A discordance analysis of apolipoprotein A-I and high-density lipoprotein-cholesterol in UK Biobank

Selin Bilgic

Division of Experimental Medicine, McGill University, Montreal

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ABSTRACT

Atherosclerotic cardiovascular disease (ASCVD) remains a leading cause of death worldwide. Multiple lines of evidence have now established that apolipoprotein B (apoB) is the most accurate marker of ASCVD risk, superior in predictive power to both low-density lipoprotein-cholesterol (LDL-C) and non-high-density lipoprotein-cholesterol (non-HDL-C). However, evidence as to whether apolipoprotein A-I (apoA-I), the primary apolipoprotein in HDL-C, is a better marker of ASCVD than HDL-C is limited. To address this gap in the literature, I will first categorize the relation of HDL-C and apoA-I to ASCVD risk, and then compare the relative strengths of HDL-C and apoA-I to risk by residual discordance analysis, a novel statistical method developed to compare the predictive power of highly correlated variables. I also investigate the relation between apoB, LDL-C, and non-HDL-C to ASCVD risk at different levels of apoA-I. Part 1 includes a sample of 291,995 UK Biobank adults, median age 56, 42.1% men, free of cardiovascular disease and lipid-lowering medication, with a median follow-up for new onset ASCVD of 11 years. Residuals of apoA-I and HDL-C were constructed after regressing each variable onto the other. Pearson correlation coefficients between apoA-I, HDL-C, non-HDL-C, apoB, LDL-C, log-transformed triglycerides, and both residuals were calculated. These markers were used as predictors in Cox proportional hazards regression models for new onset ASCVD, adjusted for standard risk factors, such as smoking. Part 2 of the study divides the cohort of participants into quintiles of apoA-I and repeats the steps in Part 1 within each quintile. In the main cohort, HDL-C, apoA-I, the HDL-C residual, and the apoA-I residual were significantly associated with new onset ASCVD (hazard ratio (HR)=0.85, 0.85, 0.98, 0.96, respectively; $p<0.05$). Across apoA-I quintiles, the concentrations of apoB, LDL-C, and non-HDL-C remained consistent, while their HRs significantly decreased from the lowest apoA-I

quintile to the highest (1.20 to 1.06, 1.21 to 1.04, and 1.23 to 1.05 for apoB, LDL-C, and non-HDL-C, respectively; $p<0.05$ for apoB and non-HDL-C). Interaction between apoA-I (or HDL-C) and apoB (or LDL-C or non-HDL-C) was determined by adding an interaction term to the proportional hazards regression models used in the main cohort. This analysis demonstrates that both HDL-C and apoA-I are significant markers of ASCVD risk and they appear to be of similar value. A novel finding in this study is the interaction between apoA-I (or HDL-C) and apoB (or LDL-C or non-HDL-C), in which the atherogenic risk predicted by apoB relates inversely to the concentration of apoA-I.

RÉSUMÉ

Les maladies cardiovasculaires liées à l'athérosclérose demeurent parmi les principales causes de mortalité dans le monde. De nombreuses données établi l'apolipoprotéine B (apoB) comme l'indicateur le plus fiable pour déterminer le risque d'athérosclérose, supérieur au cholestérol des lipoprotéines de basse densité (LDL-C) et des lipoprotéines de non-haute densité (non-HDL-C). Cependant, les données disponibles pour déterminer si l'apolipoprotéine A-I (apoA-I), la principale apolipoprotéine du HDL-C, est un meilleur marqueur que le HDL-C sont limitées. Je commencerai par classer la relation entre le HDL-C et l'apoA-I et le risque d'athérosclérose, puis je comparerai les forces relatives du HDL-C et de l'apoA-I par rapport au risque par utiliser l'analyse de la discordance résiduelle, une méthode statistique développée pour comparer le pouvoir prédictif de variables fortement corrélées. J'étudie également la relation entre l'apoB, le LDL-C et le non-HDL-C avec le risque d'athérosclérose à différents niveaux d'apoA-I. La première partie de cette étude utilise un échantillon de 291 995 adultes de la UK Biobank, 56 ans d'âge médian, 42,1 % d'hommes, sans maladie cardiovasculaire et médicaments hypolipidémiants, avec un suivi médian d'environ 11 ans pour l'apparition d'athéroscléroses. Les résidus de l'apoA-I et du HDL-C ont été construits après la régression de chacun sur l'autre. Les coefficients de corrélation de Pearson entre l'apoA-I, le HDL-C, le non-HDL-C, l'apoB, le LDL-C, les triglycérides log-transformés et les deux résidus ont été calculés. Ces marqueurs ont été utilisés comme prédicteurs dans les modèles de régression des risques proportionnels pour l'apparition de nouvelles maladies athéroscléroses, ajustés pour les facteurs de risque standard, tels que le tabagisme. La deuxième partie divise la cohorte en quintiles d'apoA-I et répète les étapes de la première partie dans chaque quintile. Dans la cohorte principale, le HDL-C, l'apoA-I, le résidu du HDL-C et le résidu de l'apoA-I étaient

significativement associés à l'apparition d'athéroscléroses (ratio de risque (HR)=0,85, 0,85, 0,98, 0,96, respectivement ; $p<0,05$). Dans les quintiles d'apoA-I, les concentrations d'apoB, le LDL-C et le non-HDL-C étaient constantes, tandis que leurs HRs ont diminué du quintile le plus bas au plus élevé (1,20 à 1,06, 1,21 à 1,04, 1,23 à 1,05 pour l'apoB, le LDL-C et le non-HDL-C respectivement ; $p<0,05$ pour l'apoB et le non-HDL-C). L'interaction entre l'apoA-I (ou le HDL-C) et l'apoB (ou le LDL-C ou le non-HDL-C) a été déterminée en ajoutant un terme d'interaction aux modèles de la cohorte principale. Cette analyse démontre que le HDL-C et l'apoA-I sont des marqueurs significatifs de l'athérosclérose, avec une valeur similaire. L'interaction entre l'apoA-I (ou le HDL-C) et l'apoB (ou le LDL-C ou le non-HDL-C) constitue une nouveauté de cette étude, dans laquelle le risque athérogène prédit par l'apoB est inversement proportionnel à la concentration de l'apoA-I.

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CONTRIBUTION OF AUTHORS

Dr. Allan Sniderman established the main goal of this thesis. Selin Bilgic conducted all analyses, which were reviewed by Line Dufresne when necessary. Selin Bilgic drafted all chapters of the thesis, which were reviewed by Dr. Sniderman.

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ABBREVIATIONS

ABCA1: ATP-Binding Cassette Transporter A1

ABCG1: ATP-Binding Cassette Subfamily G Member 1

ACC: American College of Cardiology

AHA: American Heart Association

ApoA-I: Apolipoprotein A-I

ApoA-II: Apolipoprotein A-II

ApoA-IV: Apolipoprotein A-IV

ApoB: Apolipoprotein B

ApoC: Apolipoprotein C

ApoD: Apolipoprotein D

ApoE: Apolipoprotein E

ApoF: Apolipoprotein F

ApoH: Apolipoprotein H

ApoJ: Apolipoprotein J

ApoL-I: Apolipoprotein L-I

ApoM: Apolipoprotein M

ApoO: Apolipoprotein O

ASCVD: Atherosclerotic Cardiovascular Disease

ATF3: Activating Transcription Factor 3

BMI: Body Mass Index

C3: Complement Component 3

C4: Complement Component 4

C9: Complement Component 9

CAD: Coronary Artery Disease

CETP: Cholesterol Ester Transfer Protein

CHD: Coronary Heart Disease

CI: Confidence Interval

COPD: Chronic Obstructive Pulmonary Disease

CVD: Cardiovascular Disease

HbA1c: Hemoglobin A_{1c}

HDL: High-Density Lipoprotein

HDL-C: High-Density Lipoprotein-Cholesterol

HR: Hazard Ratio

IDL: Intermediate-Density Lipoprotein

LCAT: Lecithin-Cholesterol Acyltransferase

LDL: Low-Density Lipoprotein

LDL-C: Low-Density Lipoprotein-Cholesterol

Lp(a): Lipoprotein (a)

LPS: Lipopolysaccharide

LXR: Liver X Receptor

Non-HDL-C: Non-High-Density Lipoprotein-Cholesterol

NPC1L1: Niemann-Pick C1-Like 1

OR: Odds Ratio

PAD: Peripheral Artery Disease

PAF: Platelet-Activating Factor

PAFAH: Platelet-Activating Factor Acetylhydrolase

PCE: Pooled Cohort Risk Estimator Plus

PCSK9: Proprotein Convertase Subtilisin/Kexin Type 9

PLTP: Phospholipid Transfer Protein

PON-1: Paraoxonase-1

PPAR: Peroxisome Proliferator-Activated Receptor

RXR: Retinoid X Receptor

SAA: Serum Amyloid A

SBP: Systolic Blood Pressure

SR-B1: Scavenger Receptor Class B Member 1

TC: Total Cholesterol

VLDL: Very Low-Density Lipoprotein

1.0 INTRODUCTION

Atherosclerotic cardiovascular disease (ASCVD) is a leading cause of death worldwide, accounting for 31% of deaths in 2015.[1] ASCVD is characterized by plaque buildup in the arteries, which is formed by the complex biological reaction to the deposition of cholesterol within the arterial wall.[2,3] Based on the arteries affected, the condition can be referred to by different names like coronary artery disease (CAD), peripheral artery disease (PAD), or carotid artery disease.[2] Atherosclerotic plaques can cause narrowing or obstruction of these arteries, reducing or eliminating the supply of oxygenated blood to vital organs in the body.[2,4,5]

For decades, cardiovascular researchers have focused on determining the most useful markers of ASCVD to ensure accurate diagnosis of the disease and to develop effective treatments. Research has demonstrated that high levels of low-density lipoprotein (LDL)-cholesterol (LDL-C) and low levels of high-density lipoprotein (HDL)-cholesterol (HDL-C) are associated with increased ASCVD risk.[6] LDL particles are the end-product of the metabolism of very low-density lipoprotein (VLDL) and intermediate-density lipoprotein (IDL) particles.[7] VLDLs are triglyceride-rich particles produced by the liver that transport triglycerides and cholesterol through the circulation because these lipids are insoluble in blood.[7] The removal of triglycerides from VLDL particles by virtue of lipoprotein lipase forms IDL particles, and the further removal of triglycerides from IDL particles forms LDL particles.[7] LDL particles contain most of the cholesterol in the circulation.[7] Apolipoprotein B (apoB)-100 is the main structural component of VLDL, IDL, and LDL, and is the essential player in cholesterol deposition in arterial walls.[7,8] It is the trapping of apoB particles in the arterial wall that initiates and drives the development of atherosclerosis.[9] Although LDL-C is currently the primary marker of

cardiovascular risk in clinical care, apoB has been shown to be a superior marker of ASCVD risk.[9,10]

Alternatively, HDL, produced in the liver and intestines, removes cholesterol from macrophages to oppose the progression of atherosclerosis.[8,11] The most accepted model for the anti-atherogenic effect of HDL-C is the reverse cholesterol transport model.[6] This multistep model involves the liberation of free cholesterol from macrophages to HDL via three mechanisms.[6] Cholesterol can be transferred via the interaction of apolipoprotein A-I (apoA-I), the main structural component of HDL, and ATP-binding cassette transporter A1 (ABCA1); via ATP-binding cassette subfamily G member 1 (ABCG1); and by Scavenger Receptor Class B Member 1 (SR-B1).[6] The lecithin-cholesterol acyltransferase (LCAT) enzyme prevents the backwards transfer of free cholesterol to macrophages by converting free cholesterol to cholesteryl esters.[6] Finally, SR-B1 found on the surface of hepatocytes takes free cholesterol and cholesteryl esters from HDL to be transferred to bile for intestinal excretion.[6] Another important mediator is the cholesterol ester transport protein (CETP), which transfers cholesterol esters from HDL to VLDL, chylomicrons, and LDL in exchange for triglycerides from VLDL and chylomicrons to HDL.

Despite the essential role of apoA-I in reverse cholesterol transport, most emphasis is still placed on HDL-C as an anti-atherogenic particle. Currently, researchers are divided on whether HDL-C or apoA-I is a more useful marker for ASCVD risk. The potential superiority of either HDL-C or apoA-I is not as clearly understood as the superiority of apoB over non-HDL-C and LDL-C. Some researchers have shown that apoA-I may be a more effective predictor for ASCVD,[12-15] whereas others have demonstrated the superiority of HDL-C over apoA-I.[16-19]

Some research even reveals that both apoA-I and HDL-C are similar predictors of ASCVD risk, adding little value to each other.[20]

An obstacle faced in analyzing ASCVD risk across various markers using conventional statistical methods lies in separating the effects of highly correlated variables, such as HDL-C and apoA-I, which are biologically intertwined. Discordance analysis is a novel statistical method that addresses this challenge by creating groups in which the highly correlated markers are discordant.[21] The least arbitrary, and therefore the most unbiased, of the different methods to analyze discordance is residual analysis, which calculates the differences of the "observed" values of HDL-C and apoA-I from the "expected" values obtained from the regression that relates the two.[22,23]

This study aims: first, to categorize the relation of HDL-C and apoA-I to ASCVD risk; second, to compare the relative strengths of HDL-C and apoA-I to ASCVD risk using residual discordance analysis; and third, to determine if there is any interaction between apoB (or LDL-C or non-HDL-C) and apoA-I (or HDL-C) on ASCVD risk.

2.0 BACKGROUND

2.1 Cardiovascular Disease Overview

Cardiovascular disease (CVD) encompasses a wide range of conditions involving the heart and blood vessels.[24] These various disorders can be grouped into four main categories: CAD, defined as narrowing of the coronary arteries; cerebrovascular disease, which primarily affects blood vessels of the brain, commonly leading to stroke; PAD, which affects blood flow in large- and medium-sized arteries, other than the coronary arteries and arteries to the brain; and aortic atherosclerosis, characterized by a build-up of plaque in the aorta.[25-29] Altogether, CVD is the primary cause of death worldwide, contributing to over 19 million deaths in 2020.[25,30] This number has been projected to increase to over 23 million deaths worldwide in 2030.[24,31,32] Notably, more than three-quarters of these deaths caused by CVD have ensued in low- and middle-income countries.[24] Central Europe, Eastern Europe, and Central Asia have the highest rates of CVD deaths.[33] Between 2011 and 2015, the economic burden from CVDs in low- and middle-income countries was about $3.7 trillion.[34] This economic burden places CVD as one of the most costly diseases, ahead of Alzheimer's disease and diabetes.[25]

CVD commonly occurs alongside other health conditions, known as comorbidities, which can exacerbate its burden.[35] Multiple studies demonstrate a statistically significant association between CVD and low vision, diabetes, back and neck problems, osteoarthritis, chronic obstructive pulmonary disease (COPD), and cancer.[35-39] These associations may be attributed to the overlap of risk factors between the conditions.[35] The prevalence of risk factors for CVD, such as dyslipidemia and smoking, are also high in patients who live with Type 2 Diabetes.[35] Smoking is also a risk factor for low vision, COPD, and certain types of cancer.[35,40] Comorbidities for CVD may also include additional cardiovascular disorders.[35] A cohort study using logistic

regression analysis with medical record data from the Julius General Practitioners' Network in the Netherlands identified coronary heart disease (CHD) as the most common cardiovascular comorbid condition in patients with heart failure, PAD, and stroke.[35] Other studies have employed a meta-analysis approach to reveal PAD and heart failure, as well as stroke and heart failure, as comorbid conditions.[41,42]

2.1.1 Pathophysiology

The pathophysiology of CVD remains complex and depends on the type of CVD in question. For instance, CAD manifests from the formation of an atherosclerotic plaque, which is a build-up of calcium, cholesterol, and inflammatory components, that can obstruct the coronary vessel lumen, limiting normal blood flow.[3] These plaques are initially formed by the subendothelial deposition of foam cells, lipid-laden macrophages, which ultimately create the initial atherosclerotic lesion, known as a "fatty streak".[3] The subendothelial plaque develops as more foam cells accumulate, necrose, and the cholesterol within them form a lipid core within a fibrous outer surface.[3,43] The plaque may remain stable and quiescent.[3] However, if it ruptures and is exposed to tissue factor, an initiator of blood coagulation, acute thrombosis can result, causing occlusion of the vessel lumen.[3,44] Thrombosis may then lead to death of the tissue being supplied by the vessel, producing myocardial or cerebral infarction depending on whether the heart or brain are involved.[3,45]

Cerebrovascular disease encompasses several distinct pathologies, such as ischemic stroke caused by an obstruction that reduces blood flow to the brain, transient ischemic attack which is a stroke that lasts a few minutes, and aneurysms, when a blood vessel bulges in one

spot.[46-49] Stroke is the most devastating manifestation of cerebrovascular disorders.[46] Ischemic stroke can arise from a thrombotic or embolic event that reduces oxygen-rich blood flow to the brain.[47] Similar to CAD, a thrombotic event occurs when an atherosclerotic plaque narrows the vessels supplying blood to the brain and accumulates, leading to thrombosis and causing a stroke.[50,51] Less commonly, an embolus, a blood clot or plaque debris that travels through the bloodstream until it can no longer fit through the vessel, develops elsewhere in the body and travels to a vessel in the brain, causing an embolic stroke.[51-53]

Similar to both CAD and some manifestations of cerebrovascular disease, PAD is primarily triggered by an atherosclerotic plaque in the blood vessels.[54] Generally, an atherosclerotic plaque develops in the vessels carrying blood from the heart to the legs, which narrows or blocks blood flow in this direction.[54] This may cause typical symptoms of PAD such as leg pain with physical activity, muscle weakness, or cramps with walking.[54]

Lastly, aortic atherosclerosis manifests through a progressive buildup of plaque in the aorta, the largest artery in the body, which supplies blood from the heart to the rest of the body.[55] Specifically, aortic atherosclerosis can cause two types of emboli - a thromboembolism or an atheroembolism.[55] More commonly, thromboembolisms develop as a blood clot that forms on the plaque's surface, breaks away, and travels through the blood.[55] Otherwise, an atheroembolism can develop when the cholesterol plaque itself breaks away and travels through the blood.[55] Both types of emboli block blood flow to the area in which they become stuck, leading to ischemia (shortage of oxygen-rich blood) and subsequent damage of tissues and organs in the area.[55]

Atherosclerosis is the common denominator in the pathophysiology of the great majority of cardiovascular events.[56]

2.1.2 Risk Factors

Although the direct cause of CVD is unknown, there are various risk factors that increase the risk of developing CVD.[57] It is important to recognize risk factors, monitor them, and make life-style changes with this information to manage CVD risk and burden.[58] These risk factors may be non-modifiable, such as age, sex, ethnicity, race, and genetic factors, or modifiable, such as smoking, physical inactivity, hypertension, poor diet, alcohol, and diabetes.[59]

Age

Age is one of the strongest risk factors for CVD. In both males and females, the prevalence of CVD rises substantially after the 5th decade.[60] Two different mechanisms are involved. The first is more prolonged exposure to the causes of atherosclerosis: entry and trapping of apoB particles within the arterial wall, elevated blood pressure or blood sugar, and smoking.[61] The second relates to the still poorly understood changes with aging that accelerate that atherosclerotic process, such as increased oxidative stress and inflammation.[60,61] In addition to the various processes by which age influences CVD risk, age also has complex interactions with a multitude of other risk factors of CVD, exacerbating its impact.[60,62]

Sex

Notable differences in CVD risk exist between men and women.[63] Typically, men have an elevated risk and earlier onset of CVD.[63] This difference in risk can be attributed to many factors, for instance, men generally have less favorable levels of blood pressure and cholesterol and smoke more than women.[64] Sex differences in risk have also been attributed to sex hormones.[60] The incidence of stroke significantly increases in menopausal women.[65] Therefore, many studies have investigated the protective effect of estrogen on the cardiovascular system.

