HAPTOGLOBIN PHENOTYPE, HDL-CHOLESTEROL RAISING THERAPY (FIBRATE), AND RISK OF CARDIOVASCULAR DISEASE EVENTS WITHIN THE ACTION TO CONTROL CARDIOVASCULAR RISK IN DIABETES (ACCORD) LIPID TRIAL

By

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I dedicate this thesis to my parents and my brother.

Thank you for being constant, positive role models in my life and for always encouraging me to be the best version of myself. It is through your unwavering love and support that this thesis was made possible.

Table Of Contents

List Of Tables

List Of Figures

Abstract

Background: The failure of many clinical trials aimed at preventing cardiovascular disease (CVD) by pharmacologically raising high-density lipoprotein (HDL) cholesterol has cast doubt on the idea that more HDL-cholesterol is better and that the function of HDL may be of more importance. The haptoglobin (Hp)2-2 phenotype (~40% of people) is associated with dysfunctional HDL that can become heavily oxidized in hyperglycemia. The effect of raising potentially pro-atherogenic HDL-cholesterol on risk of CVD in the Hp2-2 phenotype in hyperglycemia is unknown.

Objectives: 1) To determine whether the effect of adding fenofibrate therapy to simvastatin therapy on risk of coronary heart disease (CHD) and CVD events depends on Hp phenotype, and 2) to determine whether the association between HDL-cholesterol and cardiovascular events (CHD and CVD) depend on Hp phenotype, in type 2 diabetes mellitus (T2DM). These objectives were also examined in men and women and in primary and secondary prevention patients separately.

Methods: Haptoglobin phenotype was determined using a validated assay in 4,996 men and women with T2DM who participated in the ACCORD lipid trial with a mean follow-up of 4.7 years for CVD and 4.6 for CHD. In an intention-to-treat analysis (objective 1), multivariable Cox proportional hazards regression was used to determine the effect of fenofibrate therapy on CHD and CVD events for the two Hp phenotype groups separately. In a biomarker analysis with repeated measures over time (objective 2), multivariable Cox proportional hazards regression was used to determine the association between a 1-mg/dL increase in HDL-cholesterol and CHD and CVD events, stratifying by Hp phenotype.

Results: Fenofibrate with background simvastatin, compared to simvastatin alone, reduced the risk of CHD in Hp1 allele carriers but not in the Hp2-2 phenotype (p-value for interaction=0.009). The effects also differed by sex with a reduced risk of CHD in men who were Hp1 allele carriers and an increased risk of CHD in women with the Hp2-2 phenotype. There was a significant, inverse association between HDL-cholesterol and cardiovascular outcomes in Hp1 allele carriers but there was no significant association between HDL-cholesterol and cardiovascular outcomes in patients with the Hp2-2 phenotype, although the interaction was not significant.

Conclusion: The effect of adding fenofibrate to simvastatin on CHD and CVD risk may depend on Hp phenotype and sex. Further research is needed to understand both the relationship between fenofibrate therapy and cardiovascular events, and between HDL function and cardiovascular events in different Hp phenotypes.

List Of Abbreviations Used

ACCORD	Action to Control Cardiovascular Risk in Diabetes
BioLINCC	National Institutes of Health's Open Biologic Specimen and Data Repository Information Coordinating Center
CHD	Coronary heart disease
CVD	Cardiovascular disease
ELISA	Enzyme-linked immunosorbent assay
HDL	High-density lipoprotein
Hb	Hemoglobin
Hp	Haptoglobin
LDL	Low-density lipoprotein
MI	Myocardial infarction
PPAR-α	Peroxisome proliferator-activated receptor-α
T2DM	Type 2 diabetes mellitus
VLDL	Very-low-density lipoprotein

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Chapter 1: Introduction

Cardiovascular disease (CVD), including heart disease and stroke, is the leading cause of death in the world. Approximately 17.9 million people died from CVD in 2016, making up 31% of the total deaths globally(1). Type 2 diabetes mellitus (T2DM) is an increasingly prevalent disorder and is an important risk factor for CVD(2,3). Individuals with T2DM have a substantially higher risk of cardiovascular morbidity and mortality compared to those without diabetes and approximately 68% of people age 65 or older with diabetes die from some form of heart disease and 16% of stroke(3,4). With the steady rise in the global prevalence of T2DM, diabetes-related CVD is a primary concern of health care providers(5). Investigations to identify an optimal plan for successful treatment and prevention of CVD in T2DM would significantly reduce the overall rates of CVD death.

The increased incidence of CVD among T2DM can be attributed, at least in part, to the augmented prevalence of well-known risk factors including hyperglycemia and dyslipidemia(2,3,6). Hyperglycemia, or high blood sugar, is the hallmark of diabetes and many studies have demonstrated a positive continuous relationship between blood sugar level and CVD risk(7,8). Diabetic dyslipidemia is characterized by elevated plasma triglyceride (TG) levels and smaller denser low-density lipoprotein (LDL) particles, and low levels of high-density lipoprotein (HDL) cholesterol and is a major risk factor for atherosclerotic CVDs(6,9,10). Aggressive management of blood lipid levels is therefore generally necessary in patients with T2DM(11). Numerous studies have demonstrated the benefit of lowering LDL-cholesterol with statins in patients with and without T2DM and current guidelines recommend LDL-cholesterol management as the primary goal of lipid

therapy in T2DM(11–16). Although statins are efficacious in individuals with T2DM, they do not normalize overall dyslipidemia and rates of cardiovascular events remain high in such patients even after statin therapy(17,18). The possible benefit of the addition of other lipid-modifying agents to statin therapy to reduce the residual cardiovascular risk has attracted a great deal of interest(11).

Fibrates are drugs that act primarily to raise HDL-cholesterol and lower triglycerides. Clinical trials investigating the effect of fibrate monotherapy on incident CVD have reported inconsistent and conflicting results(19–21). Several studies have demonstrated that fibrate and statin combination therapy is better at improving overall lipid profiles relative to fibrate or statin monotherapy; however, evidence that fibrate and statin combination therapy reduces CVD events is lacking(22). The Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial was a large, multi-center study that tested the effect of intensive blood glucose control and either intensive blood pressure control or fenofibrate and simvastatin combination lipid therapy on CVD outcomes in high-risk patients with T2DM. In the lipid arm, it was found that the combination of fenofibrate and simvastatin did not reduce the rate of CVD events compared to simvastatin alone(23). Similarly, two other large trials investigating the effect of niacin (HDL-cholesterol raising and triglyceride lowering drug) and statin combination therapy on CVD outcomes found no benefit compared to statin monotherapy(24,25). The reason for the failure of HDL-cholesterol raising and triglyceride lowering therapy in most of these clinical trials is unknown and may be due to differences in unmeasured characteristics between study participants, such as genetics.

A common variation in the gene that codes for the abundant plasma protein Hp has identified individuals who may be at increased risk of CVD from hyperglycemia and altered HDL function(26–31). A major role of the Hp protein is to bind and clear free Hb in the blood, thereby preventing Hb-mediated oxidative damage to blood vessels and proteins. The common Hp variation is a copy number variant with two alleles, Hp1 and Hp2, that vary vastly in size (the Hp2 allele contains a large 1.8kb intragenic duplication of exons 3 and 4 of the Hp1 allele) and produce three structurally and functionally distinct Hp proteins (Hp1-1, Hp2-1 and Hp2-2) that determine an individual's Hp phenotype. Approximately 40% of people world-wide have the Hp2-2 genotype and produce the Hp2-2 protein which is substantially larger, more cyclic, and has repeatedly demonstrated less antioxidant function compared to the Hp1-1 and Hp2-1 proteins(32–34). In the setting of high blood sugar (often defined as glycated Hb ≥6.5%) the differences in function in these proteins are magnified, resulting in reduced ability of Hp2-2 to clear Hb and prevent oxidation of serum and cellular proteins, and HDL dysfunction because Hp tethers to HDL(35–39). There is a resulting increase in Hp-Hb complexes circulating in the blood of patients with the Hp2-2 phenotype, and Hp-Hb complex oxidatively modifies HDL, generating dysfunctional HDL that is potentially pro-atherogenic and is thought to increase susceptibility to atherosclerosis, deterioration of cardiac function and ultimately CHD(27,35,39). The effect of raising HDL-cholesterol levels in people with the Hp2-2 phenotype and high blood sugar on risk of CHD events is currently unknown, but may not have a beneficial effect and may even be harmful, whereas it may be favorable in Hp1 allele carriers (Hp1-1 and Hp2-1) in whom the functions of Hp and HDL are better preserved.

To investigate whether haptoglobin phenotype may be a useful biomarker in clinical care, the aim of this thesis was to determine if the effect of adding fenofibrate to simvastatin therapy on cardiovascular events (CHD and CVD), and the association between HDL-cholesterol and cardiovascular events (CHD and CVD), is dependent on Hp phenotype in high risk patients with T2DM who participated in the ACCORD lipid trial. Because women have higher HDL-cholesterol levels than men and sex differences may occur (40–43), and because secondary prevention patients may have a higher risk of CVD events (44,45), the above effects and associations in men and women as well as in primary and secondary prevention patients separately were also examined. This is a novel study that can help to provide an explanation for the apparent failure of HDL-raising and triglyceride lowering therapy in the prevention of CVD previously reported and can potentially help to guide clinical practice with respect to lipid management for CVD prevention.

Chapter 2: Literature Review And Rationale

2.0 Cardiovascular Disease And Type 2 Diabetes Mellitus

Cardiovascular diseases (CVDs) are the number one cause of death globally for both men and women and is an umbrella term used for diseases that affect the heart and blood vessels, resulting from the interaction of genetic, environmental and behavioral factors. Included in CVDs is coronary heart disease (CHD), such as angina or myocardial infarction (MI), stroke, heart failure, rheumatic heart disease, cardiomyopathy arrhythmia, congenital heart disease, valvular disease, peripheral artery disease, thromboembolic disease and venous thrombosis(1). Heart attacks and strokes make up 85% of CVD deaths and are usually caused by a build-up of fatty deposits on the wall of arteries, a process termed atherosclerosis(1,4). Atherosclerosis leads to a narrowing of the arteries which, in turn, leads to restricted blood flow. If a blood clot forms, the narrowing can be detrimental or fatal as blood flow to the heart and brain can cease and cause a heart attack or stroke(1). In the absence of diabetes, the prevalence of CVD is higher in men than in age-matched women and it occurs about ten years later in women(46,47). In the setting of T2DM, sex differences in CVD diminish or disappear(48–51). Patients with T2DM have 2-4 times the rate of cardiovascular mortality and also have increased rates of non-fatal MI and stroke compared to their non-diabetic counterparts(2–4). The relative risk of CVD in patients with T2DM compared to those without T2DM is higher in women than in men while the absolute risks are generally comparable(49–51).

T2DM is a chronic and complex metabolic disorder with abnormalities in carbohydrate and lipid metabolism and affects over 400 million adults globally(52). T2DM is characterized by elevated levels of insulin resistance and variable levels of circulating insulin. Insulin is a hormone made by the pancreas that regulates the amount

of glucose in the blood by signaling the uptake of glucose into tissues. In insulin resistance, the body is unable to absorb glucose properly and levels of sugar build up in the blood. Exactly why insulin resistance occurs is unknown but is associated with genetic and behavioral factors such as being overweight and a sedentary lifestyle.

In T2DM, insulin resistance leads to chronic abnormally high blood sugar levels, or hyperglycemia. Hyperglycemia is often accompanied by dyslipidemia as there is an excessive availability of energy-rich substrates (glucose and /or free fatty acids) which leads to increased hepatic secretion and impaired clearance of very-low-density lipoprotein (VLDL). Triglyceride-rich VLDL then transfers triglycerides to LDL and HDL promoting the formation of small, dense triglyceride-rich LDL and clearance of triglyceride-rich HDL, and is commonly referred to as “diabetic dyslipidemia”(53,54). However, the relationship between glucose and lipid metabolism is complex and more recently it was recognized that not only can abnormalities in glucose metabolism affect lipid metabolism but abnormalities in lipid metabolism may also impair glucose metabolism. Elevated levels of triglycerides lead to elevated levels of free fatty acids which may induce insulin resistance, although the mechanisms are not well understood(55). Both hyperglycemia and dyslipidemia are well established risk factors for CVD.

2.1 Hyperglycemia And Cardiovascular Disease Risk In T2DM

Hyperglycemia, or high blood sugar, is an inflammatory trigger that, over time, can damage blood vessels and lead to the development of endothelial dysfunction and contribute to atherosclerosis (56,57). Hyperglycemia induces multiple alterations in vascular tissue including non-enzymatic glycosylation of serum lipids and proteins,

oxidative stress and protein kinase C activation (58). All of these mechanisms lead to a common effect, an increased oxidative stress state. Hyperglycemia is also thought to increase monocyte adhesion to vascular endothelial cells, which is an important initial event in the pathogenesis of atherosclerosis(59,60). Serum glycated Hb (HbA1c) is a form of Hb covalently bound to glucose and is an established marker used to assess glycemic control in T2DM, as it reflects the average plasma glucose control over a period of 2-3 months(61). An HbA1c cut point of ≥6.5% is used to diagnose T2DM(62).

Several large prospective studies have consistently shown that there is a positive and continuous relationship between blood sugar levels (%HbA1c) and risk of CVD in T2DM(7,8). In a meta-analysis of prospective cohort studies in patients with T2DM, Zhang et al. found that a 1% increase in glycosylated hemoglobin (Hb) is associated with a 25% increase in the hazard of CVD mortality, 17% in total CVD, 15% in CHD and 11% in stroke. Accordingly, one might expect that effective glucose lowering strategies would reduce the risk of CVD. However, large clinical trials in which patients with T2DM are given treatment strategies (oral diabetes medications, insulin and behavioral interventions) that lower their blood glucose levels (targeting HbA1c of "non-diabetic" levels [<6.5%]) to potentially decrease their risk of CVD, compared to standard glucose control, have reported conflicting results with some studies reporting harm. On average, the intensive glycemic control group achieved an HbA1c level ≥1.1% lower than the standard therapy group in each study(63–66). As a result, the optimal target for glucose control in T2DM remains unclear.

2.2 Diabetic Dyslipidemia And Cardiovascular Disease Risk In T2DM

2.2.1 Atherosclerosis And Diabetic Dyslipidemia

A single layer of endothelial cells lines the inner surface of blood cells that synthesize important bioactive substances that regulate blood vessel function and structure and, in the absence of endothelial dysfunction, inhibit atherosclerosis and protect the blood vessel(67–69). The development of atherosclerosis is described in detail by James Scott(70). Briefly, atherosclerosis occurs because of endothelial dysfunction caused by irritants such as LDL-cholesterol and free radicals that cause damage to the endothelium. Once a break in the endothelial lining has been made, it becomes permeable to lymphocytes and monocytes which can then move into deeper layers of the blood vessel and a series of biochemical reactions occur that attract LDL particles. Oxidized LDL particles are then engulfed by monocytes which subsequently become foam cells or macrophages. Localized accumulation of foam cells and macrophages lead to plaque development and ultimately, atherosclerosis. The epithelial surface of the atherosclerotic plaque can become ruptured, eventually breaking away from the vessel wall where cellular debris and lipid fragments become released into the blood vessel. A coronary or cerebral blood vessel can become blocked if thrombogenic agents attach to this material to form a blood clot. A potentially fatal heart attack or stroke can result. Small, dense LDL particles are more proatherogenic as they more readily undergo oxidative modification and thus uptake by monocytes in blood vessel walls(71,72). An important risk factor for the development of atherosclerosis is atherogenic dyslipidemia.

Atherogenic dyslipidemia is the most common lipid abnormality in T2DM and consists of hypertriglyceridemia (high serum levels of triglycerides), low HDL-cholesterol and relatively normal levels of LDL-cholesterol. However, even in mildly elevated concentrations of serum triglycerides, LDL-particles are typically small and

dense and are more susceptible to oxidation. Additionally, chronic hyperglycemia promotes the glycation of LDL and both glycation and oxidation are thought to increase the atherogenicity of LDL-cholesterol(73).

2.2.2 Statins And CVD Prevention

Statins are drugs that lower LDL-cholesterol levels and are currently used as the first line of defense against CVD in patients with dyslipidemia(14,15). Many studies have consistently demonstrated the beneficial effects of statins on lowering LDL-cholesterol as well as in the primary (no evidence of established CVD) and secondary (evidence of established CVD present) prevention of CVD in patients with and without diabetes(12,13,74). In a subgroup analysis of the Heart Protection Study, which compared simvastatin 40-mg daily to placebo, patients with diabetes (n=5,963 including 615 with type 1 diabetes) who took simvastatin found a 27% reduction in major coronary events and a 25% reduction in stroke compared to those taking placebo(75). The Cholesterol Treatment Trialists' (CTT) Collaboration meta-analysis of more than 170,000 statin treated patients demonstrated a 20% reduction in CVD events for every 1 mmol/L reduction on LDL-cholesterol, regardless of baseline values. The reduction was similar in the diabetes subgroup(76). Although statins are effective in the prevention of CVD events, excess residual CVD risk remains in patients with T2DM compared to their non-diabetic counterparts. Some of the residual risk could be attributed to lipoprotein abnormalities that are not adequately managed by statins such as low HDL-cholesterol and high triglyceride levels.

2.2.3 HDL, Triglycerides And Residual CVD Risk In T2DM

HDL-cholesterol, or “good cholesterol”, has long been postulated to be cardioprotective and an inverse association between plasma HDL-cholesterol and CVD is well established in patients with and without T2DM, even in patients on optimal statin therapy(77–83). The protection conferred by HDL-cholesterol has often been attributed to its anti-atherogenic functions, which include reverse cholesterol transport and the ability to act as a potent antioxidant and anti-inflammatory particle. In general, levels of HDL-cholesterol tend to be higher in women than in men with and without T2DM(41–43). Large prospective studies have demonstrated an inverse relationship between HDL-cholesterol and the risk of CHD across a large range of HDL-cholesterol (30-60-mg/dL)(84,85). Furthermore, it has been reported that for each 1-mg/dL increment in HDL-cholesterol, the risk of CHD is reduced by 3% in women and 2% in men(79). HDL-cholesterol has also been shown to have an inverse relationship with stroke in T2DM(83). High levels of serum triglyceride rich particles, as is common in T2DM, promote the clearance of HDL-cholesterol from the plasma, thus lowering the HDL-cholesterol concentration in the blood(53,54).

Triglyceride-rich lipoproteins include VLDL and metabolites of VLDL and chylomicron remnants. The role of these lipoproteins in CVD and whether they are simply a marker for accompanying lipid abnormalities, especially low HDL-cholesterol and small dense LDL-cholesterol, remains controversial. Triglyceride concentrations vary inversely with HDL-cholesterol concentrations which, to some extent, confounds the interpretations related to the relationship between increases in triglycerides and CVD (86,87). In a meta-analysis of 17 population-based prospective studies, increased plasma triglycerides were associated increased coronary disease in both men and women after

adjusting for HDL-cholesterol(88). In another meta-analysis, adjustment for established coronary risk factors, especially HDL-cholesterol, substantially attenuated the risk associated with high triglyceride levels(89).

Although statins are aggressive LDL-cholesterol lowering drugs and are known to reduce the risk of CVD, they have little effect on HDL-cholesterol and triglyceride abnormalities and considerable cardiovascular disease risk remains in T2DM when achieving LDL-cholesterol levels at or below recommended targets(17,18). The possible improvement of CVD risk yielded by the addition of other lipid modifying drugs to statin therapy has been a topic of great interest(11).

2.2.4 Fibrates And CVD Prevention

Fibrates are drugs that are agonists of the proliferator-activated-receptor-α (PPAR-α), a master regulator of energy homeostasis, vascular inflammation and cell differentiation. Fibrates are used primarily to raise HDL-cholesterol and lower triglycerides and also have a role in reducing systemic inflammation(90–92). Clinical trials investigating the effect of fibrate monotherapy on incident CVD have reported inconsistent and conflicting results(19–21). In the Veterans Affairs HDL Intervention Trial (VA-HIT), patients randomized to receive a fibrate (gemfibrozil) had a 32% reduction (HR=0.68, 95% CI: 0.53-0.88) in the risk of major CVD events (composite endpoint of CHD death, MI or stroke), a 41% reduction in CHD death (HR= 0.59, 95% CI: 0.39-0.91) and a 40% reduction in stroke risk (HR= 0.60, 95% C.I 0.37-0.99) in men with T2DM compared to placebo(19). Reductions in incident MI were also observed in the BIP trial in men and women with metabolic syndrome who were treated with fibrate (bezafibrate) relative to placebo (HR= 0.71, 95% CI: 0.49-0.91) while no significant

reduction in total cardiac mortality was observed (HR= 0.74, 95% CI: 0.54-1.03)(20). In the Fenofibrate Intervention and Event Lowering Diabetes (FIELD) trial, in men and women with T2DM treatment with a fibrate (fenofibrate) did not significantly reduce the primary outcome of major coronary heart outcomes (CHD mortality and non-fatal MI) or stroke compared to placebo, however a significant reduction in total CVD events (HR= 0.89, 95% CI: 0.80-0.99) was observed mainly due to a reduction in non-fatal MI and other revascularizations. In a post-hoc analysis of the FIELD study, it was postulated that the non-significant findings in the primary outcome may partially be explained because there was a greater use of statin therapy in patients allocated to placebo, which may have masked the benefit of the fibrate(21).

Several studies have demonstrated that fibrate and statin combination therapy is better at improving overall lipid profiles relative to fibrate or statin monotherapy; however, evidence that combination therapy reduces CVD events is lacking(22). The Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial was a large, multi-center study that tested the effect of intensive blood glucose control and either intensive blood pressure control or fenofibrate and simvastatin combination lipid therapy on CVD outcomes in high-risk patients with T2DM. In the lipid arm of the ACCORD trial, participants were randomized to receive either fenofibrate or placebo in combination with simvastatin. Fenofibrate and simvastatin combination therapy did not significantly reduce total CVD events (HR=0.92, 95% CI: 0.79-1.08), CVD mortality (HR=0.86, 95% CI: 0.66-1.12) or major CHD events (HR=0.92, 95% CI: 0.79-1.07) compared to simvastatin treatment alone. In a pre-specified subgroup analysis among males and females separately, it was found that men seemed to benefit from fenofibrate therapy whereas

there seemed to be a trend toward harm in women(23). Similarly, two other large trials investigating the effect of niacin (HDL-cholesterol raising and triglyceride lowering drug) and statin combination therapy on CVD outcomes found no benefit compared to statin monotherapy(24,25). The reason for the failure of HDL-cholesterol raising and triglyceride lowering therapy in these clinical trials is currently unknown.

