Non-fasting remnant cholesterol as a predictor for cardiovascular disease risk in Canadians with and without diabetes mellitus in the Alberta's Tomorrow Project

by

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Abstract

Cardiovascular disease (CVD) is a leading cause of death in Canada, and its risk of development is 3 times greater in those with diabetes mellitus (DM). Fasting low-density lipoprotein cholesterol (LDL-C) is the traditional lipid biomarker used in CVD risk assessment. While lowering LDL-C does reduce CVD risk, substantial residual risk remains and underscores the complex multifactorial nature of the disease. Recent studies in Europe have demonstrated that elevated non-fasting remnant cholesterol (RC) significantly predicts CVD risk, often with greater statistical power than for LDL-C. While Canadian lipid screening guidelines do recommend the measurement of non-fasting lipids, Canadian epidemiological data on non-fasting RC and CVD risk prediction is lacking in individuals with and without DM. The first study in this thesis aimed to determine the relationship between non-fasting RC (compared to LDL-C) and CVD in the Canadian general population, and the second study aimed to determine the relationship between non-fasting RC (compared to other lipids) and CVD in Canadians with and without DM.

Alberta's Tomorrow Project (ATP) is a large prospective Canadian cohort initiated in 2000. Non-fasting lipid data collected from this cohort was linked to Alberta Health administrative databases for individual-level determination of CVD outcomes and covariates such as: statin use, diagnosis of DM, and other comorbidities. The relationship of non-fasting RC (calculated as total cholesterol – LDL-C – high-density lipoprotein cholesterol) and other lipids with a composite of incident CVD was determined by multivariate logistic regression in n=13, 988 eligible participants in the first study, and n=13, 631 eligible participants stratified by DM status in the second study.

In the first study, 69.4% of participants were female and had a mean age of 61.8±9.7 years. Mean non-fasting RC was significantly elevated in individuals with CVD (n=1,156; RC 0.87±0.40 mmol/L) compared to individuals without CVD (n=12, 832; RC 0.78±0.38 mmol/L). Conversely, mean LDL-C was moderately decreased in individuals with CVD compared to those without (2.69±0.93 mmol/L and 2.88±0.84 mmol/L, respectively). Accordingly, the odds of incident composite CVD per 1 mmol/L increase of RC were significantly elevated by 48% (adjusted odds ratio (aOR) 1.48; 95% confidence interval (CI) 1.27-1.73), and odds of CVD per 1 mmol/L increase of LDL-C were significantly decreased by 27% (aOR 0.73; 95% CI 0.68-0.79).

In the second study, 69.8% of participants were female and had a mean age of 61.6±9.7 years. Individuals with DM (n=881) had significantly higher mean non-fasting RC (0.94±0.41 mmol/L) and proportion of incident CVD diagnoses (12.2%) compared to individuals without DM (n=12, 750, RC 0.77±0.38 mmol/L, CVD 7.4%). In addition, individuals with DM had significantly lower mean LDL-C compared to those without DM (2.22±0.90 mmol/L and 2.92±0.82 mmol/L, respectively). Unit (1 mmol/L) increases of non-fasting RC were significantly associated with increased odds of CVD in individuals without DM (aOR 1.39; 95% CI 1.16-1.65) and tended to be associated with increased odds of CVD in individuals with DM (aOR 1.32; 95% CI 0.79-2.22).

Non-fasting RC significantly predicted CVD incidence in the ATP population overall and in those without DM. Similarly, non-fasting RC tended to predict increased CVD risk in participants with DM, although we recognize that the latter observation did not reach statistical significance and had relatively low event rates. Further studies are needed in Canadian populations to determine normal reference ranges for non-fasting RC. However, in light of the

rising clinical importance of treating residual CVD risk, non-fasting RC could be considered as a potential screening and treatment target; both in the general population and in those with DM.

Preface

This thesis is an original work by Olivia Weaver. The research presented in chapters 2 and 3 received ethics approval from the University of Alberta Research Ethics Board on April 13, 2018 (study title: "Non-fasting lipids to better predict heart disease using ATP cohort", study ID: Pro00073641). A version of chapter 2 has been submitted to CMAJ Open for publication, and a version of chapter 3 will also be submitted to a journal for publication.

The study design of chapter 2 represents the combined effort of Jacqueline Krysa, Dean Eurich and Spencer Proctor. Data was obtained from ATP and linked to Alberta Health data by Ming Ye and Dean Eurich. Data analysis was completed by Olivia Weaver and Jacqueline Krysa, and interpretation of results was done by Olivia Weaver, Jacqueline Krysa, Dean Eurich and Spencer Proctor. The first draft of the manuscript was written by Jacqueline Krysa and updated by Olivia Weaver with feedback from Dean Eurich and Spencer Proctor.