Clinical studies have found a high incidence of CAD in young women who have had a bilateral oophorectomy, the removal of both estrogen-producing ovaries.[60] Experiments in rodents have demonstrated the role of estrogen and its receptors in increasing angiogenesis and vasodilation and reducing oxidative stress, inflammation, and cardiomyocyte apoptosis.[66-70] Similarly, genetic research has shown that the X chromosome influences the expression of genes associated with apoptosis, lipid oxidation, and mitochondrial production of reactive oxygen species.[71] Further, the sex-determining region Y of the Y chromosome mediates transcription of tyrosine hydroxylase, the rate-limiting enzyme in catecholamine (e.g., noradrenaline) synthesis, resulting in a sex-specific difference in sympathetic activity, making men more predisposed to hypertension than women.[72,73] Despite the strong evidence for estrogen as a cardioprotective agent, multiple studies investigating hormone-replacement therapy have yielded mixed results.[74-77] For instance, the Heart and Estrogen/Progestin Replacement Study found no significant differences in CHD death, nonfatal myocardial infarction, coronary revascularization, stroke, PAD, congestive heart failure, resuscitated cardiac arrest, or unstable angina when randomly treating 3,000 postmenopausal women with either placebo or combination hormone therapy (estrogen and medroxyprogesterone), after a four-year follow-up.[77] The Women's Health Initiative study even revealed a higher incidence of cardiovascular events, thromboembolic events, and stroke in postmenopausal women who received combination hormone therapy, leading to the trial's termination.[75]

Ultimately, sex differences in CVD risk may stem from a complex interaction between environmental, hormonal, and genetic factors.

Race and ethnicity

Racial and ethnic disparities in cardiovascular health have been documented extensively, emphasizing the disproportionate effect of CVD on ethnic minority groups.[78] In the United States, research has consistently underscored higher rates of CVD mortality among Black adults compared to White adults, with many potential reasons for this difference in risk.[79-81] Studies have demonstrated a higher body mass index (BMI), systolic blood pressure (SBP), hemoglobin A_{1c} (HbA1c), and prevalence of obesity, diabetes, and hypertension, as well as a lower socioeconomic status, in Black populations compared to White populations.[80,82,83] Furthermore, an earlier age of onset of CVDs among African Americans may be attributable to the higher prevalence of CVD risk factors, such as hypertension, diabetes, obesity, and ASCVD risk, in these populations.[84] South Asian populations also face increased CVD risk compared to White populations.[78,85,86] South Asians have been shown to have stronger associations of BMI, triglycerides, and HbA1c with CVD compared to White populations.[85]

Genetic factors

Some CVDs may be linked to genetic causes and can therefore be inherited from one generation to the next.[87] For instance, familial hypercholesterolemia is mainly caused by mutations within the LDL receptor, apoB, and proprotein convertase subtilisin/kexin type 9 (PCSK9) genes.[88] LDL receptor mutations are the most frequent, and there are about 1,000 LDL receptor mutations that lead to familial hypercholesterolemia.[88] These mutations in the LDL receptor gene cause a significant increase of LDL-C levels in the plasma, which greatly increases ASCVD risk.[89] Thus, genetics can influence the varying levels of lipoproteins in the blood, which can, in turn, lead to increased CVD risk.[90] Another example of this involves apolipoprotein E (apoE), which is found in many lipoproteins and binds tightly to the LDL receptor.[91] The *APOE* gene is polymorphic (i.e., its DNA sequence can vary across individuals),

generating three alleles that encode three corresponding isoforms of the final protein.[91,92] The E2 isoform is linked to lower LDL-C levels than the E3 isoform, whereas the E4 isoform is linked to higher LDL-C levels.[91] Consequently, those with an E4 isoform have been shown to have a higher risk of CHD compared to individuals who are homozygous for E3.[91]

Smoking

Tobacco use was the cause of over 3 million cardiovascular deaths in 2021 and remains one of the leading causes of cardiovascular death today.[93] Smoking is linked to early onset atherosclerosis, which underlies the development of most CVDs.[56,94] Experimental studies have associated hydrophilic cigarette smoke fractions (e.g., nicotine, metals, and various oxidants and free radicals) with the oxidation of endothelial cell structures (e.g., microtubules, cytoskeleton, intermediate filaments), LDL oxidation, and endothelial cell apoptosis, necrosis, contraction, and leakiness.[95-99] Though there may be various mechanisms by which the different components of cigarette smoke promote CVD risk, the endothelium is a clear target of smoking.[94] The onset of atherosclerosis begins with vascular endothelial dysfunction.[94,100]

Physical inactivity

Physical activity is known to have a positive influence on cardiovascular health.[101] Conversely, physical inactivity, has been associated with increased CVD risk.[101-104] Approximately 7% of CVD deaths and 6% of CHD prevalence worldwide can be attributed to physical inactivity.[102,105] The World Health Organization also identifies physical inactivity as the cause for about 30% of ischemic heart disease burden.[106] Physical inactivity may cause CVD burden via different mechanisms, such as reducing popliteal artery flow-mediated dilation and

increasing endothelial cell apoptosis.[107] Furthermore, physical inactivity is strongly linked to metabolic disorders, like impaired glucose metabolism, which significantly elevates CVD risk.[101]

Hypertension

High SBP accounted for almost 11 million cardiovascular deaths in 2021.[93] High blood pressure, coined hypertension, and defined as an SBP of 130 mmHg or higher and/or a diastolic blood pressure of 80 mmHg or higher, is a modifiable CVD risk factor with the most compelling evidence linking it to causation.[108-110] Meta-analyses and cohort studies reveal a log-linear relationship between blood pressure and CVD, increasing significantly with age.[111-113] The harmful actions of hypertension on CVD risk may be associated to damaged endothelial function, likely related to oxidative stress.[114] This endothelial dysfunction plays a significant role in the development of atherosclerosis, leading to CVD.[94,100,114] Decreasing blood pressure is one of the best approaches to lowering CVD burden.[115] Research has demonstrated that a 10 mmHg decrease in SBP can decrease the risk of major CVD events by 20%, CHD by 17%, and stroke by 27%.[112] Randomized controlled trials have verified the effectiveness of antihypertensive drugs like thiazides, beta blockers, calcium channel blockers, angiotensin receptor blockers, and angiotensin-converting-enzyme inhibitors in decreasing CVD risk.[116]

Diet

In the United States, it is estimated that poor diet is linked to over half of the deaths due to CHD and stroke.[117] Diet has a significant impact on many CVD components, such as diabetes, obesity, and hypertension, ultimately increasing CVD risk.[118] There are many foods that have been clearly linked with negative impacts on CVD components and should be avoided. Foods with a high-glycemic index, like ultra-processed foods, are associated with a higher risk of Type

2 Diabetes.[117-119] Red meat and refined grains are associated with greater inflammation.[120,121] Excessive sodium consumption (e.g., through commercially processed foods) is linked to a higher risk of hypertension, through mechanisms such as perturbing renal sodium homeostasis or direct effects on the vascular wall.[117] Conversely, many foods have been linked to having beneficial effects on CVD risk. Fruits, vegetables, whole grains, nuts, seeds, fiber, olive oil, and legumes are associated with lower inflammation.[117,120] Nuts have also been linked to weight loss and decreased LDL-C, hypertension risk, and Type 2 Diabetes risk.[119,120] Magnesium, which may be found in whole grains, nuts, and vegetables, has also been shown to lower the risk of Type 2 Diabetes.[119] Higher potassium intake is associated with a lower risk of CVD mortality.[117] Moreover, specific diets such as the Mediterranean diet, involving fish, olive oil, fruits, vegetables, whole grains, legumes, nuts, and moderate alcohol consumption, have been linked to lower inflammation and protection against risk factors like waist circumference, lipids, glucose, and blood pressure.[120,122] However, the optimal diet to enhance cardiovascular health is still under investigation.

Alcohol

Alcohol consumption presents a complex relationship with CVD risk. Excessive alcohol intake greater than 60 g/day in men and 40 g/day in women is significantly associated with CVD burden and death.[123] Excessive alcohol consumption is associated with other risk factors of CVD as well, including hypertension and diabetes.[124-126] However, alcohol consumption appears to form a J-shaped relationship with CVD risk, as low to moderate alcohol consumption leads to lower CVD risk compared to no alcohol intake or excessive alcohol intake.[123] Alcohol is thought by some researchers to initially confer a positive effect on the endothelial-nitric oxide-generating system, then a negative effect.[127] Notably, endogenous nitric oxide synthase expression and nitric

oxide production, which helps mediate vascular tone and contributes a protective effect to the cardiovascular system, was increased in the aortic vascular wall of rats that consumed low to moderate amounts of ethanol for six weeks.[127,128] In another study, endogenous nitric oxide synthase expression was decreased in rats that consumed high concentrations of ethanol.[129]

Diabetes

Diabetic adults have a cardiovascular risk that is two to four times higher than non-diabetic adults.[130,131] Diabetes is strongly associated with incident CVD in all age groups, and this may be due to a multitude of factors.[62] First, diabetes and CVD share many risk factors, like obesity, insulin resistance, dyslipidemia, and inflammation.[132] Furthermore, the relationship between diabetes and CVD can be explained by pathophysiological mechanisms such as hyperglycemia and hyperinsulinemia.[130] Hyperglycemia, high blood glucose, is required for diabetes diagnosis and has been shown to cause endothelial dysfunction through oxidative stress.[133-135] Hyperglycemia can also increase the concentrations of pro-atherothrombotic biomarkers, including adhesion molecules VCAM-1, E-selectin, and ICAM-1.[135-139] Hyperinsulinemia, high levels of insulin in the blood, is linked to cardiomyocyte hypertrophy via diverse pathways like the PI3Kα/Akt-1 pathway.[140] Cardiomyocyte hypertrophy is the heart's early response to stress and may eventually lead to heart failure via increased left ventricle thickness.[141]

2.2 Atherosclerotic Cardiovascular Disease Overview

Atherosclerosis is an inflammatory condition, characterized by the buildup of plaques in the arteries, and is the primary cause of CVDs.[142,143] ASCVD mainly constitutes ischemic heart

disease and cerebrovascular disease (e.g., stroke), whereas aortic atherosclerosis and PAD are other less prevalent ASCVDs.[142-144] Clinically, plaques can be visualized via non-invasive imaging procedures, such as magnetic resonance tomography, computer tomography, positron emission tomography, and single-photon emission computed tomography.[145] Angiography is also a common method for atherosclerotic lesion imaging; however, it is an invasive procedure used for higher-risk patients.[142] Furthermore, physicians can assess risk scores through a variety of methods. For instance, the 2018 Multi-Society Cholesterol Management Guidelines and the 2019 American College of Cardiology (ACC)/American Heart Association (AHA) Primary Prevention of Cardiovascular Disease Guidelines recommend the Pooled Cohort Risk Estimator Plus (PCE) for ASCVD risk assessment for those without diagnosed CVD or familial hypercholesterolemia.[146] The PCE considers risk factors, such as age, sex, SBP, treatment for hypertension, total cholesterol (TC), diabetes, and smoking, to provide a 10-year risk estimation of future ASCVD, categorized into low (<5%), borderline (5-<7.5%), intermediate (7.5-<20%), and high (≥20%) risk.[146]

Despite the complexity of the pathologic process leading to atherosclerosis, a commonly accepted model involves endothelial dysfunction adding to LDL retention and oxidation by reactive oxygen species in the intima.[5,147] Oxidized LDL particles and other atherogenic factors help activate endothelial cells, resulting in the recruitment of monocytes within the intima.[5] Monocytes, which attach to the vessel wall, transmigrate into the arterial wall and mature into macrophages.[148] Such monocyte differentiation into pro-inflammatory or anti-inflammatory macrophages is influenced by local metabolic signals, such as lactate that can promote an anti-inflammatory environment or HIF-1α that can promote inflammatory macrophages, and the availability of cytokines.[149-151] Pro-inflammatory macrophages release inflammatory cytokines,

mainly IL-1β, IL-6, TNFα, and CCL2, and produce reactive oxygen species, which further promote monocyte recruitment and inflammatory response propagation.[5,149] Additionally, macrophages accumulate lipids via cholesterol uptake by scavenger receptors (e.g., SR-A1 and CD36) on their surface and turn into foam cells, lipid-laden macrophages existing at all stages of disease progression.[149] The accumulation of foam cells, and thus cholesterol, forms an atherosclerotic lesion.[5] Overtime, foam cells die and form a necrotic core in the lesion.[152,153] The necrotic core is covered by a "fibrous cap", which acts as a barrier between the necrotic core and circulating coagulation factors and platelets.[5] The thickness of the fibrous cap is inversely related to the vulnerability of the plaque to rupture.[5] If the fibrous cap weakens, for instance from its degradation by metalloproteases released by foam cell apoptosis, the plaque can rupture, and a thrombus forms, leading to complications such as CAD or ischemic stroke as described in section 2.1.1.[5,152,154]

2.2.1 Biomarkers of Atherosclerosis

Extensive research has identified numerous biomarkers associated with atherosclerosis. In fact, the many components of atherosclerosis development provide several different types of biomarkers. As described in section 2.2, inflammation is an integral part in the development of atherosclerosis.[155] Therefore, many inflammatory markers of atherosclerosis exist, including high sensitivity C-reactive protein, secreted in response to IL-6 by macrophages, and cytokines (e.g., IL-1β, IL-6, TNFα, and CCL2), which are released by macrophages during atherosclerosis development.[155] Oxidative stress constitutes another important process in atherosclerosis, thus oxidized LDL, resulting from the action of reactive oxygen species, is a biomarker for atherosclerosis.[147,155] Matrix metalloproteases are also biomarkers of oxidative stress in

atherosclerosis.[155] Metalloproteases like MMP-2 and MMP-9 degrade extracellular matrix, weakening the fibrous cap of the atherosclerotic plaque, leading to plaque rupture.[155]

The trapping of apoB lipoprotein particles in the arterial wall is the key process that triggers and sustains the entire atherosclerotic process.[156] ApoB lipoproteins include chylomicrons, chylomicron remnants, VLDL, IDL, LDL, and lipoprotein(a) (Lp(a)).[156] Chylomicrons are the largest of the apoB particles.[7] These triglyceride-rich particles are produced in the intestine and contain one apoB-48 molecule each.[7] In peripheral tissues, lipoprotein lipase removes triglycerides from chylomicrons, resulting in chylomicron remnants.[7] Intact chylomicrons are the only apoB particles too large to enter the arterial wall.[9] All the rest can enter the wall and, therefore, all the rest are atherogenic.[9] Similar to chylomicrons, VLDL particles are triglyceride-rich; however, they are made by the liver and each contains one apoB-100 molecule.[7] When triglycerides are removed from VLDL by lipoprotein lipase, IDL particles are formed.[7] Further removal of triglycerides from IDL results in LDL particles, which carry most of the cholesterol in the circulation.[7,157] LDL particles vary in size and density, depending on their lipid content, and small dense LDL particles are more atherogenic than large LDL particles.[7,158] Small dense LDL particles might be more atherogenic because they are more rapidly oxidized, they bind more strongly to proteoglycans in the subendothelial space, they have a lower binding affinity for the LDL receptor, and they have a longer half-life.[7,158-160] Finally, Lp(a) is another atherogenic form of LDL that contains an apolipoprotein(a) molecule connected to its apoB molecule through a disulfide bond.[9] Like hypertension, apoB particles are causal factors for atherosclerosis.[161] Lowering their concentration in plasma is a primary objective of prevention.[162] LDL-C, triglycerides, and non-HDL-C have been the conventional biomarkers used clinically.[9,163] However, insufficient emphasis is placed on apoB, the common denominator

of all apoB particles. The α3 domain of apoB binds to the LDL receptor, initiating the clearance of LDL from the plasma.[162] Thus, loss-of-function mutations in either the LDL receptor or the apoB found on LDL can lead to high LDL-C in the plasma, and thus familial hypercholesterolemia and atherosclerosis.[162] ApoB also possesses binding sites for proteoglycans in the endothelial wall, and this binding is essential for LDL retention in the subendothelium.[162] The evidence is now overwhelming that apoB is a more accurate marker of atherosclerotic risk than LDL-C or non-HDL-C.[14,15,164-171]