More recently, with the several apparently failed clinical trials that aimed to reduce CVD events by pharmacologically raising HDL-cholesterol and lowering triglycerides, research has cast doubt on the idea that more HDL-cholesterol is better with the hypothesis that the absolute level of HDL-cholesterol may be less important compared to its functional capacity(93). In fact, recent insights in the complex structural and functional properties of HDL have shown that under certain circumstances, particularly in inflammation, HDL is prone to oxidative modifications and can become dysfunctional and transition from an anti-atherogenic to a pro-atherogenic particle(94). Accordingly, although two individuals may have the same concentration of serum HDL-cholesterol, the quality of their HDL, and as a result their CVD risk profile, may differ. Unmeasured differences between study participants, such as genetics, that affect HDL function could provide an explanation for inconsistent results previously reported in trials that aimed to pharmacologically raise HDL-cholesterol.

2.3 Haptoglobin Phenotype, HDL Dysfunction And CVD Risk In T2DM

2.3.1 Haptoglobin

Haptoglobin (Hp) is a circulating blood protein that binds to and aids in the clearance of free hemoglobin (Hb) in the blood. When blood cells lyse, Hb is released into the blood stream and promotes the formation of hydroxyl radicals and oxidative

damage of serum and cellular proteins. Hp protects against oxidative damage by binding to Hb with a very high affinity, allowing the removal of the Hb-Hp complex from the blood to the liver via macrophage/ monocyte CD163 receptor, which is the only method of removing Hb from the extravascular compartment(32,33). Additional functions of Hp which are not yet well understood, are angiogenesis and vasodilation, and may be related to blood pressure(95,96).

A mutation (a copy number variant) in the gene that codes for Hp gave rise to two alleles, Hp1 and Hp2, that vary vastly in size (the Hp2 allele contains a large 1.8kb intragenic duplication of exons 3 and 4 of the Hp1 allele) and produce three structurally and functionally distinct Hp proteins (Hp1-1, Hp2-1 and Hp2-2) that determine an individual's Hp phenotype. Approximately 40% of people world-wide have the Hp2-2 genotype and produce the Hp2-2 protein which is substantially larger, more cyclic, and has repeatedly demonstrated less antioxidant function compared to the Hp1-1 and Hp2-1 proteins (32–34). The frequencies of Hp phenotypes vary based on racial origin and geographic region but both the Hp1 and Hp2 alleles have been found in every population to date(28). Previous studies in mostly White cohorts have found a Hp2-2 frequency of ~35-40%, Hp2-1 frequency of ~45-50% and a Hp1-1 frequency of ~15% (29,30,33). The Hp polymorphism and the Hp2 allele is thought to have arisen in early human evolution due to its protective effect against infectious diseases. In modern times, the Hp2 allele can increase the risk of several non-inflammatory, chronic diseases(28).

2.3.2 HDL Dysfunction And Risk Of CHD In The Hp2-2 Phenotype

It has previously been established that people with the Hp2-2 phenotype may be at increased risk of CHD from hyperglycemia, potentially due to altered HDL

function(26–31). In three independent prospective cohorts, participants with both the Hp2-2 phenotype and HbA_{1c} ≥6.5% had a pronounced risk of CHD compared to those with the Hp1-1 or Hp2-1 phenotype and HbA_{1c}<6.5%(29). Participants with an Hp1 allele and elevated HbA1c were not at significantly increased risk of CHD when compared to the same reference group. A subsequent observational study in which data from two large prospective nested case-control studies were modelled to mimic a randomized controlled trial study design of intensive glycemic control reported that the risk of CHD associated with HbA1c ≥6.5% is pronounced in the Hp2-2 phenotype, particularly in earlier onset cases(30) More recently, using data from the ACCORD trial, Carew et al. demonstrated a significant benefit from intensive glycemic control for CVD prevention among patients with the Hp2-2 phenotype, but there was no significant effect in Hp1 allele carriers. In contrast, an analysis of CVD among participants in the longitudinal Bruneck study found that HbA1c ≥6.5% in the Hp2-2 phenotype was not predictive of the risk of CVD compared to Hp1 allele carriers(97). This inconsistency could be a result of several differences in the studies. Most importantly, the authors combined CHD with stroke to make CVD as their outcome of interest. Stroke has been associated with the Hp1-1 phenotype rather than the Hp2-2 phenotype(98,99). It was hypothesized that the relationship between Hp2-2 and CHD is related to the function of Hp as a scavenger of free Hb, while the function of Hp in angiogenesis may confer a protective effect of Hp2-2 against stroke(100,101). Therefore, CHD and stroke should be separated in an analysis examining the effect of Hp2-2 on CVD. Additionally, in the Bruneck study they included participants with prevalent disease at baseline whereas only patients without prevalent disease were included in the other studies. The Bruneck study

also had a small number of cases during follow up(123) with only a small portion attributed to CHD (48).

The mechanism for the impairment of HDL-cholesterol function in Hp2-2 in the setting of hyperglycemia (often defined as HbA1c ≥6.5%) has been published previously (Figure 2.1). In hyperglycemia, CD163 expression is reduced. Further, the differences in function of the Hp proteins are magnified, resulting in reduced ability of Hp2-2 to clear Hb and prevent oxidation of serum and cellular proteins, and HDL dysfunction because Hp tethers to HDL(35–39). There is a resulting increase in Hp-Hb complexes circulating in the blood of patients with the Hp2-2 phenotype, and the Hp-Hb complex oxidatively modifies HDL and its related components (apolipoprotein A, glutathione peroxidase and lecithin-cholesterol acyltransferase), generating dysfunctional HDL that is potentially pro-atherogenic and is thought to increase susceptibility to atherosclerosis, deterioration of cardiac function and ultimately CHD(27,35,39).

In a subset of participants in the Atherothrombosis Intervention in Metabolic Syndrome with Low HDL/High Triglycerides: Impact on Global Health Outcomes (AIM-HIGH) study who had diabetes, Asleh et al. found that niacin improved HDL-cholesterol antioxidant function in individuals with Hp1-1 phenotype but worsened HDL-cholesterol antioxidant function in individuals with the Hp2-2 phenotype, but the study was not powered to investigate if these changes were related to clinical events(102). Further research is needed to determine if the effect of pharmacologically raising HDL-cholesterol depends on Hp phenotype.

Figure 2.1. Proposed biological mechanism of the relationship between Hp2-2 and CVD risk in T2DM

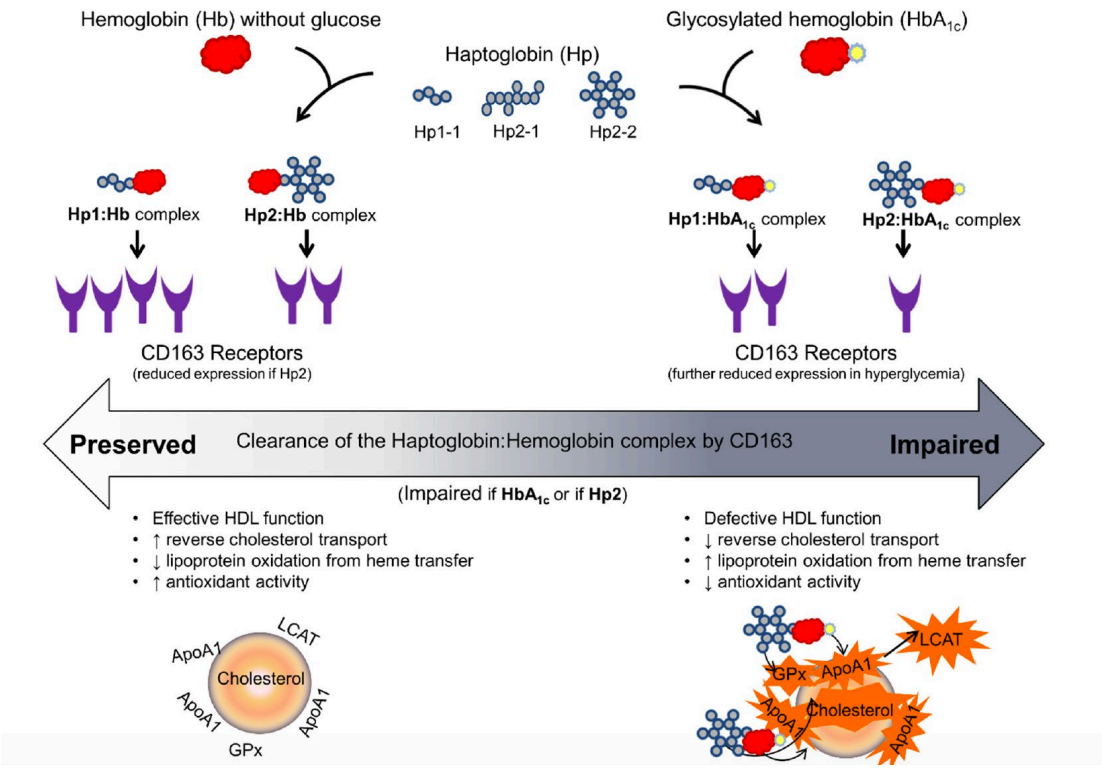


Figure Legend Free hemoglobin (Hb) is bound to haptoglobin (Hp) and cleared from the bloodstream via scavenger receptor CD163. CD163 receptor expression is reduced in hyperglycemic conditions. The clearance of Hp-Hb is further attenuated in patients with Hp2-2, resulting in increased circulating Hp-Hb complexes. Glycosylated Hp-Hb complexes impair the antioxidant activity of Hp and oxidizes HDL-cholesterol and its related components. The anti-atherogenic function of HDL-cholesterol is thus inhibited, paradoxically turning it into a pro-atherogenic molecule (this figure has been previously published(30)).

2.4 Other Predictors Of CVD And HDL-Cholesterol

CVD in both patients with and without T2DM can be influenced by other genetic, lifestyle and environmental factors including: age, hypertension, antihypertensive treatment, cigarette smoking, obesity, previously established CVD, family history of CVD, ethnicity/race, geographic area, diabetes duration, education and alcohol consumption. Other factors that are associated with HDL-cholesterol include: age, sex, hormone replacement therapy, cigarette smoking, obesity, ethnicity/race and alcohol consumption. For more information and specific literature references regarding the above predictors of CVD and/or HDL-cholesterol, see Appendix 1.

2.5 Research Gap

Dyslipidemia is a major CVD risk factor in T2DM and a residual risk in these patients, compared to their non-diabetic counterparts, remains after optimal statin therapy. Despite the established CVD risk associated with low HDL-cholesterol and high triglycerides, inconsistent results have been reported in trials that aimed to reduce CVD events by therapeutically raising HDL-cholesterol and lowering triglycerides as monotherapy and, in combination with statins, trials have failed to demonstrate an additional benefit(19–21,23–25). A gap in knowledge exists about why therapeutically raising HDL-cholesterol and lowering triglycerides has not consistently been demonstrated to prevent CVD and why there is no evidence of added clinical benefit when already on optimal statin therapy.

Chapter 3: Objectives

3.0 Objectives

The present study is a re-analysis of data from the ACCORD lipid trial while incorporating haptoglobin phenotype to address the following two objectives:

Objective 1: To determine whether the effect of adding fenofibrate to simvastatin therapy on risk of CHD and total CVD events in high risk patients with T2DM depends on Hp phenotype (Hp2-2 or Hp1 allele carrier), and to determine these effects in

i) Men and women separately

ii) Primary and secondary prevention patients separately

Objective 2**:** To determine whether the association between HDL-cholesterol and risk of CHD and total CVD events in high risk patients with T2DM depends on Hp phenotype (Hp2-2 or Hp1 allele carrier), and to determine these effects in

i) Men and women separately

ii) Primary and secondary prevention patients separately

3.1 Summary

The aim for objective 1 (Chapter 4) was to replicate the original ACCORD lipid trial analysis as closely as possible with stratification by Hp phenotype. This allowed for observation of what the results of the ACCORD lipid trial would have been if it had been conducted in each phenotype group separately, and to test whether the effects within the Hp phenotype groups were different from each other. Therefore, the analysis was

conducted following the intention-to-treat principal, with assignment to fenofibrate therapy as the exposure variable.

In objective 2 (Chapter 5), the aim was to investigate the association between the actual repeated measures of HDL-cholesterol concentrations and each of the outcomes of interest directly over the duration of the study. Therefore, the updated measures of HDL-cholesterol over time serve as the exposure variable for this time-dependent biomarker analysis.

Chapter 4: Haptoglobin Phenotype, Fenofibrate Therapy, And Risk Of Cardiovascular Disease Events Within The Action To Control Cardiovascular Risk In Diabetes (ACCORD) Lipid Trial

4.0 Abstract

Background: The Hp2-2 phenotype (~40% of people) is associated with dysfunctional HDL because HDL bound to Hp2-2 can become heavily oxidized in hyperglycemia. The effect of medications that raise potentially pro-atherogenic HDL-cholesterol on risk of CVD in the Hp2-2 phenotype in hyperglycemia is unknown.

Objective: To determine whether the effect of adding fenofibrate therapy to simvastatin therapy on risk of coronary heart disease (CHD) and total CVD events in type 2 diabetes mellitus (T2DM) depends on Hp phenotype (Hp2-2 or Hp1 allele carrier) in the ACCORD lipid randomized trial.

Methods: Haptoglobin phenotype was determined using a validated assay in 4,996 men and women who participated in the ACCORD lipid trial with a mean follow-up of 4.7 years for CVD and 4.6 for CHD. Multivariable-adjusted hazards ratios and 95% confidence intervals from Cox proportional hazards regression were used to quantify the relationship between fenofibrate therapy and incident CHD and CVD, stratifying by Hp phenotype. Further stratifications by sex and CVD history were also performed.

Results: Compared to simvastatin therapy alone, combination therapy with fenofibrate lowered the risk of CHD in Hp1 allele carriers (HR=0.74, CI: 0.60-0.90) but not in patients with the Hp2-2 phenotype (1.16, 0.87-1.56, p, interaction=0.009). A similar but non-significant pattern was observed for risk of total CVD events with HRs of 0.82 (0.66-1.03) in Hp1 allele carriers and 1.02 (0.75-1.37) in patients with the Hp2-2 phenotype (p, interaction=0.20). Interactions between treatment and sex were observed for both Hp phenotypes, including HRs of 0.64, 0.50-0.81 for CHD and 0.74, 0.57-0.96 for CVD in men who were Hp1 allele carriers and of 2.55, 1.27-5.12 (CHD) and 1.89, 0.99-3.59 (CVD) in women with the Hp2-2 phenotype.

Conclusion: The effect of adding fenofibrate to simvastatin on CHD and CVD risk depends on Hp phenotype and sex in the ACCORD lipid randomized trial.

4.1 Introduction

People with type 2 diabetes mellitus (T2DM) have a substantially higher risk of CVD morbidity and mortality compared to those without diabetes(3). With the steady rise in the global prevalence of T2DM, diabetes-related CVD is a primary concern of health care providers(103). The increased incidence of CVD among T2DM is largely due to the augmented prevalence of well-known risk factors including hyperglycemia and dyslipidemia(2,3,6). Many studies have demonstrated a positive continuous association between hyperglycemia and CVD risk(7,8). Diabetic dyslipidemia is characterized by elevated plasma triglyceride (TG) levels, small dense low-density lipoprotein (LDL) particles, and low levels of high-density lipoprotein (HDL) cholesterol and is a major risk factor for atherosclerotic CVDs(6,9,10). Aggressive management of blood lipid levels is therefore generally necessary in patients with T2DM, and current guidelines recommend LDL-cholesterol management as the primary goal of therapy in T2DM(11,14–16). Although statins are efficacious in lowering LDL-cholesterol and reducing CVD risk in T2DM, they do not normalize overall lipid levels, and rates of CVD events remain high in T2DM even after statin therapy(17,18). The possible benefit of the addition of other lipid-modifying agents to statin therapy to reduce the residual cardiovascular risk has therefore attracted a great deal of interest(11).

Fibrates are drugs that raise HDL-cholesterol and lower triglycerides, however; clinical trials investigating the effect of fibrate monotherapy on incident CVD in T2DM have reported inconsistent results(19–21). Moreover, while several studies have demonstrated that fibrate and statin combination therapy is better at improving overall lipid profiles relative to fibrate or statin monotherapy, evidence that the combination

therapy reduces CVD events is lacking(22). The Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial was a large multi-center study that tested the effect of intensive blood glucose control and either intensive blood pressure control or fenofibrate and simvastatin combination lipid therapy on CVD outcomes in high-risk patients with T2DM. It was found that the combination of fenofibrate and simvastatin did not reduce the rate of CVD events compared to simvastatin alone(23). Similarly, two other large trials investigating the effect of niacin (HDL-cholesterol raising and triglyceride lowering drug) and statin combination therapy on CVD outcomes found no benefit compared to statin monotherapy(24,25). The reason for the failure of HDL-cholesterol raising and triglyceride lowering therapy in most of these clinical trials is unknown and may be due to differences in unmeasured characteristics between study participants, such as genetics.

A common variation in the gene that codes for the abundant plasma protein haptoglobin (Hp) has identified individuals who may be at increased risk of CHD from hyperglycemia and altered HDL-cholesterol function (26–31). The Hp protein functions to bind and clear free Hb in the blood, thereby preventing Hb-mediated oxidative damage to blood vessels and proteins. The common Hp variation is a copy number variant with two alleles, Hp1 and Hp2, that vary vastly in size (the Hp2 allele contains a large 1.8kb intragenic duplication of exons 3 and 4 of the Hp1 allele) and produce three structurally and functionally distinct Hp proteins (Hp1-1, Hp2-1 and Hp2-2) that determine an individual's Hp phenotype. Approximately 40% of people world-wide produce the Hp2-2 protein which is substantially larger, more cyclic, and has less antioxidant function compared to Hp1-1 and Hp2-1(32–34). In the setting of high blood sugar (often defined as glycated Hb≥ 6.5%) these effects are magnified, resulting in reduced ability of Hp to

clear Hb and prevent oxidation of serum and cellular proteins, and HDL-cholesterol dysfunction because Hp tethers to HDL-cholesterol(35–39). There is a resulting increase in pro-oxidant Hp-Hb complexes circulating in the blood of patients with the Hp2-2 phenotype, thus generating dysfunctional HDL that is potentially pro-atherogenic and is thought to increase susceptibility to atherosclerosis, deterioration of cardiac function and ultimately CHD(27,35,39). The effect of raising HDL-cholesterol levels in people with the Hp2-2 phenotype and hyperglycemia on risk of CHD events is currently unknown, but may not have a beneficial effect and may even be harmful, whereas it may be favorable in Hp1 allele carriers (Hp1-1 and Hp2-1) in whom the functions of Hp and HDL-cholesterol are better preserved.

The objective of the present study was to conduct a hypothesis driven re-analysis of the ACCORD lipid trial to determine the effect of adding fenofibrate therapy versus placebo to simvastatin on the risk of CHD and total CVD events in each of the Hp phenotype groups. Because women have higher HDL-cholesterol levels than men and sex differences may occur (40–43), and because secondary prevention patients may have a higher risk of CVD events (44,45), the effects in men and women separately as well as in primary and secondary prevention patients were also examined.

4.2 Methods

4.2.1 Study Design And Participants

A re-analysis of data from the ACCORD lipid trial with the addition of Hp phenotype was undertaken to assess the effect of fenofibrate therapy on CVD outcomes among each of the Hp phenotype groups. The design, methods, and major findings of the ACCORD trial (ClinicalTrials.gov Identifier: NCT00000620) have been reported

previously (23,104). Briefly, the ACCORD trial was a large-scale multi-center (77 clinical sites in Canada and the USA) double-blind 2x2 factorial design randomized control trial (RCT) designed to examine the effect of strict glycemic control and either intensive blood pressure control or fenofibrate and simvastatin combination lipid therapy on cardiovascular outcomes in 10,251 (men and women) high risk patients with T2DM. All participants in the trial had to have a glycated Hb level of ≤7.5% and were aged between 40-79 years if there was evidence of clinical CVD or between 55-79 years if there was anatomical evidence of significant atherosclerosis, albuminuria, left ventricular hypertrophy, or at least two additional risk factors for CVD at baseline. Participants were randomly assigned to either intensive glycemic control (targeting a glycated Hb level <6.0%) or standard therapy (targeting a glycated Hb level of 7.0 to 7.9%). In the lipid arm of the ACCORD trial, 5,518 patients were further randomized to receive either fenofibrate or placebo in addition to open-label background simvastatin over a mean follow-up of 4.7 years. Participants were eligible for the lipid trial if they had an LDL-cholesterol level of 60-180-mg/dL, HDL-cholesterol of <55-mg/dL for women and Blacks or less than 50-mg/dL for all others and a serum TG level of less than 750-mg/dL if not on a lipid medication or less than 400-mg/dL if on a lipid medication(23,66,104,105).

All participants in the ACCORD trial provided consent for future research. The ACCORD study was completed in 2009 and all collected specimens and data have since become available to non-ACCORD researchers through the National Institutes of Health's Open Biologic Specimen and Data Repository Information Coordinating Center (BioLINCC).

4.2.2 Haptoglobin Phenotyping

There is a 1:1 correspondence between Hp phenotype (determined based on size and shape of the Hp protein in the blood) and Hp genotype (Hp1-1, Hp2-1 and Hp2-2 determined based on DNA). The Hp phenotype of all ACCORD patients was determined using a previously validated high throughput enzyme linked immunosorbent assay (ELISA) with a sensitivity and specificity of 99% and 98.1% respectively(106). The ELISA can detect different Hp phenotypes in the serum (50 µl) based on shape and size, which is determined by the presence (Hp2 allele) or absence (Hp1 allele) of a copy number variant (CNV) polymorphism (a 1.7 kb partial in-frame intragenic duplication of the exons 3 and 4)(106). Hp phenotype does not change over time and therefore a blood sample from either baseline or a follow-up visit was used for each participant. Of the 5,518 ACCORD lipid participants, Hp phenotype was determined for 4,996 (90.5%). The loss of 522 participants occurred because serum samples from these participants had previously been depleted from measuring other biomarkers.

4.2.3 Cardiovascular Events

Outcome variables were major CHD events (defined as fatal CHD, non-fatal MI or unstable angina) and total CVD events (nonfatal MI, non-fatal stroke or CVD death), as in the original ACCORD lipid trial(23). Pre-specified definitions for MI, nonfatal stroke, CVD death and major CHD events were described in the ACCORD study protocol and can be found in Appendix 2(23).