The study design of chapter 2 represents the combined effort of Olivia Weaver, Dean Eurich and Spencer Proctor. Data was obtained from ATP and linked to Alberta Health data by Ming Ye and Dean Eurich. Data analysis was completed by Olivia Weaver, and interpretation of results was done by Olivia Weaver, Dean Eurich and Spencer Proctor. The manuscript was written by Olivia Weaver with feedback from Dean Eurich and Spencer Proctor.

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List of Abbreviations

ABC: Alberta Blue Cross

ACS: Acute coronary syndrome

AIS: Acute ischemic stroke

aOR: Adjusted odds ratio

apoA1: Apolipoprotein A1

apoB: Apolipoprotein B

apoB48: Apolipoprotein B48

apoB100: Apolipoprotein B100

apoE: Apolipoprotein E

ASCVD: Atherosclerotic cardiovascular disease

ATP: Alberta's Tomorrow Project

BMI: Body mass index

CABG: Coronary artery bypass graft

CanPath: Canadian Partnership for Tomorrow's Health

cCHS: Combined Copenhagen Heart Study

CCHS: Copenhagen City Heart Study

CCS: Canadian Cardiovascular Society

CE: Cholesteryl ester

CGPS: Copenhagen General Population Study

CI: Confidence interval

CIHDS: Copenhagen Ischemic Heart Disease Study

CM: Chylomicron

CMr: Chylomicron remnant

CVD: Cardiovascular disease

DAD: Discharge Abstract Data

DM: Diabetes mellitus

ECM: Extracellular matrix

EPA: Eicosapentanoic acid

GLP1-RA: glucagon-like peptide-1 receptor agonist

HDL: High-density lipoprotein

HDL-C: High-density lipoprotein cholesterol

HF: Heart failure

HMG-CoA: 3-hydroxy-3-methylglutaryl coenzyme A

HR: Hazard ratio

HSL: Hormone sensitive lipase

ICD: International Classification of Diseases

IDL: Intermediate-density lipoprotein

IHD: Ischemic heart disease

IPE: Icosapent ethyl

LDL: Low-density lipoprotein

LDL-C: Low-density lipoprotein cholesterol

LDL-R: Low-density lipoprotein receptor

LPL: Lipoprotein lipase

MACE: Major adverse cardiovascular events

MI: Myocardial infarction

NMR: Nuclear magnetic resonance

Non-HDL-C: Non-high-density lipoprotein cholesterol

OR: Odds ratio

PCI: Percutaneous coronary intervention

PCSK9: proprotein convertase subtilisin/kexin type 9

PHN: Personal Health Number

PIN: Pharmaceutical Information Network

REDUCE-IT: Reduction of Cardiovascular Events with Icosapent Ethyl-Intervention Trial

RC: Remnant cholesterol

SD: Standard deviation

SGLT2i: sodium-glucose cotransporter-2 inhibitor

TC: Total cholesterol

T1DM: Type 1 diabetes mellitus

T2DM: Type 2 diabetes mellitus

TG: Triglyceride

TIA: Transient ischemic attack

VLDL: Very low-density lipoprotein

Chapter 1: Introduction

1.1 Global and national cardiovascular disease burden

Globally, there were 523 million prevalent cases of total cardiovascular diseases (CVD) in 2019 (1). Despite a decreasing rate of death attributed to CVD, it is still the primary cause of chronic disease-related death in the world (1). In Canada, 2.6 million individuals had existing ischemic heart disease (IHD) in 2017. While the age-standardized prevalence rate gradually decreased in the 10 years prior to this, the prevalence and prevalence rate of IHD increased overall (2). The number of deaths due to IHD have decreased over the past 20 years in Canada, but heart diseases have nonetheless remained among the top causes of mortality (3-5).

1.2 Lipoproteins and lipid metabolism in normal physiological conditions

Triglycerides (TG) and cholesteryl esters (CE) consumed in a meal are absorbed into enterocytes as their component parts: monoglycerides, fatty acids and cholesterol before being reassembled and transported throughout the body in chylomicrons (CM). CM are large lipoproteins that allow for transport of these hydrophobic lipids in aqueous plasma, and are composed of cholesterol and several apolipoproteins (including apolipoproteins B48 and E (apoB48 and apoE)) embedded in a phospholipid monolayer (6, 7). Lipoprotein lipase (LPL) hydrolyzes TG, and the cleaved fatty acids are removed from circulation by receptor mediated uptake mechanisms in tissues. CM thus become smaller, more cholesterol dense chylomicron remnants (CMr) (6). The liver is responsible for the majority of removal of CMr via the LDL-receptor (LDL-R) which can bind the ligands apoE and apolipoproteinB100 (apoB100) (see additional detail below) (6, 7) (Figure 1.1).

