=29) reduced absolute VO_2 max while 65% of subjects (n=57) improved absolute VO_2 max. Twenty-five percent of subjects who participated in the diet intervention and 89% of subjects who participated in an exercise intervention improved their absolute VO_2 max. Characteristics of subjects who improved absolute VO_2 max and subjects who reduced absolute VO_2 max are summarized in **Table 8 / Figure 6**. As expected, subjects in these groups had unequal variance between groups as assessed by Levene's Test for Equality of Variance ($p<0.05$). Interestingly, the variance existed predominately in the subjects' lipoprotein subfractions distribution in LDL and HDL regions and not within Total LDL AUC and Total HDL AUC as might be captured by cholesterol measurements. Nevertheless, the violation of homogeneity was expected due to heterogeneity observed using arbitrary fluorescent pixel units and, more prominently, when unequal group sizes exist. Outliers were visually identified using a boxplot, and two outliers were removed from the analysis from both groups. A modified T-Test (Welch's T-Test) was utilized to account for the heterogeneity of variance.

Significant differences in weight loss (-8.2 % versus -1.4 %), fat mass reduction (-21.8 % versus -6.3 %), lean mass accrual (-0.8 % versus +2.2 %), and trunk fat reduction (-25.7 % versus +6.3 %) exist between subjects who increased absolute VO_2 max and subjects who reduced absolute VO_2 max ($p<0.05$). Significant differences with lipid profiles also were found between subjects who increased absolute VO_2 max and subjects who reduced absolute VO_2 max: TRL AUC (-15.7% versus +17.5%), LDL-5 AUC (-24.6% versus +7.3%), HDL-3b AUC (-17.3% versus -0.3%) and HDL-3c AUC

Absolute VO_2 Max Change	Negative	Positive
Absolute VO_2 max	-7.6546	14.4188*
Weight	-8.1294	-1.5161*
DXA	-15.3738	-5.8238*
BMI	-8.1275	-1.5622*
Lean Mass	-0.7900	2.0721*
Fat Mass	-21.9624	-6.3274*
Trunk Fat	-25.9532	-7.6616*
TRL	-19.2447	18.9331*
LDL-1	0.7656	21.9970*
LDL-2	-6.5481	-2.1253
LDL-3	0.1413	6.3644
LDL-4	0.1322	-2.0622
LDL-5	-24.5051	6.4895*
Total LDL	-10.0976	-0.5152
HDL-2b	0.5051	-0.1038
HDL-2a	-3.2998	-10.3821
HDL-3a	-12.6616	-11.2316
HDL-3b	-19.3863	-1.7316*
HDL-3c	-20.1349	7.4957*
Total HDL	-11.5110	-6.6766

Table 8. Comparison of positive and negative absolute VO_2 max. Overall metrics associated with those who increased absolute VO_2 max and those who decreased absolute VO_2 max. All data expressed as percent change from baseline. * indicates statistically significant difference between groups at 12 weeks ($p<0.05$).