4.2.4 Statistical Analysis

All statistical analyses were conducted using Stata/IC software version 15.1 (StataCorp. 2017. *Stata Statistical Software: Release 15.1*. College Station, TX:

StataCorp LP) at a 2-tailed alpha level of 0.05. With the exception of when the Hardy-Weinberg equilibrium testing was performed, Hp1-1 and Hp2-1 phenotypes were combined to form the group 'Hp1 allele carriers' which is a common approach because of the low frequency of the Hp1-1 phenotype (~15%) and the structure and function of the different Hp proteins(29,30,107,108).

Hardy-Weinberg equilibrium was tested using a permutation-based chi-square test. Participants were grouped based on a combination of their treatment assignment and Hp phenotype and baseline characteristics were compared using t-tests, one-way analysis of variance or Kruskal-Wallis tests for continuous variables and chi-square tests for categorical variables.

The analysis was kept as similar to the ACCORD lipid trial as possible with stratification by Hp phenotype(23). As such, the relationship between fenofibrate therapy and each of the two outcomes of interest was determined using Cox proportional hazards regression according to the intention-to-treat principle. The occurrence of events between treatment groups was compared using adjusted hazard ratios (HRs) and 95% confidence intervals for Hp1 allele carriers and Hp2-2s separately. Multivariable Cox regression models were adjusted for the same covariates (Appendix 3) that were included in the original ACCORD lipid analyses including: the seven clinical networks; assignment to intensive glycemic control; and a history of CVD at baseline (Appendix 2F). Additional adjustment was made for age, sex, ethnicity, and two variables that differed between treatment groups: baseline angiotensin receptor blocker use and baseline aspirin use. Further stratification was performed by sex and by previous CVD at baseline. Less than 3% of data were missing for any of the baseline variables so a complete case analysis was

used. Interactions were tested between treatment group and Hp phenotype, and then when stratified by Hp phenotype, between treatment group and sex as well as between treatment group and CVD history at baseline. Follow-up time was defined as the time from randomization to date of documented outcome (major CHD event or total CVD event), or until they were censored at 7 years after randomization if no event occurred.

4.3 Results

The distribution of Hp phenotype frequencies was 17.9% Hp1-1, 46.2% Hp2-1 and 35.9% Hp2-2 and was in Hardy-Weinberg equilibrium (HWE) (data not shown). Among the Hp1 allele carriers (n=3,201), 1,595 were randomized to receive fenofibrate and 1,606 to placebo in combination with simvastatin (Table 1). The mean age was 62.7±6.5, 33.5% were women and 34.6% had a history of CVD at baseline. Among those who had the Hp2-2 phenotype (n=1,795), 919 were in the fenofibrate group and 876 were in the placebo group, the mean age was 62.8±6.4, 31.2% were women and 35.9% had a history of CVD at baseline. Baseline characteristics are described according to Hp phenotype and treatment allocation. Hp1 allele carrier and Hp2-2 treatment groups differed in baseline triglycerides. Among patients with the Hp2-2 phenotype, the treatment groups had different baseline angiotensin receptor blocker and aspirin use. Lipid changes over the course of the study are shown in Figure 4.1. Of note, among Hp1 allele carriers, the mean HDL-cholesterol levels went from 38.5-mg/dL to 40.6-mg/dL in the placebo group and from 38.0-mg/dL to 41.2-mg/dL in the fenofibrate group. Among patients with the Hp2-2 phenotype, mean HDL-cholesterol levels went from 38.1-mg/dL to 40.6-mg/dL in the placebo group and from 38.1 to 41.2-mg/dL in the fenofibrate group.

Incidence rates of both major CHD events and total CVD events are presented for each Hp phenotype group and subgroups in Table 4.2. There was a significant interaction effect between Hp phenotype and treatment for CHD (p-value=0.009) but not for CVD (p-value=0.20) (Table 4.3). In multivariable adjusted Cox models, allocation to receive fenofibrate compared to placebo was associated with a 26% lower risk of major CHD events (HR= 0.74, 95% CI:0.60-0.90) and resulted in a non-significant HR of 0.82 (0.66-1.03) for total CVD prevention among the Hp1 allele carriers. Among patients with the Hp2-2 phenotype, there was no significant difference in the risk of major CHD events (1.16, 0.87-1.56) and total CVD events (1.02, 0.75-1.37) for the fenofibrate group compared to the placebo group. In a sensitivity analysis conducted among the largest ethnicity group (whites) only, the results for Hp1 allele carriers and patients with the Hp2-2 phenotype were not materially altered.

When the Hp1 allele carriers were stratified by sex (p-value for sex interaction= 0.02 for CHD and 0.10 for CVD), fenofibrate treatment assignment was associated with a 36% lower risk of major CHD events (0.64, 0.50-0.81) and a 26% lower risk of total CVD events (0.74, 0.57-0.96) compared to placebo in men, while there was no significant difference in the rates in women. Secondary prevention Hp1 allele carriers (history of clinically established CVD at baseline) who were randomized to fenofibrate had a 30% and 27% lower risk of major CHD events (0.70, 0.53-0.91) and total CVD events (0.73, 0.54-1.00) respectively while there was no significant effect in primary prevention (no history of clinically established CVD at baseline) patients. For the Hp2-2 phenotype stratified by sex, there was no significant difference in rates between treatment groups for both outcomes in men, however an increased risk of major CHD events (2.55, 1.27-5.12)

and a nonsignificant trend towards increased risk of CVD (1.89, 0.99-3.59) was observed in women allocated to receive fenofibrate (p-values for interaction= 0.002 for CHD and 0.007 for CVD). There was no significant difference in risk between treatment groups in primary or secondary prevention patients with the Hp2-2 phenotype.

4.4 Discussion

The current study is the first to investigate the effect of adjunct fenofibrate with simvastatin therapy on CVD events in T2DM by Hp phenotype. Using data from the ACCORD lipid trial, fenofibrate with background simvastatin, compared to simvastatin alone, reduced the risk of CHD in Hp1 allele carriers but not in the Hp2-2 phenotype. In prespecified subgroup analyses, there was a significantly reduced risk of CHD in men who were Hp1 allele carriers and an increased risk of CHD in women with the Hp2-2 phenotype. Among Hp1 allele carriers, fenofibrate reduced the risk of CVD in men and a nonsignificant trend towards harm in women was observed. When stratified by CVD history at baseline, there a significant benefit from fenofibrate in Hp1 allele carriers with a history of CVD only; however, the interaction effect was not statistically significant.

The original ACCORD lipid trial analysis did not reveal that fenofibrate and simvastatin combination therapy reduced the risk of CHD (0.92, 0.79-1.07) or CVD (0.92, 0.79-1.08) compared to simvastatin alone(23). The results of the present study suggest that the effect of adding fenofibrate to simvastatin on cardiovascular outcomes may have been confounded by Hp phenotype in the original trial. Had the original study been conducted in Hp1 allele carriers only, fenofibrate and simvastatin would likely have been reported to reduce the effect of CHD compared to simvastatin alone whereas it may have had no effect had the trial been conducted in only people with the Hp2-2 phenotype.

The main findings of this study support previous research on the role of Hp phenotype in HDL-cholesterol dysfunction and CHD risk in hyperglycemia(35–39,102). In several *in vitro* and *in vivo* studies, individuals with the Hp2-2 phenotype show reduced ability to protect against Hb-mediated oxidative damage resulting in increased inflammation, oxidative stress and dysfunctional HDL-cholesterol(35–38). In a subset of participants in the Atherothrombosis Intervention in Metabolic Syndrome with Low HDL/High Triglycerides: Impact on Global Health Outcomes (AIM-HIGH) study who had diabetes, Asleh et al. found that niacin improved HDL-cholesterol antioxidant function in individuals with Hp1-1 but worsened HDL-cholesterol antioxidant function in individuals with the Hp2-2 phenotype(102). More recently, Asleh et al. demonstrated a significant association between the Hp2-2 phenotype and microvascular endothelial dysfunction and increased HDL-cholesterol bound Hb in patients with early atherosclerotic disease and diabetes(39). Taken together, the results of these studies suggest that a pronounced risk of CHD in the Hp2-2 phenotype with hyperglycemia may be due, at least in part, to dysfunctional HDL prevalent in this setting. In the present study, it was demonstrated that HDL-cholesterol raising and triglyceride lowering therapy (fenofibrate) with background simvastatin was in fact only effective in preventing CHD compared to simvastatin alone in Hp1 allele carriers but not in the Hp2-2 phenotype in the setting of T2DM and it is hypothesized that this finding may be a result of dysfunctional HDL in the Hp2-2 phenotype.

Overall, there was no effect of added fenofibrate to simvastatin in either Hp phenotype for the total CVD outcome and may be because the composite CVD outcome includes CHD and stroke. Stroke is an endpoint that has been associated with the Hp1-1

phenotype rather than Hp2-2(98,99). It has been suggested that protection against stroke conferred by the Hp2-2 phenotype may be connected to the role of Hp phenotype in angiogenesis whereas protection against CHD has been linked to the function of Hp as a Hb scavenger and antioxidant(100,101). However, this study was underpowered to examine Hp1-1 participants alone or to detect the effect of fenofibrate on CVD in Hp1 allele carriers.

Sex differences in CVD are not well understood because women have typically been underrepresented in research(109,110). In the present study, it was not found that adding fenofibrate to simvastatin had a significant effect in women who were Hp1 allele carriers. Women have naturally higher levels of HDL-cholesterol than men, which may offer a potential explanation for why fenofibrate significantly reduced the risk of CHD among Hp1 allele carriers who were men but not in women(41,42). It is possible that fenofibrate therapy may have raised HDL-cholesterol enough to lower the risk in men but not in women. Men and women may also differ in their response to fibrates, as one study reported a significantly higher increase in HDL-cholesterol in women who took gemfibrozil compared to men(111). In a previous study on Hp phenotype and CHD risk, Cahill et al found the highest risk in patients with the Hp2-2 phenotype with glycated Hb≥6.5% (compared to Hp1 allele carriers with HbA1c <6.5) in the Nurses' Health Study, a cohort made up of entirely women and with relatively high HDL-cholesterol(29).

Among Hp1 allele carriers, a benefit from adding fenofibrate to simvastatin for CHD and total CVD prevention in patients with a history of CVD and a nonsignificant trend towards benefit in those without a history of CVD (interaction not significant) was

found. Secondary prevention patients have a higher risk of CVD events compared to those without a history of vascular disease(44,45) and power limited the ability to detect the effect in primary prevention patients.

Currently available genome-wide association study technologies have identified single nucleotide polymorphisms in partial linkage with the Hp polymorphism, but these polymorphisms have not been able to determine Hp2-2 genotypes or phenotype, explaining why the Hp polymorphism has not been identified in the previous genetic analyses of ACCORD participants, which only included single nucleotide polymorphisms(112–115).

The present findings are in accordance with the current literature on the biological mechanism linking Hp phenotype and HDL dysfunction in CHD. Free intravascular Hb is bound to Hp with high affinity, creating an Hp-Hb complex that is cleared from the bloodstream via scavenger receptor CD163, thereby preventing Hb-mediated oxidative damage to serum and cellular proteins(32,33). CD163 receptor expression is reduced in hyperglycemic conditions. Further, Hp2-2 is the largest and bulkiest of the Hp phenotypes and is less able to remove Hb from the blood compared to Hp1-1 and Hp2-1, resulting in an increased amount of Hb-Hp complexes left circulating in the bloodstream of people with the Hp2-2 phenotype and hyperglycemia(32). The impairment of Hp2-2 function is magnified in T2DM due to the associated increase in glycated Hb caused by hyperglycemia(35,36). Glycation of Hb hinders the antioxidant ability of Hp2-2, promoting pro-oxidant Hp-Hb complexes(27,35). Pro-oxidant Hp-Hb binds to and oxidizes HDL and its related components such as apolipoprotein A, glutathione peroxidase and lecithin-cholesterol acyltransferase. As a result, in people who have the

Hp2-2 phenotype and hyperglycemia, the ability of HDL to promote cholesterol efflux and prevent oxidization by free Hb is impaired, paradoxically making it pro-atherogenic(27,35). Increasing HDL-cholesterol may therefore be ineffective or counterproductive in preventing CHD in those with hyperglycemia and Hp2-2 because HDL function may be impaired and it may increase oxidative stress. However, increasing HDL-cholesterol in the Hp1 allele carriers may be beneficial because Hp and HDL function are better preserved.

4.5 Strengths And Weaknesses

The strengths of this study include that it was a double-blind randomized control trial, with a large sample size, minimal missing data, a validated Hp phenotyping method, and stratification by sex. However, the participants were all middle-aged and elderly individuals who were mostly non-Hispanic white, were at a high risk for CVD and who had chronic T2DM and hyperglycemia, and so it remains unknown whether these results are generalizable to other populations. This study was also underpowered to detect the effect of fenofibrate in combination with simvastatin in some of the subgroup analyses. Additionally, HDL-cholesterol levels and function were not examined directly in this study, for the analysis was conducted according to the intention-to-treat principle in order to facilitate comparison of results to the original ACCORD lipid trial results. Also, fenofibrate not only affects HDL but also lowers triglycerides, increases the size of LDL particles and has several other non-lipid effects including a reduction in systemic inflammation(90–92). Therefore, it can only be hypothesized that these results may be due to prominent HDL-dysfunction in patients with the Hp2-2 phenotype.

4.6 Future Directions And Implications

The next step is to examine the relationship between serum HDL-cholesterol and CHD and CVD in the different Hp phenotype groups to determine if there is a difference by Hp phenotype and sex. Additionally, replication in other trials examining the effect of HDL-cholesterol raising and triglyceride lowering drugs in T2DM is required to confirm the findings from this study. In future research, the effect of HDL-cholesterol raising and triglyceride lowering therapy within each phenotype group should also be explored in different ethnic groups and for stroke as a separate outcome. If the results of this study are found to replicate, then a trial of HDL-cholesterol raising and triglyceride lowering drugs in combination with statin therapy could be planned incorporating Hp phenotype into the study design to determine if Hp phenotype serves as a useful biomarker in clinical care.

Currently, statins are the first line of therapy in patients with T2DM and dyslipidemia, and guidelines do not support fibrate therapy for CVD prevention in patients who are already meeting LDL-cholesterol targets(11). The results of the present study suggest that fenofibrate therapy with background simvastatin may be beneficial for CHD prevention in some subgroups, especially men who are Hp1 allele carriers with hyperglycemia, but there is no evidence to suggest a benefit in patients with the Hp2-2 phenotype and hyperglycemia and may even be harmful in women with this phenotype. These findings provide an explanation for the failure of HDL-cholesterol raising and triglyceride lowering therapy previously reported and if replicated in future studies, Hp phenotype could potentially serve as a simple and inexpensive one-time test used to

personalize treatment of lipid abnormalities for greater precision of CVD prevention in people with T2DM.

Table 4.1 Baseline characteristics stratified by haptoglobin (Hp) phenotype*

Characteristic	Hp1 Allele Carriers			P-value	Hp2-2			P-value	Overall P-value
	All (n=3,201)	Fenofibrate (n=1,595)	Placebo (n=1,606)		All (n=1795)	Fenofibrate (n=919)	Placebo (n=876)		
Age (year)	62.7±6.5	62.6±6.4	62.9±6.5	0.37	62.8±6.4	62.7±6.4	62.8±6.4	0.87	0.84
Female sex- no.(%)	1073 (33.5)	529 (33.2)	544 (33.9)	0.67	560 (31.2)	288 (31.3)	272 (31.1)	0.90	0.40
Race or ethnic group- no.(%)				0.97				0.16	<0.001
White	2093 (65.4)	1044 (65.5)	1049 (65.3)		1198 (66.7)	616 (67.0)	582 (66.4)		
Black	549 (17.2)	269 (16.9)	280 (17.4)		181 (10.1)	80 (8.7)	101 (11.5)		
Hispanic	252 (7.9)	128 (8.0)	124 (7.7)		105 (5.9)	60 (6.5)	45 (5.1)		
Other	307 (9.6)	154 (9.7)	153 (9.5)		311 (17.3)	163 (17.7)	148 (16.9)		
Education- no.(%)				0.40				0.79	0.10
Less than high school	450 (14.1)	233 (14.6)	217 (13.5)		213 (11.9)	111 (12.1)	102 (11.6)		
High-school graduate or GED	840 (26.2)	418 (26.2)	422 (26.3)		467 (26.0)	234 (25.5)	233 (26.6)		
Some college	1063 (33.2)	509 (31.9)	544 (34.5)		573 (31.9)	288 (31.3)	285 (32.5)		
College degree or higher	845 (26.4)	433 (27.2)	412 (25.7)		542 (30.2)	286 (31.1)	256 (29.2)		
Previous cardiovascular event- no.(%)	1108 (34.6)	550 (34.5)	558 (34.7)	0.88	644 (35.9)	335 (36.5)	309 (35.3)	0.60	0.78
Previous congestive heart failure- no.(%)	184 (5.6)	99 (6.2)	85 (5.3)	0.27	76 (4.2)	38 (4.1)	38 (4.3)	0.83	0.08
Smoking status- no.(%)				0.23				0.79	0.49
Current	394 (12.3)	208 (13.0)	186 (11.6)		201 (11.2)	107 (11.6)	94 (10.7)		
Former	1466 (45.8)	740 (46.4)	726 (45.2)		852 (47.5)	437 (47.6)	415 (47.4)		
Never	1341 (41.9)	647 (40.6)	694 (43.2)		742 (41.3)	375 (40.8)	367 (41.9)		
Weight- kg	94.9±18.2	94.7±17.7	95.2±18.6	0.45	94.3±18.7	93.8±18.9	94.9±18.4	0.21	0.34
Body-mass index (kg/ m^2)	32.4±5.3	32.4±5.2	32.5±5.4	0.52	32.3±5.4	32.1±5.5	32.5±5.3	0.12	0.31

Characteristic	Hp1 allele carriers				Hp2-2s				
	All (n=3,201)	Fenofibrate (n=1,595)	Placebo (n=1,606)	P-value	All (n=1795)	Fenofibrate (n=919)	Placebo (n=876)	P-value	Overall P-value
Blood Pressure (mm Hg)									
Systolic	134.2±17.9	134.4±17.8	134.1±17.9	0.73	133.5±17.9	132.9±17.7	134.1±18.1	0.16	0.25
Diastolic	74.1±10.7	74.0±10.5	74.1±10.9	0.94	74.0±10.9	73.8±10.8	74.2±10.9	0.42	0.88
Medications- no.(%)									
Insulin	1108 (34.7)	562 (35.4)	546 (34.1)	0.45	586 (32.8)	295 (32.2)	291 (33.4)	0.61	0.43
Metformin	2091 (65.3)	1035 (64.9)	1056 (65.8)	0.61	1191 (66.4)	609 (66.3)	582 (66.4)	0.97	0.84
Any sulfonylurea	1744 (54.5)	872 (54.7)	872 (54.3)	0.83	1004 (55.9)	509 (55.4)	495 (56.5)	0.65	0.74
Any thiazolidinedione	649 (20.3)	309 (19.4)	340 (21.2)	0.21	364 (20.3)	193 (21.0)	171 (19.5)	0.43	0.53
Angiotensin-converting–enzyme inhibitor	1678 (52.4)	826 (51.8)	852 (53.1)	0.49	943 (52.5)	477 (51.9)	466 (53.2)	0.61	0.86
Angiotensin-receptor blocker	501 (15.7)	245 (15.4)	256 (15.9)	0.67	284 (15.8)	129 (14.0)	155 (17.7)	0.043	0.20
Aspirin	1816 (56.7)	913 (57.2)	902 (56.2)	0.55	1008 (56.2)	538 (58.5)	470 (53.7)	0.032	0.16
Beta-blocker	1041 (32.5)	527 (33.0)	514 (32.0)	0.52	572 (31.9)	294 (32.00)	278 (31.7)	0.88	0.88
Any thiazide diuretic	868 (27.1)	449 (28.2)	419 (26.1)	0.19	481 (26.8)	233 (25.4)	248 (28.3)	0.17	0.29
Any anti-hypertensive agent	2593 (81.0)	1289 (80.8)	1304 (81.2)	0.81	1448 (80.7)	729 (79.3)	719 (82.1)	0.16	0.54
Statin	1950 (60.9)	964 (60.4)	986 (61.4)	0.58	1126 (62.7)	576 (62.7)	550 (62.8)	0.98	0.59
Any lipid-lowering agent	2109 (65.9)	1051 (65.9)	1058 (65.9)	0.99	1196 (66.6)	607 (66.1)	589 (67.2)	0.64	0.92
Duration of diabetes (years)				0.81				0.77	0.90
Median	10	10	9		9	9	9.5		
Interquartile range	5 to 15	5 to 15	5 to 15		5 to 15	5 to 15	5 to 15		
Glycated hemoglobin (%)				0.15				0.59	0.50
Mean	8.3±1.0	8.3±1.0	8.2±1.0		8.3±1.0	8.3±1.1	8.3±1.0		
Median	8.1	8.1	8		8.1	8.1	8.1		
Interquartile range	7.6-.8	7.6-8.9	7.5-8.8		7.5-8.8	7.5-8.8	7.5-8.8		

Characteristic	Hp1 allele carriers				Hp2-2s				
	All (n=3,201)	Fenofibrate (n=1,595)	Placebo (n=1,606)	P-value	All (n=1795)	Fenofibrate (n=919)	Placebo (n=876)	P-value	Overall P-value
Plasma cholesterol (mg/dL)									
Total	175.2±37.2	174.8±37.2	175.7±37.2	0.50	177.2±38.0	176.0±37.1	178.4±38.9	0.19	0.15
Low-density lipoprotein	100.8±30.8	100.2±30.7	101.5±30.8	0.21	101.2±30.9	100.2±30.2	102.1±31.6	0.19	0.34
High-density lipoprotein				0.11					
Mean	38.3±7.9	38.0±8.1	38.5±7.7		38.0±7.5	38.1±7.2	38.1±7.9	0.89	0.37
Median	38	37	38		37	38	37		
IQR	33-43	32-43	33-43		33-43	33-43	33-43		
Plasma triglycerides (mg/dL)				0.07				0.76	<0.001
Median	159	162	157		169	170.5	168		
Interquartile range	111 to 227	113 to 231	109 to 224		120 to 241	119 to 241.5	121 to 241		
Triglycerides ≥204-mg/dL & High-density lipoprotein ≤34-mg/dL- no.(%)	531 (16.6)	282 (17.7)	249 (15.5)	0.10	326 (18.2)	160 (17.4)	166 (18.9)	0.40	0.14

* Plus–minus values are means ±SD. Percentages may not total 100 because of rounding. CVD=cardiovascular disease; GED= general equivalency diploma; Hp=haptoglobin; IQR= interquartile range.