The liver is also responsible for de novo lipogenesis, where the enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase plays an important role in maintaining

cholesterol homeostasis (6, 8). Endogenously synthesized as well as exogenously sourced TG and cholesterol (from CMr) are secreted from the liver in very-low density lipoproteins (VLDL). Structurally, VLDL resembles CM but is smaller and contains apoB100. Similar to CM, during circulation fatty acids are taken up by tissues after LPL hydrolyzes TG, and VLDL become smaller, more cholesterol dense intermediate-density and low-density lipoproteins (IDL and LDL), that are also returned to the liver by hepatic LDL-R (6) (Figure 1.2).

Finally, high-density lipoproteins (HDL) containing apolipoprotein A1 (apoA1), among other apolipoproteins, are secreted from the liver and intestine. These transport cholesterol from tissues, other lipoproteins and macrophages back to the liver (6).

CMr, VLDL and IDL share similarities in size (approximately 25-80nm), density and direction of cholesterol transport compared to other lipoproteins (CM, LDL and HDL) which allow them to contribute uniquely to atherosclerosis (6, 9, 10). They are collectively classified as 'remnant' lipoproteins, and the cholesterol carried within is termed 'remnant cholesterol' (RC) (10).

1.3 Pathophysiology of atherosclerotic cardiovascular diseases

IHD refers to arterial narrowing due to atherosclerotic plaque specifically in susceptible regions of the heart (11). This in turn can lead to reduced blood flow to the heart (angina) as well as the central nervous system (transient ischemic attack (TIA)), causing chest pain during exertion and temporary neurological dysfunction, respectively (11-13). Full or severe elimination of blood flow to the heart and brain due to atherosclerotic plaques results in myocardial infarction (MI) and acute ischemic stroke (AIS), and may result in death. MI may also cause damage to the heart itself and result in improper cardiac functioning (heart failure (HF)) (14-16).

Procedures such as coronary artery bypass grafting (CABG) and percutaneous coronary intervention (PCI) are able to physically circumvent these arterial blockages (17).

The role of lipids in the development of IHD is complex. Intestinally-derived CMr, and hepatically derived VLDL, IDL and LDL containing TG and cholesterol all influx and efflux from the arterial wall during normal lipid metabolic pathways (18). However, when lipoproteins delivered to the arterial wall are trapped due to their size and/or binding to arterial extracellular matrix (ECM) proteoglycans, an atherogenic inflammatory cascade of events is initiated (9, 18, 19). This prevailing concept has been termed the 'response to retention' hypothesis (20). The retention and accumulation of excess lipids within the vascular wall eventually leads to plaque formation and clinical CVD events (19).

Interestingly, there is evidence to suggest that LDL particles can influx and efflux from the arterial wall more readily than other larger lipoproteins because of their smaller size (21). In contrast, remnant lipoproteins also all enter the arterial wall, but are larger in size which appears to prevent them from exiting the arterial wall as readily (21, 22). CMr (which are present in higher concentrations during the non-fasted state) (9) are of additional importance as apoB48 has been reported to bind more readily to arterial proteoglycans within the ECM compared to apoB100 (23).

1.4 Risk factors for atherosclerotic cardiovascular diseases

Risk factors for CVD include a variety of lifestyle, genetic and metabolic components such as inactive lifestyles, poor diet, smoking, family history of CVD, obesity, hypertension and dyslipidemia. Diabetes mellitus (DM) (often itself associated with many of these) is also an important risk factor for CVD (24-26).

It is also important to note that CVD risk factors differ by sex, resulting in different disease outcomes (27, 28). For instance, risk of CVD is elevated in females during perimenopause, and compared to males this often aligns with CVD incidence later in life (29). Therefore, it is important to analyze novel risk factors for each sex separately.

1.5 First line interventions for cardiovascular disease management in Canada

In Canada, after a multifaceted approach to screening and determination of CVD risk level, the first line intervention in management of the disease is to improve changes in diet and lifestyle, followed by (and/or in conjunction with) pharmacological intervention (30).

The primary recommended pharmacotherapy is the 'statin' class of compounds (30), which inhibit HMG-CoA reductase in the liver and result in the downregulation of endogenous cholesterol synthesis. Reduced hepatic concentrations of cholesterol can lead to an upregulation of LDL-R expression, increasing low-density lipoprotein (LDL-C) uptake by the liver and reducing overall circulating concentrations (31). Fasting plasma RC can also be reduced by statins, although due to the mechanism of action, not as efficiently as LDL-C (32-34). This is also likely true of RC specifically in the non-fasted state, as statins have been shown to facilitate compensatory increases in cholesterol absorption by the intestine (35, 36).