HDL-C levels also predict atherosclerotic risk.[172] Specifically, low HDL-C levels are associated with a higher risk of CVD.[172-177] HDL is produced in the liver and intestines and is believed to remove cholesterol from foam cells to counteract the progression of atherosclerosis.[8,11] This reverse cholesterol transport pathway is considered the primary process by which HDL decreases atherosclerotic burden.[178] However, HDL may confer additional cardioprotective effects through its anti-inflammatory activity, its anti-oxidant activity, modulation of coagulation, alteration of platelet function, interaction with the metabolism of triglyceride-rich lipoproteins, and enhancement in endothelial function.[178] Despite substantial research revealing the possible cardioprotective effects of HDL-C, more recent research has demonstrated that very high levels of HDL-C predict increased risk of cardiovascular mortality, creating a "U-shaped" curve.[179-181] The Copenhagen City Heart Study and the Copenhagen General Population Study each examined non-overlapping patient cohorts with low risks for CVD.[182] This combined cohort of 110,000 men and women revealed a significantly increased risk of all-cause mortality at HDL-C levels over 2.51 mmol/L in men and 3.50 mmol/L in women and less than 1.04 mmol/L in both men and women. Cardiovascular death also had a U-shaped association with HDL-C values in both sexes.[182] Evidence remains mixed as other studies have

demonstrated that extremely high levels of HDL-C are not linked to such poor outcomes and Mendelian randomization studies have not confirmed a causal role for HDL as a determinant of cardiovascular risk.[183-185] This paradoxical association between HDL-C and CVD remains unclear, but researchers have suggested several potential explanations.[180] First, the increase in adverse events in relation to raised HDL-C may be associated with other CVD risks factors.[180] This relationship may also be linked to genetic variation in specific genes like CETP, ABCA1, or SR-B1, which are linked to increased HDL-C when mutated.[180,182] Research examining patients with these mutated alleles has not reported a clear increased risk of cardiovascular events.[182] Furthermore, a higher risk of mortality could be related to HDL size and function.[180,186] For instance, Martin et al. found an increase of greater than 50% in mortality and myocardial infarction risk in participants with lower HDL_3-C (i.e., small HDL) levels, while HDL-C and HDL_2-C (i.e., large HDL) levels had no significant relationships.[187] HDL subclasses may also be associated with age and sex, adding another complex layer to the paradox. A study by Freedman et al. describes a steady decrease in HDL_2 particles with age in women, while men show a U-shaped trend.[188] HDL_3 particles appeared to exhibit an inverted U-shaped curve with age in both men and women.[188] Therefore, it may be advantageous to differentiate between HDL subgroups and gain a deeper understanding of their functions in relation to cardiovascular outcomes.[186] Lastly, it is important to keep in mind the multifunctional role of HDL in processes like inflammation, oxidation, thrombosis, and immunity.[180] Like apoB lipoprotein particles, HDL has its own primary apolipoprotein molecule, apoA-I.[189] ApoA-I is an important mediator of reverse cholesterol transport as it interacts with ABCA1 to transfer free cholesterol from macrophages to HDL.[6,189] ApoA-I is also a cofactor for LCAT, enhancing its activity and preventing the backwards transfer of free cholesterol to macrophages by converting free cholesterol to

cholesteryl esters.[6,178,189] Furthermore, apoA-I can inhibit neutrophil and endothelial cell activation, an important step in the development of atherosclerosis.[147,190] However, like HDL-C, Faaborg-Andersen et al. have observed a U-shaped association between apoA-I and CVD, whereby very low and very high apoA-I levels are related to higher cardiovascular mortality.[191] This may be partly due to participants with very high apoA-I levels being older, having a higher prevalence of smoking and alcohol use, and having high TC levels.[191] Another proposed mechanism involves molecular modifications to apoA-I that modify its function in response to environmental stressors, like oxidative stress and inflammation, at high concentrations.[191]

Although specific lipid levels are key for the prediction of ASCVD risk, lipid ratios have also been used in risk prediction. The LDL-C:apoB ratio can be helpful in distinguishing between large buoyant LDL from small dense LDL, which are more atherogenic.[160] Specifically, an LDL-C:apoB ratio below 1.2 was found to be the cutoff between large buoyant LDL and small dense LDL particles.[160] Furthermore, a greater HDL-C:apoA-I ratio may reflect the hindered capacity of dysfunctional HDL to uptake excess cholesterol from tissues and atherosclerotic plaques.[192] Researchers have shown the positive association between increasing HDL-C:apoA-I ratio and CVD mortality.[192] In general, the associations between lipid ratios such as the TC:HDL-C ratio and the apoB:apoA-1 ratio with cardiovascular risk are stronger than between single lipid measurements and risk. Kastelein et al. suggest that ratios of proatherogenic to antiatherogenic lipid measurements are better associated with cardiovascular risk than single proatherogenic lipid measurements.[193] In some, but not all studies, the apoB:apoA-I ratio is more strongly associated with cardiovascular risk than the TC:HDL-C ratio.[193] Indeed, across all nationalities, the INTERHEART study found the apoB:apoA-I ratio to be the most important risk

factor for myocardial infarction.[194] By contrast, the Framingham Heart study found the TC:HDL-C ratio to be superior to the apoB:apoA-1 ratio.[19]

2.3 High-Density Lipoprotein

2.3.1 Structure of High-Density Lipoprotein and Size Variation

HDL particles consist of a lipid core, which includes cholesterol and triglycerides surrounded by a monolayer of phospholipids. Within this monolayer are multiple apolipoproteins, such as apoA-I and apolipoprotein A-II (apoA-II) as well as enzymes like LCAT.[195] HDL is highly heterogeneous and can be classified into different subclasses based on density, size, shape, and lipid and protein composition.[196] Generally, HDL is separated by ultracentrifugation based on density and classified as either HDL_2, the less dense (1.063-1.125 g/mL) and more lipid-rich form of HDL, or HDL_3, the more dense (1.125-1.210 g/mL) and protein-rich form.[197] Non-denaturing polyacrylamide gradient gel electrophoresis can be used to separate HDL_2 and HDL_3 into further subclasses based on size: HDL_{3c} (7.2-7.8 nm in diameter), HDL_{3b} (7.8-8.2 nm), HDL_{3a} (8.2-8.8 nm), HDL_{2a} (8.8-9.7 nm), and HDL_{2b} (9.7-12.0 nm).[197] Furthermore, agarose gel electrophoresis can separate HDL into α-migrating particles and pre-β-migrating particles based on surface charge and shape.[197] Pre-β-migrating HDL particles represent nascent HDL particles, which are less abundant in the plasma and can be found in a discoidal shape. A-migrating HDL particles are the predominant form found in plasma and are present in spherical form.[196,198]

HDL is formed when apoA-I is secreted by hepatocytes or intestinal mucosa and interacts with the 220-240 kDa transporter ABCA1 in peripheral cells to gain free cholesterol and

phospholipids, creating the small discoidal particle referred to as pre-β-HDL or nascent HDL.[196,199,200] Apart from de novo synthesis and secretion, apoA-I can be found in circulation from mature HDL catabolism or apoB lipoprotein lipolysis.[196] The free cholesterol on the maturing HDL's surface acts as a substrate for LCAT, whereas the apoA-I activates the enzyme, converting free cholesterol into cholesteryl esters, which penetrate the phospholipid monolayer of discoidal HDL to accumulate in its hydrophobic core.[196] This prevents the backwards transfer of free cholesterol to the tissues and transforms the discoidal HDL into spherical HDL.[196] While discoidal HDL is small, lipid-poor, and formed from apolipoprotein in a monolayer of phospholipid and cholesterol, spherical HDL is larger, formed from discoidal HDL, and has a hydrophobic core of cholesteryl esters.[201] Mature HDL can further facilitate cholesterol efflux via ABCG1 and ABCG4.[201] Research has found that smaller HDL_3 particles perform more efficient cholesterol efflux via ABCA1 compared to larger particles; however, these smaller and larger particles are equally as effective via ABCG1.[201] Small, dense HDL_3 has also been shown to be more effective at protecting against LDL oxidation than HDL_2 and appear to have a stronger anti-inflammatory effect.[202-204]

While apoA-I is the predominant apolipoprotein found on HDL, making up for about 70% of total HDL protein, apoA-II can also be found on HDL, making up for about 20% of HDL protein.[195] Briefly, apoA-I has important functions in reverse cholesterol transport, the assembly of HDL, inflammation, and infection, whereas apoA-II plays important roles in cholesterol efflux, HDL remodelling, and stabilizing HDL structure.[205] HDL particles can contain only apoA-I (referred to as LpA-I) or both apoA-I and apoA-II (referred to as LpA-I:A-II).[205] Most proteins associated with LpA-I have roles in hemostasis, metal ion binding, protease inhibition, inflammatory responses, and immune responses, whereas most proteins associated with LpA-

I:A-II are important for lipid transport.[205] Current evidence predominantly agrees that LpA-I has superior anti-atherogenic effects compared to LpA-I:A-II. Research has shown that LpA-I facilitates cholesterol efflux through both slow, nonspecific and fast, specific, energy-dependent mechanisms, thus being more active in the reverse cholesterol transport chain.[206] Furthermore, expression of human apoA-I in transgenic mice has been shown to increase cholesterol efflux from macrophages, whereas murine apoA-II in transgenic mice did not.[207] A similar study by Chiesa et al. demonstrated that the expression of human apoA-I, but not apoA-II, improved cholesterol efflux in apoA-I-deficient mice.[208] Some researchers have also shown that transgenic mice overexpressing apoA-I and apoA-II had an increased susceptibility for developing atherosclerosis compared to mice that overexpressed only human apoA-I.[209] Additionally, apoA-II is able to displace apoA-I from HDL since apoA-II possesses a higher affinity for lipids.[210,211] Ultimately, it is important to keep in mind this complexity of HDL structure, resulting in different cardioprotective activities.[212]

2.3.2 Proteome of High-Density Lipoprotein

The HDL proteome refers to the complete set of proteins found in HDL.[196] There have been hundreds of proteins identified to-date, including various types of apolipoproteins, enzymes, lipid transfer proteins, complement system components, and protease inhibitors.[196] The protein:lipid ratio in HDL can range from 10:1 in pre-β-HDL to 1:2 in large HDL_2.[196] Importantly, changes in HDL protein content can act as a marker, or even mediator, of ASCVD.[213] Compared to the LDL proteome, HDL is more complex in its protein composition. Most sources describe the LDL proteome to consist of less than 100 proteins.[214] Given the complexities of the composition of HDL particles from the large number of differing

constituents, and therefore differing properties of HDL particles, there is an almost infinite number of ways discordance could be described and tested. As the primary protein of HDL, accounting for 70% of the proteome, apoA-I is a clear first choice for a discordance analysis with HDL.

Apolipoproteins

Apolipoproteins are serum proteins with four general functions.[7] They can help maintain particle structure, serve as ligands for lipoprotein receptors, guide lipoprotein formation, and act as activators or inhibitors of enzymes that serve roles in lipoprotein metabolism.[7] HDL contains several types of apolipoproteins in its proteome, including apoA-I, apoA-II, apolipoprotein A-IV (apoA-IV), apolipoprotein C (apoC), apolipoprotein D (apoD), apoE, apolipoprotein F (apoF), apolipoprotein H (apoH), apolipoprotein J (apoJ), apolipoprotein L-I (apoL-I), apolipoprotein M (apoM), and apolipoprotein O (apoO).[197]

Accounting for approximately 70% of total HDL protein, apoA-I is the primary structural and functional protein of HDL.[197] ApoA-I is approximately 28 kDa, composed of 243 amino acids, glycosylated, and can be found as an antiparallel dimer.[215-218] Its secondary structure consists of repeating amphipathic α-helical domains, which help the apolipoprotein associate with lipids in aqueous blood.[217] These amphipathic helices in addition to non-helical residues provide apoA-I with flexibility and plasticity to self-associate with other elements of HDL, conferring its receptor binding ability and catalytic activity.[189] ApoA-I plays a multifunctional role in reverse cholesterol transport, cellular cholesterol homeostasis, inflammation, apoptosis, and viral and bacterial infection.[189] During reverse cholesterol transport, apoA-I interacts with various molecules like ABCA1, LCAT, and SR-B1 to initiate and progress the pathway.[219] As the first step of the pathway, free apoA-I binds directly to the extracellular domain of the ABCA1

dimer to accept lipids and form nascent pre-β-HDL.[219] ApoA-I is also essential for the maturation of nascent HDL through its activation of LCAT, which transfers the sn-2 acyl of phosphatidylcholine to cholesterol to form a cholesteryl ester.[219] When these cholesteryl esters move to the hydrophobic core of nascent HDL, they cause the nascent discoidal HDL to transform into spherical α-HDL.[219] As the last step of the reverse cholesterol transport pathway, apoA-I binds to SR-B1, the primary receptor for HDL, and acts as a bridge that connects SR-B1 and HDL, mediating the uptake of HDL-cholesteryl ester and regulating HDL-C plasma levels.[219] Aside from these reverse cholesterol transport roles of apoA-I, apoA-I can also exhibit anti-inflammatory activities, such as interfering with the T-cell signalling of monocytes, therefore inhibiting the production of the pro-inflammatory cytokines TNF-α and IL-1β, and transferring lipopolysaccharide (LPS) from LPS-binding protein to HDL instead of CD14 on the surface of monocytes, thus inhibiting the activation of monocytes by LPS.[189] Conversely, oxidized and dysfunctional apoA-I has been shown to lose its ability to accept cholesterol and behaves as pro-inflammatory, initiating atherogenesis.[189,213] ApoA-I also plays a role in apoptosis by decreasing survivin, which is an inhibitor of apoptosis and a promoter of the cell cycle that typically plays a role in the poor prognosis of melanoma.[189] Furthermore, apoA-I can serve anti-bacterial functions by conjugating with and neutralizing bacterial endotoxins, which are LPS residing in the outer membrane of gram-negative bacteria that can cause septic reactions, and lipoteichoic acid, which is a polymer found in the cell wall of gram-positive bacteria.[189,220,221] Mice deficient in apoA-I can develop increased atherosclerosis, even in well maintained HDL-C levels.[222] This finding strengthens the importance of apoA-I as an anti-atherogenic particle that helps HDL-C against ASCVD.[222] Moore et al. further demonstrated that the absence of apoA-I impairs macrophage reverse cholesterol transport and HDL anti-inflammatory and anti-oxidant

functions, despite preserved levels of HDL-C.[223] In humans, apoA-I deficiency, also known as familial hypoalphalipoproteinemia, is a rare lipoprotein metabolism disorder that has only been described in about 30 families.[224] Biochemically, this condition is defined by the absence of apoA-I and very low HDL-C in the plasma, due to mutations in the *APOA1* gene.[224] In this case, apoA-I and HDL-C, given that HDL-C is also almost completely absent in apoA-I deficiency, significant discordance is not possible. Clinically, the condition can manifest as corneal opacities which can cause vision loss, xanthomas which are localized accumulations of cholesterol-laden histiocytes, and premature CHD.[224-227] Not only is apoA-I vital for HDL functioning and protection from atherosclerosis, but it continues to be investigated for its diverse functions that may play a role in cancer, infectious diseases, neurological diseases like Alzheimer's disease, and diabetes.[189,228]

ApoA-II is the second most abundant HDL protein.[217] It is composed of 77 amino acids as a homodimer and has amphipathic α-helix domains like apoA-I.[217] ApoA-II is synthesized in the liver and the intestines, similar to apoA-I, and may be a monomer or homo/heterodimer through disulfide bonds with itself, apoD, or apoE.[217] Mainly, apoA-II serves a role in HDL remodelling, HDL structure stabilization, and cholesterol efflux.[205] However, apoA-II is more poorly understood than apoA-I and findings are mixed regarding its cardioprotective functions.[205] For instance, one study examining apoA-II knock-in rabbits produced positive results.[229] Knock-in rabbits on a standard diet had low plasma triglycerides resulting from increased clearance of triglyceride-rich particles and lipoprotein lipase activity compared to control rabbits.[229] Furthermore, knock-in rabbits on a cholesterol-rich diet were resistant to diet-induced hypertriglyceridemia, leading to fewer atherosclerotic lesions.[229] Conversely, Schultz et

al. used human apoA-II transgenic mice and discovered an increased susceptibility of atherosclerosis.[209]

ApoA-IV is a 46 kDa apolipoprotein secreted by enterocytes of the small intestine, and may be found on chylomicrons, HDL, or in free form in the plasma.[230-232] Though human studies on apoA-IV are limited, research from animal and in vitro studies suggest that apoA-IV activates chylomicron lipolysis, enhances reverse cholesterol transport, protects from LDL oxidation, helps glucose homeostasis, reduces inflammation, and inhibits platelet aggregation.[230]

ApoCs compose a family of apolipoproteins synthesized in the liver that range in size from 6-9 kDa.[233,234] The smallest apoC, apoC-I, associates with HDL and VLDL and activates LCAT.[197,233] ApoC-II is associated with HDL and VLDL and acts as a cofactor for lipoprotein lipase.[197] ApoC-III is the most abundant apoC, predominantly found in VLDL but also in some HDL, and inhibits lipoprotein lipase and hepatic lipase.[197,233] HDL with apoC-III is recognized as dysfunctional and is associated with a higher risk of CHD.[235]

ApoD is a 20 kDa glycoprotein primarily associated with HDL_3.[197,236] ApoD is part of the lipocalin family of small lipid transfer proteins.[197] This apolipoprotein is involved in LCAT regulation and functions against oxidative stress and inflammation.[236]

ApoE is a 34 kDa glycoprotein that is mainly synthesized in the liver and intestine.[233,237] ApoE plays important roles in the regulation of cholesterol efflux and metabolism of apoB lipoproteins.[238] Mainly, apoE appears to interact with LDL and VLDL receptors to promote lipoprotein clearance.[238] ApoE and apoC-III have an antagonistic relationship whereby apoC-III hinders VLDL binding to its receptors, which shifts VLDL metabolism away from clearance and

toward its conversion to LDL.[239] The cardioprotective effects of apoE are diminished by the copresence of apoC-III on HDL.[235]

ApoF is a 29 kDa sialoglycoprotein mostly found in large HDL particles, but it also binds to LDL.[240,241] ApoF overexpression has been shown to increase the rate of HDL-C clearance from the circulation.[241]

ApoH is a 43-50 kDa glycoprotein expressed by the liver that may play a role in inhibiting blood coagulation by binding to phospholipids on damaged cells.[197,242] ApoH can be found on HDL, chylomicrons, VLDL, and LDL.[243]

ApoJ is a 70 kDa glycoprotein that may be found unbound or on HDL.[244,245] Although apoJ is not well understood, decreased levels of apoJ are found in CHD and the protein may promote cholesterol efflux from foam cells.[244]

ApoL-I is a 39-42 kDa protein found in HDL_3 with paraoxonase-1 (PON-1) and apoF, and is only present in humans and a few primate species.[246,247] Its specific protein domains suggest potential roles in ion transport and apoptosis.[247] However, apoL-I on HDL is most notably responsible for inhibiting infections by parasites like tryposomes and Leishmania.[248,249]

ApoM is a 26 kDa lipocalin, like apoD, that is only found in about 5% of HDL particles.[250,251] HDL that contains apoM enhances cholesterol efflux, protects endothelial cells from apoptosis, and decreases TNF-α release from macrophages by neutralizing LPS.[250,252]

ApoO associated with HDL is a 55 kDa glycoprotein.[253] The physiological function of apoO is not well understood as it serves as a cholesterol acceptor in vitro, like apoA-I, but its overexpression in transgenic mice does not affect cholesterol transport.[253]