Table 4.2. Unadjusted rate, person-years of follow-up and incidence of outcomes occurring up to 7 years from randomization for the two haptoglobin (Hp) phenotype groups separately

	Hp 1 allele carriers		Hp2-2s	
	Fenofibrate	**Placebo**	**Fenofibrate**	**Placebo**
CHD				
Total	**n=1595**	**n=1606**	**n=919**	**n=876**
No. of events (%)	169 (10.6)	219 (13.6)	104 (11.3)	83 (9.5)
Person-years	7356.6	7232.7	4260.6	4112.0
Incidence rate†	229.7	302.8	244.1	201.8
Men	**n=1066**	**n=1062**	**n=631**	**n=604**
No. of events (%)	117 (11.0)	166 (15.6)	69 (10.9)	71 (11.75)
Person-years	4943.1	4737.7	2960.9	2840.5
Incidence rate†	236.7	350.4	233.0	250.0
Women	**n=529**	**n=544**	**n=288**	**n=272**
No. of events (%)	52 (9.8)	53 (9.7)	35 (12.2)	12 (4.4)
Person-years	2413.5	2494.9	1299.7	1271.5
Incidence rate†	215.5	212.4	269.3	94.4
Previous CVD	**n=550**	**n=558**	**n=335**	**n=309**
No. of events (%)	96 (17.5)	132 (23.7)	62 (18.5)	49 (15.9)
Person-years	2450.8	2364.4	1516.3	1369.4
Incidence rate†	391.7	558.3	408.9	357.8
No previous CVD	**n=1045**	**n=1048**	**n=584**	**n=567**
No. of events (%)	73 (7.0)	87 (8.3)	42 (7.2)	34 (6.0)
Person-years	4905.8	4868.3	2744.4	2742.6
Incidence rate†	148.8	178.7	153.0	124.0
CVD				
Total	**n=1545**	**n=1606**	**n=919**	**n=876**
No. of events (%)	141 (8.8)	167 (10.4)	93 (10.1)	82 (9.4)
Person-years	7442.6	7390.0	4297.9	4139.7
Incidence rate†	189.4	226.0	216.4	198.1
Men	**n=1066**	**n=1062**	**n=631**	**n=604**
No. of events (%)	102 (9.6)	129 (12.2)	60 (9.5)	67 (11.1)
Person-years	4993.2	4848.7	2993.0	2866.2
Incidence rate†	204.3	266.1	200.5	233.8
Women	**n=529**	**n=544**	**n=288**	**n=272**

	Hp 1 allele carriers		Hp2-2s	
	Fenofibrate	**Placebo**	**Fenofibrate**	**Placebo**
No. of events (%)	39 (7.4)	38 (7.0)	33 (11.1)	15 (5.5)
Person-years	2449.4	2541.3	1305.0	1273.5
Incidence rate†	159.2	149.5	245.2	117.8
Previous CVD	**n=550**	**n=558**	**n=335**	**n=309**
No. of events (%)	74 (13.5)	99 (17.7)	54 (16.1)	45 (14.6)
Person-years	2524.3	2475.2	1537.1	1396.9
Incidence rate†	293.2	400.0	351.3	322.1
No previous CVD	**n=1045**	**n=1048**	**n=584**	**n=567**
No. of events (%)	67 (6.4)	68 (6.5)	39 (6.7)	37 (6.5)
Person-years	4918.4	4914.8	2760.9	2742.9
Incidence rate†	136.2	138.4	141.3	134.90

CHD= coronary heart disease; CVD =cardiovascular disease; Hp= haptoglobin

†Incidence rate is per 10,000 person-years and is not adjusted for covariates

Table 4.3. Multivariable-adjusted hazard ratios of outcomes if given fenofibrate therapy compared to placebo for the separate haptoglobin (Hp) phenotype groups.

	Hp1 allele carriers		Hp2-2		P-value for interaction**
	aHR* (95% CI)	P-value for interaction†	aHR* (95% CI)	P-value for interaction†	
CHD events	0.74 (0.60-0.90)		1.16 (0.87-1.56)		0.009
By Sex		0.02		0.002	
Men	0.64 (0.50-0.81)		0.90 (0.65-1.27)		
Women	1.11 (0.75-1.65)		2.55 (1.27-5.12)		
By CVD History		0.39		0.71	
No	0.82 (0.60-1.12)		1.24 (0.78-1.98)		
Yes	0.70 (0.53-0.91)		1.07 (0.73-1.56)		
CVD events	0.82 (0.66-1.03)		1.02 (0.75-1.37)		0.20
By Sex		0.10		0.007	
Men	0.74 (0.57-0.96)		0.80 (0.56-1.14)		
Women	1.23 (0.78-1.94)		1.89 (0.99-3.59)		
By CVD History		0.17		0.78	
No	0.99 (0.70-1.39)		1.05 (0.66-1.67)		
Yes	0.73 (0.54-1.00)		0.94 (0.62-1.41)		

aHR= adjusted hazard ratio; CHD= coronary heart disease; CI= confidence interval; CVD= cardiovascular disease; Hp= haptoglobin

*Adjusted hazard ratios compare fenofibrate therapy to the reference group of participants who received placebo. Models were adjusted for age, sex, the seven clinical centre networks, assignment to intensive glycemic control, history of CVD at baseline, ethnicity, baseline triglycerides, baseline use of angiotensin receptor blockers and baseline use of aspirin.

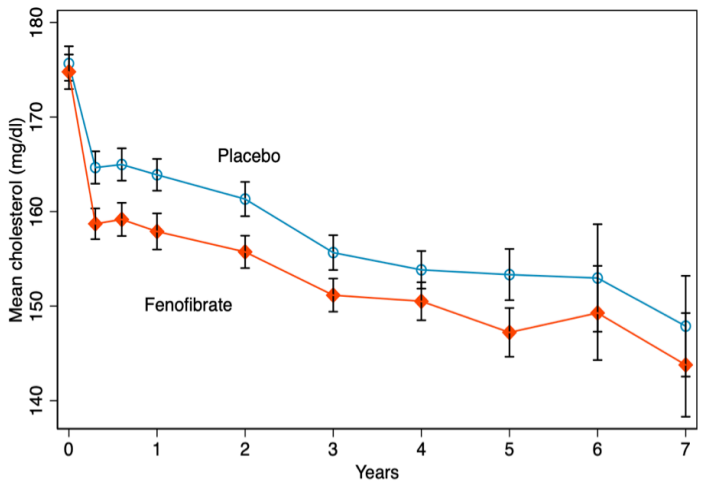
**P-values for interaction between fenofibrate treatment Hp phenotype.

†P-values for interaction between fenofibrate treatment and either sex or history of CVD at baseline within Hp phenotypes.

Figure 4.1. Lipid values by treatment in each phenotype group over time

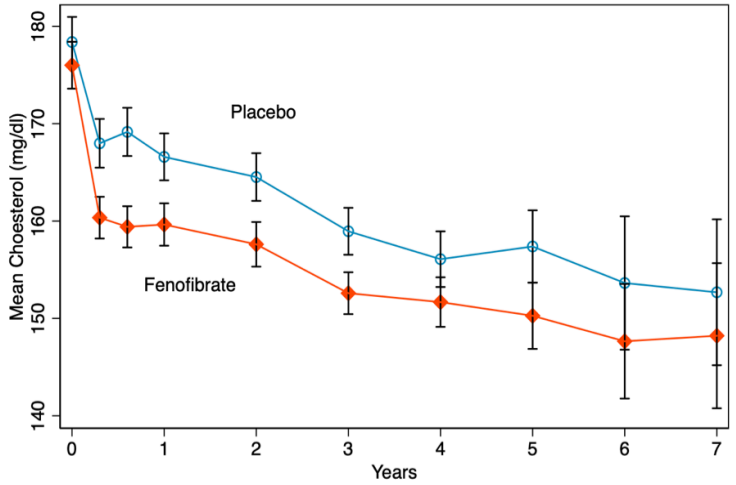
A) Mean total cholesterol

Hp1 allele carriers



No. of Patients								
Fenofibrate	1590	1512	1473	1413	1057	591	177	144
Placebo	1600	1534	1493	1418	1069	592	172	145

Hp2-2s

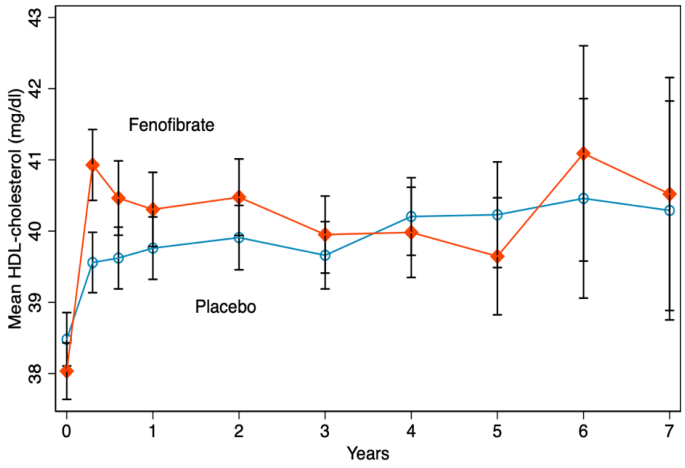


No. of Patients								
Fenofibrate	912	878	850	830	630	359	105	86
Placebo	869	839	807	782	608	355	97	80

Fenofibrate Placebo

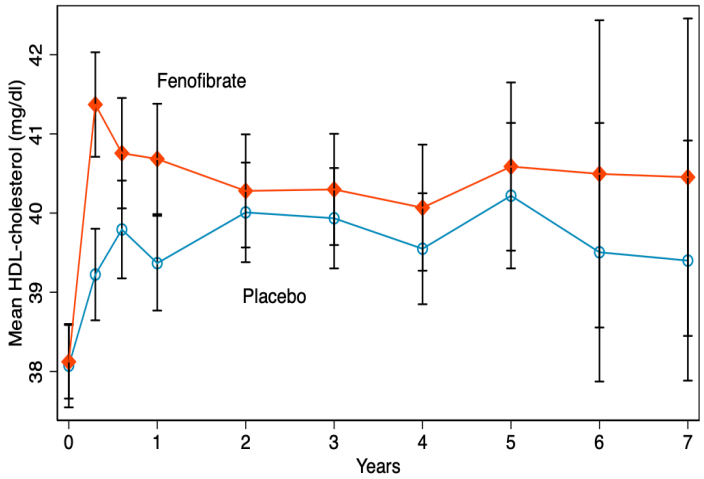
B) Mean HDL-cholesterol

Hp1 allele carriers



No. of Patients								
Fenofibrate	1590	1512	1473	1413	1057	590	177	144
Placebo	1601	1534	1493	1418	1069	592	172	145

Hp2-2s

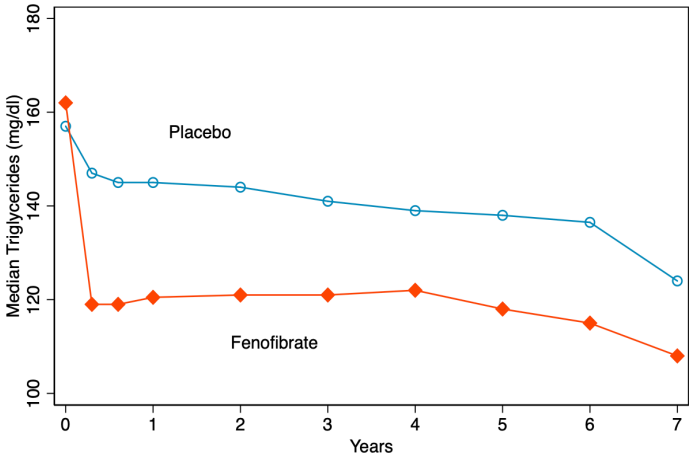


No. of Patients								
Fenofibrate	912	878	850	830	630	359	105	86
Placebo	869	839	807	782	608	355	97	80

Fenofibrate Placebo

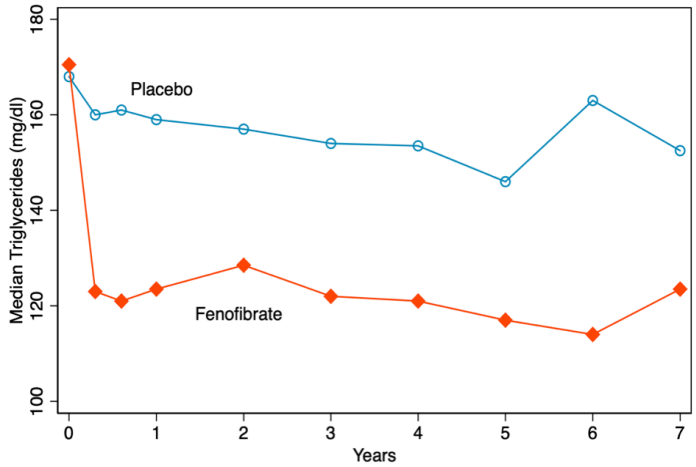
C) Median triglycerides

Hp1 allele carriers



No. of Patients								
Fenofibrate	1590	1512	1473	1413	1057	591	177	144
Placebo	1600	1534	1493	1418	1069	592	172	145

Hp2-2s

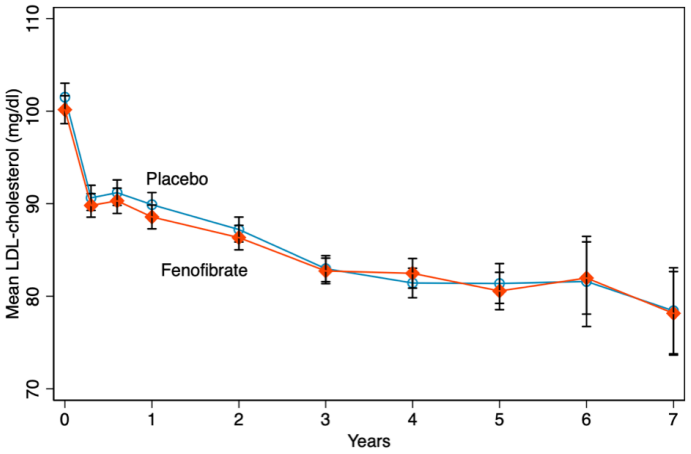


No. of Patients								
Fenofibrate	912	878	850	830	630	359	105	86
Placebo	869	839	807	782	608	355	97	80

Fenofibrate Placebo

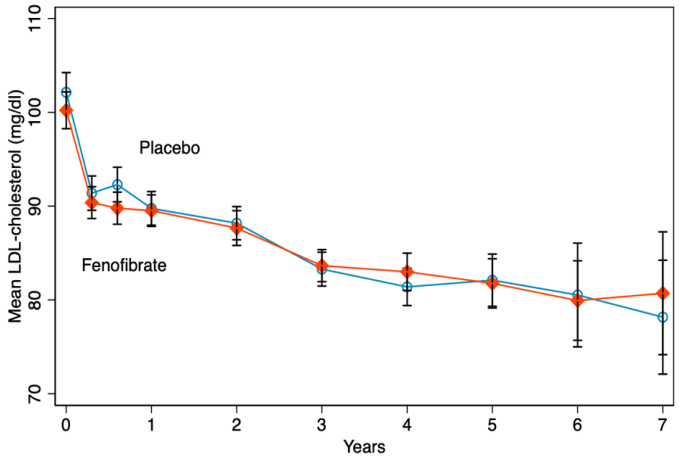
D) Mean LDL-cholesterol

Hp1 allele carriers



No. of Patients								
Fenofibrate	1590	1512	1473	1413	1057	590	177	144
Placebo	1600	1534	1493	1418	1069	592	172	145

Hp2-2s



No. of Patients								
Fenofibrate	912	878	850	830	630	359	105	86
Placebo	869	839	807	782	608	355	97	80

Fenofibrate Placebo

Figure Legend. Shown are mean plasma levels of total cholesterol (A), HDL-cholesterol (B), median triglycerides (C) and mean low-density lipoprotein (LDL) cholesterol at baseline, 4 months, 8 months, 1 year, and annually thereafter for Hp 1 allele carriers and patients with the Hp2-2 phenotype separately. P-values for differences between study groups at 4 months and the end of the study among Hp 1 allele carriers were, respectively: total cholesterol, P<0.001 and P = 0.12; LDL cholesterol, P = 0.39 and P = 0.23; HDL cholesterol, P<0.001 and P=0.13; and triglycerides, P<0.001 for both comparisons with the use of nonparametric tests. P-values for differences between study groups at 4 months and the end of the study among patients with the Hp2-2 phenotype, respectively: total cholesterol, P<0.001 and P = 0.03; LDL cholesterol, P = 0.43 and P = 0.76; HDL cholesterol, P<0.001 and P=0.25; and triglycerides, P<0.001 for both comparisons with the use of nonparametric tests. End-of-study visits were those that occurred in early 2009 and included follow-up at years 4, 5, 6, and 7.

Chapter 5: Haptoglobin Phenotype And The Association Between HDL-Cholesterol And Cardiovascular Disease Events Within The Action To Control Cardiovascular Risk In Diabetes (ACCORD) Lipid Trial

5.0 Abstract

Background: The Hp2-2 phenotype (~40% of people) is associated with dysfunctional high-density lipoprotein (HDL) because HDL bound to Hp2-2 can become heavily oxidized in hyperglycemia. The association between HDL-cholesterol and CVD events in the Hp2-2 phenotype in hyperglycemia is unknown.

Objective: To determine whether the association between HDL-cholesterol and CVD events (CHD and total CVD) in type 2 diabetes mellitus (T2DM) depends on Hp phenotype (Hp2-2 or Hp1 allele carrier) in the ACCORD lipid randomized trial.

Methods: Haptoglobin phenotype was determined using a validated assay in 4,996 men and women who participated in the ACCORD lipid trial with a mean follow-up of 4.7 years for CVD and 4.6 for CHD. Multivariable-adjusted hazards ratios and 95% confidence intervals from Cox proportional hazards regression with time-dependent covariates were used to quantify the relationship between a 1-mg/dL increase in incident CHD and CVD events, stratifying by Hp phenotype. Further stratifications by sex and history of CVD were also performed.

Results: Among Hp1 allele carriers, a 1-mg/dL increase in HDL-cholesterol was associated with a 1.8-3.3% and a 2.2-3.1% significantly reduced risk of CHD and CVD respectively (p= <0.001-0.04) depending on sex and CVD history, and these associations were generally attenuated with additional adjustment for triglycerides, although in some subgroups the association was unaffected by triglyceride adjustment. Among patients with the Hp2-2 phenotype, there were no significant associations observed, however; the interactions between Hp phenotype and HDL-cholesterol for the outcomes of interest were not significant (all p-interaction values ≥0.2).

Conclusion: These results do not support a significant interaction effect between HDL-cholesterol and Hp phenotype on risk of incident CHD or CVD in hyperglycemia. However, a significant inverse association between HDL-cholesterol and risk of CHD and CVD events was not present among participants with the Hp2-2 phenotype, suggesting that a cardioprotective association between HDL-cholesterol concentration and cardiovascular outcomes may only exist in Hp1 allele carriers.

5.1 Introduction

People with type 2 diabetes mellitus (T2DM) suffer higher rates of CVD compared to those without T2DM, largely due to a high prevalence of CVD risk factors in this population including dyslipidemia(2,3,6). The most common form of dyslipidemia in T2DM consists of hypertriglyceridemia, elevated plasma levels of small dense low-density lipoprotein (LDL) cholesterol, and low high-density lipoprotein (HDL) cholesterol levels(6,9,10). Numerous studies have demonstrated the benefit of lowering LDL-cholesterol with statins in patients with and without T2DM(12,13). However, rates of CVD remain high in treated patients, and the potential benefit of modifying other lipids to reduce the residual risk has attracted a great deal of interest(11).

HDL-cholesterol has long been postulated to be cardio-protective and has a well-established inverse association with CVD in patients with and without T2DM, even in patients on optimal statin therapy(77–83). The protection conferred by HDL-cholesterol has often been attributed to its anti-atherogenic functions which include reverse cholesterol transport and the ability to act as a potent antioxidant and anti-inflammatory. Implicit in this view is that the level of HDL-cholesterol in the blood is a biomarker of the ability of HDL to mediate anti-atherogenic function. However, the results of human genetic studies and the failure of recent clinical trials aiming to reduce CVD events by pharmacologically raising HDL-cholesterol have cast doubt on the HDL hypothesis(23,24,116–119). Mendelian randomization studies have generally not found a lower risk of CVD among individuals with a predisposition for high HDL-cholesterol(116). Existing drugs that raise HDL-cholesterol by inhibiting cholesterol ester transfer protein or by using extended release niacin have not impacted clinical outcomes

in several large clinical trials(117–119). In the ACCORD lipid trial, compared to statin monotherapy, combination therapy with fenofibrate did not reduce the risk of CVD outcomes(23). It is now recognized that the quantity of HDL-cholesterol does not necessarily reflect its quality or anti-atherogenic function, and that although two individuals may have the same concentration of serum HDL-cholesterol, the quality of their HDL, and as a result their CVD risk profile, may differ(94).

A common variation in the gene that codes for the abundant plasma protein Hp has identified individuals who may be at increased risk of CHD from hyperglycemia and altered HDL-cholesterol function(26–31). The Hp protein is abundant in human plasma, performing several functions with its main role being to bind and clear free Hb, thereby preventing Hb-mediated oxidative damage to blood vessels and proteins. Due to a copy number variant in the Hp gene with two alleles, Hp1 and Hp2, three Hp phenotypes exist (Hp1-1, Hp2-1 and Hp2-2), that each produce a structurally and functionally distinct Hp protein. Approximately 40% of people world-wide produce the Hp2-2 protein which is substantially larger, more cyclic, and has repeatedly demonstrated less antioxidant function compared to Hp1-1 and Hp2-1(32–34). In the setting of hyperglycemia (often defined as glycated Hb $\geq$ 6.5%) these effects are magnified, resulting in reduced ability of Hp to clear Hb and prevent oxidation of serum and cellular proteins, and HDL-cholesterol dysfunction because Hp tethers to HDL-cholesterol(35–39). There is a resulting increase in pro-oxidant Hp-Hb complexes circulating in the blood of patients with the Hp2-2 phenotype, thus generating dysfunctional HDL-cholesterol that is potentially pro-atherogenic and is thought to increase susceptibility to atherosclerosis, deterioration of cardiac function and ultimately CHD(27,35,39). The association of HDL-

cholesterol levels and CHD risk in individuals with the Hp2-2 phenotype and high blood sugar is currently unknown, but it may not be positive whereas higher levels may be favorable in Hp1 allele carriers (Hp1-1 and Hp2-1) in whom the functions of Hp and HDL-cholesterol are better preserved.