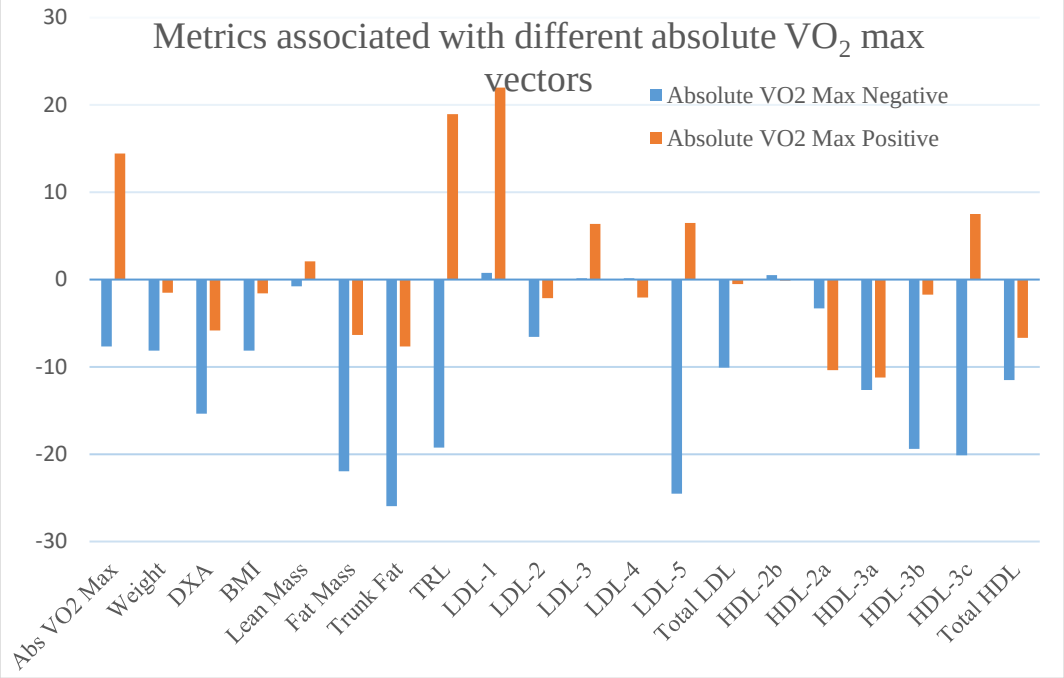

Figure 6. Visualization of metrics associated with positive and negative percent change in absolute VO_2 max. All data expressed as percent change from baseline.

(-17.6% and +10.3%).

To determine whether age or gender of the subjects impacted the results attributed to changes in absolute VO_2 max, a MANCOVA with age categories and gender as covariates was conducted (Table X). The influence of absolute VO_2 max percent change on TRL AUC, LDL-5 AUC, HDL-3b AUC, and HDL-3c AUC percent change remained significant after controlling for age, gender, body fat, and a combination of the aforementioned variables. Although the assumption of homogeneity of covariance was violated, as assessed by a Box's M test ($p<0.001$), the robust nature of *F* statistic and using allowed the analysis to continue. Additionally, to account for the lack of homoscedasticity Pillai's Trace was used rather than Wilk's λ, primarily due to its ability to account for unequal variance (62). The results of this test demonstrated that absolute VO_2 max percent change maintains a significant influence on LDL-5 AUC percent change and HDL-3c AUC percent change when controlling for the effects of age and gender ($p<0.001$). Gender did influence HDL-3b AUC percent change significantly, $F(4, 80) = 1.221$, $p<0.05$, Pillai's Trace = 0.194, $\eta^2 = 0.194$.

A follow-up univariate analysis of variance (ANOVA) was conducted to determine the influence of age on LDL-5 AUC percent change, HDL-3c AUC percent change, and absolute VO_2 max measurements and care was taken to identify differences between groups utilizing Bonferroni post hoc analysis and Bonferroni adjustment, the latter which can be being described α / m, where m = the number of ANOVA tests conducted. Although significance with age was found in the MANCOVA, multiple ANOVA and post hoc analysis determined a difference existed only between age

categories and relative LBM VO_2 max, not lipid profiles. The difference in age categories was found between age group 20-29 and age group 60-69 ($p<0.017$).

Significant correlations

In order to standardize units, all variables were correlated using their percent change values which were calculated as *(final measurement – baseline measurement) / baseline measurement x 100.* Over 125 variables were significantly correlated with each other as assessed by Pearson Correlation. Most noteworthy, significant positive correlations were found between weight and total LDL, weight and total HDL, weight and LDL-5, and weight and small HDL ($p<0.05$). Percent change of body fat was positively correlated to the same variables as percent weight change, though the HDL fraction of HDL-3a was not. Absolute VO_2 max percent change was positively correlated with total LDL AUC, LDL-5 AUC, and HDL-3c AUC. Fat mass percent change was significantly correlated with TRL percent change, LDL percent change, HDL-3b percent change, and HDL-3c percent change.