Enzymes

HDL enzymes play important roles for many of HDL's activities, including protection against LDL oxidation and facilitating reverse cholesterol transport. PON-1, LCAT, and platelet-activating factor acetylhydrolase (PAFAH) are key examples of HDL enzymes with anti-oxidant properties.[213] PON-1 is a 43 kDa calcium-dependent glycoprotein that hydrolyzes biologically active lipid peroxides to prevent LDL oxidation, counteracting the development of atherosclerosis.[254,255] Most of this important PON-1 activity can be found in small HDL_3.[255] Similarly, PAFAH, a 45 kDa hydrophobic serine lipase, also protects LDL from oxidation.[256,257] PAFAH degrades platelet-activating factor (PAF), which is normally produced during LDL oxidation, leading to endothelial dysfunction, and promotes the release of reactive oxygen species, leading to additional LDL oxidation.[258,259] By hydrolyzing the sn-2 ester bond of PAF, PAFAH degrades PAF into its inactive form, lyso-PAF.[258,259] As introduced in section 2.3.1, LCAT converts free cholesterol to cholesteryl esters to prevent the backwards transfer of free cholesterol on HDL to macrophages during reverse cholesterol transport.[6,256,258] LCAT is a 67 kDa secretory protein with two catalytic activities to esterify cholesterol: (1) its phospholipase A2 activity cleaves fatty acids from the sn-2 position of phosphatidylcholine, among other phospholipids, and (2) its transesterification activity moves the newly cleaved fatty acid to the hydroxyl group present on the A-ring of cholesterol.[260] These activities require apoA-I as a co-factor.[260] Not only does LCAT prevent the backwards exchange of cholesterol from HDL to macrophages, but its formation of cholesteryl esters transforms small, discoidal pre-β-HDL into larger, spherical α-HDL.[260] HDL's increase in size stabilizes the lipoprotein, preventing its removal via renal clearance.[260] Modifications to HDL enzymes can impair the cardioprotective functions of HDL.[213] In fact, HDL dysfunctionality, in which HDL can no longer prevent

atherosclerosis, is commonly measured by the loss of anti-inflammatory and anti-oxidative function.[255]

Lipid Transfer Proteins

HDL is primarily associated with two types of lipid transfer proteins, CETP and phospholipid transfer protein (PLTP). CETP is a 74 kDa glycoprotein that mediates the transfer of cholesteryl esters from HDL to LDL and triglyceride-rich lipoproteins like VLDL, in addition to the transfer of triglycerides from triglyceride-rich lipoproteins to HDL and LDL.[6,261,262] The tunnel hypothesis describes a ternary complex with a tunnel for transfer whereby the N-terminal domain of CETP penetrates the core of HDL and the C-terminal domain binds to LDL or VLDL.[198] Alternatively, the shuttle hypothesis describes the potential for HDL to act as a shuttle that transfers triglycerides between LDLs and VLDLs.[198] Overall, CETP activity results in a decrease in HDL-C, which may increase CVD risk.[261] Similar to CETP, PLTP activity is a CVD risk factor, and it is highly expressed in atherosclerotic lesions.[263] PLTP is an 80 kDa glycoprotein with hydrophobic pockets that bind acyl chains of phospholipids.[198,264] PLTP has a high affinity for binding triglyceride-rich HDLs, transferring phospholipids between HDL particles and promoting their fusion to create larger HDLs during HDL remodelling.[198] PLTP also transfers excess phospholipids from VLDL to HDL.[198] Ultimately, PLTP plays a role in reverse cholesterol transport by helping to generate and mature HDL particles, and may potentially promote ABCA1-mediated cholesterol efflux.[198]

Complement System Proteins

The complement system consists of about 40 serum proteins, with enzymatic, receptor, or regulatory functions, that work with the innate and adaptive immune systems to eliminate

pathogens and protect from infection.[265] The complement system even helps mediate tissue damage from acute myocardial infarction.[213] Many important complement factors associate with HDL, such as complement component 3 (C3), complement component 4 (C4), complement component 9 (C9), and vitronectin.[213] C3, a 190 kDa glycoprotein, is essential for the activation of the complement system.[197,266] C3 has an inverse relationship with large HDL particles and a positive relationship with small HDL particles.[267] Interestingly, HDL from CAD patients appear to be enriched in C3 and C4, a 200 kDa glycoprotein that is important for the activation of the classical pathway of the complement system.[197,268,269] Furthermore, C9 is a 70 kDa glycoprotein associated with apoA-I and apoA-II.[270,271] The increased binding affinity of apoA-I and apoA-II to endothelial cells treated with the complex C5b-9 depends on the presence of polymeric C9.[270,271] In addition to complement components, vitronectin is a 65-75 kDa polypeptide that can also associate with HDL and serves a role in complement system regulation.[197,272] Vitronectin is an extracellular matrix protein, thus some HDL components may be derived from noncellular sources or cells other than those in the liver and intestine that form apoA-I.[213]

Protease Inhibitors

Plaque rupture may be influenced by the proteolysis of structural proteins in atherosclerotic lesions.[213] Therefore, protease inhibitors in HDL may play a key role in protecting against CAD.[213] HDL is enriched with protease inhibitors, mainly serine protease inhibitors, which inhibit proteases with diverse roles in inflammation, hemostasis, and the complement system.[270,273] HDL may potentially serve as a shuttle to transport serine protease inhibitors into parts of the vasculature that they are unable to reach.[274] For instance, HDL may be able to transport alpha-1-antitrypsin, a serine protease inhibitor, to areas of inflammation across the endothelium so that it can prevent damage of extracellular matrix by proteases such as

elastase.[274] Normally, this destruction of extracellular matrix can lead to atherosclerotic plaque rupture.[274]

2.3.3 Mechanism of Reverse Cholesterol Transport

The accumulation of cholesterol in peripheral cells via the uptake of apoB particles and de novo cholesterol synthesis is an important step in atherosclerosis development.[2,3,7] However, most cells, other than those capable of synthesizing steroid hormones from cholesterol, do not have processes for catabolizing cholesterol.[7] Therefore, reverse cholesterol transport plays a key role in combatting atherosclerosis by reducing the cholesterol content within cells.[7]

The reverse cholesterol transport pathway begins with the formation of HDL, the main lipoprotein involved (Figure 1).[200] ApoA-I secreted into the bloodstream by the liver or intestine interacts with ABCA1 in various cell types, like hepatocytes and macrophages, which facilitates the movement of free cholesterol and phospholipids from peripheral tissues to apoA-I and forms nascent (or pre-β) HDL that continues to accumulate cholesterol and phospholipids.[196,200] LCAT ensures that there is no backwards transfer of cholesterol by turning free cholesterol into cholesteryl ester.[196] Cholesteryl esters penetrate the phospholipid monolayer of HDL to accumulate in the hydrophobic core, which transforms the discoidal-shaped HDL into spherical HDL, the main form of HDL in the plasma that is ready for transport.[196,275]

Cholesterol efflux from foam cells in atherosclerotic plaques to HDL can occur through three different mechanisms.[275,276] First, ABCA1 integrated in the membrane can mediate the ATP-dependent unidirectional transfer of cholesterol from foam cells to apoA-I found on HDL.[275,276] As described above, this mechanism is essential for the formation of pre-β-HDL.

LCAT can then catalyze the transformation of cholesterol to cholesteryl ester.[275,276] The specific method of interaction between ABCA1 and apoA-I is still to be determined.[276] However, several methods have been proposed, such as direct apoA-I binding to extracellular ABCA1 loop domains, apoA-I binding to protrusions caused by ABCA1 floppase activity, and apoA-I binding to a phosphatidylserine that was translocated outward by ABCA1 floppase activity.[276] The second mechanism of cholesterol efflux involves the ATP-dependent unidirectional transfer of cholesterol from foam cells to HDL via ABCG1, a 644-785 amino acid transporter that requires homo/heterodimerization to function.[276] Following this cholesterol transfer, LCAT can transform cholesterol into cholesteryl ester.[276] The exact transport mechanism of ABCG1 in transferring cholesterol to HDL is still under investigation.[277] However, a recent study by Xu et al. used cryo-electron microscopy to observe human ABCG1 in complex with cholesterol and sphingomyelin, suggesting that sphingomyelin may be important for ABCG1-mediated cholesterol efflux.[277] Specifically, they found a cholesterol-binding cavity formed by a cluster of conserved hydrophobic residues and two sphingomyelins, which was closed by three pairs of conserved phenylalanine residues.[277] This structure creates a hydrophobic path for cholesterol release.[277] A third mechanism of cholesterol efflux is the bidirectional passive diffusion of cholesterol via SR-B1 and HDL interaction.[275] When HDL binds to SR-B1 found on the cell surface, free cholesterol can be transferred from cells to HDL, or cholesteryl esters from HDL can be selectively taken up by the cell.[278] This process does not internalize the HDL particle.[278,279]

The efficiency of cholesterol efflux can be mediated by various mechanisms, such as increased apoA-I levels and increased ABCA1 and ABCG1 expression at the plasma membrane.[276] The production and release of apoA-I by the liver and intestine are transcriptionally and post-transcriptionally regulated by various hormones and second

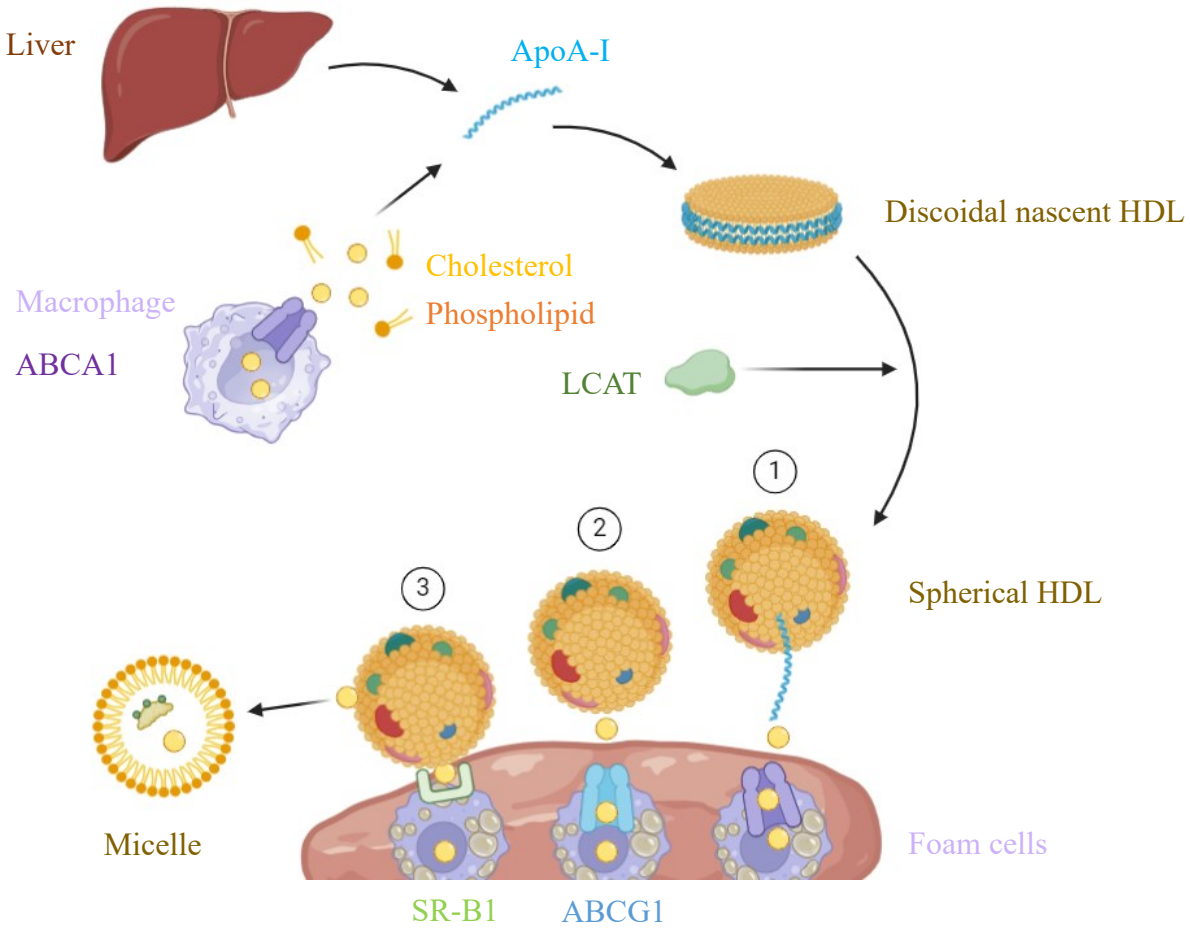
messengers like retinoids, insulin, thyroid hormone, and sex hormones.[280] For example, insulin has been shown to increase apoA-I gene expression, whereas glucose inhibits expression.[281,282] ABCA1 and ABCG1 transcription can be regulated by liver X receptor (LXR), retinoid X receptor (RXR), and peroxisome proliferator-activated receptors (PPARs).[276] LXRs are cellular cholesterol sensors that are activated by the accumulation of cholesterol's oxidized derivatives oxysterols.[276] Once activated, LXR forms a heterodimer with RXR. This LXR/RXR complex binds to the LXR response element and initiates transcription of various target genes, including ABCA1 and ABCG1.[276] Similarly, PPARs form heterodimers with RXR once they become activated by fatty acid metabolites.[276] This PPAR/RXR complex binds to isotope-specific peroxisome proliferator response elements in target genes, such as apoA-1 and LXR.[276] Therefore, PPARs can increase apoA-I transcription as well as ABCA1 and ABCG1 expression via increased LXR transcription.[276]

Once cholesterol is loaded onto HDL through cholesterol efflux, HDL travels through the bloodstream to the liver.[275] SR-B1 on the cell surface of hepatocytes selectively takes up cholesteryl ester from HDL, as described earlier.[275] Cholesteryl esters can also be transferred from HDL to apoB lipoprotein particles by CETP.[275] The LDL receptor can then take up these transferred cholesteryl esters.[275] HDL containing apoE may also be recognized by the LDL receptor for cholesteryl ester uptake.[275]

Finally, cholesterol in the hepatocytes will either be transferred to bile for intestinal excretion or used to maintain the lipid membrane.[6,275] ABCB11, an ATP bile salt export pump, pumps bile salts out of the cell where they may interact with the extracellular canalicular membrane, which contains ABCB4 and heterodimers of ABCG5 and ABCG8.[275] These ATP-dependent canalicular membrane transporters facilitate biliary excretion of cholesterol and

phospholipids.[275] A micelle is then formed from the interaction of bile salts, cholesterol, and phospholipids.[275] This micelle has a hydrophobic core covered by hydrophilic head groups to facilitate its transport through the digestive system.[275]

Figure 1. Visual Depiction of the Reverse Cholesterol Transport Pathway. Reverse cholesterol transport begins with the formation of discoidal HDL via the association of apoA-I from the liver with cholesterol and phospholipids from peripheral cells, with the help of ABCA1. LCAT converts free cholesterol on discoidal HDL to cholesteryl esters, which penetrate into the core of the HDL particle, forming a spherically shaped HDL. HDL can facilitate cholesterol efflux via three different mechanisms: 1) unidirectional transfer of cholesterol from foam cells to apoA-I on HDL via ABCA1; 2) unidirectional transfer of cholesterol from foam cells to HDL via ABCG1; 3) bidirectional passive diffusion of cholesterol via SR-B1 and HDL interaction. Eventually, the cholesterol loaded onto HDL is transported to hepatocytes where it can interact with bile salts and phospholipids to form a micelle that travels through the digestive system to be excreted.



2.3.4 Other roles of High-Density Lipoprotein

Although HDL is primarily known for its vital role in reverse cholesterol transport, it can also play a pivotal role in inflammation, oxidation, thrombosis, reproduction, and bacterial, viral, and parasitic infections.[178]

Inflammation

HDL plays several roles in inflammation, which are mainly anti-inflammatory.[283] HDL is capable of promoting the expression and activation of activating transcription factor 3 (ATF3).[283]

ATF3 is a transcriptional modulator that negatively feeds back onto Toll-like receptor signalling, which normally promotes the expression of pro-inflammatory cytokines from macrophages.[283] Furthermore, HDL inhibits cytokine-induced expression of the endothelial cell adhesion molecules E-selectin, ICAM-1, and VCAM-1.[190,252,284] HDL can also reduce CD11b expression, a monocyte-adhesion molecule, and MCP-1, a chemokine that recruits monocytes into the subendothelial space.[190,252,285,286] These anti-inflammatory roles of HDL can be cardioprotective as monocyte attachment to the vessel wall and transmigration into the subendothelial space are important steps in foam cell formation and atherosclerosis development.[148] Furthermore, HDL inhibits the release of pro-inflammatory cytokines TNF-α and IL-6.[252] Specifically, apoM in the HDL proteome can bind and neutralize LPS, which decreases TNF-α release from macrophages.[252] Smaller HDLs have a higher apoM content, which may influence their greater potential to inhibit adhesion molecule expression.[252] ApoE may also confer anti-inflammatory activity to HDL as HDLs containing apoE have been shown to promote the survival of anti-inflammatory regulatory T lymphocytes.[252]

However, HDL may switch from anti-inflammatory to pro-inflammatory in patients with autoimmune diseases.[283] Primarily, apoA-I in HDL is displaced by serum amyloid A (SAA), and PON-1 activity is reduced.[283] Oxidation of HDL can also cause HDL to exhibit pro-inflammatory activity.[283,287] In this case, oxidized HDL can induce the upregulation of platelet-derived growth factor receptor β, which increases monocyte chemotaxis and TNF-α release.[283]

Oxidation

Oxidative stress plays an important role in the pathophysiology of several diseases, including atherosclerosis, diabetes, and Alzheimer's disease.[252,288] This stress can induce endothelial dysfunction, pro-inflammatory pathways in the vascular wall, and lipoprotein

modifications.[252] HDL possesses many anti-oxidant functions to counteract the development of disease.[252] Mainly, HDL can protect LDL from oxidation by free radicals and copper ions.[252] Normally, oxidized LDLs promote inflammation and oxidative stress in blood vessels by promoting monocyte recruitment, pro-inflammatory cytokine release, reactive oxygen species formation, and the disruption of endothelial cell homeostasis and nitric oxide production.[252] The anti-oxidant role of HDL may involve removing or neutralizing lipid hydroperoxides that oxidize LDL through the specific action of apoA-I.[252,286] The methionine residues at positions 112 and 148 of apoA-I can convert lipid hydroperoxides into redox-inactive lipid hydroxides. HDL also carries enzymes, such as PON-1 and paraoxonase-3, that destroy lipid hydroperoxides.[252,286] PON-1 induces lipid peroxide breakdown into carboxylic acids, instead of aldehydes or ketones, which prevents oxidized LDL uptake by macrophages and smooth muscle cells.[252] CETP has also been found to enhance the ability of HDL to inhibit LDL oxidation, possibly through promoting the transfer of lipid peroxides to HDL.[252] Interestingly, HDL size appears to be negatively correlated to HDL's effectiveness in protecting LDL from oxidation.[252]