The objective of the present study was to conduct a hypothesis driven re-analysis of data from the ACCORD lipid trial to determine the association between HDL-cholesterol and risk of CHD and total CVD events in each of the Hp phenotype groups separately. Additionally, the above association was also investigated in men and women separately as well as in primary and secondary prevention patients.

5.2 Methods

5.2.1 Study Design And Participants

A re-analysis of data from the ACCORD lipid trial with the addition of Hp phenotype was undertaken to determine the relationship between HDL-cholesterol and CVD outcomes among each of the Hp phenotype groups. The design, methods, and major findings of the ACCORD trial (ClinicalTrials.gov Identifier: NCT00000620) have been reported previously (23,66,104,105). Briefly, the ACCORD trial was a large-scale multi-center (77 clinical sites in Canada and the USA) double-blind 2x2 factorial design randomized control trial (RCT) designed to examine the effect of strict glycemic control and either intensive blood pressure control or fenofibrate and simvastatin combination lipid therapy on cardiovascular outcomes in 10,251 (men and women) high risk patients with T2DM. All participants in the trial had to have a glycated Hb level of ≤7.5% and were aged between 40-79 years if there was evidence of clinical CVD or between 55-79 years if there was anatomical evidence of significant atherosclerosis, albuminuria, left

ventricular hypertrophy, or at least two additional risk factors for CVD at baseline. Participants were randomly assigned to either intensive glycemic control (targeting a glycated Hb (HbA1c) level <6.0%) or standard therapy (targeting a HbA1c level of 7.0 to 7.9%). In the lipid arm of the ACCORD trial, 5,518 patients were further randomized to receive either fenofibrate or placebo in addition to open-label background simvastatin over a mean follow-up of 4.7 years. Participants had a baseline LDL-cholesterol level of 60-180-mg/dL, HDL-cholesterol of <55-mg/dL for women and Black participants or otherwise <50-mg/dL, and a serum TG level <750-mg/dL if not on a lipid medication or <400-mg/dL if on a lipid medication. Lipid and HbA1c determinations were performed at the ACCORD central laboratory. A fasting plasma lipid profile was measured at baseline; 4, 8, 12 months post randomization; annually thereafter; and at study end. HbA1c was measured at baseline; every 4 months thereafter; and at study end(23,66,104).

All participants in the ACCORD trial provided consent for future research. The ACCORD study was completed in 2009 and all collected specimens and data have since become available to non-ACCORD researchers through the National Institutes of Health's Open Biologic Specimen and Data Repository Information Coordinating Center (BioLINCC).

5.2.2 Haptoglobin Phenotyping

The Hp phenotype of all ACCORD patients was determined using a previously validated high throughput enzyme linked immunosorbent assay (ELISA) with a sensitivity and specificity of 99% and 98.1% respectively(106). The ELISA can detect different Hp phenotypes in the serum (50 µl) based on shape and size, which is determined by the presence (Hp2 allele) or absence (Hp1 allele) of a copy number variant

(CNV) polymorphism (a 1.7 kb partial in-frame intragenic duplication of the exons 3 and 4)(106). Hp phenotype does not change over time and therefore a blood sample from either baseline or a follow-up visit was used for each participant. Of the 5,518 ACCORD lipid participants, Hp phenotype was determined for 4,996 (90.5%). The loss of 522 participants occurred because serum samples from these participants had previously been depleted from measuring other biomarkers.

5.2.3 Cardiovascular Events

Outcome variables were major CHD events (defined as fatal CHD, non-fatal MI, or unstable angina) and total CVD events (nonfatal MI, non-fatal stroke or CVD death), as in the original ACCORD lipid trial(23). Pre-specified definitions for MI, nonfatal stroke, CVD death and major CHD events were described previously in the ACCORD study protocol and can be found in Appendix 2(23).

5.2.4 Statistical Analysis

All statistical analyses were conducted using Stata/IC software version 15.1 (StataCorp. 2017. *Stata Statistical Software: Release 15.1*. College Station, TX: StataCorp LP) at a 2-tailed alpha level of 0.05. With the exception of when Hardy-Weinberg equilibrium testing was conducted, Hp1-1 and Hp2-1 phenotypes were combined to form the group 'Hp1 allele carriers' which is a common approach because of the low frequency of the Hp1-1 phenotype (~15%) and the structure and function of the different Hp proteins(29,30,107,108).

Hardy-Weinberg equilibrium was tested using a permutation-based chi-square test. Participants were grouped based on Hp phenotype and baseline characteristics were compared using t-tests and Kruskal-Wallis tests for continuous variables and chi-square

tests for categorical variables. Adjusted hazard ratios with 95% confidence intervals estimated from Cox proportional hazards regression models were used to quantify the relationship between a 1-mg/dL increase in HDL-cholesterol and CVD outcomes in each phenotype group separately. Covariable catergorization can be found in Appendix 3. Time-independent covariables recorded at baseline included: age, sex, ethnicity, alcohol consumption (drinker/ non-drinker), diabetes duration (≤10 years/ >10 years), a history of CVD (present/ absent, Appendix 2F), family history of CVD (absent/ premature heart disease or stroke/ heart disease or stroke at an unknown age/ unknown), education (high school/ less than high school/ some college/ college degree or higher), glycemic control group assignment (standard/ strict), lipid treatment assignment (placebo/ fenofibrate) and baseline statin use (present/ absent). Time-varying covariables included HDL-cholesterol (mg/dL), LDL-cholesterol (mg/dL), triglycerides (mg/dL), cigarette smoking status (current/ past/ never), glycated Hb (%HbA1c), hypertension (present/ absent) and anti-hypertensive medication use (present/ absent). Time-varying covariables and last observation carried forward were used to relate the most recent measure for each of those variables to incident CVD outcomes to avoid potential bias from using a single baseline measurement. Robust variance estimates adjusting for within-subject correlation of repeated measures were used. Base models were adjusted for age and sex (model 1) and two models with progressive adjustment were employed (model 2: additional adjustment for LDL-cholesterol, cigarette smoking status, hypertension, anti-hypertensive medication use, HbA1c, ethnicity, alcohol consumption, diabetes duration, CVD history at baseline, family history of CVD, education, glycemic control assignment, lipid treatment assignment and baseline statin use; model 3: additional adjustment for

triglycerides). Other variables that were considered but were not included because they did not influence the outcome in univariable analyses (p-value > 0.20) for either outcome in either phenotype group included: BMI (kg/m^2), clinical network and hormone replacement therapy.

Analyses stratified by sex and previous CVD at baseline were also performed in each phenotype group to determine the relationship between HDL-cholesterol and CVD outcomes in men and women and in primary (no history of clinically established CVD at baseline) and secondary prevention patients (history of clinically established CVD at baseline) separately. Interactions were tested between treatment group and Hp phenotype, and then when stratified by Hp phenotype, between treatment group and sex as well as between treatment group and CVD history at baseline. Follow-up time was defined as the time from randomization to date of documented outcome (major CHD events or total CVD events), or until they were censored at 7 years after randomization if no event occurred.

5.3 Results

The distribution of Hp phenotype frequencies was 17.9% Hp1-1, 46.2% Hp2-1 and 35.9% Hp2-2 and was in Hardy-Weinberg equilibrium (HWE) (data not shown). Baseline characteristics are described according to Hp phenotype group (Table 5.1). Among Hp1 allele carriers (n=3,201), the mean age was 62.7±6.5, 33.5% were women and 34.6% had a history of CVD at baseline. Among patients with the Hp2-2 phenotype (n=1,795), the mean age was 62.8±6.4, 31.2% were women and 35.9% had a history of CVD at baseline. Hp1 allele carriers and patients with the Hp2-2 phenotype had significantly different ethnic profiles, education, clinical network distribution and

baseline triglycerides. Triglycerides were skewed and so a log transformation was performed for multivariable analyses. Less than 3% of data were missing for any of the baseline variables. Time-varying characteristics of participants over the follow-up period are shown in Table 5.7.1 (Supplementary Material). By the end of the study, mean HDL-cholesterol in the Hp1 allele carriers increased from 38.3-40.9-mg/dL and from 38.1-40.9-mg/dL in patients with the Hp2-2 phenotype (Table 5.7.1, Figure 5.1).

Incidence rates of both major CHD events and total CVD events are presented for each Hp phenotype group and subgroups in Table 5.2. There was no significant interaction effect between Hp phenotype and HDL-cholesterol for CHD or CVD in any models (all p-values ≥0.20, data not shown). When adjusting for age and sex among Hp1 allele carriers (model 1 in Table 5.3), a 1-mg/dL increase in HDL-cholesterol was significantly associated with a 2.7% and 2.5% decreased risk of CHD and CVD respectively. The results did not materially change upon further adjustment for LDL-cholesterol, cigarette smoking status, hypertension, anti-hypertensive medication use, HbA1c, ethnicity, alcohol consumption, diabetes duration, CVD history at baseline, family history of CVD, education, glycemic control assignment, lipid treatment assignment and baseline statin use (model 2). Additional adjustment for triglycerides (model 3) resulted in a 1.8% and 2.0% significantly decreased risk of CHD and CVD respectively in Hp1 allele carriers. Among Hp2-2s, in model 1 (Table 5.4), a 1-mg/dL increase in HDL-cholesterol was significantly associated with a 2.4% and 2.1% decreased risk of CHD and CVD respectively; however, there was no association between HDL-cholesterol and either CHD or CVD upon further adjustment in models 2 and 3. In a sensitivity analysis conducted among the largest ethnicity group (whites) only,

the results for Hp1 allele carriers and patients with the Hp2-2 phenotype were not materially altered.

When Hp1 allele carriers were stratified by sex, a 1-mg/dL increase in HDL-cholesterol was associated with a 2.1% and 1.9% decreased risk of CHD and CVD respectively in men when adjusting for age. Results were similar with additional adjustment in model 2. Upon further adjustment for triglycerides (model 3), there was no association between HDL-cholesterol and CHD and CVD. In women who were Hp1 allele carriers, a 1-mg/dL increase in HDL-cholesterol was associated with a 3.9% and 3.7% decreased risk of CHD and CVD respectively when adjusting for age. Upon further adjustment, a 1-mg/dL increase in HDL-cholesterol was associated with a 3.0% and 2.9% (model 2) and a 2.6% and 3.2% (model 3) decreased risk of CHD and CVD respectively. Among men with the Hp2-2 phenotype, a 1-mg/dL increase in HDL-cholesterol was associated with a 2.8% decreased risk of CHD when adjusting for age, but there was no association with CVD. Upon further adjustment in models 2 and 3, there was no association between CHD or CVD and HDL-cholesterol. Among women with the Hp2-2 phenotype, a 1-mg/dL increase in HDL-cholesterol was associated with a 3.7% decreased risk of CVD when adjusting for age but the association was eliminated in models 2 and 3. There was no association between CHD and HDL-cholesterol in any of the models in women with the Hp2-2 phenotype.

In Hp1 allele carriers without a history of CVD at baseline, a 1-mg/dL increase in HDL-cholesterol was significantly associated with a 3.2% and a 3.3% decreased risk of CHD and CVD respectively in model 1 and similar results were observed in model 2. Upon additional adjustment for triglycerides in model 3, a 1-mg/dL increase in HDL-

cholesterol was significantly associated with a 2.3% decreased risk of CHD and the association with CVD was eliminated. In secondary prevention patients who were Hp1 allele carriers, a 1-mg/dL increase in HDL-cholesterol was significantly associated with a 1.7% decreased risk of CHD in model 1 and results did not materially change in model 2, however there was no significant association in model 3. There was no association observed between HDL-cholesterol and CVD in secondary prevention Hp1 allele carriers. Among patients with the Hp2-2 phenotype, there was no association between HDL-cholesterol and CVD observed in primary or secondary prevention patients, or between CHD and HDL-cholesterol in secondary prevention patients. There was a significant inverse association observed between HDL-cholesterol and CHD primary prevention patients with the Hp2-2 phenotype in model 1 but not in models 2 and 3.

5.4 Discussion

The current study is the first to investigate the relationship between HDL-cholesterol and incident CVD outcomes in T2DM by Hp phenotype. Among Hp1 allele carriers, a 1-mg/dL increase in HDL-cholesterol was associated with a significantly reduced risk of CHD and CVD, and among patients with the Hp2-2 phenotype there were no significant associations observed between HDL-cholesterol and risk of CHD or CVD. However, there was no evidence of an interaction between a 1-mg/dL increase HDL-cholesterol and Hp phenotype on CHD and CVD outcomes.

The results in Hp1 allele carriers are in accordance with previous studies that have concluded that there is an inverse relationship between HDL-cholesterol and CVD events, even in patients on optimal statin therapy(77–83). Adjustment for triglycerides attenuated the association and may be due to the fact that metabolism of HDL-cholesterol

and triglycerides are closely interrelated and therefore both variables, to a certain degree, may measure similar metabolic abnormalities(86,87). Among patients with the Hp2-2 phenotype, there were no significant associations between HDL-cholesterol and CHD or CVD events after adjustment for important covariables. There was no significant interaction between Hp phenotype and HDL-cholesterol on the outcomes in any model, however; we were underpowered to detect the effect for a 1-mg/dL increase in HDL-cholesterol among patients with the Hp2-2 phenotype and may not have been powered to detect a significant interaction effect as interactions often require more power than main effects(120). Had the ACCORD lipid study been conducted in only Hp1 allele carriers, a significant association between HDL-cholesterol and CHD and CVD would likely have been found whereas had the trial been conducted in patients with the Hp2-2 phenotype there may not have been a significant association or a weaker association may have been found.

The results in men and women separately are in agreement with previous epidemiological studies that, when adjusting for age, blood pressure, smoking, body mass index, and LDL-cholesterol, found that a 1-mg/dL increase in HDL-cholesterol was associated with a 2% and 3% reduced CHD risk in men and women respectively who were free of clinical evidence of CHD at baseline(79). The non-significant results observed in men in the present study after adjustment for triglycerides are likely due to sample size as this study was underpowered to detect those effects. Sex differences in CVD are not well understood because women have typically been underrepresented in research(109,110). However, the higher risk reduction with increasing HDL-cholesterol demonstrated in women than in men is consistent with the current body of literature and

it has been suggested that low levels of HDL-cholesterol may be more predicative of CHD in women than in men(79,121,122).

Among patients with the Hp2-2 phenotype, there were no significant associations between HDL-cholesterol and CHD or CVD in men or women after adjustment for important covariables. For the CHD outcome, a trend towards a null association among women was observed, although the interaction effect was not significant. Women have naturally higher levels of HDL-cholesterol than men which may serve as a potential explanation for the observed trend in CHD(41,42). Higher levels of HDL-cholesterol may translate to an increased amount of potentially pro-oxidant HDL and thus endothelial damage and decreased protection against CHD. For the CVD outcome, a trend towards a null association among men was observed. These findings were not expected, but CHD is the only outcome that has been associated with Hp2-2 and HDL-dysfunction in hyperglycemia. Stroke has been associated with the Hp1-1 phenotype rather than the Hp2-2 phenotype(98,99). It was hypothesized that the relationship between Hp2-2 and CHD is related to the function of Hp as a scavenger of free Hb, while the function of Hp in angiogenesis may confer a protective effect of Hp2-2 against stroke, although the mechanism and sex differences are not well understood(100,101).

In Hp1 allele carriers stratified by history of CVD at baseline, there was a 3.3% reduced risk of CHD in primary prevention patients when adjusting for covariables in model two and the association was attenuated when adjusting for triglycerides. In secondary prevention patient patients, there was a borderline significant 1.8% reduced risk of CHD that became non-significant when adjusting for triglycerides. Similarly, larger CVD effect sizes were also reported for primary prevention patients than in

secondary prevention patients. Several studies that demonstrated an inverse relationship between HDL-cholesterol and CVD events did so in patients free of CVD at baseline(78,79). Furthermore, Silbernagel et al. have reported a strong association between HDL-cholesterol and cardiovascular mortality in patients without established coronary artery disease but not in patients with stable or unstable coronary artery disease(123). There is evidence to suggest that the anti-inflammatory and anti-oxidant properties of HDL may be reduced in coronary artery disease and may serve as an explanation for these findings(124,125). Another possible explanation could be that multimodal treatment of CVD and co-morbidity may have blunted the relationship between HDL-cholesterol and CVD events.

Overall, adjusting for triglycerides among Hp1 allele carriers and among patients with the Hp2-2 phenotype was attenuated and is in accordance with the idea that both triglycerides and HDL-cholesterol are associated with CVD and that their metabolism is interrelated. However, when stratified by sex; adjusting for triglycerides had the largest effect on the association between HDL-cholesterol and cardiovascular events in men who were Hp1 allele carriers while there was little effect in men with the Hp2-2 phenotype. In women, adjusting for triglycerides had little effect on the association between HDL-cholesterol and cardiovascular events in Hp1 allele carriers and between HDL-cholesterol and CHD in Hp2-2s, while there was a larger effect on the association between HDL-cholesterol and CVD in women with the Hp2-2 phenotype. These results do not have an immediate explanation and warrant further investigation in future studies.

The present findings are in accordance with the current literature on the biological mechanism linking Hp phenotype and HDL dysfunction in CHD. Free intravascular Hb is

bound to Hp with high affinity, creating an Hp-Hb complex that is cleared from the bloodstream via scavenger receptor CD163, thereby preventing Hb-mediated oxidative damage to serum and cellular proteins(32,33). CD163 receptor expression is reduced in hyperglycemic conditions. Further, Hp2-2 is the largest and bulkiest of the Hp phenotypes and is less able to remove Hb from the blood compared to Hp1-1 and Hp2-1, resulting in an increased amount of Hb-Hp complexes left circulating in the bloodstream of people with the Hp2-2 phenotype and hyperglycemia(32). The impairment of Hp2-2 function is magnified in T2DM due to the associated increase in glycated Hb caused by hyperglycemia(35,36). Glycation of Hb hinders the antioxidant ability of Hp2-2, promoting pro-oxidant Hp-Hb complexes(27,35). Pro-oxidant Hp-Hb binds to and oxidizes HDL and its related components such as apolipoprotein A, glutathione peroxidase and lecithin-cholesterol acyltransferase. As a result, in people who have the Hp2-2 phenotype and hyperglycemia, the ability of HDL to promote cholesterol efflux and prevent oxidization by free Hb is impaired making it potentially pro-atherogenic as it is thought to increase susceptibility to atherosclerosis, deterioration of cardiac function and ultimately CHD (27,35,39). Therefore, raising HDL-cholesterol may not reduce the risk of CHD in those with hyperglycemia and Hp2-2 because their HDL function may be impaired and it may increase oxidative stress if their HDL concentrations are increased. However, increasing HDL-cholesterol in the Hp1 allele carriers may be beneficial because Hp and HDL function are better preserved.

It is important to note that although this study examined HDL-cholesterol concentrations, HDL particles are very complex and heterogeneous in composition and function, and plasma HDL-cholesterol may not be a reliable indicator of the vascular

protective function of HDL within each phenotype group. Broadly, HDL particles can be classified into two subclasses: HDL2-cholesterol which constitutes large HDL particles, and HDL3-cholesterol which constitutes small and medium particles(126). HDL subclasses may differ in their ability to perform anti-atherogenic functions, but and there is conflicting evidence on which subclass may offer more cardioprotective effects(127). Whether their effects vary by Hp phenotype is unknown.

5.5 Strengths And Weaknesses

Strengths of this study include the ability to adjust for a wide range of CVD risk factors, the inclusion of time-varying variables, a validated Hp phenotyping method, minimal missing baseline data, and stratification of results by sex. A main limitation to this study is that HDL function was not assessed. HDL particles are very complex and heterogeneous in composition and function and this study was limited in that information on HDL particle subclass was not available. Power was also an issue in some subgroups in this study, particularly in patients with the Hp2-2 phenotype and may have also limited the ability to detect significant interactions. Another limitation is the use of discrete measurements for continuous variables, especially for the exposure (HDL-cholesterol) variable. Time-dependent variables with the last observation carried forward was used in an attempt to mitigate bias from the use of a single measurement, however; HDL-cholesterol (and other time-dependent continuous variable) changes that may occur between visits that may influence outcome events were not captured. Further, participants who did not have at least one measurement for any variable included in the model would have been excluded from the analysis which may have biased the results if missingness is related to the outcomes. However, less than 3% of subjects were excluded in either

phenotype group. Additionally, although this study is in the context of a randomized controlled trial, it is observational in nature and there may be unmeasured confounders that could influence the association between HDL-cholesterol and cardiovascular events such as diet and physical activity. The participants were also all middle-aged and elderly individuals who were mostly non-Hispanic white, at a high risk for CVD and who had chronic T2DM and hyperglycemia, and so it remains unknown whether these results are generalizable to other populations.

5.6 Future Directions Implications

The present study presents new information about the relationship between HDL-cholesterol and cardiovascular events among patients with different Hp phenotypes and replication of these findings in other cohorts of patients with hyperglycemia are needed to confirm the association and if it varies by sex and/or the presence of established CVD. Additionally, future studies should also take HDL subclass and assessment of HDL anti-atherogenic function (anti-oxidant and reverse cholesterol efflux capability) into consideration where possible as HDL-cholesterol may not be a reliable indicator of the vascular protective function of HDL. Further investigation on the association between triglycerides and CVD events, and their effect on the association between HDL-cholesterol and different CVD events, especially among the different sexes, in each Hp phenotype group is also warranted. In future research, the association of HDL-cholesterol and cardiovascular events in each phenotype group should also be explored in different ethnic groups and for stroke as a separate outcome. If, in future studies, there is evidence to support variation in the relationship between HDL and CHD and CVD events by Hp phenotype, that evidence could not only help to provide an explanation for inconsistent

results previously reported in clinical trials that aimed to pharmacologically raise HDL-cholesterol, but could also be used to help to develop more targeted prevention and treatment strategies for CVD in T2DM.

In conclusion, the results support a significant inverse relationship between HDL-cholesterol and CHD and CVD among Hp1 and no significant associations between HDL and cardiovascular events in the Hp2-2 phenotype. Further studies are needed to understand the relationship between increased HDL-cholesterol, HDL dysfunction and CVD events in T2DM, as well as relationship between other serum lipids (such as triglycerides) and cardiovascular events, among the different Hp phenotypes.