A partial correlation was also conducted to determine if fat mass percent change explained the changes observed with VO_2 max (**Table 9**). The partial correlation indicated that when fat mass percent change was controlled, significant correlations continued to exist between absolute VO_2 max and LDL5, and absolute VO_2 max and HDL3c.

Linear regression

Linear regression using the aforementioned significant correlations was utilized in an effort to construct a prediction equation that would predict a lipoprotein

Control Variables			abs VO_2 max
Fat mass	Rel LBM VO_2 max	Correlation	0.927
		Significance (2-tailed)	0.000**
	TRL	Correlation	0.079
		Significance (2-tailed)	0.468
	LDL5	Correlation	0.239
		Significance (2-tailed)	0.026*
	HDL3b	Correlation	0.069
		Significance (2-tailed)	0.526
	HDL3c	Correlation	0.240
		Significance (2-tailed)	0.025*

Table 9. Partial correlation analysis controlling for fat mass change.

subfraction profile percent change in response to an absolute VO_2 max percent change (when baseline is known). Various iterations of the equation were attempted to include body fat percent change, weight percent change, and covariates such as age categories and gender but the universally significant variable to consistently predict changes to the HDL-3c AUC was absolute VO_2 max percent change. A simple linear regression was constructed to identify how HDL-3c AUC percent change is predicted when absolute VO2 max percent change is known. A significant regression equation was found using an R^2 of 0.108, $F(1, 87) = 10.537$, $p<0.002$. Predicting HDL-3c AUC percent change is -5.244 + 0.838 (absolute VO_2 max percent change) when absolute VO_2 max is measured as a percent change from baseline. Participants HDL-3c percent change increased **0.838** for every percent change in absolute VO_2 max.

CHAPTER IV

SUMMARY OF FINDINGS AND FUTURE DIRECTION

The phrase “diet and exercise” is used to describe the most common lifestyle interventions to reduce ASCVD risk, the research presented here shows that changes in select lipoprotein subclass occurred in response to changes in VO_2 max regardless of the means used to achieve that change. This research also shows the disparity that may exist between classic cholesterol panels and subfraction measurements.

The anthropometric data demonstrates the similar responses to body weight, body fat, and BMI scores that result from consistent lifestyle intervention. The cardiorespiratory markers however trended differently despite similar anthropometric modulations. It appears that changes in HDL-3c are closely if not directly related to changes in absolute VO_2 max. Research indicates that small dense HDL particles are associated with an increased risk for ASCVD (13, 186, 227). For example, Salonen et al. (186) reported an inverse correlation between large HDL-C and acute myocardial infarction using preparative ultracentrifugation and tube slicing. Xu et al. (227) compared HDL-C levels in subjects with and without ASCVD, then subdivided HDL-C into subfractions by gel electrophoresis; they reported positive correlations between ASCVD and small HDL-C particles.

There are several plausible reasons why our research appears at odds with these findings. Our subjects engaged in a lifestyle intervention, which the clinical endpoints used in the aforementioned studies did not assess. We did not clinically-diagnose the

presence of ASCVD in our subjects while the majority of studies reporting a relationship between small HDL particles and ASCVD risk did assess. Finally, we utilized lipoprotein subfractions density distributions rather than lipoprotein cholesterol profiles. Our methodology is more specific to density fractional modifications and thus may not be replicable using different methodologies.

Other studies have indicated that exercise does support improved antioxidant capacity in HDL-3 subfractions, but to date none have assessed or speculated S1P content as the mechanism. High-density lipoproteins have an increase in PON1 activity following an exercise intervention in subjects with metabolic syndrome (29). Casella-Filho et al. (29) found that although HDL-C did not improve with exercise in subjects with MetS, PON1 activity increased in the smallest HDL subfraction. Ribeiro et al. (174) found that no changes occurred in HDL-C following 18 weeks of exercise in subjects with type 2 diabetes, but HDL-3 improved antioxidant capacity as assessed via oxLDL generation.