Thrombosis

Thrombosis, a blood clot formed in blood vessels limiting blood flow, often underlies ischemic heart disease, ischemic stroke, and venous thromboembolisms.[289,290] HDL exhibits many anti-thrombotic properties, mainly promoting blood flow, reducing thrombin production, and attenuating endothelial and platelet activation.[289] For instance, HDL promotes blood flow by increasing the production of nitric oxide and prostacyclin, which stimulates vasodilation and inhibits platelet aggregation.[289,291] Platelet activation can also be inhibited by HDL's downregulation of the release of PAF and production of thromboxane A2, a platelet aggregation factor and vasoconstrictor.[289,292] Similarly, HDL can induce the production of nitric oxide and

inhibit the apoptosis of endothelial cells, formation of microparticles, and expression of tissue factor, P-selectin, and E-selectin to ultimately attenuate endothelial activation.[289] HDL can also reduce thrombin production, a procoagulant and platelet adhesion factor, by enhancing activated protein C and protein S activity within the anticoagulant protein C pathway.[289,293] HDL sphingolipids, such as glucosylceramide and glycosphingolipids, can also act as cofactors for activated protein C.[289] Moreover, HDL is important for platelet count and morphology.[294] Specifically, mice deficient in SR-B1 exhibit low platelet count and large, abnormally shaped platelets due to an increased content of unesterified cholesterol.[294]

Reproduction

Although the role of HDL is traditionally seen in the context of ASCVD defense, researchers have also associated HDL to reproduction. In many mammalian species, such as mice and humans, HDL is the only lipoprotein class that is substantially found in follicular fluid, the proper environment for oocyte development.[295,296] HDL appears to execute multiple roles in female fertility.[295] For instance, after ovulation, HDL-C in the follicular fluid is taken up by SR-B1 to support progesterone synthesis by luteal cells, a hormone that prepares the endometrium for fertilized egg implantation in the uterus.[295,297] This role of HDL metabolism in female fertility has been demonstrated by SR-B1 knockout mice.[295] Knockout mice have abnormally structured HDL that are large, cholesterol-rich, and high in apoA-I, apoE, phospholipids, and unesterified cholesterol.[295] Compared to normal mouse HDL, SR-B1 knockout mouse HDL was dysfunctional and displayed defective cholesterol transport ability.[295] These female SR-B1 knockout mice were infertile, whereas males with the same knockout were not.[295] To strengthen this association, fertility was restored in SR-B1 knockout mice by giving probucol, a cholesterol-lowering drug, or inactivating the apoA-1 gene, which normalizes HDL-C content.[295] Another

key protein in reverse cholesterol transport, ABCA1, may be important for female fertility.[298] ABCA1 knockout females have significantly decreased TC, HDL lacking cholesterol, abnormal phospholipid composition, reduced fertility, lower pregnancy rates, and smaller litter sizes.[298] Moreover, oxidative stress negatively affects reproductive fitness, as seen in females with polycystic ovarian syndrome and endometriosis.[296] Interestingly, researchers have demonstrated an association between higher follicular fluid HDL anti-oxidative capacity and reduced odds of the oocyte being properly fertilized.[296] This may be due to the potentially harmful effects of both extremely low and extremely high levels of oxidative stress for oocyte maturation and embryo quality.[296] One study has suggested that a certain amount of oxidative stress might be needed for normal reproduction, which again highlights HDL's potential role in fertility.[299]

HDL also plays a role in male fertility.[300] HDL was found to be the primary source of cholesterol used by Sertoli cells, which maintain cholesterol homeostasis via reverse cholesterol transport, in rodents.[300] In fact, ABCA1 is expressed highly in the testis and Sertoli cells in the seminiferous tubules.[300] ABCA1 knockout mice accumulated lipid in their Sertoli cells and had decreased intra-testicular testosterone levels and sperm counts, diminishing their fertility.[300]

Infection

HDL plays a multifunctional role against infectious diseases, including bacterial, viral, and parasitic infections. Regarding bacterial infections, HDL has the ability to neutralize gram-negative bacterial LPS and gram-positive bacterial lipoteichoic acid through apoA-I activity.[189,248] HDL also promotes the uptake, and thus clearance, of LPS via SR-B1.[248] Normally, LPS and lipoteichoic acid can induce cytotoxic actions that lead to sepsis and possibly death.[248] Low levels of HDL-C are inversely related to the severity of septic disease and related to exaggerated systemic inflammation.[248] HDL is also effective in protecting against leprosy,

caused by *Mycobacterium leprae*, a bacterium dependent on host metabolic pathways, which includes host-derived oxidized phospholipids.[248] The addition of normal HDL, which can scavenge oxidized phospholipids, has been shown to reverse the inhibition of innate immune responses by mycobacterial infection.[248]

HDL can play both beneficial and detrimental roles in the context of viral infections. For instance, HDL can remove cholesterol from lipid rafts, in which the ACE2 receptor, the receptor for SARS-CoV-2, is localized.[301] This reduction in cholesterol may induce a change in configuration of ACE2 in the lipid raft, decreasing SARS-CoV-2 entry and the severity of the infection. On the other hand, the main HDL receptor, SR-B1, can facilitate viral entry by binding to the S1 subunit of the SARS-CoV-2 spike protein.[301] Similarly, SR-B1 plays a pivotal role in the entry of hepatitis B virus, hepatis C virus, dengue virus, and Zika virus. Researchers have demonstrated the effective use of anti-SR-B1 antibodies to block some of these infections.[302,303]

HDL also serves an important role during parasitic infections. Mainly, apoL-I found on HDL can traffic to the lysosome of trypanosomes, dangerous blood parasites, where acidic pH leads to conformational change, activating anion channels in the N-terminus of apoL-I and resulting in lysosomal swelling and killing of the trypanosome.[248] ApoL-I and haptoglobin-related protein, a plasma protein associated with HDLs that contain apoL-I, can also inhibit infection by Leishmania, another parasite.[249,304]

2.3.5 High-Density Lipoprotein-Cholesterol versus Apolipoprotein A-I

The link between LDL-C and ASCVD risk has been strengthened by numerous epidemiological studies, Mendelian randomization studies, randomized clinical trials with LDL-

C lowering drugs, observations from familial hypercholesterolemia, and experimental studies, such as those inducing LDL receptor knockouts.[305-324] LDL-C has thus traditionally served as the main indicator of CVD risk attributable to apoB lipoprotein particles.[9] More recently, evidence, including epidemiological studies, randomized clinical trials, and discordance analyses, has grown remarkably in favor of apoB as a more accurate marker of ASCVD risk than LDL-C and non-HDL-C.[14,15,164-171] In fact, multiple discordance analyses have demonstrated that apoB is a more accurate marker of cardiovascular risk than LDL-C or non-HDL-C, the most detailed study being an analysis of UK Biobank that confirmed apoB is a more accurate marker of cardiovascular risk overall than non-HDL-C.[325]

Epidemiological studies have also strengthened the association between HDL-C and ASCVD, leading to the notion of HDL-C as "good cholesterol".[173,326-328] Furthermore, in vivo experimental studies demonstrate the association between high HDL and apoA-I levels with protection against atherosclerotic lesion development.[329,330] Despite an extensive focus on HDL-C as a marker for atherosclerosis, apoA-I has not been as thoroughly considered. Similar to the relationship between apoB and non-HDL-C, apoA-I acts as the primary structural component of HDL-C and the two are highly correlated.[7] As described, the acceptance of apoB as a better marker for ASCVD risk than non-HDL-C is growing. However, comparisons between apoA-I and HDL-C are limited and lack a clear consensus. The current literature includes both sides of the matter. The INTERHEART Study analyzed 21,465 participants with logistic regression and found that apoA-I was associated with a 33% decrease in myocardial infarction risk whereas HDL-C was associated with a 15% decrease in risk.[14] On the same side of the argument, the AMORIS Study analyzed 175,553 participants with Cox proportional hazards regression.[15] They also found that apoA-I was a stronger predictor of myocardial infarction risk than TC or

triglycerides.[15] Conversely, Benoit Lamarche and colleagues analyzed 2,155 males with Cox proportional hazards regression and found that although apoA-I was associated with a decreased risk of ischemic heart disease, it was not statistically significant.[18] The same study revealed that HDL-C was associated with a greater decrease in ischemic heart disease risk than apoA-I, which was statistically significant.[18] Similarly, Erik Ingelsson and colleagues analyzed 3,322 participants from the Framingham Heart Study with Cox proportional hazards regression and found that apoA-I was not significantly associated with CHD risk while HDL-C was associated with reduced risk.[19] Other researchers have shown that both apoA-I and HDL-C are similar predictors of ASCVD that add minimal value to each other.[20]

Although multiple discordance analyses have shown that apoB is a better marker of ASCVD risk than non-HDL-C, a discordance analysis of HDL-C and apoA-I has not been carried out. This gap in the literature led to the rationale of the first two aims of the present study: 1) to categorize the relation of HDL-C and apoA-I to ASCVD risk; 2) to compare the relative strengths of HDL-C and apoA-I to ASCVD risk using residual discordance analysis.

2.4 Current Treatments of Atherosclerosis

Promoting a healthy lifestyle throughout life is the key to preventing ASCVD.[331] Current recommendations underscore the importance of controlling body weight, alcohol intake, smoking, and diet.[163] Weight loss (≥5% change from baseline BMI) is associated with cardiovascular event risk reduction.[332,333] Alcohol intake has been shown to have a J- or U-shaped association with CVD risk, representing a reduced risk of CVD with light to moderate alcohol consumption compared to no alcohol or heavy alcohol intake.[334-336] Studies have also

demonstrated reductions in CVD risk in those who stopped smoking.[337-341] One study examining the Framingham Heart Study cohort revealed that smoking cessation was related to a substantial decline in CVD risk within 5 years of cessation (hazard ratio (HR)= 0.61).[337] Regarding a healthy diet, the ACC/AHA Nutrition and Diet Recommendations for Prevention of ASCVD promotes the intake of vegetables, fruits, legumes, nuts, whole grains, and fish, as well as a lower intake of sodium, cholesterol, processed meats, refined carbohydrates, and trans fats to minimize ASCVD risk.[342] However, adopting a healthy lifestyle is not always enough and many individuals may require medicinal treatments, which focus on lowering LDL, triglycerides, or Lp(a) and increasing levels of HDL.[163]

Atherosclerosis was first characterized by high levels of LDL-C, making statins, a lipid-lowering therapy, an important line of defense against the condition.[6,343] Investigations into statins began in 1959 with triparanol, an inhibitor of cholesterol synthesis, and overcame many challenges to finally achieve FDA approval of lovastatin in 1987.[344] The active form of lovastatin, metabolized in the stomach, inhibits HMG-CoA reductase, an essential enzyme in the rate-limiting step of cholesterol synthesis.[345] In addition to lowering cholesterol levels by directly inhibiting its synthesis, HMG-CoA reductase inhibitors like lovastatin can reduce high-sensitivity C-reactive protein levels, help endothelial function, decrease inflammation at plaque sites, limit platelet aggregation, and inhibit coagulation.[345-348] Lovastatin has been FDA-approved to prevent and treat CHD, hypercholesterolemia, and adolescents with heterozygous familial hypercholesterolemia.[345] One clinical trial reports that lovastatin decreased the incidence of coronary events, myocardial infarction, unstable angina, and cardiovascular events in participants.[310]

By 2010, simvastatin, pravastatin, fluvastatin, atorvastatin, rosuvastatin, and pitavastatin were available on the market.[344] These statins serve as HMG-CoA reductase inhibitors like lovastatin, reducing the biosynthesis of cholesterol.[349-354] The type of statin used for each patient generally depends on their 10-year ASCVD risk estimation determined by the PCE, recommended by the 2018 Multi-Society Cholesterol Management Guidelines and the 2019 ACC/AHA Primary Prevention of Cardiovascular Disease Guideline.[146,355] Such guidelines recommend high-intensity statin treatment for patients with the highest ASCVD risk.[355] In the ACC/AHA guidelines, statins are categorized into high-intensity statins (rosuvastatin 20 mg or 40 mg, and atorvastatin 40 mg or 80 mg) which reduce LDL-C by greater than 50%, moderate-intensity statins (atorvastatin 10 mg or 20 mg, pravastatin 40 mg or 80 mg, lovastatin 40 mg or 80 mg, fluvastatin XL 80 mg, fluvastatin 40 mg BID, and pitavastatin 1-4 mg) which reduce LDL-C by 30 to 49%, and low-intensity statins (simvastatin 10 mg, pravastatin 10 mg or 20 mg, lovastatin 20 mg, and fluvastatin 20 mg or 40 mg) which reduce LDL-C by less than 30%.[343]

Newer non-statin lipid-lowering treatments have emerged, such as ezetimibe and PCSK9 inhibitors.[356] Ezetimibe is the most common non-statin lipid-lowering drug and acts by targeting the NPC1L1 transporter in the jejunal brush border in the intestine.[356] This reduces cholesterol absorption by 50% and less cholesterol travels to the liver.[356] LDL receptor expression is increased as a result, decreasing LDL-C levels in the plasma by about 20%.[356] Ezetimibe may be added on top of statin therapy in very high ASCVD risk patients where the LDL-C level remains ≥70 mg/dL.[144,357] If the LDL-C level remains ≥70 mg/dL even after statin and ezetimibe therapy, a PCSK9 inhibitor may be used.[357] PCSK9 is a serine protease that signals LDL receptors for lysosomal degradation, resulting in less LDL receptors available on hepatocytes and, therefore,

less clearance of LDL particles.[356] PCSK9 inhibitors, such as alirocumab and evolocumab, inhibit LDL receptor degradation and lead to lower LDL-C levels.[356]

HDL-C-increasing drugs have also been explored. Niacin, also known as nicotinic acid or vitamin B3, is the most potent HDL-C-elevating drug used in the clinic.[358] Clinical studies over the years have revealed the beneficial effects of niacin alone and in combination with other drugs. Niacin treatment on its own has been linked to a 27% decrease in nonfatal myocardial infarction, whereas niacin treatment in combination with colestipol or simvastatin decreased cardiovascular events by up to 90%.[359-362] Niacin can also decrease triglyceride and LDL-C levels in addition to increasing HDL-C.[358] Several studies have proposed mechanisms of action of niacin to increase HDL-C. Jin et al. found that niacin selectively inhibited the uptake of HDL-apoA-I, but not HDL cholesterol esters, thus increasing apoA-I-containing HDL particles, in a human hepatocyte model system.[363] Specifically, Zhang et al. demonstrated the downregulation of β-chain adenosine triphosphate synthase expression, associated with mediating hepatic HDL holoparticle endocytosis, resulting in decreased hepatic removal of HDL.[364] Therefore, niacin's mechanism of action may involve HDL holoparticle catabolism receptors, rather than SR-B1 which is selective to HDL cholesterol esters.[365] Niacin also appears to favor LpA-I over LpA-I:A-II as it has been shown to significantly inhibit the uptake of LpA-I particles by human hepatocytes, while having no significant effect on LpA-I:A-II uptake.[366] This inhibition may increase LpA-I particles in the circulation and enhance reverse cholesterol transport, as LpA-I is believed to be more efficient than LpA-I:A-II at delivering cholesterol esters.[366] Furthermore, niacin decreases CETP gene expression and hepatic VLDL output which decreases the exchange of triglycerides in VLDL with cholesteryl esters in HDL via CETP.[367] This increases HDL-C by turning HDL into HDL2, the more cholesterol-rich form.[367] Niacin may also promote ABCA1,

stimulating cholesterol efflux from macrophages to HDL.[367] Although niacin appears to be a promising drug for elevating HDL-C levels and protecting against CVD, some randomized clinical trials have failed to demonstrate that niacin can reduce CVD incidence.[368,369] The reason why research regarding niacin has produced mixed results may be due to a change in the HDL proteome associated with niacin administration.[370] Gordon et al. found that niacin administration was associated with a decrease in apoJ, apoL-I, and apoA-II, as well as an increase in SAA and angiotensinogen.[370] Importantly, SAA is able to displace apolipoproteins from HDL, making dysfunctional HDL with damaged cholesterol efflux and reverse cholesterol transport.[370]

CETP inhibitors were thought to provide another avenue for cardioprotection via HDL-C elevation.[371] By decreasing the rate of cholesteryl ester transfer from HDL to triglyceride-rich lipoproteins, CETP inhibitors increase HDL-C and decrease non-HDL-C.[371] Though the REVEAL study with 30,449 ASCVD participants showed a significant decrease in coronary death, myocardial infarction, and coronary revascularization with administration of the CETP inhibitor anacetrapib, other CETP inhibitors that significantly increase HDL-C levels, such as torcetrapib and dalcetrapib, did not reduce CVD risk in clinical trials.[372,373] A study by Furtado et al. revealed that treatment with evacetrapib and torcetrapib elevated total apoA-I concentration.[235] Mainly, apoA-I concentration in HDL that contains apoC-III, a dysfunctional HDL subclass, was elevated the most of all subclasses.[235] Elevating HDL-C should not be about elevating total HDL-C, but researchers must consider whether the HDL is functional.[235] These CETP inhibitors were also able to increase apoA-I in HDL with cardioprotective apoE.[235] However, the cardioprotective effects of apoE are counteracted by the copresence of apoC-III.[235]

As apoA-I is recognized as the major protein conferring HDL's beneficial effects, apoA-I mimetic peptides have been investigated for the development of potential CVD treatments. Most

of these apoA-I mimetic peptides are composed of amphipathic helices with no primary amino acid homology to apoA-I.[374] The mechanism of action for anti-atherogenic and anti-inflammatory apoA-I mimetic peptides involves binding non-oxidized lipids, associating closely to their head group rather than their lipid acyl chain, and activating LCAT.[375] Researchers have suggested that the association of apoA-I mimetic peptides to lipid head groups creates a microenvironment that favours the sequestration of pro-inflammatory lipids.[375] 4F peptides are the most studied of the apoA-I mimetic peptides.[375] In vitro, 4F has been found to increase the generation of pre-β-HDL with enhanced PON-1 activity, enhance cholesterol efflux, and decrease the oxidation of lipoproteins.[375,376] 4F also associates with HDL to recruit phospholipids and apoA-I into the HDL particle, which form LpA-I particles that are good anchors for PON-1, inhibit LPS-induced inflammation, and enhance endothelial function.[376] Though there are no apoA-I mimetic peptides being used in the clinic, 4F has been tested in two phase 2 clinical studies.[374] In the first, 36 men and 14 postmenopausal women with CHD or CHD equivalent conditions were randomized into groups receiving one oral dose of D-4F (4F made with D-amino acids) at 30, 100, 300, or 500 mg or placebo.[377] These single doses were well tolerated, absorbed rapidly, and doses of 300 or 500 mg improved the HDL inflammatory index, a measure of HDL's ability to modify inflammatory responses promoted by LDL.[377] The second study merged two trials, one with participants with CHD or CHD equivalents receiving daily L-4F (4F made with L-amino acids) intravenously for seven days, and the other receiving L-4F subcutaneously for 28 days.[378] Though doses were tolerated, there were no improvements in HDL inflammatory index or PON-1 levels.[378] Furthermore, one apoA-I therapy named CSL-112 was recently tested in a phase 3 trial, AEGIS-II.[379] CSL-112 is an apoA-I derived from the plasma that has been remodeled into disk-shaped lipoproteins with phosphatidylcholine and stabilized with sucrose.[380]

In the trial, 18,219 participants were split into either an experimental group receiving four weekly infusions of 6 g of CSL-112 or a placebo group.[379] However, after a 90-day follow-up, no significant differences in the risk of myocardial infarction, stroke, or cardiovascular death were found between the two groups.[379] Therefore, further research leading to more promising clinical trials is needed to consider apoA-I peptides for clinical use against CVD.