Table 5.1. Baseline characteristics stratified by haptoglobin (Hp) phenotype*

Characteristic	Hp 1 allele carriers (n=3,201)	Hp2-2s (n=1795)	p-Value
Age (year)	62.74±6.47	62.76±6.39	0.93
Female sex- no.(%)	1073 (33.52)	560 (31.20)	0.09
Race or ethnic group- no.(%)			<0.001
White	2093 (65.39)	1198 (66.74)	
Black	549 (17.15)	181 (10.08)	
Hispanic	252 (7.87)	105 (5.85)	
Other	307 (9.59)	311 (17.33)	
Education- no.(%)			0.01
Less than high school	450 (14.06)	213 (11.87)	
High-school graduate or GED	840 (26.24)	467 (26.02)	
Some college	1063 (33.21)	573 (31.92)	
College degree or higher	845 (26.40)	542 (30.19)	
Network- no.(%)			<0.001
Site 1	541 (16.90)	363 (20.22)	
Site 2	504 (15.57)	341 (19.00)	
Site 3	444 (13.87)	251 (13.98)	
Site 4	402 (12.56)	229 (12.76)	
Site 5	363 (11.34)	159 (8.86)	
Site 6	489 (15.28)	233 (12.98)	
Site 7	458 (14.31)	219 (12.20)	
Previous cardiovascular event- no.(%)	1108 (34.61)	644 (35.88)	0.37
Family history of CVD- no.(%)			0.48
No family history (Ref.)	1588 (49.61)	896 (49.92)	
Family history of premature CVD	938 (29.30)	517 (28.80)	
Family history at unknown age	569 (17.78)	308 (17.16)	
Unknown	106 (3.31)	74 (4.12)	
Smoking status- no.(%)			0.34
Current	394 (12.31)	201 (11.20)	
Former	1466 (45.80)	852 (47.47)	
Never	1341 (41.89)	742 (41.34)	
Alcohol- no.(%)			0.27
Nondrinker	2417 (75.51)	1330 (74.09)	
Drinker	783 (24.46)	464 (25.86)	
Weight- kg	94.92±18.18	94.33±18.65	0.29
Body-mass index (kg/ m^2)	32.41±5.29	32.29±5.41	0.44

Characteristic	Hp 1 allele carriers (n=3,201)	Hp2-2s (n=1795)	p-Value
Hypertension- no.(%)	1964 (61.4)	1056 (58.8)	0.08
Medications- no.(%)			
Insulin	1108 (34.72)	586 (32.79)	0.17
Metformin	2091 (65.32)	1191 (66.39)	0.45
Any sulfonylurea	1744 (54.48)	1004 (55.93)	0.31
Any thiazolidinedione	649 (20.27)	364 (20.28)	0.99
Any anti-hypertensive agent	2593 (81.01)	1448 (80.67)	0.72
Statin	1950 (60.92)	1126 (62.73)	0.20
Any lipid-lowering agent	2109 (65.89)	1196 (66.63)	0.61
Aspirin	1816 (56.73)	1008 (56.16)	0.67
Glycemic control group- no.(%)			0.62
Standard glycemic control	1594 (49.80)	907 (50.53)	
Intensive glycemic control	1607 (50.20)	888 (49.47)	
Duration of diabetes (years)			0.50
Median	10	9	
Interquartile range	5 to 15	5 to 15	
Glycated hemoglobin (%)			0.90
Mean	8.27±1.00	8.26±1.03	
Median	8.1	8.1	
Interquartile range	7.60 to 8.8	7.5 to 8.8	
Plasma cholesterol (mg/dL)			
Total	175.22±37.19	177.16±37.96	0.08
Low-density lipoprotein	100.84±30.77	101.16±30.90	0.73
High-density lipoprotein			0.48
Mean	38.26±7.87	38.01±7.54	
Median	38	37	
IQR	33 to 43	33-43	
Plasma triglycerides (mg/dL)			<0.001
Median	159	169	
Interquartile range	111 to 227	120 to 241	
Triglycerides ≥204-mg/dL & HDL ≤ 34-mg/dL	531 (16.59)	326 (18.16)	0.16

* Plus–minus values are means ±SD. Percentages may not total 100 because of rounding. CVD=cardiovascular disease; GED= general equivalency diploma; Hp=haptoglobin; IQR= interquartile range.

Table 5.2. Unadjusted rate, person-years of follow-up and incidence of outcomes occurring up to 7 years from randomization for the two haptoglobin (Hp) phenotype groups separately

	Hp 1 allele carriers	Hp2-2s
CHD		
Total	**n=3201**	**n=1795**
No. of events (%)	388 (12.1)	187 (10.4)
Person-years	14589.3	8372.627
Incidence rate†	265.9	223.3
Men	**n=2128**	**n=1235**
No. of events (%)	283 (13.3)	140 (11.3)
Person-years	9680.8	5801.4
Incidence rate†	292.3	241.3
Women	**n=1073**	**n=560**
No. of events (%)	105 (9.8)	47 (8.4)
Person-years	4908.4	2571.3
Incidence rate†	213.9	182.8
Previous CVD	**n=1108**	**n=644**
No. of events (%)	228 (20.7)	111 (17.2)
Person-years	4815.2	2885.7
Incidence rate†	473.5	384.7
No previous CVD	**n=2093**	**n=1151**
No. of events (%)	160 (7.6)	76 (6.6)
Person-years	9774.1	5486.9
Incidence rate†	163.7	138.5
CVD		
Total	**n=3201**	**n=1795**
No. of events (%)	308 (9.6)	175 (9.7)
Person-years	14832.7	8437.7
Incidence rate†	207.6	207.4
Men	**n=2128**	**n=1235**
No. of events (%)	231 (10.9)	127 (10.3)
Person-years	9841.9	5859.2
Incidence rate†	234.7	216.8
Women	**n=1073**	**n=560**
No. of events (%)	77 (7.2)	48 (8.6)

	Hp 1 allele carriers	Hp2-2s
Person-years	4990.8	2578.5
Incidence rate†	154.29	186.2
Previous CVD	**n=1108**	**n=644**
No. of events (%)	173 (15.6)	99 (15.4)
Person-years	4999.5	2933.9
Incidence rate†	346.0	337.4
No previous CVD	**n=2093**	**n=1151**
No. of events (%)	135 (6.5)	76 (6.6)
Person-years	9833.2	5503.7
Incidence rate†	137.3	138.1

CHD= coronary heart disease; CVD =cardiovascular disease; Hp= haptoglobin

†Incidence rate is per 10,000 person-years and is not adjusted for covariates.

Table 5.3. Multivariable-adjusted hazard ratios of outcomes for every 1-mg/dL increase in HDL-cholesterol among Hp1 allele carriers.

	Hp1 allele carriers								
	Model 1*			Model 2**			Model 3***		
Outcome	**aHR (95% CI)**	**P-value**	**P-value for interaction†**	**aHR (95% CI)**	**P-value**	**P-value for interaction†**	**aHR (95% CI)**	**P-value**	**P-value for interaction†**
CHD									
Overall	0.973 (0.962-0.984)	<0.001		0.974 (0.962-0.987)	<0.001		0.982 (0.969-0.996)	0.01	
By Sex			0.12			0.68			0.54
Men	0.979 (0.965-0.992)	0.002		0.976 (0.961-0.992)	0.003		0.986 (0.968-1.004)	0.14	
Women	0.962 (0.946-0.978)	<0.001		0.970 (0.952-0.989)	0.002		0.974 (0.954-0.994)	0.01	
By CVD history			0.08			0.09			0.10
No	0.968 (0.953-0.983)	<0.001		0.967 (0.949-0.985)	<0.001		0.977 (0.956-0.998)	0.03	
Yes	0.983 (0.968-0.999)	0.04		0.982 (0.965-1.000)	0.045		0.990 (0.973-1.009)	0.30	
CVD									
Overall	0.975 (0.963-0.987)	<0.001		0.976 (0.962-0.990)	0.001		0.980 (0.964-0.995)	0.01	
By Sex			0.16			0.57			0.52
Men	0.981 (0.966-0.996)	0.01		0.978 (0.961-0.995)	0.01		0.985 (0.966-1.00)	0.12	
Women	0.963 (0.943-0.983)	<0.001		0.971 (0.948-0.995)	0.02		0.968 (0.943-0.993)	0.01	

	Hp1 allele carriers								
	Model 1*			Model 2**			Model 3***		
Outcome	**aHR (95% CI)**	**P-value**	**P-value for interaction†**	**aHR (95% CI)**	**P-value**	**P-value for interaction†**	**aHR (95% CI)**	**P-value**	**P-value for interaction†**
By CVD history			0.03			0.09			0.09
No	0.967 (0.950-0.984)	<0.001		0.969 (0.949-0.989)	0.002		0.980 (0.957-1.00)	0.09	
Yes	0.987 (0.969-1.00)	0.16		0.985 (0.965-1.00)	0.14		0.984 (0.962-1.01)	0.16	

aHR= adjusted hazard ratio; CHD= coronary heart disease; CI= confidence interval; CVD= cardiovascular disease; Hp= haptoglobin

*Presented are adjusted hazard ratios for a 1-mg/dL increase in HDL-cholesterol. In the main analysis, model 1 was adjusted for age and sex

** Presented are adjusted hazard ratios for a 1-mg/dL increase in HDL-cholesterol. In the main analysis, model 2 was adjusted for age, sex, LDL-cholesterol, cigarette smoking status, hypertension, anti-hypertensive medication use, HbA1c, ethnicity, alcohol consumption, diabetes duration, CVD history at baseline, family history of CVD, education, glycemic control assignment, lipid treatment assignment and baseline statin use.

*** Presented are adjusted hazard ratios for a 1-mg/dL increase in HDL-cholesterol. In the main analysis, model 2 was adjusted for the same variables in model 2 with the addition of triglycerides.

†P-values for interaction between HDL-cholesterol and either sex or history of CVD at baseline.

Table 5.4. Multivariable-adjusted hazard ratios of outcomes for every 1-mg/dL increase in HDL-cholesterol among Hp2-2s.

	Hp2-2s								
	Model 1*			**Model 2****			**Model 3*****		
Outcome	**aHR (95% CI)**	**P-value**	**P-value for interaction†**	**aHR (95% CI)**	**P-value**	**P-value for interaction†**	**aHR (95% CI)**	**P-value**	**P-value for interaction†**
CHD									
Overall	0.976 (0.960-0.992)	0.004		0.988 (0.969-1.006)	0.19		0.992 (0.970-1.014)	0.47	
By Sex			0.40			0.43			0.45
Men	0.972 (0.955-0.989)	0.001		0.984 (0.964-1.004)	0.12		0.986 (0.963-1.011)	0.27	
Women	0.990 (0.953-1.03)	0.59		1.004 (0.960-1.049)	0.87		1.002 (0.957-1.050)	0.92	
By CVD history			0.27			0.26			0.26
No	0.976 (0.954-1.000)	0.045		0.983 (0.957-1.009)	0.19		0.985 (0.956-1.016)	0.34	
Yes	0.987 (0.964-1.010)	0.26		0.991 (0.966-1.017)	0.50		0.997 (0.967-1.027)	0.82	
CVD									
Overall	0.979 (0.962-0.995)	0.01		0.990 (0.972-1.009)	0.30		0.994 (0.973-1.016)	0.60	
By Sex			0.23			0.82			0.86
Men	0.987 (0.970-1.005)	0.15		0.999 (0.979-1.02)	0.93		0.999 (0.974-1.02)	0.93	
Women	0.962 (0.926-0.999)	0.05		0.969 (0.928-1.01)	0.16		0.980 (0.936-1.03)	0.38	

	Hp2-2s								
	Model 1*			Model 2**			Model 3***		
Outcome	**aHR (95% CI)**	**P-value**	**P-value for interaction†**	**aHR (95% CI)**	**P-value**	**P-value for interaction†**	**aHR (95% CI)**	**P-value**	**P-value for interaction†**
By CVD history			0.16			0.79			0.55
No	0.985 (0.961-1.01)	0.23		0.992 (0.965-1.019)	0.55		0.998 (0.970-1.028)	0.92	
Yes	0.984 (0.962-1.007)	0.17		0.988 (0.964-1.014)	0.37		0.990 (0.96-1.021)	0.54	

aHR= adjusted hazard ratio; CHD= coronary heart disease; CI= confidence interval; CVD= cardiovascular disease; Hp= haptoglobin

*Presented are adjusted hazard ratios for a 1-mg/dL increase in HDL-cholesterol. In the main analysis, model 1 was adjusted for age and sex

** Presented are adjusted hazard ratios for a 1-mg/dL increase in HDL-cholesterol. In the main analysis, model 2 was adjusted for age, sex, LDL-cholesterol, cigarette smoking status, hypertension, anti-hypertensive medication use, HbA1c, ethnicity, alcohol consumption, diabetes duration, CVD history at baseline, family history of CVD, education, glycemic control assignment, lipid treatment assignment and baseline statin use.

*** Presented are adjusted hazard ratios for a 1-mg/dL increase in HDL-cholesterol. In the main analysis, model 2 was adjusted for the same variables in model 2 with the addition of triglycerides.

†P-values for interaction between HDL-cholesterol and either sex or history of CVD at baseline.

Figure 5.1. Mean HDL-cholesterol (mg/dL) over time stratified by Hp phenotype.

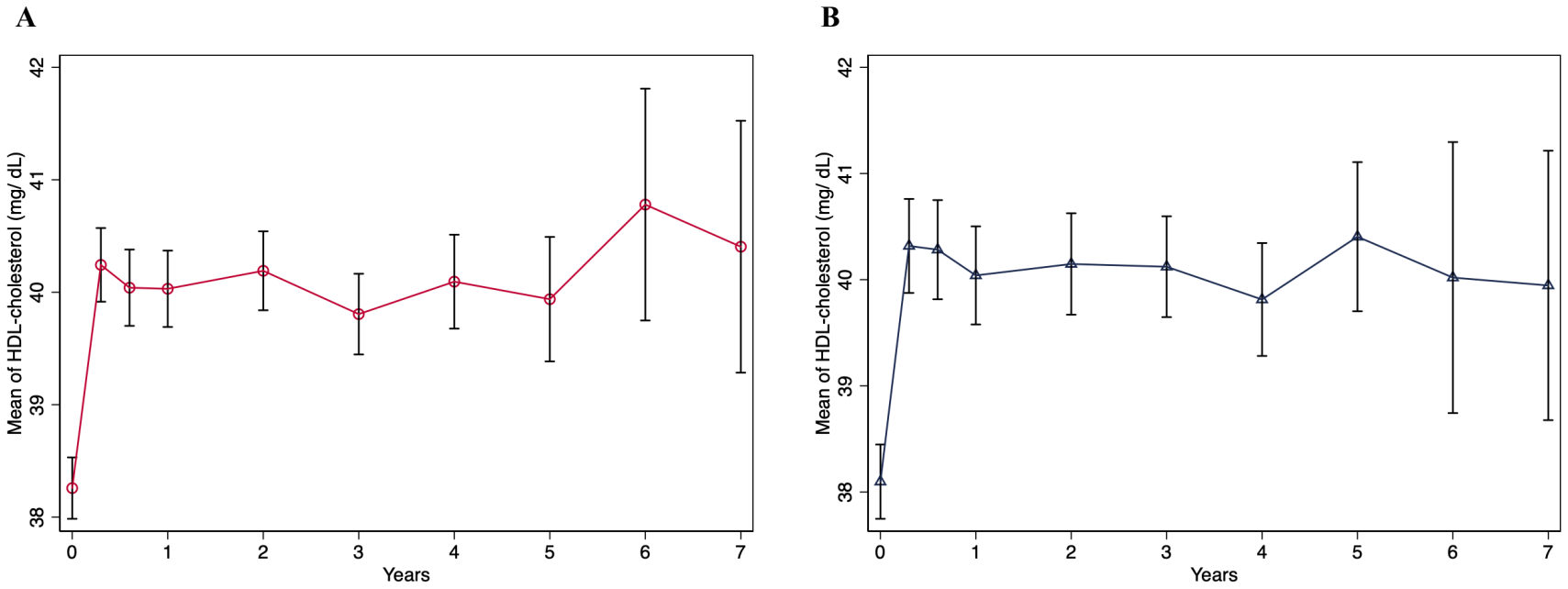


Figure Legend. Shown are mean plasma levels of HDL-cholesterol in Hp1 allele carriers (panel A) and patients with the Hp2-2 phenotype (panel B) at baseline, 4 months, 8 months, 1 year, and annually thereafter.

5.7 Supplementary Material

Table 5.7.1. Time-dependent characteristics at different time points stratified by haptoglobin phenotype*

Characteristic	4 Months			8 Months		
	Hp1 allele carriers(n=3,177)	**Hp2-2s (n=1,769)**	**p-Value**	**Hp1 allele carriers(n=3,160)**	**Hp2-2s (n=1,766)**	**p-Value**
HDL-cholesterol (mg/dL)	40.2±9.3	40.3±9.4	0.79	40.0±9.5	40.3±9.8	0.41
Missing	97 (3.05)	38 (2.15)		128 (4.05)	60 (3.40)	
LDL-cholesterol (mg/dL)	90.2±26.4	90.9±26.5	0.41	90.8±27.2	91.0±26.5	0.75
Missing	97 (3.05)	38 (2.15)		128 (4.05)	60 (3.40)	
Triglycerides (mg/dL)	160.3 (92-195.5)	168.8 (98-201)	0.003	131 (94-193)	140 (96-207)	0.005
Missing	97 (3.05)	38 (2.15)		128 (4.05)	60 (3.40)	
Glycated hemoglobin (%)	7.2±1.0	7.2±0.9	0.7	7.1±1.0	7.1±1.0	0.39
Missing	188 (5.92)	82 (4.64)		249 (7.88)	129 (7.30)	
Hypertension- no.(%)	1364 (42.9)	768 (43.4)	0.82	1333 (42.2)	751(42.5)	0.95
Missing	467 (15.0)	254 (14.6)		496 (15.7)	262 (14.8)	
Body-mass index (kg/m^2)	32.5±5.4	32.3±5.5	0.38	32.5±5.4	32.3±5.5	0.38
Missing	67 (2.11)	36 (2.04)		95 (3.01)	38 (2.15)	
Any anti-hypertensive agent-no(%)	N/A	N/A	N/A	N/A	N/A	N/A
Missing	N/A	N/A	N/A	N/A	N/A	N/A
Smoking status- no.(%)						
Current	N/A	N/A	N/A	N/A	N/A	N/A
Former	N/A	N/A	N/A	N/A	N/A	N/A
Never	N/A	N/A	N/A	N/A	N/A	N/A
Missing	N/A	N/A	N/A	N/A	N/A	N/A

Characteristic	Year 1			Year 2		
	Hp1 allele carriers(n=3,167)	**Hp2-2s (n=1,768)**	**p-Value**	**Hp1 allele carriers(n=3,125)**	**Hp2-2s (n=1,744)**	**p-Value**
HDL-cholesterol (mg/dL)	40.0±9.6	40.0±9.8	0.97	40.2±9.8	40.1±9.9	0.89
Missing	121 (3.82)	51 (2.88)		159 (5.1)	87 (5.0)	
LDL-cholesterol (mg/dL)	89.2±25.8	89.6±25.9	0.61	86.8±26.2	87.9±26.6	0.16
Missing	121 (3.82)	51 (2.88)		159 (5.1)	87 (5.0)	
Triglycerides (mg/dL)	132 (93-197)	142 (97-207)	0.003	132 (91-194)	143 (100-204)	<0.001
Missing	121 (3.82)	51 (2.88)		159 (5.1)	87 (5.0)	
Glycated hemoglobin (%)	7.1±1.0	7.1±0.99	0.91	7.1±1.1	7.0±1.1	0.37
Missing	374 (11.8)	182 (10.3)		450 (14.4)	268 (15.4)	
Hypertension- no.(%)	1532 (48.5)	833 (47.2)	0.24	1491 (47.7)	802 (46.0)	0.19
Missing	132 (4.2)	57 (3.2)		186 (5.6)	97 (5.6)	
Body-mass index (kg/m^2)	32.8 ±5.5	32.7±5.7	0.54	32.9±5.7	32.9±5.8	0.77
Missing	133 (4.20)	52 (2.94)		176 (5.6)	94 (5.4)	
Any anti-hypertensive agent- no(%)	2432 (76.8)	1364 (77.1)	0.31	2434 (78.6)	1321 (76.3)	0.02
Missing	154 (4.9)	52 (2.9)		146 (4.7)	76 (4.4)	
Smoking status- no.(%)			0.6			0.4
Current	348 (11.0)	181(10.2)		321 (10.3)	159 (9.1)	
Former	1461 (46.1)	842 (47.6)		1461 (46.8)	836 (47.9)	
Never	1223 (38.6)	696 (39.4)		1189 (38.0)	662 (38.0)	
Missing	126 (4.0)	45 (2.5)		154 (4.9)	87 (5.0)	

Characteristic	Year 3			Year 4		
	Hp1 allele carriers (n=3,057)	**Hp2-2s (n=1,724)**	**p-Value**	**Hp1 allele carriers (n=2,286)**	**Hp2-2s (n=1,332)**	**p-Value**
HDL-cholesterol (mg/dL)	39.8±9.7	40.1±9.7	0.3	39.8±9.5	40.1±9.8	0.42
Missing	226 (7.4)	112 (6.5)		160 (7.00)	94 (7.1)	
LDL-cholesterol (mg/dL)	82.9±26.8	83.5±25.4	0.45	82.0±26.6	82.2±25.2	0.78
Missing	226 (7.4)	112 (6.5)		160 (7.00)	94 (7.1)	
Triglycerides (mg/dL)	131 (92-190)	136 (98-198.5)	0.003	130 (94-188)	136 (99-196)	0.01
Missing	226 (7.4)	112 (6.5)		160 (7.00)	94 (7.1)	
Glycated hemoglobin (%)	7.2±1.1	7.1±1.1	0.43	7.2±1.2	7.2±1.1	0.98
Missing	515 (16.9)	301 (17.5)		428 (18.7)	250 (18.8)	
Hypertension- no.(%)	1400 (45.8)	778 (45.1)	0.55	1060 (46.4)	645 (48.4)	0.34
Missing	217 (7.1)	115 (6.7)		180 (7.9)	93 (7.0)	
Body-mass index (kg/m^2)	33.0±5.8	33.0±6.0	0.91	32.7±5.7	32.8±5.9	0.74
Missing	197 (6.4)	90 (5.2)		172 (7.5)	85 (6.4)	
Any anti-hypertensive agent-no(%)	2352 (76.9)	1330 (77.1)	0.15	1756 (76.8)	1005 (75.5)	0.03
Missing	217 (7.1)	84 (4.9)		187 (8.3)	87 (6.6)	
Smoking status- no.(%)			0.04			0.14
Current	300 (9.8)	133 (7.7)		211 (9.2)	100 (7.5)	
Former	1447 (47.3)	856 (49.7)		1092 (47.8)	671 (50.3)	
Never	1153 (37.7)	659 (38.2)		848 (37.1)	494 (37.1)	
Missing	114 (5.1)	76 (4.4)		135 (5.9)	67 (5.0)	