It is highly plausible that HDL-S1P content is responsible for the improved function of HDL-3 subfractions through increased generation of NO and other benefits of HDL modulation following exercise, but current methodologies do not assess this (208). Our subjects did not significantly change HDL-2b and thus a HDL-C panel would not be able to indicate the changes we were able to assess via lipoprotein density distributions. As previously mention, S1P is most prominently carried on the HDL-3c subfraction (83, 84, 121). Our study found a significant linear relationship between improved absolute VO_2 max and increased HDL-3c subfraction AUC and indicates that

the subfraction may be a strong prognostic indicator of exercise efficacy in relation to cardiorespiratory fitness.

This study utilized a promising technology that can rapidly assess biological adaptations to lifestyle interventions and reveal the inefficacious nature of HDL-C if measuring effects of lifestyle interventions outside of lipoprotein cholesterol for ASCVD risk reduction. This methodology allowed the measurement of specific lipoprotein density gradients which revealed far more predictive power that may have been overlooked. Finally, this method detected modification to lipoprotein density distributions that would have remained obscure if using traditional lipoprotein cholesterol assessment methods, especially given the covariates and cofactors of weight, body fat, age, and gender.

Most importantly, this study demonstrates the ubiquitous and complex characteristics of lipoprotein subfractions. It highlights the importance of standardizing assessment methodologies and characterizing lipoprotein profiles for a specific population rather than overgeneralizing findings. The transcending reductionism in diet and exercise research may further complicates rather than clarify the complex biochemical and physiological adaptations observed following lifestyle intervention.

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APPENDIX A: NOMENCLATURE

AHA	American Heart Association
$apoA_1$	apolipoprotein A_1
$apoA_2$	apolipoprotein A_2
$apoB_{100}$	apolipoprotein B_{100}
$apoB_{48}$	apolipoprotein B_{48}
$apoC_{II}$	apolipoprotein C_{II}
$apoC_{III}$	apolipoprotein C_{III}
apoE	apolipoprotein E
apoM	apolipoprotein M
ASCVD	atherosclerotic cardiovascular disease
CE	cholesterol esters
CETP	cholesterol:ester transfer protein
Chol	cholesterol
CR	chylomicron remnant
CVD	cardiovascular disease
FABP	fatty acid binding protein
FAT/CD36	fatty acid translocase/cluster of determination 36
HDL	high-density lipoprotein
HDL-C	high-density lipoprotein cholesterol
HDL-S1P	high-density lipoprotein-associated sphingosine 1-phosphate

HDL-x	high-density lipoprotein, subclass/subfraction x
HMG-CoA	3-hydroxy-3-methylglutaryl-coenzyme A
HPLDP	high performance lipoprotein density profiling
HSGP	heparin sulfate proteoglycan
ICAM-1	intracellular adhesion molecule-1
IDL	intermediate-density lipoprotein
LBM	lean body mass or non-fat mass
LDL	low-density lipoprotein
LDL-C	low-density lipoprotein cholesterol
LDL-x	low-density lipoprotein, subclass/subfraction x
LRP	lipoprotein remnant receptor protein
MetS	metabolic syndrome
MRFIT	Multiple Risk Factor Intervention Trial
NHLBI	National Heart, Lung, and Blood Institute
NO	nitric oxide
NMR	nuclear magnetic resonance
oxLDL	oxidized low-density lipoprotein
PCSK9	proprotein convertase subtilisin/kexin 9
PL	phospholipids
PLTP	phospholipid transfer protein
S1P	sphingosine 1-phosphate
sdLDL	small dense low-density lipoproteins

SMC	smooth muscle cells
SR-B1	scavenger receptor, type B, class 1
TAG	triacylglycerides
TC	total cholesterol
VCAM-1	vascular cell adhesion protein-1
VLDL	very-low-density lipoprotein
VEGF	vascular endothelial growth factor
$\dot{V}O_2$ max	volume of oxygen in liters consumed per minute