2.5 Approaches to Estimate the Impact of Variables on Atherosclerosis

To determine the impact of different variables (i.e., markers) on disease risk, researchers often utilize regression analyses. Typically, the choice is between three types of regression analyses: linear, logistic, and multiple regression.[381] Linear regression determines the relationship between one continuous (e.g., age) or categorical (e.g., smoking vs non-smoking) independent variable and one dependent continuous variable.[381] Logistic regression uses a categorical dependent variable, and multiple regression uses one or more continuous dependent variables.[381] Regression analyses quantify the direction and strength of the relationship by providing a regression coefficient, which describes the average change in the dependent variable for each 1-unit change in an independent variable.[382] The odds ratio (OR) is one of the main measurements of interest produced by a regression analysis and can be obtained by the exponential function of the regression coefficient ($OR = e^{\text{regression coefficient}}$).[383] For example, a logistic regression model with age as the independent variable and CVD as the dependent variable, resulting in an OR of 1.98, means that a 1-unit increase in age increases the odds of CVD by a factor of 1.98. An OR above 1 indicates that the independent variable is associated with higher odds of the dependent variable occurring, whereas an OR below 1 indicates that the independent variable is associated with lower odds of the dependent outcome.[383] Another core

statistical method, Cox regression, also known as proportional hazards regression, is a survival analysis that describes the relationship between exposures (independent variables) and the occurrence of an outcome (dependent variable) after a certain follow-up time in a cohort of participants.[384] Cox regression is the analytic technique chosen in the present study as ASCVD incidence is the outcome of interest. The main estimate of interest provided by Cox regression is the HR, which is the magnitude of the hazard rate change when a continuous independent variable increases, for instance by one unit.[384] HRs are interpreted in a similar manner to ORs, where an HR above 1 indicates an increased risk of the outcome and an HR below 1 suggests a decreased risk of the outcome.[385] The farther the HR is from 1, in either direction, the larger the effect of the exposure variable on the outcome.[384]

A considerable challenge arises in both multiple and Cox regression when two or more independent variables in the model are correlated, a situation known as multicollinearity.[386,387] This phenomenon can produce a change in the coefficients, and thus HRs, obtained by the regression analysis.[386] The standard errors for predictors' coefficients may also be inflated, which can result in the insignificance of predictors when they should be significant.[387] Ultimately, multicollinearity can lead to wrong interpretations and conclusions, and should be avoided. Typically, multicollinearity can be solved by omitting one of the correlated variables.[387] However, if the research aim involves analyzing the effects of all highly correlated variables on an outcome, such as HDL-C and apoA-I on ASCVD incidence in this study, researchers must turn to another method. Discordance analysis is a statistical method that addresses the challenge of multicollinearity and separates the effects of highly correlated variables by creating groups in which the highly correlated markers are discordant.[21] These groups can be formed by residual discordance analysis, the method chosen for this study, which calculates the differences between

the observed level of each variable (i.e., HDL-C and apoA-I) and the expected level from the regression relating the two markers.[22,23]

3.0 METHODOLOGY

There are two main parts to this thesis.

Part 1

From the full cohort of 502,413 participants in UK Biobank, those with CVD or taking lipid-lowering therapy at baseline examination were excluded. Participants with missing records for HDL-C, apoA-I, LDL-C, non-HDL-C, apoB, triglycerides, HbA1c, BMI, or SBP baseline measurements as well as missing records for sex, smoking history, hypertension treatment, or diabetes treatment were also excluded. Furthermore, participants with triglycerides ≥ 400 mg/dL, apoB <20 mg/dL and >400 mg/dL, or LDL-C ≥ 250 mg/dL were excluded. The final analytic sample included 291,995 participants aged 38 to 73 years old.

R version 4.2.2 was used to perform all statistical analyses. First, residuals of HDL-C and apoA-I were created due to the high correlation between the two variables. The residual of HDL-C was formed by regressing HDL-C on apoA-I, and the residual of apoA-I was formed by regressing apoA-I on HDL-C. The residual of HDL-C represents the amount of HDL-C that is not explained by apoA-I, and the residual of apoA-I represents the amount of apoA-I that is not explained by HDL-C. Triglycerides were log-transformed in order to create a less-skewed distribution of the variable. Next, correlations between HDL-C, LDL-C, non-HDL-C, log-transformed triglycerides, apoB, apoA-I, the HDL-C residual, and the apoA-I residual were measured by obtaining Pearson correlation coefficients, which represent high correlation between two variables the farther the coefficient is from 0. Negative correlation coefficients represent inverse relationships between variables. Finally, the effect of exposure variables (HDL-C, LDL-C, non-HDL-C, triglycerides, apoB, apoA-I, the HDL-C residual, and the apoA-I

residual) on ASCVD incidence was estimated using Cox proportional hazards models. Two separate models were created: one with HDL-C and the apoA-I residual, and the other with apoA-I and the HDL-C residual. SBP, HbA1c, age, sex, BMI, hypertension treatment, diabetes treatment, and smoking status were adjusted for by including them in both models. Cox proportional hazards models provide HRs, a 95% confidence interval (CI) for the HRs, and p-values. If the HR for a variable is below 1, the variable reduces the risk of ASCVD incidence. If the HR is above 1, the variable increases the risk of ASCVD incidence. The farther the HR is from 1, the larger the effect. All HRs were expressed per 1 standard deviation. Note that due to the high correlation between apoB, LDL-C, and non-HDL-C, they were each run in separate models. The values presented for all other variables were obtained from the model that included apoB.

Plots of apoA-I concentration against HDL-C concentration and apoB concentration against non-HDL-C concentration were also created to help visualize the differences in these relationships. Furthermore, plots were created to display the relationship of apoA-I to ASCVD risk and HDL-C to ASCVD risk, controlled for age, sex, apoB, log-transformed triglycerides, HbA1c, BMI, SBP, blood pressure medication, diabetes medication, and smoking.

Part 2

The main cohort of 291,995 participants was divided into quintiles of apoA-I. The first quintile included participants with apoA-I ≥42.9 mg/dL and <132.9 mg/dL, the second quintile included apoA-I ≥132.9 mg/dL and <146.6 mg/dL, the third quintile included apoA-I ≥146.6 mg/dL and <159.7 mg/dL, the fourth quintile included apoA-I ≥159.7 mg/dL and <176.5 mg/dL, and the fifth quintile included apoA-I ≥176.5 mg/dL and ≤250 mg/dL. Similar to Part 1, HDL-C and apoA-I residuals were created, Pearson correlation coefficients were obtained between HDL-

C, LDL-C, non-HDL-C, log-transformed triglycerides, apoB, apoA-I, the HDL-C residual, and the apoA-I residual, and Cox proportional hazards models were used to measure the effects of the same exposure variables as Part 1 on ASCVD incidence in each apoA-I quintile. Note that HDL-C, apoA-I, and both residuals were included in the Cox proportional hazards models, but their HRs are not reported in the results because their values within the quintiles are within a small range and are not normally distributed. Nevertheless, the concentrations of apoA-I and HDL-C over the whole distribution of their values are powerful and consistent predictors of risk, as tested in Part 1.

An interaction term between apoA-I and apoB, also expressed as the apoA-I:apoB ratio, was added to the Cox proportional hazards model used within the full cohort in Part 1 to investigate potential biological interaction. As apoA-I and HDL-C are highly correlated, an HDL-C to apoB interaction term was also added in a separate model to determine whether HDL-C and apoB have a significant biological interaction. Similarly, interaction terms between apoA-I and LDL-C, apoA-I and non-HDL-C, HDL-C and LDL-C, and HDL-C and non-HDL-C were analyzed in separate models. These tests output HRs, 95% CIs, and p-values as usual, but the p-values become the focus for interaction. A significant p-value ($p<0.05$) suggests a significant interaction between apoA-I (or HDL-C) and apoB (or LDL-C or non-HDL-C).

4.0 RESEARCH FINDINGS

Part 1

The median age of the main cohort of participants at baseline was 56 years, and 42.1% of these participants were male (Table 1). Over a median follow-up of 11 years, there were 19,849 ASCVD events. Correlation was high (Pearson correlation coefficient ≥0.50) between apoA-I and HDL-C, apoB and non-HDL-C, apoB and LDL-C, non-HDL-C and LDL-C, non-HDL-C and log-transformed triglycerides, the HDL-C residual and log-transformed triglycerides, and the HDL-C residual and apoA-I residual (Table 2). Interestingly, log-transformed triglycerides were moderately correlated with HDL-C but less strongly with apoA-I.

Table 1. Participant Characteristics in UK Biobank Cohort

Variables	Values [SD] (%)
Participants (N)	291995
Age (years)	56.00 [8.11]
Male (N)	123057 (42.1)
Incidence ASCVD (N)	19849 (6.8)
Follow-up (years)	10.96 [2.06]
Body Mass Index (kg/m^2)	26.27 [4.59]
Systolic Blood Pressure (mmHg)	135.00 [18.61]
Blood Pressure Medication (N)	36020 (12.3)
Ever Smoked (N)	170628 (58.4)
HbA1c (%)	5.33 [0.46]
Diabetes Medication (N)	2379 (0.8)
Total Cholesterol (mg/dL)	225.06 [39.73]
HDL-C (mg/dL)	55.68 [14.19]
Non-HDL-C (mg/dL)	167.52 [38.18]
LDL-C (mg/dL)	141.57 [30.66]
TG (mg/dL)	125.59 [71.17]
ApoB (mg/dL)	104.80 [22.67]
ApoA-I (mg/dL)	153.00 [26.89]
HDL-C: ApoA-I Ratio	0.36 [0.04]

Table 2. Pearson Correlation Coefficients for Lipids and Residuals in UK Biobank Cohort

	HDL-C	Non-HDL-C	ApoB	LDL-C	lnTG	ApoA-I Residual SD	HDL-C Residual SD
ApoA-I	0.92 (p<0.001)	-0.02 (p<0.001)	-0.08 (p<0.001)	0.04 (p<0.001)	-0.28 (p<0.001)	0.39 (p<0.001)	0 (p=1.00)
HDL-C		-0.07 (p<0.001)	-0.11 (p<0.001)	0.02 (p<0.001)	-0.47 (p<0.001)	0 (p=1.00)	0.39 (p<0.001)
Non-HDL-C			0.96 (p<0.001)	0.98 (p<0.001)	0.51 (p<0.001)	0.12 (p<0.001)	-0.14 (p<0.001)
ApoB				0.96 (p<0.001)	0.42 (p<0.001)	0.06 (p<0.001)	-0.10 (p<0.001)
LDL-C					0.38 (p<0.001)	0.07 (p<0.001)	-0.05 (p<0.001)
lnTG						0.39 (p<0.001)	-0.54 (p<0.001)
ApoA-I Residual SD							-0.92 (p<0.001)

In the Cox proportional hazards model with HDL-C, the apoA-I residual, apoB, and log-transformed triglycerides (adjusted for SBP, HbA1c, age, sex, BMI, hypertension treatment, diabetes treatment, and smoking status), HDL-C and the apoA-I residual were significantly associated with new onset ASCVD with HR=0.85 (95% CI: 0.83, 0.87; p<0.0001) and HR=0.96 (95% CI: 0.94, 0.97; p<0.0001), respectively (Table 3). In the Cox proportional hazards model adjusted for the same predictors but including apoA-I, the HDL-C residual, apoB, and log-transformed triglycerides, apoA-I and the HDL-C residual were significantly associated with new onset ASCVD with HR=0.85 (95% CI: 0.83, 0.86; p<0.0001) and HR=0.98 (95% CI: 0.96, 0.99; p<0.01), respectively (Table 3).

Table 3. Hazard Ratios, 95% Confidence Intervals, and p-values for Incident Atherosclerotic Cardiovascular Disease in Cox Proportional Hazards Models with Lipid Parameters and Risk Factors

Variable	Hazard Ratio	95% Hazard Ratio Confidence Limits		P-value
ApoA-I	0.85	0.83	0.86	< 0.0001
HDL-C Residual	0.98	0.96	0.99	0.0098
HDL-C	0.85	0.83	0.87	< 0.0001
ApoA-I Residual	0.96	0.94	0.97	< 0.0001
ApoB	1.14	1.12	1.16	< 0.0001
LDL-C[1]	1.13	1.12	1.15	< 0.0001
Non-HDL-C[1]	1.15	1.13	1.17	< 0.0001
lnTG	1.03	1.01	1.05	0.0107
SBP	1.19	1.17	1.20	< 0.0001
HbA1c	1.08	1.07	1.09	< 0.0001
Age	1.07	1.06	1.07	< 0.0001
Male	1.70	1.65	1.75	< 0.0001
BMI	1.08	1.07	1.10	< 0.0001
BP Medication	1.52	1.46	1.57	< 0.0001
Diabetes Medication	1.57	1.42	1.74	< 0.0001
Smoking	1.24	1.20	1.28	< 0.0001

[1]LDL-C and non-HDL-C were included in separate models in substitution for apoB. All other numbers are taken from the models with apoB.

The plot of apoA-I concentration against HDL-C concentration looks very different from the plot of apoB concentration against non-HDL-C concentration (Figures 2 and 3). The apoA-I and HDL-C plot fans out at higher concentrations of apoA-I. Therefore, the variance in HDL-C for a given value of apoA-I gets larger and larger as apoA-I increases. However, this is not seen in the apoB and non-HDL-C plot. The variance in non-HDL-C stays relatively the same throughout the range of apoB. The difference between these relationships led to the idea of dividing the main cohort of participants based on quintiles of apoA-I.

Figure 2. Plot of High-Density Lipoprotein-Cholesterol (HDL-C) Concentration (mg/dL) Against Apolipoprotein A-I (ApoA-I) Concentration (mg/dL)

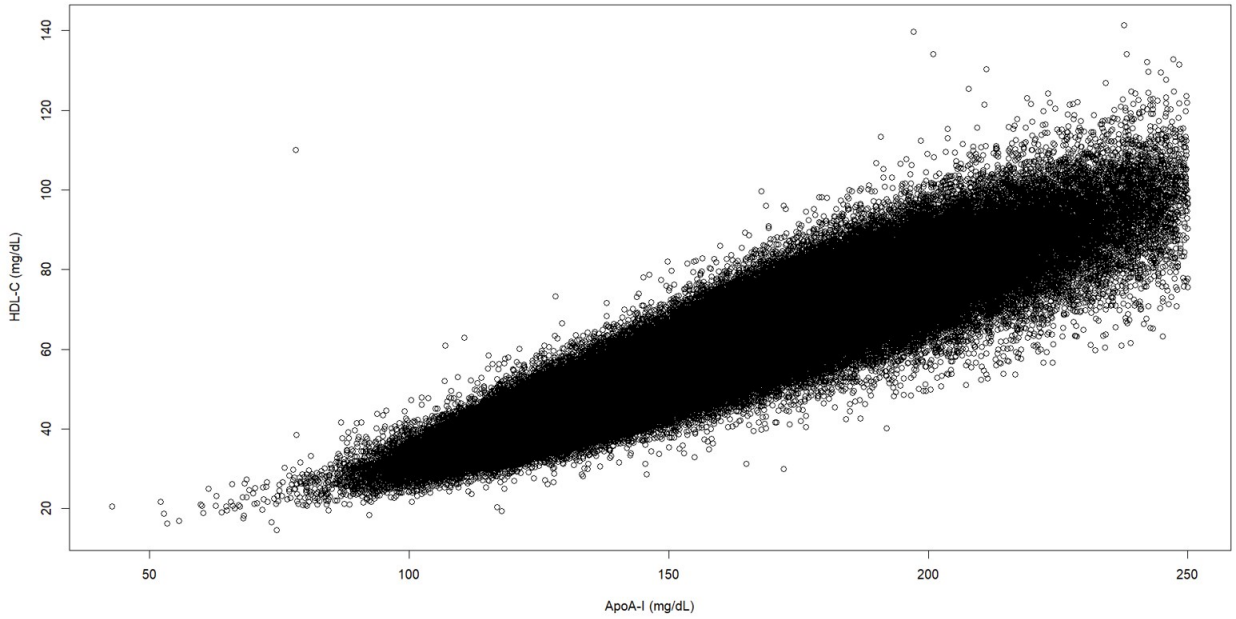
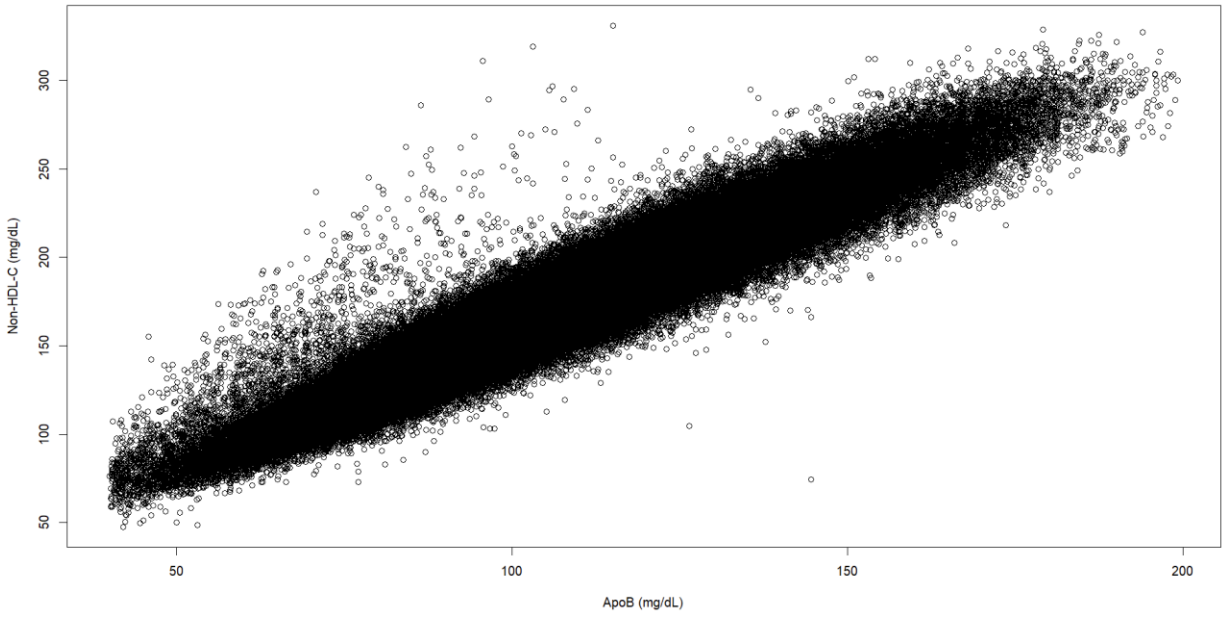


Figure 3. Plot of Non-High-Density Lipoprotein-Cholesterol (Non-HDL-C) Concentration (mg/dL) Against Apolipoprotein B (ApoB) Concentration (mg/dL)



Furthermore, the plotted relationships of apoA-I and HDL-C to ASCVD risk are curvilinear (Figures 4 and 5), suggesting that the relation of apoA-I and HDL-C to risk is not constant over their entire range of concentrations.