Characteristic	Year 5			Year 6		
	Hp1 allele carriers (n=1,318)	**Hp2-2s (n=776)**	**p-Value**	**Hp1 allele carriers (n=383)**	**Hp2-2s (n=215)**	**p-Value**
HDL-cholesterol (mg/dL)	39.9±9.7	40.4±9.6	0.31	40.8±9.8	40.0±9.3	0.36
Missing	136 (10.3)	62 (8.0)		34 (8.9)	13 (6.1)	
LDL-cholesterol (mg/dL)	81.9±26.0	81.0±25.9	0.43	81.8±29.6	80.2±25.0	0.51
Missing	136 (10.3)	62 (8.0)		34 (8.9)	13 (6.1)	
Triglycerides (mg/dL)	127 (90-183)	133 (96-194)	0.02	126 (87-174)	131 (90-190)	0.28
Missing	135 (10.2)	62 (8.0)		34 (8.9)	13 (6.1)	
Glycated hemoglobin (%)	7.3±1.1	7.4±1.2	0.07	7.1±7.0	7.2±7.0	0.70
Missing	263 (20.0)	127 (16.4)		79 (20.6)	32 (14.9)	
Hypertension- no.(%)	571 (43.3)	392 (50.5)	0.003	180 (47.00)	108 (50.2)	0.66
Missing	126 (9.6)	61 (7.9)		37 (9.7)	15 (7.0)	
Body-mass index (kg/m^2)	32.6±5.8	32.2±5.8	0.21	32.2±5.9	32.2±5.7	0.93
Missing	112 (8.5)	54 (7.0)		35 (9.1)	12 (5.6)	
Any anti-hypertensive agent- no(%)	1030 (78.1)	596 (76.8)	0.02	296 (77.2)	168 (78.1)	0.53
Missing	118 (9.0)	48 (6.2)		34 (8.9)	12 (5.6)	
Smoking status- no.(%)			0.79			0.27
Current	100 (7.6)	54 (7.0)		18 (4.7)	13 (6.1)	
Former	652 (49.5)	399 (51.4)		185 (48.3)	117 (54.4)	
Never	479 (36.3)	288(37.1)		154 (40.2)	74 (34.4)	
Missing	87 (6.6)	35(4.5)		26 (6.8)	11 (5.1)	

Characteristic	Year 7			Exit Visit†		
	Hp1 allele carriers (n=332)	Hp2-2s (n=182)	p-Value	Hp1 allele carriers (n=2,894)	Hp2-2s (n=1,695)	p-Value
HDL-cholesterol (mg/dL)	40.4±9.7	39.9±8.3	0.6	40.9±9.9	40.9±9.9	0.99
Missing	43 (13.0)	16 (8.8)		189 (6.5)	94 (5.7)	
LDL-cholesterol (mg/dL)	78.3±28.0	79.5±29.3	0.67	80.0±26.1	80.7±26.3	0.38
Missing	43 (13.0)	16 (8.8)		189 (6.5)	94 (5.7)	
Triglycerides (mg/dL)	116 (89-168)	131 (97-190)	0.01	130 (91-187)	135 (95-190)	0.05
Missing	43 (13.0)	16 (8.8)		189 (6.5)	94 (5.7)	
Glycated hemoglobin (%)	7.2±1.2	7.2±1.1	0.89	7.6±1.2	7.6±1.2	0.31
Missing	81 (24.2)	49 (26.9)		187 (6.5)	94 (5.7)	
Hypertension- no.(%)	136 (41.0)	81(44.5)	0.54	2339 (80.8)	1337 (80.6)	0.19
Missing				136 (4.7)	54 (3.3)	
Body-mass index (kg/m^2)	32.0±5.6	32.1±5.9	0.78	32.6±5.8	32.6±6.0	0.87
Missing	45 (13.6)	21 (11.5)		183 (6.3)	94 (5.7)	
Any anti-hypertensive agent- no(%)	251 (75.6)	136 (74.7)	0.76	1360 (47.0)	753 (45.4)	
Missing	35 (10.5)	19 (10.4)		218 (7.5)	119 (7.2)	
Smoking status- no.(%)			0.35			
Current	12 (3.6)	6 (3.3)		242 (8.4)	117 (7.1)	0.25
Former	159 (47.9)	98 (53.8)		1464 (50.6)	863 (52.0)	
Never	132 (39.8)	29 (8.7)		1106 (38.2)	644 (38.8)	
Missing	29 (8.7)	17 (9.3)		82 (2.8)	35 (2.1)	

* Plus–minus values are means ±SD. Median and interquartile range is presented for triglycerides. Percentages may not total 100 because of rounding. Hp=haptoglobin

† 'Exit Visit' measurements were allocated among annual visits in year 4, 5, 6 and 7.

Chapter 6: Conclusions

6.0 Summary Of Overall Findings For Objectives 1 And 2

The present study was the first to determine whether the effect of adding fenofibrate to simvastatin on cardiovascular events and the association between HDL-cholesterol and cardiovascular events, depends on Hp phenotype. Using data from the ACCORD lipid trial, this thesis demonstrates a difference in the effect of fenofibrate in combination with simvastatin therapy for CHD prevention by Hp phenotype and sex in patients with T2DM, that is hypothesized to be related to HDL dysfunction (objective 1). Fenofibrate with background simvastatin, compared to simvastatin alone, reduced the risk of CHD in Hp1 allele carriers but not in the Hp2-2 phenotype with a significant interaction effect (objective 1). Further, in prespecified subgroup analyses, a reduced risk of CHD in men who were Hp1 allele carriers and an increased risk of CHD in women with the Hp2-2 phenotype was found. In objective 2, there was a significant inverse association between HDL-cholesterol and cardiovascular outcomes in Hp1 allele carriers and no significant association was observed in patients with the Hp2-2 phenotype, although the interaction was not significant. Among Hp1 allele carriers, for every 1-mg/dL increase in HDL-cholesterol the risk of CHD decreased by 1.8-3.3% and the risk of CVD decreased by 2.2-3.1%. The association was generally attenuated by triglyceride adjustment, however; in some subgroups, the association was unaffected by triglyceride adjustment. A greater risk reduction was observed among women than in men, although the interaction was not significant. There were no significant associations between HDL-cholesterol and cardiovascular events in the Hp2-2 phenotype and interactions by sex were also insignificant.

6.1 Combining Intention-To-Treat (Objective 1) Findings And HDL-Cholesterol Biomarker (Objective 2) Findings

The overall results for objective 1 were in accordance with the proposed hypothesis that HDL dysfunction is prominent in the Hp2-2 phenotype in hyperglycemia and thus pharmacologically raising HDL-cholesterol in these patients would not be beneficial whereas it would be beneficial in Hp1 allele carriers in whom the function of Hp and HDL are better preserved. Although there were no significant interactions in objective 2, a significant association between a 1-mg/dL increase in HDL-cholesterol and CHD and CVD was observed in Hp1 allele carriers only and no significant results were observed in the patients with the Hp2-2 phenotype, which still supports the idea that HDL dysfunction is prominent in the Hp2-2 phenotype. Together, these results suggest that raising HDL-cholesterol may only have cardioprotective benefit in patients who are Hp1 allele carriers. Some similarities and some differences between objective 1 and objective 2 were observed in subgroup analyses.

In objective 1, among Hp1 allele carriers, a significant benefit in CHD and CVD prevention from fenofibrate added to simvastatin therapy, compared to simvastatin alone, was observed in men and in secondary prevention patients only. In objective 2, in multivariable adjusted models, the association between a 1-mg/dL increase in HDL-cholesterol and CHD and CVD was stronger in women and in primary prevention patients. In patients with the Hp2-2 phenotype, men and women randomized to fenofibrate did not have a reduced risk of CHD or CVD and the risk of CHD was increased in women. Similarly, there were no significant associations between a 1-mg/dL increase in HDL-cholesterol and CHD or CVD outcomes in men or women after adjusting for important covariates and there appeared to be trend towards a null

association between HDL-cholesterol and CHD among women. However, for the CVD outcome, the trend towards a null association was observed in men. It is important to consider potential explanations for why there were inconsistencies between objective 1 and objective 2.

It is possible that the results observed in objective 1 may not be related to HDL but may instead be related to other actions of fenofibrate that could potentially have some unknown relationship with Hp phenotype and CVD risk and explain any discrepancies in results between objective 1 and objective 2. Fenofibrate is a potent PPAR-α agonist that not only affects HDL but also lowers triglycerides, increases the size of LDL particles and has several other non-lipid effects including a reduction in systemic inflammation(90–92). Additionally, HDL-cholesterol only changed by a few mg/dL in this study which further supports the idea that HDL-cholesterol levels alone may not be the reason for the present findings.

HDL particles are very complex and heterogeneous in composition and function, and HDL-cholesterol concentrations may not be a direct indicator of HDL function within each phenotype group. Plasma HDL particles are heterogeneous and have recently been discovered to be divisible into subclasses that consist of different sizes, densities, apolipoprotein composition and lipid content (128). HDL can be classified into two general subclasses, by density: HDL2-cholesterol and HDL3-cholesterol with HDL2-cholesterol constituting large HDL particles and HDL3-cholesterol constituting small and medium particles(126). HDL particle subclasses may differ in their biological functions, including in their role in reverse cholesterol transport, as well as anti-inflammatory and antioxidant functions(127).There is no consensus yet on the relationship between HDL

specific subclasses and CVD risk. Some researchers have confirmed that large HDL particles have a protective effect on CHD while others have found that the small, dense, protein rich HDL have superior cardioprotective activities(127). Of note, in one study bezafibrate (a fibrate) has been demonstrated to significantly increase HDL3-cholesterol(129). It remains unclear if HDL subclass information can provide additional information on cardiovascular risk, and whether their effects vary by Hp phenotype is unknown.

6.2 Haptoglobin Phenotype: A Potential Explanation For Results Observed In Previous Trials That Aimed To Pharmacologically Raise HDL-Cholesterol And Lower Triglycerides

Regardless of whether the results of this study are due to HDL function, it was still demonstrated that the effect of fenofibrate added to simvastatin therapy differed according to Hp phenotype and sex among high risk patients with T2DM who participated in the ACCORD lipid trial and this finding can offer an explanation for the inconsistencies previously reported in clinical trials that aimed to pharmacologically raise HDL-cholesterol and reduce triglycerides.

The VA-HIT, BIP and FIELD studies were all clinical trials that looked at the use of fenofibrate monotherapy relative to placebo(19). In the VA-HIT trial, patients randomized to fibrate (gemfibrozil) had a 32% risk reduction in a composite CVD outcome, compared to placebo, in patients with diabetes(19). The VA-HIT trial was a study conducted entirely in men, for whom this study has demonstrated a 36% and 26% risk reduction of CHD and CVD respectively when adding fenofibrate to simvastatin therapy in Hp1 allele carriers and no effect in men with the hp2-2 phenotype and T2DM. Differences in risk reduction may be related to differing responses to treatment, as

patients with diabetes who took gemfibrozil in the VA-HIT study had a higher increase in HDL-cholesterol, or may be related to the fact that background simvastatin was used in the ACCORD lipid trial. In the BIP trial, men and women with metabolic syndrome who were randomized to bezafibrate experienced 29% risk reduction in any MI compared to placebo(20). However, only 11% of the participants in this study were women and patients were not required to have T2DM or high blood sugar to be in the study. Baseline mean fasting glucose was 107 ± 20-mg/dL (20). A fasting blood sugar level of 126-mg/dL is the cut point for diagnosing T2DM(62). In the FIELD study, among 9795 patients with T2DM (37% women), patients who were randomized to fenofibrate therapy did not significantly reduce CHD (HR= 0.89, 0.75-1.05). Had the FIELD study been stratified by Hp phenotype, a reduction in CHD may have been observed in Hp1 allele carriers. Similarly, two other large trials investigating the effect of niacin (HDL-cholesterol raising and triglyceride lowering drug) and statin combination therapy on CVD outcomes found no benefit compared to statin monotherapy(24,25). In the AIM-HIGH trial, the addition of niacin to simvastatin did not reduce CVD events compared to simvastatin alone (1.02, 0.87-1.21). Of note, ~34% of patients in the niacin group had T2DM and ~15% were women(24). In a subset of the AIM-HIGH study who had diabetes, Asleh et al. found that niacin improved HDL-cholesterol antioxidant function in individuals with Hp1-1 but worsened HDL-cholesterol antioxidant function in individuals with the Hp2-2 phenotype, but the study was not powered to investigate if these changes were related to clinical events (102). In the HP2S-THRIVE study, compared to statin alone, the use of extended-release niacin did not reduce composite CVD events (0.96,

0.90-1.03), however; ~17% of the study population was female and ~33% had diabetes(25).

This was the first study to determine if the effect of HDL-cholesterol raising and triglyceride lowering drugs are dependent on Hp phenotype and so these results cannot be directly compared to the literature, but they indicate that further research is necessary.

6.3 Future Directions For Research

Replication of the results from the intention-to-treat analysis in objective 1 are needed in other trials examining the effect of therapeutically raising HDL-cholesterol by inhibiting cholesterol ester transfer protein or by using extended release niacin to confirm these results. Investigation of these effects in in clinical trials aimed at raising HDL-cholesterol via nutritional interventions, such as consumption of omega-3 polyunsaturated fatty acids, is also warranted. The effects should also be explored in different ethnic groups as this study was not powered to do so, and in different stages of cardiometabolic disease progression as the results may not be generalizable to all populations with type 2 diabetes. Women made up only 33.5% and 31.2% of Hp1 allele carriers and patients with the Hp2-2 phenotype respectively, and few gendered variables were available in this study. Future studies with more women and gender information are needed to confirm and understand the role of sex and gender in these results. The effect of fenofibrate on different CVD outcomes (including stroke as a separate outcome) in each of the three phenotypes separately should also be explored if there is a large enough sample size to accommodate the less frequent Hp1-1 phenotype. If the results are replicated, then a clinical trial testing the effect of HDL-cholesterol raising and triglyceride lowering drugs with background statin while incorporating Hp phenotype to

determine if it is a useful biomarker in clinical care to fine-tune lipid-altering treatment and provide a more personalized and evidence-based approach to patient care.

Further investigations on the relationship between HDL and CHD and CVD events in each phenotype group in T2DM are needed to determine if the effect of fenofibrate with background statin observed in objective 1 is related to prominent HDL dysfunction in the Hp2-2 phenotype. Future studies investigating this relationship should consider HDL subclass (HDL2 or HDL3) as well as markers of HDL function (anti-oxidant and reverse cholesterol efflux capability) directly in relation to cardiovascular events (including stroke as a separate outcome) in each Hp phenotype group to fully understand the relationship between HDL and CVD and if there is a difference by Hp phenotype. Although many important variables were included in the current analysis, future studies should also include information of diet and physical activity which are both important CVD risk factors and influence HDL-cholesterol. Investigation of the other effects of fenofibrate, such as raising triglycerides and changing LDL composition, on CHD and CVD events in each phenotype group in T2DM should also be considered.

6.4 Implications

The results of this study have potential implications for clinical practice. Statins are the first line of therapy for dyslipidemia in T2DM, and current guidelines do not support added fibrate therapy due to the failure of clinical trials to demonstrate additional benefit of pharmacologically raising HDL-cholesterol and lowering triglycerides in patients on optimal statin therapy(130–132). This study is the first to investigate and determine a difference in the effect of added fibrate therapy to statin by Hp phenotype in hyperglycemia and suggests that fenofibrate therapy with background simvastatin may be

beneficial for CHD prevention in some subgroups, especially men who are Hp1 allele carriers with hyperglycemia, but there is no evidence to suggest a benefit in patients with the Hp2-2 phenotype and hyperglycemia and may even be harmful in women with this phenotype. If these results are found to replicate in other studies aimed at pharmacologically raising HDL-cholesterol and lowering triglycerides, Hp phenotype could potentially serve as a simple and inexpensive one-time blood test used to personalize treatment of lipid abnormalities for greater precision of CVD prevention in people with T2DM. The results of this study may also serve to help explain the inconsistencies previously reported on the effect of HDL-cholesterol raising and triglycerides lowering drugs when used as monotherapy and the failure when used in combination with statins.

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Appendix 1: Predictors Of CVD And HDL-Cholesterol

Predictors of CVD

Age

Age is a well-known non-modifiable CVD risk factor. As age increases, the exposure to other CVD risk factors is cumulative. However, when these risk factors are incorporated into multivariable regression models, age remains an independent risk factor(11). There is a marked increase in CVD risk with age(133).

Hypertension

Hypertension, or high blood pressure is one of the most important risk factors for CVD. High blood pressure is more common in those with T2DM compared those without T2DM, with it being reported in more than two-thirds of T2DM patients(51). The risk conferred by the presence of both diabetes and hypertension is additive. Results from several prospective studies have demonstrated that the relationship between blood pressure and CVD events is positive, continuous and graded in the setting of T2DM(134). The ACC/AHA guidelines define the high blood pressure cut-point as systolic blood pressure ≥130 mmHg or diastolic blood pressure of ≥80 mmHg and the current recommended treatment target for people with T2DM is <130/80 mm Hg(135,136).

Antihypertensive Treatment

Many randomized controlled trials have demonstrated the benefit of blood pressure lowering (antihypertensive) medications on the reduction of risk of CVD morbidity and mortality(137,138). Treatment of high blood pressure appears to confer a greater benefit in people with diabetes compared to those without diabetes(139) In the Hypertension Optimal Treatment (HOT) clinical trial, the diabetes subgroup (n=1,501) had a 51% reduction in major cardiovascular events in the group with the lower blood pressure (diastolic blood pressure target of <80 mmHg) target compared to those in the higher blood pressure target group (diastolic blood pressure target of 85-90 mmHg)(140,141). In the UKPD study (n=1,148), more intensive blood pressure lowering led to a 44% reduction in strokes (p=0.013) and a 37% reduction in microvascular endpoints (p=0.009). Although it was not statistically significant, all-cause mortality was reduced by 18% and MI by 21%(142). In the placebo-controlled Systolic Hypertension in Europe (Syst-Eur) trial, participants with type 2 diabetes and isolated systolic hypertension (n=492) who had a reduced mean systolic blood pressure from 175 to 153 mmHg, were reported to have significant reductions in CVD mortality, CHD events and stroke(143).

Cigarette Smoking

Cigarette smoking is a major cause of CVD and past reports of the Surgeon General have reviewed the relevant evidence(141). The general mechanisms by which smoking results in CVD include contribution to the development of atherosclerotic plaques and thrombosis(144).

In patients with T2DM, smoking has been shown to increase the risk of CVD. In the UKPD study, was shown to be a significant and independent risk factor for CHD in patients with type 2 diabetes. Compared to non-smokers, smokers had a higher risk of

coronary artery disease including fatal and non-fatal events (HR=1.41, 95% CI: 1.06-1.88)(144). In women with type 2 diabetes who were enrolled in the Nurses' health study, it was found that that cigarette smoking was associated with increased CHD and mortality in a dose-dependent manner(9). Compared to Nurses who had never smoked, the RR for CHD was 1.66 (95% CI: 1.10-2.52) for current smokers of 1-14 cigarettes per day and 2.68 (95%CI: 2.07-3.48) for those who smoke 15 or more cigarettes per day(145,146). Smoking has also been linked to stroke in T2DM, although the relationship is not as strong as the relationship with CHD. In the UKPD study, cigarette smoking was identified as an independent and significant risk factor for stroke(145). In the Nurses' Health study, in women who smoked 1-14 cigarettes the relative risk for stroke was not significant. In those who smoked more than 15 cigarettes per day, the relative risk for stroke was 1.84 (95% CI: 1.21-2.81)(9).
A large prospective cohort study has examined the effects of smoking cessation on CVD risk and found that it reduces mortality among T2DM patients however the risk remains high for several years after quitting and is dependent on the duration of smoking history(145).

Obesity
An abundance of clinical and epidemiological evidence has linked obesity to CVD, including both CHD and stroke, and is considered a major modifiable CVD risk factor(147)**.** Overweight and Obesity, defined as the excessive accumulation of body fat, can increase the risk of CVD morbidity and mortality both directly and indirectly. The direct effects of obesity on CVD include structural and functional changes of the cardiovascular system and cytokine-mediated inflammation and thrombosis. The indirect effects are mediated by associated CVD risk factors including insulin resistance, hypertension, dyslipidemia and T2DM(148,149).
Body mass index (BMI), defined as body weight in kilograms divided by height in meters squared, is the most widely used measure of generalized obesity and has been linked with CVD risk. The National Heart Lung and Blood Institute defines BMI categories as follows: underweight (BMI <18.5 kg/m^2), normal (18.5 ≤ BMI ≤24.9 kg/m^2), overweight (25.0 ≤ BMI ≤29.9 kg/m^2) and obese (BMI >30)(150). In the ACCORD Lipid trial, the mean BMI in each of the fibrate and placebo group was ~32kg/m^2(151). The use of BMI has been criticized due to its inability to distinguish between fat and lean body mass as well as its inability to incorporate different patterns of body composition(23). Measures of central obesity such as waist circumference (WC) and waist-to-height (WHtR) have been suggested as alternative measures of CVD risk.
Current obesity guidelines define a waist circumference of ≥40 inches (102 cm) for males and ≥35 inches (88 cm) for females as being linked with a higher CVD risk and an important clinical compliment to BMI measurements(152). However, different cut-points have been recommended for various racial/ethnic groups(153). Additionally, WC measurements have not been well adapted in clinical settings and therefore errors in measurement and inconsistency of technique are common(154). In the general population, WHtR has been shown to be a better predictor of CVD compared to WC and BMI(155,156). Among the general population, in previous studies a WHtR cut-point of 0.5 has been shown to be effective at predicting and increased CVD risk across different ages, regions and ethnic groups (157–159). However, there is evidence to suggest that

this cut-point is too low in the setting of T2DM and an established WHtR cut-point for predicting CVD in this population is lacking(158,160–162).

Previous CVD

Individuals with clinical manifestations of vascular disease have a higher risk of CVD events, including MI, stroke and death, compared to those without a CVD history(163). Patients with a history of stroke have a 10-year stroke risk of 19% and a combined risk of stroke MI and vascular death of 43%(44,164). Individuals with evidence of CHD or arteriosclerosis (hardening and narrowing) of arteries other than the heart have a 10-year CHD risk of >20%, which is considered high risk(46).