Figure 4. Relationship of Apolipoprotein A-I (ApoA-I) and Atherosclerotic Cardiovascular Disease (ASCVD) Incidence

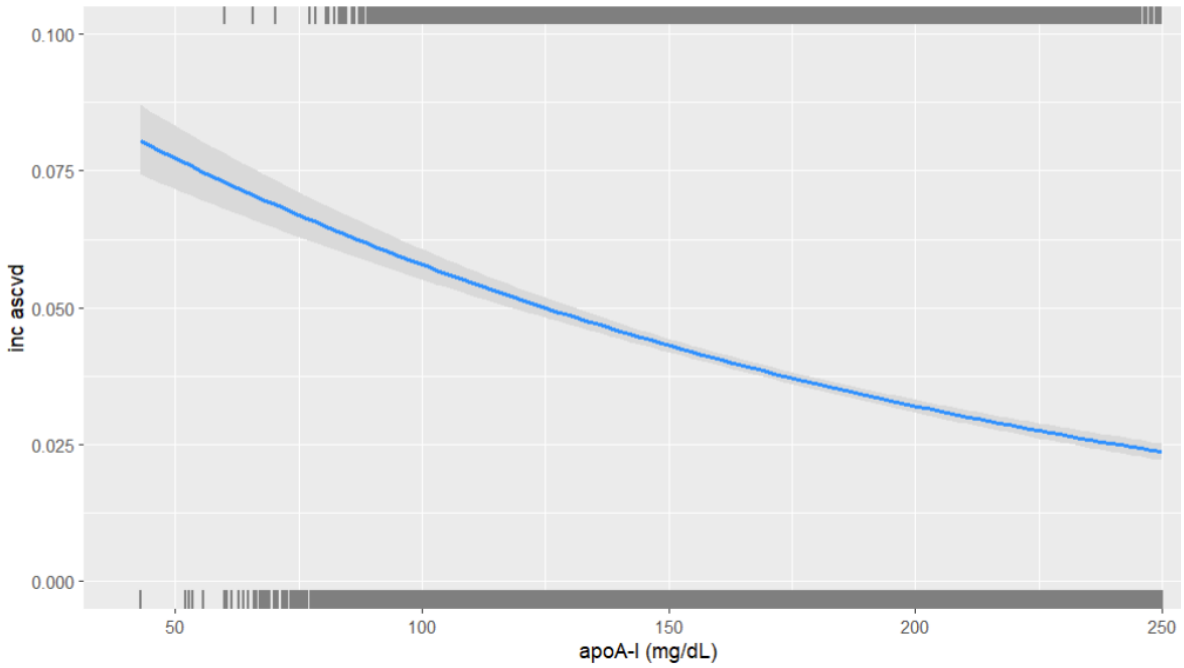
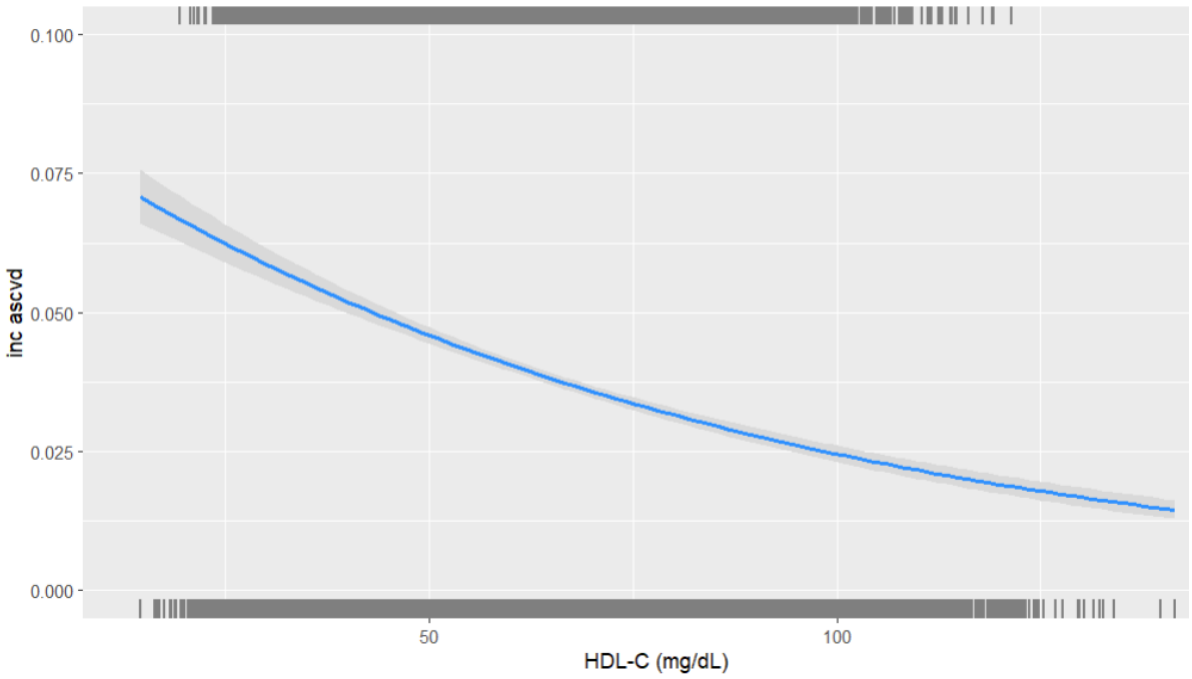


Figure 5. Relationship of High-Density Lipoprotein-Cholesterol (HDL-C) and Atherosclerotic Cardiovascular Disease (ASCVD) Incidence



Part 2

Quintile 1 includes 58,353 participants with apoA-I ≥ 42.9 mg/dL and <132.9 mg/dL. These participants had a median age of 54 years, and 69.4% of these participants were male (Table 4). Over a median follow-up of about 11 years, there were 5,502 ASCVD events. Quintile 2 includes 58,048 participants with apoA-I ≥ 132.9 mg/dL and <146.6 mg/dL. These participants had a median age of 55 years, and 54.5% of these participants were male. Over a median follow-up of 11 years, there were 4,455 ASCVD events. Quintile 3 includes 58,434 participants with apoA-I ≥ 146.6 mg/dL and <159.7 mg/dL. These participants had a median age 56 years, and 41.2% of these participants were male. Over a median follow-up of 11 years, there were 3,742 ASCVD events. Quintile 4 includes 58,741 participants with apoA-I ≥ 159.7 mg/dL and <176.5

mg/dL. These participants had a median age 57 years, and 28.7% of these participants were male. Over a median follow-up of 11 years, there were 3,285 ASCVD events. Quintile 5 includes 58,419 participants with apoA-I ≥ 176.5 mg/dL and ≤ 250 mg/dL. These participants had a median age 58 years, and 17.2% of these participants were male. Over a median follow-up of 11 years, there were 2,865 ASCVD events. Overall, similar patterns in correlation strength between variables seen in the main cohort were observed in each of the apoA-I quintiles (Tables 5-9). However, within the first quintile, apoA-I and the apoA-I residual are highly correlated (as well as HDL-C and the HDL-C residual) whereas they are weakly correlated in the main cohort. The same observation holds in the second quintile, in addition to both residuals being more weakly inversely correlated to each other and apoA-I and HDL-C being more weakly correlated to each other than they are within the main cohort. The correlations in quintiles 3 and 4 are similar to those in quintile 2, and quintile 5 is similar to quintile 1.

Table 4. Participant Characteristics in Apolipoprotein A-I Quintiles

	1st Quintile	2nd Quintile	3rd Quintile	4th Quintile	5th Quintile
N	58353	58048	58434	58741	58419
Age (years)	54.00 [8.40]	55.00 [8.25]	56.00 [8.07]	57.00 [7.87]	58.00 [7.57]
Male (N)	40500 (69.4)	31615 (54.5)	24053 (41.2)	16846 (28.7)	10043 (17.2)
Incidence ASCVD (N)	5502 (9.4)	4455 (7.7)	3742 (6.4)	3285 (5.6)	2865 (4.9)
Follow-up (years)	10.93 [2.33]	10.95 [2.12]	10.97 [2.02]	10.97 [1.92]	10.95 [1.86]
Body Mass Index (kg/m2)	27.89 [4.74]	27.09 [4.63]	26.32 [4.50]	25.55 [4.29]	24.57 [3.96]
Systolic Blood Pressure (mmHg)	134.50 [17.39]	135.00 [18.25]	135.00 [18.62]	134.50 [19.06]	135.50 [19.63]
Blood Pressure Medication (N)	8231 (14.1)	7561 (13.0)	7081 (12.1)	6703 (11.4)	6444 (11.0)
Ever Smoked (N)	34676 (59.4)	33662 (58.0)	33582 (57.5)	33924 (57.8)	34784 (59.5)
HbA1c (%)	5.35 [0.56]	5.34 [0.48]	5.33 [0.45]	5.33 [0.41]	5.32 [0.37]
Diabetes Medication (N)	971 (1.7)	533 (0.9)	402 (0.7)	270 (0.5)	203 (0.4)
Total Cholesterol (mg/dL)	208.05 [38.80]	218.18 [38.42]	224.44 [38.10]	230.78 [37.38]	241.84 [37.31]
HDL-C (mg/dL)	40.87 [5.42]	49.00 [4.83]	55.69 [5.37]	63.46 [6.30]	76.03 [10.34]
TG (mg/dL)	159.52 [80.44]	138.97 [73.72]	125.77 [67.69]	113.81 [61.72]	102.56 [54.73]
ApoB (mg/dL)	106.80 [22.94]	106.30 [23.11]	105.50 [22.82]	103.90 [22.23]	101.60 [21.86]
ApoA-I (mg/dL)	123.60 [9.02]	140.10 [3.90]	152.90 [3.77]	167.20 [4.79]	192.00 [16.59]

Table 5. Pearson Correlation Coefficients for Lipids and Residuals in Quintile 1

	HDL-C	Non-HDL-C	ApoB	LDL-C	lnTG	ApoA-I Residual	HDL-C Residual
ApoA-I	0.70 (p<0.001)	0.10 (p<0.001)	0.05 (p<0.001)	0.13 (p<0.001)	-0.06 (p<0.001)	0.71 (p<0.001)	0 (p=1.00)
HDL-C		0.08 (p<0.001)	0.08 (p<0.001)	0.17 (p<0.001)	-0.40 (p<0.001)	0 (p=1.00)	0.71 (p<0.001)
Non-HDL-C			0.95 (p<0.001)	0.98 (p<0.001)	0.51 (p<0.001)	0.06 (p<0.001)	0.01 (p=0.004)
ApoB				0.96 (p<0.001)	0.40 (p<0.001)	-0.01 (p=0.034)	0.07 (p<0.001)
LDL-C					0.38 (p<0.001)	0.01 (p=0.017)	0.11 (p<0.001)
lnTG						0.31 (p<0.001)	-0.50 (p<0.001)
ApoA-I Residual							-0.70 (p<0.001)

Table 6. Pearson Correlation Coefficients for Lipids and Residuals in Quintile 2

	HDL-C	Non-HDL-C	ApoB	LDL-C	lnTG	ApoA-I Residual	HDL-C Residual
ApoA-I	0.40 (p<0.001)	0 (p=0.99)	-0.01 (p=0.022)	0.01 (p=0.002)	-0.06 (p<0.001)	0.92 (p<0.001)	0 (p=1.00)
HDL-C		-0.11 (p<0.001)	-0.06 (p<0.001)	-0.01 (p<0.001)	-0.55 (p<0.001)	0 (p=1.00)	0.92 (p<0.001)
Non-HDL-C			0.96 (p<0.001)	0.98 (p<0.001)	0.55 (p<0.001)	0.05 (p<0.001)	-0.12 (p<0.001)
ApoB				0.97 (p<0.001)	0.44 (p<0.001)	0.02 (p<0.001)	-0.06 (p<0.001)
LDL-C					0.43 (p<0.001)	0.02 (p<0.001)	-0.02 (p<0.001)
lnTG						0.17 (p<0.001)	-0.57 (p<0.001)
ApoA-I Residual							-0.40 (p<0.001)

Table 7. Pearson Correlation Coefficients for Lipids and Residuals in Quintile 3

	HDL-C	Non-HDL-C	ApoB	LDL-C	lnTG	ApoA-I Residual	HDL-C Residual
ApoA-I	0.38 (p<0.001)	-0.01 (p=0.009)	-0.02 (p<0.001)	0 (p=0.82)	-0.06 (p<0.001)	0.93 (p<0.001)	0 (p=1.00)
HDL-C		-0.17 (p<0.001)	-0.12 (p<0.001)	-0.08 (p<0.001)	-0.58 (p<0.001)	0 (p=1.00)	0.93 (p<0.001)
Non-HDL-C			0.96 (p<0.001)	0.98 (p<0.001)	0.55 (p<0.001)	0.06 (p<0.001)	-0.18 (p<0.001)
ApoB				0.97 (p<0.001)	0.45 (p<0.001)	0.03 (p<0.001)	-0.12 (p<0.001)
LDL-C					0.44 (p<0.001)	0.03 (p<0.001)	-0.09 (p<0.001)
lnTG						0.17 (p<0.001)	-0.60 (p<0.001)
ApoA-I Residual							-0.38 (p<0.001)

Table 8. Pearson Correlation Coefficients for Lipids and Residuals in Quintile 4

	HDL-C	Non-HDL-C	ApoB	LDL-C	lnTG	ApoA-I Residual SD	HDL-C Residual
ApoA-I	0.41 (p<0.001)	-0.01 (p=0.04)	-0.02 (p<0.001)	0 (p=0.64)	-0.06 (p<0.001)	0.91 (p<0.001)	0 (p=1.00)
HDL-C		-0.19 (p<0.001)	-0.15 (p<0.001)	-0.10 (p<0.001)	-0.58 (p<0.001)	0 (p=1.00)	0.91 (p<0.001)
Non-HDL-C			0.96 (p<0.001)	0.98 (p<0.001)	0.53 (p<0.001)	0.08 (p<0.001)	-0.20 (p<0.001)
ApoB				0.97 (p<0.001)	0.43 (p<0.001)	0.05 (p<0.001)	-0.16 (p<0.001)
LDL-C					0.42 (p<0.001)	0.05 (p<0.001)	-0.12 (p<0.001)
lnTG						0.20 (p<0.001)	-0.61 (p<0.001)
ApoA-I Residual							-0.41 (p<0.001)

Table 9. Pearson Correlation Coefficients for Lipids and Residuals in Quintile 5

	HDL-C	Non-HDL-C	ApoB	LDL-C	lnTG	ApoA-I Residual	HDL-C Residual
ApoA-I	0.68 (p<0.001)	-0.01 (p=0.03)	-0.03 (p<0.001)	-0.02 (p=0.66)	-0.07 (p<0.001)	0.73 (p<0.001)	0 (p=1.00)
HDL-C		-0.15 (p<0.001)	-0.15 (p<0.001)	-0.08 (p<0.001)	-0.48 (p<0.001)	0 (p=1.00)	0.73 (p<0.001)
Non-HDL-C			0.96 (p<0.001)	0.98 (p<0.001)	0.47 (p<0.001)	0.09 (p<0.001)	-0.17 (p<0.001)
ApoB				0.96 (p<0.001)	0.39 (p<0.001)	0.07 (p<0.001)	-0.16 (p<0.001)
LDL-C					0.36 (p<0.001)	0.05 (p<0.001)	-0.09 (p<0.001)
lnTG						0.20 (p<0.001)	-0.59 (p<0.001)
ApoA-I Residual							-0.68 (p<0.001)

ApoB and sex were the only variables with a clear and statistically significant ($p<0.05$) trend across apoA-I quintiles. Despite the concentration of apoB remaining relatively constant across apoA-I quintiles, the HR of apoB decreased from 1.20 (first quintile) to 1.06 (fifth quintile). As expected, the HRs of LDL-C and non-HDL-C showed the same pattern as apoB across apoA-I quintiles. The HR of LDL-C decreased from 1.21 to 1.04. The HR of non-HDL-C decreased from 1.23 to 1.05. The HR for being male increased from 1.56 (first quintile) to 1.88 (fifth quintile). To address potential concerns about whether being male acts as a confounding variable influencing the observed diminishing effect of apoB on ASCVD risk, the same quintile analysis was performed separately in males and females (Supplemental Materials 1 and 2). The effect of apoB on ASCVD risk decreases as apoA-I concentration increases in both males and females. Although the effect is stronger in males, it is still evident in females.

Table 10. Hazard Ratios, 95% Confidence Intervals, and p-values for Incident Atherosclerotic Cardiovascular Disease in Cox Proportional Hazards Models with Lipid Parameters and Risk Factors in Apolipoprotein A-I Quintiles

	1st Quintile	2nd Quintile	3rd Quintile	4th Quintile	5th Quintile
Variable	**Hazard Ratio (95% Confidence Limits)**				
ApoB	1.20 (1.16,1.24) ***	1.18 (1.14,1.22) ***	1.14 (1.10,1.18) ***	1.10 (1.06,1.14) ***	1.06 (1.02,1.10) *
LDL-C[1]	1.21 (1.18,1.25) ***	1.18 (1.14,1.22) ***	1.14 (1.10,1.18) ***	1.09 (1.05,1.13) ***	1.04 (1.00,1.08)
Non-HDL-C[1]	1.23 (1.19,1.27) ***	1.19 (1.15,1.23) ***	1.15 (1.11,1.20) ***	1.10 (1.06,1.15) ***	1.05 (1.01,1.10) *
lnTG	0.98 (0.94,1.01)	1.00 (0.95,1.04)	1.04 (0.99,1.09)	1.04 (0.99,1.09)	1.11 (1.06,1.17) ***
SBP	1.15 (1.12,1.18) ***	1.18 (1.15,1.22) ***	1.20 (1.16,1.24) ***	1.22 (1.17,1.26) ***	1.19 (1.14,1.23) ***
HbA1c	1.10 (1.07,1.12) ***	1.10 (1.07,1.12) ***	1.06 (1.04,1.09) ***	1.09 (1.06,1.12) ***	1.06 (1.04,1.09) ***
Age	1.07 (1.06,1.07) ***	1.07 (1.06,1.07) ***	1.07 (1.06,1.07) ***	1.07 (1.06,1.07) ***	1.07 (1.06,1.08) ***
Male	1.56 (1.46,1.67) ***	1.68 (1.57,1.79) ***	1.68 (1.57,1.80) ***	1.74 (1.62,1.87) ***	1.88 (1.73,2.04) ***
BMI	1.08 (1.05,1.11) ***	1.07 (1.04,1.11) ***	1.10 (1.06,1.13) ***	1.07 (1.03,1.11) ***	1.07 (1.03,1.11) ***
BP Medication	1.45 (1.35,1.54) ***	1.47 (1.37,1.58) ***	1.45 (1.34,1.57) ***	1.62 (1.49,1.76) ***	1.70 (1.55,1.86) ***
Diabetes Medication	1.50 (1.28,1.76) ***	1.73 (1.40,2.13) ***	1.62 (1.23,2.13) ***	1.40 (0.99,1.99)	2.05 (1.41,2.98) ***
Smoking	1.32 (1.25,1.40) ***	1.23 (1.16,1.31) ***	1.20 (1.12,1.28) ***	1.18 (1.10,1.27) ***	1.19 (1.10,1.29) ***

* $p<0.05$ (statistically significant)

*** $p<0.001$ (high statistical significance)

[1]LDL-C and Non-HDL-C were included in separate models in substitution for apoB. All other numbers are taken from the models with apoB.

Finally, the interaction term between apoA-I and apoB in the Cox proportional hazards models used in the main cohort produced a p-value <0.001 (highly statistically significant). The interaction terms between HDL-C and apoB, HDL-C and LDL-C, HDL-C and non-HDL-C,

apoA-I and LDL-C, and apoA-I and non-HDL-C (in separate models) were also significant. Therefore, there is significant interaction between apoA-I (or HDL-C) and apoB (or LDL-C or non-HDL-C), meaning that apoA-I and apoB are effect modifiers of each other. Furthermore, a significant interaction between apoA-I and apoB is observed separately in both males and females.

5.0 DISCUSSION

The debate as to whether HDL-C or apoA-I is a more useful marker of ASCVD risk continues. This study found no significant difference between the two markers. Both HDL-C and apoA-I are significant markers of ASCVD incidence as demonstrated in both Cox proportional hazards models in the main cohort. However, the HDL-C residual and the apoA-I residual appeared to add similar but relatively minor value to apoA-I and HDL-C, respectively, as indicated by their HRs being close to 1. Virtually all the apoA-I in the circulation is associated with HDL, and with a Pearson correlation coefficient of 0.92, it is clear that apoA-I and HDL-C are highly linked, and they function tightly together.[388] To the best of my knowledge, this is the first discordance analysis between apoA-I and HDL-C, adding a new line of evidence to previous research similarly revealing no significant difference between the two markers of ASCVD risk.

This thesis also presents the novel finding that apoA-I is related to a decreased risk of ASCVD associated with apoB, as shown by the decreasing effect of apoB on ASCVD risk in the Cox proportional hazards models across apoA-I quintiles. As apoA-I concentration increases, the effect of apoB on ASCVD incidence appears to weaken. This general trend is observed in both males and females. That an interaction between apoA-I and apoB is biologically credible, demonstrated by the statistical significance of the apoA-I:apoB interaction term in the main Cox proportional hazards models, strengthens this finding. Cholesterol can only be deposited in the arterial wall within apoB particles.[156] Conversely, ApoA-I is postulated to remove cholesterol from macrophages in the arterial wall via its interaction with ABCA1.[389] Thus, net apoB-induced cholesterol deposition may depend on the rate or amount of cholesterol removal by apoA-I. This is only one possible mechanism of how apoA-I and apoB may interact to impact ASCVD risk.

Interestingly, residual apoA-I does not appear to strongly affect the risk of ASCVD directly, as demonstrated by an HR of 0.96 in the main cohort. However, apoA-I clearly confers its impact in an indirect manner, as its concentration influences the effect of apoB on ASCVD risk.

To avoid toxicity, excess cholesterol within macrophages is moved to the extracellular matrix or stored in cytosolic lipid droplets as cholesteryl esters.[390] Researchers have confirmed the role of both apoA-I and HDL in cholesterol efflux to the extracellular matrix by demonstrating the loss of extracellular and cell surface-associated cholesterol microdomains of macrophages when incubated with apoA-I and HDL.[391] Therefore, reverse cholesterol transport also happens in the extracellular matrix, and apoA-I and HDL may mediate cholesterol efflux through this additional mechanism to counteract the effects of apoB.[391]

HDL has a complex structure, with particles varying in size and lipid composition, leading to different levels of cardiovascular protection. This creates an almost infinite number of hypotheses as to how it might impact atherosclerosis. The observational relations between HDL and ASCVD risk relate to HDL-C and apoA-I. These markers must, therefore, be operational surrogates for whatever pathway might actually be involved, such as the reverse cholesterol transport pathway or potential inflammatory pathways.

Although this thesis shows the interaction of apoA-I and apoB at a fairly constant level of apoB (101.60 mg/dL in the highest apoA-I quintile to 106.80 in the lowest quintile), one experiment using rodent hepatocytes finds another mechanism by which apoA-I and apoB may interact, not specifically at a constant concentration of apoB. This study by Sahoo et al. demonstrated decreased cholesterol availability for apoB lipoprotein particle secretion by the liver caused by the diversion of hepatocyte cholesterol into the reverse cholesterol transport

pathway by apoA-I.[392] Thus, it appears that the upregulation of reverse cholesterol transport by apoA-I reduces apoB lipoprotein particle secretion and thus, cholesterol deposition in the arterial wall. It would be interesting to investigate this mechanism further via in vivo experiments observing the changes in apoB cholesterol deposition with gradually increasing or decreasing reverse cholesterol transport via the apoA-I/ABCA1 pathway.

As ABCA1 is an essential component of the reverse cholesterol transport pathway alongside apoA-I, future research can investigate whether there is also an interaction between apoB and ABCA1. Uncovering more about the relationship between apoB and apoA-I (or potentially ABCA1) may help scientists find new targets for the prevention and treatment of ASCVD.

HDL particle size influences ASCVD risk and should be considered in future research involving HDL's protective role against atherosclerosis. Small, dense HDL_3 are thought to be more effective at protecting against LDL oxidation than HDL_2 and to have a stronger anti-inflammatory effect.[202-204] However, larger HDL particles are able to carry more cholesterol.[372] HDL subclasses have also been associated with age and sex. Thus, future research could explore the impact of elevating specific HDL subgroups across different populations, considering differences in age and sex.

It is important to acknowledge the limitations of this study. Although discordance analysis eliminates the challenge of separating the effects of highly correlated variables, such as HDL-C and apoA-I, posed by conventional statistical methods, it does not establish causality. Rather, Cox proportional hazards models provide information about the association between the time to an event (e.g., ASCVD) and various explanatory variables (e.g., apoA-I and HDL-C).[393] Therefore, this thesis reveals significant associations between apoA-I and ASCVD, as well as

HDL-C and ASCVD; however, these results cannot be interpreted as apoA-I or HDL-C directly causing the prevention of ASCVD. Notably, several clinical trials employing HDL-C-elevating therapies have failed to demonstrate a decrease in CVD risk.[372] CETP inhibitors that significantly increase HDL-C levels, such as torcetrapib, dalcetrapib, and evacetrapib, did not reduce CVD risk in clinical trials.[372,373] Even niacin, the most widely used medication to elevate HDL-C levels, has failed to reduce CVD incidence in randomized controlled trials.[368,369] Therefore, it is imperative to refer to multiple lines of evidence before resulting to causality when dealing with statistical methods like Cox proportional hazards models. The use of methods that are capable of determining causality, such as Mendelian randomization and randomized controlled trials, may be advantageous for future research investigating the relationships between apoA-I and ASCVD, HDL-C and ASCVD, as well as apoB and apoA-I in association with ASCVD.[394,395]

As described in Section 2.1.2, there are various risk factors associated with CVD. Future research may benefit from investigating the influence of different risk factors that were not included in this thesis. As CVD has a disproportionate effect on ethnic minority groups, it would be interesting to see if the same observations from the work in this thesis hold in different ethnic groups. First, researchers can explore whether HDL-C and apoA-I are similarly significant markers of ASCVD risk across various ethnicities or whether one marker demonstrates superiority over the other in specific ethnic groups. A similar quintile analysis can follow to determine whether the effect of apoB on ASCVD risk diminishes at higher concentrations of apoA-I in each ethnic group. Biological interaction between apoB and apoA-I can then be examined in each group to determine if any variability exists depending on ethnicity. UK Biobank provides researchers with information about participant ethnicity (e.g., Asian, South Asian, Black, Black African, Black Caribbean, Chinese, White, White British, White Irish,

Mixed, etc.); therefore, this future research will be possible in the UK Biobank cohort used for this study. The results from this ethnicity-focused research may inform the future development of preventions and treatments for diverse ethnic groups, as treatments are not typically "one-size-fits-all".

Another finding to make note of is the stronger correlation observed between log-transformed triglycerides and HDL-C in comparison to the weaker correlation obtained between log-transformed triglycerides and apoA-I. This may be because HDL-C decreases as triglycerides are exchanged from a chylomicron or LDL particle, as triglyceride-rich HDL particles are cleared more rapidly.[396] Thus, one difference between HDL-C and apoA-I may be that triglycerides do not seem to be a major determinant of apoA-I, whereas they are important for HDL-C. Future research may delve into what role triglycerides may play in regulating the reverse cholesterol transport pathway, to see whether and to what extent they influence HDL-C and apoA-I. This research could help determine whether triglycerides would be important targets for ASCVD prevention and treatment in the context of the reverse cholesterol transport mechanism.

As ASCVD remains a leading cause of death worldwide, it is critical to conduct research that advances our knowledge on the markers and causes of the disease.[1] By understanding whether current diagnostic tools are actually effective, guideline improvements can be made. This thesis demonstrates the effectiveness of HDL-C and apoA-I as ASCVD markers and supports HDL-C and apoA-I as equivalent markers. As equivalent markers, the apoA-I:apoB and HDL-C:apoB ratios should be equivalent. Thus, it is not necessary to measure apoA-I, saving time and money in the clinic. These findings, alongside similar work demonstrating the similarity of HDL-C and apoA-I as markers of ASCVD risk, should be considered when

formulating new guidelines for risk prediction.[20] This work will also help inform future avenues of research, including the novel ideas described in this section.

6.0 CONCLUSION

Today, CVD diminishes both the quality of life and life expectancy, while also placing substantial financial burdens on health systems worldwide.[24] Identifying and utilizing the most accurate biomarkers in the clinic for ASCVD risk prediction is essential to enhance CVD prevention and treatment. This thesis explores the individual relationships of apoA-I and HDL-C to ASCVD risk, and reveals the novel biological interaction between apoB and apoA-I. In two separate Cox proportional hazards models within the full cohort (one with HDL-C and the apoA-I residual, one with apoA-I and the HDL-C residual, and both adjusted for apoB, log-transformed triglycerides, SBP, HbA1c, age, sex, BMI, hypertension treatment, diabetes treatment, and smoking status), the HDL-C residual and the apoA-I residual appeared to add similar value to apoA-I and HDL-C, respectively. HDL-C and apoA-I are both individually significant markers of ASCVD ($p<0.001$). However, neither of the two markers shows superiority over the other in predicting risk. With these findings, there appears to be no benefit in switching to using apoA-I as a risk predictor over HDL-C in a clinical setting.

The third objective of this thesis investigated the interaction between apoB (or LDL-C or non-HDL-C) and apoA-I (or HDL-C) on ASCVD risk. The same discordance analysis used in the main cohort of this study, but with an interaction term added between apoB and apoA-I, revealed a significant interaction between the two markers. Thus, future research can delve deeper into this biological interaction to aid in the development of potential ASCVD treatments. This novel finding adds to a strong foundation of research aimed at preventing and treating ASCVD to ultimately enhance quality of life and life expectancy worldwide.

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8.0 SUPPLEMENTAL MATERIAL

Supplemental Material 1. Hazard Ratios for Incident Atherosclerotic Cardiovascular Disease (ASCVD) in Cox Proportional Hazards Models with Lipid Parameters and Risk Factors in Apolipoprotein A-I (ApoA-I) Quintiles in Males

	1st Quintile	2nd Quintile	3rd Quintile	4th Quintile	5th Quintile
Variable	**Hazard Ratio (95% Confidence Limits)**				
ApoB	1.19 (1.14,1.24) ***	1.24 (1.19,1.30) ***	1.18 (1.12,1.23) ***	1.19 (1.14,1.25) ***	1.10 (1.05,1.15) ***
lnTG	0.99 (0.94,1.04)	0.95 (0.90,1.00)	0.97 (0.92,1.03)	1.01 (0.95,1.07)	1.08 (1.02,1.14) *
SBP	1.16 (1.12,1.20) ***	1.11 (1.07,1.15) ***	1.18 (1.14,1.23) ***	1.21 (1.16,1.26) ***	1.23 (1.18,1.28) ***
HbA1c	1.09 (1.06,1.13) ***	1.09 (1.06,1.13) ***	1.11 (1.07,1.14) ***	1.07 (1.04,1.10) ***	1.08 (1.04,1.12) ***
Age	1.06 (1.06,1.07) ***	1.07 (1.07,1.08) ***	1.07 (1.06,1.08) ***	1.06 (1.06,1.07) ***	1.06 (1.05,1.06) ***
BMI	1.07 (1.03,1.11) *	1.08 (1.03,1.12) ***	1.08 (1.04,1.13) ***	1.07 (1.02,1.12) *	1.06 (1.02,1.11) *
BP Medication	1.47 (1.34,1.61) ***	1.46 (1.32,1.61) ***	1.36 (1.22,1.51) ***	1.42 (1.27,1.59) ***	1.60 (1.44,1.79) ***
Diabetes Medication	1.57 (1.27,1.94) ***	1.42 (1.08,1.87) *	1.51 (1.11,2.04) *	1.49 (1.03,2.17) *	1.71 (1.14,2.58) *
Smoking	1.32 (1.22,1.43) ***	1.16 (1.08,1.26) ***	1.19 (1.09,1.31) ***	1.13 (1.03,1.24) *	1.23 (1.12,1.36) ***

* $p<0.05$ (statistically significant)

*** $p<0.001$ (high statistical significance)

Supplemental Material 2. Hazard Ratios for Incident Atherosclerotic Cardiovascular Disease (ASCVD) in Cox Proportional Hazards Models with Lipid Parameters and Risk Factors in Apolipoprotein A-I (ApoA-I) Quintiles in Females

	1st Quintile	2nd Quintile	3rd Quintile	4th Quintile	5th Quintile
Variable	**Hazard Ratio (95% Confidence Limits)**				
ApoB	1.15 (1.09,1.21) ***	1.10 (1.04,1.16) ***	1.06 (1.00,1.12)	1.06 (1.00,1.12)	1.05 (0.99,1.11)
lnTG	1.04 (0.97,1.11)	1.08 (1.00,1.16) *	1.07 (1.00,1.16)	1.07 (1.00,1.15)	1.10 (1.02,1.18) *
SBP	1.18 (1.12,1.23) ***	1.18 (1.13,1.24) ***	1.20 (1.14,1.26) ***	1.18 (1.12,1.24) ***	1.18 (1.12,1.25) ***
HbA1c	1.10 (1.06,1.14) ***	1.08 (1.03,1.13) *	1.07 (1.03,1.12) ***	1.10 (1.05,1.15) ***	1.05 (1.01,1.09) *
Age	1.06 (1.06,1.07) ***	1.05 (1.01,1.10) ***	1.07 (1.06,1.08) ***	1.08 (1.07,1.09) ***	1.08 (1.07,1.09) ***
BMI	1.08 (1.03,1.13) *	1.10 (1.05,1.16) ***	1.09 (1.04,1.15) ***	1.07 (1.02,1.12) *	1.05 (0.99,1.11)
BP Medication	1.54 (1.39,1.70) ***	1.52 (1.36,1.70) ***	1.62 (1.44,1.83) ***	1.59 (1.40,1.80) ***	1.70 (1.49,1.94) ***
Diabetes Medication	1.59 (1.19,2.12) *	1.96 (1.32,2.90) ***	1.66 (1.04,2.65) *	1.93 (1.11,3.37) *	0.84 (0.99,3.45)
Smoking	1.37 (1.25,1.50) ***	1.37 (1.24,1.51) ***	1.12 (1.02,1.24) *	1.25 (1.12,1.38) ***	1.16 (1.04,1.29) *

* $p<0.05$ (statistically significant)

*** $p<0.001$ (high statistical significance)