Early studies of cardiovascular risk in diabetes concluded that adults with diabetes and no history of MI had the same risk for future MI as adults without diabetes who had a history of MI. As a result, the 2001 National Cholesterol Education Program (NECP) Adult Treatment Panel (ATP) III guidelines recommended that all individuals with diabetes be considered as a "coronary heart disease risk equivalent"(165). The assertion that all patients with diabetes are CHD risk equivalent has since been debated based on controversial results published in subsequent studies(166). In a large prospective cohort study of 1,586,061 individuals over 9 years, Rana et al., compared the risk of CHD among individuals with and without a history of diabetes or CHD. The results demonstrated across age and sex strata the risk of CHD was the highest in patients with both diabetes and a history of CHD (HR=3.9, 95 % CI: 3.8–4.0) followed by prior CHD alone (HR=2.8, 95 % CI: 2.7–2.85) and then diabetes alone (HR=1.7, 95 % CI: 1.66–1.74), compared to those with no diabetes and no prior CHD(167). These finding support the 2013 ACC/AHA risk assessment guideline which considers diabetes as a predictor rather than automatic CHD equivalent.

Family History of CVD

A family history of heart disease increases the risk of CVD. In particular, the relationship between family history and risk of CVD is the most prominent in those with a family history of premature CVD in first degree relatives (defined as aged <55 in men and ≤65 in women)(45). In the Framingham Heart Study, participants with first degree relatives who had a validated premature CVD event had approximately double the risk of CVD controlling for other risk factors(168–170). In the Women's Health Study, compared to patients with diabetes without a family history of CVD, the incidence in those with at least 1 first degree relative with a history of CVD was 50% higher (HR = 1.50, 95% CI: 1.20–1.87) when adjusting for other CVD risk factors(169,170). The American Diabetes Association considers a family history of premature CHD to be an important risk factor for CVD(171).

Ethnicity/ Race

Many people of racial and ethnic minority groups face a higher burden of CVD and CVD related risk factors. In the United States, Blacks have an increased incidence of CHD, stroke and overall CVD mortality compared to whites(172). Compared to white males, Hispanics have an overall lower CVD mortality rate, however; the incidence rates of CHD and stroke appear to be higher(46,173). Studies have shown consistently higher mean number of ideal cardiovascular health factors and behaviors in whites compared to

people of color(46,174). People of color are less likely to be in the ideal category for blood pressure, physical activity, BMI, diet, and blood glucose while they are more likely to be in the ideal category for smoking status and total cholesterol(175). There is additional variation upon stratification by sex. Although it is less well-documented, ethnic disparities in the risk of CVD have also been observed in the setting of T2DM. A 2005 systematic review concluded that Blacks, Hispanic Americans and Asian Americans had a lower risk for developing cardiovascular complications of diabetes compared to whites, although Asians had similar rates of CHD compared to whites(176,177). Despite the lower or equal rates in diabetic CVD, Gentile et Seftchick. demonstrated a higher mortality rate for from stroke in American Hispanics compared to whites, while Blacks appear to have a higher rate of CVD mortality compared to whites(178). In the Strong Heart Study, Native Americans are shown to have a risk of CVD disease that is more than double that of the general population(179,180).
The reasons for the differences in CVD risk among different ethnic groups is not fully understood and are likely complex and multi-factorial including sociocultural, environmental, genomic and treatment-disparity factors.

Geographic Area
Geographic variations in both incidence of CVD and prevalence of CVD risk factors is a recognized current challenge. According to the 2018 Heart Disease and Stroke Statistics report, the burden of CVD in the United States differs geographically, with the southeast having particularly higher prevalence of stroke, CHD, angina, and higher rates of CVD mortality(181). Regional differences in the prevalence of CVD have also been described in Canada(46). Variations in the prevalence of traditional CVD risk factors account only partially for geographic disparities in cardiovascular events, suggesting that other factors may be contributing.
In the Cardiovascular Health in Ambulatory Care Research Team (CANHEART) Regional Variation study of a large cohort of 5.5 million adults in Ontario, Canada, the authors show an almost two-fold variation in rates of cardiovascular events between regions of lowest and highest incidence. The rates ranged from 2.1 per 1000 per-years for women in low-risk regions to 7.7 per 1000 per-years for men in high-risk regions. While the majority of the variation could be attributed to socioeconomic and demographic factors, ethnic composition and other traditional CVD risk factors, approximately 16% of the variation was accounted for by health system factors that differed between health networks. In regions with the highest event rates, which consisted of mainly isolated and sparsely populated areas, had poorer blood pressure control, fewer visits to family physicians, less lipid screening and less use of lipid medications in older adults with diabetes(182).

Diabetes Duration
The duration and onset of diabetes may also contribute to the progression and severity of CVD. Although the date of T2DM onset can be difficult to determine, increasing duration of diabetes diagnosis has been associated with increasing CVD risk. Longitudinal data from the Framingham Heart Study suggests that the risk of CHD mortality is higher (RR=1.86, 95% CI: 1.17–2.93)

for each 10-year increase in diabetes duration, adjusting for age, sex and other traditional CVD risk factors. The adjusted risk of CHD morbidity for each 10-year increment in diabetes duration was not statistically significant (RR=1.38 95% CI: 0.99–1.92)(183). Other studies have also shown a positive association between diabetes duration and CVD mortality(184). Some studies did not observe a relationship between diabetes duration and CVD(185–187). More recent studies have shown diabetes duration of >10 years to increase the risk of CHD morbidity and mortality while controlling for traditional and novel CVD risk factors(188,189).

Education

In developed countries, previous observational studies have demonstrated an inverse causal association between education and CVD events and mortality(45,190). Education has also been shown to be inversely associated with CVD risk factors including smoking, obesity, blood pressure and diabetes(191–193). Therefore, the effect of education on CVD risk is at least partially mediated by other CVD risk factors. The National Health and Nutrition Examination Survey from 1988 to 2008 demonstrated an overall improved reduction in CVD risk factors among patients with T2DM, but the reductions were significantly higher among those with higher levels of education. Notably, they observed no improvement of smoking rates and poor glycemic control among participants with less than a high school degree. Individuals who had at least some college were also less likely to smoke and more likely to have better blood pressure control(194–197).
However, in the Women's Health Study, education remained an independent predictor of CVD events even after adjustment for other traditional and non-traditional CVD risk factors(198). In a prospective cohort study of patients with type 2 diabetes, compared to those with a college graduate degree or more, participants with less than a high school graduate degree were at a higher risk of CVD mortality while adjusting for other CVD risk factors (HR= 1.47, 95% CI: 1.01-2.15)(192).

Alcohol consumption

Considerable research describes the cardiovascular effects of habitual moderate and heavy alcohol consumption. Moderate alcohol consumption has been linked with improved cardiovascular health in various studies in patients with and without diabetes and they suggest a cardiovascular benefit of up to 1 drink per day for women and 2 for men. Moderate alcohol consumption has been shown to decrease the risk of CHD, stroke and CVD mortality(199). Heavy drinking increases the risk of CVD(200–202). The National Institute of Alcohol Abuse and Alcoholism defines moderate drinking as up to four drinks for men and three for women in any given day or fourteen drinks for men and seven drinks for women per week, whereas excessive drinking is anything larger than that(201,202).

Other Predictors of HDL-cholesterol

Age

Aging is often accompanied by dysregulation of the body's cholesterol metabolism. A clinical manifestation of this process is age-related changes in serum lipoproteins. Total cholesterol tends to increase with age in young or middle-aged adults. In those aged ≥

65, total cholesterol tends to decrease with age(203). The relationship between HDL cholesterol and age is less clear and does not appear to change as much during adulthood, although some studies have reported a decrease in HDL cholesterol with age in mostly middle-aged subjects(204–206). Cross-sectional reports of HDL tend to be higher in older age groups however several longitudinal studies in the elderly have reported conflicting results(207–209).
Data on the effect of aging on HDL-cholesterol quality are limited however functional impairment of HDL-cholesterol has been reported with increasing age. HDL-cholesterol from elderly subjects have shown increased susceptibility to oxidation and a significant reduction in their anti-oxidant ability(205,208,210–213). Additionally, Berrougui et al. have demonstrated a reduction in the capacity of HDL-cholesterol to mediate reverse cholesterol transport in the elderly(214,215).

Hormone Replacement Therapy
Hormone replacement therapy affects plasma HDL-cholesterol levels. All regimens with estrogen replacement only have been shown to increase HDL-cholesterol levels. Estrogen-progestogen replacement combination decreases or abolishes the effect of estrogen on HDL-cholesterol(216). Further studies are needed to assess the direct effects of hormone replacement therapy on HDL function.

Cigarette Smoking
Many studies have shown that smoking affects HDL-cholesterol metabolism. Smokers experience an increase in triglycerides and non-HDL-cholesterol as well as a decrease in HDL-cholesterol(217). In a meta-analysis, Craig et al demonstrated a 5.7% reduction of HDL cholesterol in smokers compared to non-smokers(218,219). Studies have also reported that smoking cessation leads to normalization of HDL-cholesterol levels to quantities similar to non-smokers, or a value in-between those of smokers and non-smokers(218).
Cigarette smoking can also affect HDL function. HDL-cholesterol can be oxidatively modified by cigarette smoking which can lead to dysfunctional HDL and promote atherosclerosis(220–222). Ueyama et al demonstrated that cigarette smoke significantly reduced the cholesterol efflux activity of HDL-cholesterol to the same level of HDL-cholesterol that has been oxidatively modified by copper ion(223,224). Therefore, cigarette smoking negatively affects the anti-atherogenic properties of HDL which may contribute to the increased CVD risk associated with cigarette smoking.

Obesity
Obesity is associated with abnormal metabolism and a low concentration of HDL-cholesterol. In obese individuals, an increase in serum levels of triglycerides is thought to cause the decrease in HDL-cholesterol levels. Epidemiological data from the large, cross-sectional multinational Lipid Research Clinics Program Prevalence study found a significant inverse relationship between BMI and HDL-cholesterol in Caucasian adult men and women while controlling for other confounding variables of HDL-cholesterol concentration(224). In a meta-analysis of 58 prospective cohort studies, BMI was significantly inversely associated with HDL-cholesterol in developed countries(225).

In addition to the reduction of HDL-cholesterol quantity, obesity is associated with changes in HDL function related to reverse-cholesterol transport and inflammation. In an in vitro study by Sasahaa et al., BMI was negatively correlated with the capacity of HDL-cholesterol to promote reverse cholesterol transport(226). Studies have also demonstrated a reduction in HDL-cholesterol antioxidant capabilities in obese individuals(227).

Ethnicity/ Race

Ethnic differences in lipid profile have been documented and for the first time, the 2018 ACC/AHA cholesterol guidelines describe race and ethnic backgrounds as "risk-enhancing factors" for cholesterol profiles and state that all ethnic minority groups appear to be at a greater risk for dyslipidemia. The reasons for racial/ethnic differences in lipid profiles are complex and are attributed to, in part, genetic, socioeconomic and lifestyle differences. Black Americans have higher levels of HDL-cholesterol and lower levels of triglycerides compared to whites and Hispanics. Asian Americans tend to have lower levels of HDL-cholesterol and higher levels of triglycerides compared to whites. Hispanic women tend to have a higher prevalence of HDL-cholesterol compared to Hispanic men(228,229). Further studies are needed to assess the effect of ethnicity on HDL function.

Alcohol Consumption

Alcohol intake is known to increase HDL-cholesterol. However, unlike the U-shaped relationship observed between alcohol intake and CVD risk, greater alcohol consumption has been linked with HDL-cholesterol concentration in a dose-dependent manner(165). Studies on the effect of alcohol consumption and HDL-cholesterol function and subclass distribution are limited and inconsistent. Moderate and heavy alcohol consumption has been demonstrated to enhance the first steps in reverse cholesterol transport function of HDL-cholesterol, although this relationship was not observed in all studies(230,231). However, the ability of HDL cholesterol to complete reverse cholesterol transport and deliver cholesterol to liver cells may be impaired in both moderate and heavy drinkers(232–234).

Appendix 2: Pre-Specified Outcome Definitions

The pre-specified definitions of ACCORD outcomes and previously established CVD can be found below. In order to maintain the integrity of the data, these definitions have not been changed or reworded.

The primary endpoint for the original ACCORD was the composite outcome of death from
cardiovascular causes, nonfatal myocardial infarction, or nonfatal stroke (total CVD). Cardiovascular deaths are defined in section A, myocardial infarctions are defined in section B, and strokes are defined in Section C.

A) Cardiovascular Death

a.1 Unexpected death: Unexpected death presumed to be due to ischemic cardiovascular disease, occurring within 24 hours of the onset of symptoms without confirmation of cardiovascular disease, and without clinical or post mortem evidence of other etiology.

a.2 Fatal Myocardial infarction (MI): death within 7 days of the onset of documented MI (see
below for definition of MI).

a.3 Congestive heart failure (CHF): death due to clinical, radiological or postmortem evidence
of CHF without clinical or postmortem evidence of an acute ischemic event (cardiogenic shock
to be included).

a.4 Death after invasive cardiovascular interventions: death associated with the intervention,
i.e., within 30 days of cardiovascular surgery, or within 7 days of cardiac catheterization, arrhythmia ablation, angioplasty, atherectomy, stent deployment, or other invasive coronary or
peripheral vascular intervention.

a.5 Documented arrhythmia: death due to bradyarrhythmias or tachyarrhythmias not associated
with an acute cardiac ischemic event.

a.6 Death following non-cardiovascular surgery: death due to cardiovascular causes as defined
above within 30 days of surgery.

a.7 Stroke: death due to stroke occurring within 7 days of the signs and symptoms of a stroke
(see below for definition of stroke).

a.8 Other cardiovascular diseases: death due to other vascular diseases including pulmonary
emboli and abdominal aortic aneurysm rupture.
a.9 Presumed cardiovascular death: Suspicion of cardiovascular death with supporting clinical
evidence that may not fulfill criteria otherwise stated. Example: Patient admitted with typical
chest pain of 3 hours duration and treated as an MI, but without ECG and enzymatic documentation to meet usual criteria.

B) Myocardial Infarction

b.1 Q-wave MI: Diagnosis based on the occurrence of a compatible clinical syndrome with
prolonged ischemic symptoms, associated with the development of new significant Q waves
(defined in the ECG Reading Center Manual of Procedures). Diagnostic elevation of cardiac
enzymes will include: increase in CK-MB mass to a level > twice the upper limit of normal,
and/or and increase in Troponin T or I to a level that indicates myonecrosis in the laboratory
performing the study.
b.2 Non Q-wave MI: Diagnosis based on the occurrence of a compatible clinical syndrome with
prolonged ischemic symptoms, associated with elevation of serum enzymes, as for Q-wave MI.
Only in the case that both Troponin and CK-MB mass measurements are not available, would
the elevation of total CK to > twice the upper limit of normal qualify for diagnosis.

b.3 Silent (unrecognized) MI: development of new significant Q waves without other evidence of
myocardial infarction (the date of event will be assigned halfway between the date of discovery
and last normal ECG).

b.4 Probable non Q-wave MI: Diagnosis based on the occurrence of a compatible clinical syndrome with prolonged ischemic symptoms, without documentation of cardiac enzyme elevation, but associated with the development of new and persistent significant ST-T changes
(>24 hr in duration). (Changes are defined in the ECG Reading Center Manual of Procedures).
MI after cardiovascular invasive interventions Diagnosis based upon the occurrence of CK-MB

(or Troponin) elevations to a level increased 3-5 times normal for the laboratory performing the
studies, occurring within 7 days of cardiac catheterization, arrhythmia ablation, angioplasty,
atherectomy, stent deployment or other invasive coronary, carotid or peripheral vascular intervention.

b. 5 MI after coronary bypass graft surgery: Diagnosis based upon the occurrence of CK-MB (or
Troponin) elevations to a level increased > 5-10 times normal for the laboratory performing the
studies, occurring within 30 days of cardiac surgery.
MI after non-cardiovascular surgery: MI (as defined above), occurring within 30 days of noncardiovascular
surgery.

C) Stroke

c.1 Definite ischemic stroke: CT or MRI scan within 14 days of onset of a focal neurological
deficit lasting more than 24 hours with evidence of brain infarction (mottled cerebral pattern or
decreased density in a compatible location), no intraparenchymal hemorrhage by CT/MRI, no
significant blood in the subarachnoid space by CT/MRI or by lumbar puncture, or autopsy
confirmation. A nonvascular etiology must be absent. 15

c.2 Definite primary intracerebral hemorrhage: Focal neurological deficit lasting more than 24
hours. Confirmation of intraparenchymal hemorrhage in a compatible location with CT/MRI
scan within 14 days of the deficit onset, or at autopsy, or by lumbar puncture.

c.3 Subarachnoid hemorrhage: Sudden onset of a headache, neck stiffness, loss of consciousness.
There may be a focal neurological deficit, but neck stiffness is more prominent. Blood in the
subarachnoid space by CT/MRI or lumbar puncture or intraventricular by CT/MRI.
Stroke of unknown type etiology: Definite stroke of unknown etiology when CT, MRI, or autopsy are not done. Information is inadequate to diagnose ischemic (infarction), intracerebral
hemorrhage, or subarachnoid hemorrhage.

c.4 Non-fatal stroke after cardiovascular invasive interventions: stroke associated to the intervention within 30 days of cardiovascular surgery, or within 7 days of cardiac

catheterization, arrhythmia ablation, angioplasty, atherectomy, stent deployment or other invasive coronary or peripheral vascular interventions.

c.5 Non-fatal stroke post non-cardiovascular surgery: stroke (as defined above) occurring within
30 days of non-cardiovascular surgery.

D) Major Coronary Heart Disease Events

Fatal events (defined in Section a.1 through a.6 and a.8 through a.9), nonfatal myocardial infarction (defined in Section B) and unstable angina (defined in Section E).

E) Unstable Angina

New onset exertional angina, accelerated or rest angina, or both, and at least 1 of the following
(Downs 1998):
a) at least 1-mm ST segment deviation and reversible defect on stress perfusion study, or
b) angiographic findings of at least 90% epicardial coronary artery or at least 50% stenosis in the left main coronary artery, or
c) at least 1-mm ST segment deviation with pain on ECG stress testing and/or rest ECG and evidence of at least 50% stenosis in a major epicardial coronary artery

F) Previous CVD

CVD History (most recent must be > 3 months ago): For each item marked 'Yes', supportive evidence of the diagnosis should be kept in the participant's chart.

- Myocardial infarction - Documentation of an old or age-indeterminate myocardial infarction (MI), may be by one of the following: Q-waves on an ECG; akinesis or dyskinesis on echocardiogram, MUGA, or ventriculogram; prior hospital discharge diagnosis, significant cardiac enzyme test results. If enzyme tests for this particular MI were performed on more than one date, document the date of the total CPK, CK-MB, or Troponin-I that first became significant for occurrence of an MI, or verification from the primary or consulting physician that a MI has occurred
- Stroke (or CVA) – Documentation of stroke may be by hospital discharge diagnosis, or by infarct on CT scan, an MRI, or verification from the primary or consulting physician that a stroke has occurred. Supportive evidence of the diagnosis (history and physical, discharge summary, or CT or MRI report) should be kept in the participant’s chart.
- Angina and/or ischemic changes (ECG) on Graded Exercise Tolerance Test or positive imaging - Documentation of angina and/or ischemic changes may be identified with the noninvasive cardiac diagnostic procedures such as Exercise testing (ST depression ≥ 1mm for ≥1 minute); Stress echocardiography (reversible wall motion abnormality); and Stress thallium (reversible or fixed ischemia, or SPECT).
- Coronary revascularization procedures: CABG; PTCI/PTCA/Atherectomy (with or without stenting) - Identification of the specific type of coronary

revascularization should be listed in the source documents with supportive evidence filed in the participant's chart. Some examples would be coronary artery bypass graft (CABG) surgery, stent placement, percutaneous transluminal coronary angioplasty (PTCA), rotoablation, or laser (LEAD) atherectomy.
• Other revascularization procedures: Carotid Artery Revascularization; Peripheral Artery Revascularization; AAA Repair; Other - This is defined as documented carotid endarterectomy, LEAD (leg) atherectomy, peripheral artery bypass, abdominal aortic aneurysm repair, or revascularization of other peripheral artery. Copies of the associated hospital discharge summary procedure report(s) should be kept in the participant's chart.

Appendix 3: Covariable Names And Categorization

Covariate	**ACCORD Variable(s)**	**Coding of Variable**
Age	baseline_age	Continuous
Sex	female	1= Female 0= Male (Ref)
Cigarette smoking	x4smoke quityrs	0= Nonsmoker (Ref) 1= Current smoker 2= Past smoker
Hypertension	sbp dbp	0= No hypertension (Ref) 1= Hypertension
antihypertensive medication use	loop thiazide ksparing potassium a2rb acei dhp_ccb nondhp_ccb alpha_blocker central_agent beta_blocker vasodilator reserpine other_bpmed	0= No anti-hypertensive medication use (Ref) 1=Hypertensive medication use
Obesity	wt_kg ht_cm	Continuous
Triglycerides	trig	Continuous
LDL-cholesterol	ldl	Continuous
Blood sugar (%HbA1c)	hba1c	Continuous
Alcohol intake	alcohol	0= Nondrinker (0 drinks weekly) (Ref) 1= Drinker
Ethnicity/race	raceclass	0= White (Ref) 1= Black 2= Hispanic 3= Other
Glycemic control group/ diabetes medication use	arm	0= Standard glycemic control (Ref) 1= Strict glycemic control
Diabetes duration	yrsdiab	0= ≤10 years (Ref) 1= >10 years
Geographic region	network	Clinical networks throughout the US and Canada coded 1-7.

Covariate	ACCORD Variable(s)	Coding of Variable
Previously established CVD at baseline	cvd_hx_baseline	0= No clinical CVD at baseline (Ref) 1= Clinical CVD at baseline
Family history of CVD	histhart	0= No family history of heart disease, heart attack or stroke (Ref) 1=History of premature heart disease, heart attack or stroke 2= Family history of heart disease, heart attack or stroke at unknown age 3= Unknown
Hormone replacement therapy	female progestin estrogen	0= Female with no estrogen/progestogen hormone replacement therapy (Ref) 1= Female with estrogen/progestogen hormone replacement therapy
Education	edu	0= High school graduate/GED (Ref) 1= Less than high school 2= Some college 3= College degree or higher
Baseline statin use	statin	0= No statin use at baseline (Ref) 1= Statin use at baseline