Secondary pharmacotherapy such as Ezetimibe, proprotein convertase subtilisin kexin 9 (PCSK9) inhibitors and icosapent ethyl (IPE) may also be recommended if lipid targets are not met through statin use alone (30) (Table 1.1). Ezetimibe and PCSK9 inhibitors function (respectively) by reducing intestinal cholesterol absorption and inhibiting LDL-R degradation to further reduce circulating cholesterol concentrations. IPE is a synthesized derivative of eicosapentanoic acid (EPA; n-3 fatty acid) and is thought to act by reducing TG synthesis in the

liver. All of these therapies have been shown to reduce CVD risk in clinical situations (30, 37, 38).

1.6 Novel pharmacotherapies for cardiovascular disease management

Several new lipid-altering drugs including inclisiran, lomitapide and evinacumab have been developed to target different parts of the lipid metabolic pathway. Inclisiran, a novel PCSK9 inhibitor, prevents PCSK9 synthesis in the liver and is efficacious in lowering circulating LDL-C, non-high-density lipoprotein cholesterol (non-HDL-C) and apolipoprotein B (apoB) concentrations (37, 39). Lomitapide inhibits the microsomal triglyceride transfer protein responsible for packaging TG into lipoproteins in the liver and intestine. VLDL and CM secretion is thus reduced, and plasma levels of atherogenic lipids including LDL-C, non-HDL-C and TG (as well as apoB) are lowered (37, 40). Evinacumab targets angiopoietin-like protein 3 (an LPL inhibitor) in order to increase LPL activity and TG hydrolysis. In additional to plasma TG-lowering effects, evinacumab has also been shown to reduce circulating concentrations of LDL-C, non-HDL-C and apoB (37, 41). The efficacy of each of these pharmacotherapies in reducing CVD risk remains to be determined (37, 39-41).

1.7 Residual cardiovascular disease risk

Elevated LDL-C is causally associated with CVD (42-44) and thus has been (and continues to be) used as a CVD screening tool and treatment target in Canada and worldwide (30, 45, 46). However, CVD events still occur despite statin-reduced LDL-C levels (widely recognized as residual CVD risk) (47, 48). This is particularly evident in individuals with DM who often have greater CVD risk before and after statin treatment compared to individuals without DM (please also see section 1.8) (49, 50). Lipid abnormalities other than elevated LDL-C (such as elevated non-fasting RC), likely contribute to residual risk, highlighting the need to

establish screening protocols and treatment targets that reflect the complexity of CVD risk factors (47, 51, 52). Other lipid biomarkers are now being investigated as screening tools in Canada (30).

1.8 Novel lipid screening biomarkers: non-HDL-C, total apoB and non-fasting RC

Recently, the Canadian Cardiovascular Society (CCS) has embraced lipid screening in the non-fasted state (30). Despite minimal changes to traditional lipid levels after normal meal consumption (i.e LDL-C), non-fasting lipid panels may better reflect CVD risk by including lipid proportions from remnant-containing lipoproteins (9, 53). The CCS lipid screening guidelines currently recommend measuring non-HDL-C and total apoB in the fasted or non-fasted state (30). Both biomarkers are shown to be associated with CVD incidence and are assessments of lipid risk factors beyond LDL-C which may contribute to residual risk (54).

Non-HDL-C is a simple, cost-effective calculation from a standard lipid profile (total cholesterol (TC) – high-density lipoprotein cholesterol (HDL-C)). It represents the cholesterol concentration from all sources of atherogenic lipoproteins, including LDL-C and intestinally derived lipoproteins in the non-fasted state. It is not a direct measure of particle concentration despite being highly correlated with apoB in most cases (30, 53, 54). Total apoB is a direct measure of both apoB48 (in the non-fasted state) and apoB100 concentration, and thus of all atherogenic lipoprotein *particles* (since each contains only one apoB molecule). However it is acknowledged that while apoB can provide greater clinical insight in some forms of dyslipidemia, it does not provide information on cholesterol concentration per se and adds cost to screening as it is not included in a standard lipid profile (9, 30, 53, 54).

Similar to non-HDL-C, non-fasting RC is a simple, cost effective calculation from a standard non-fasting lipid profile (TC – HDL-C – LDL-C). It does not provide information on

particle concentration, but does provide information on cholesterol concentration in CMr, VLDL and IDL (9, 53). While it is not a direct measure and may not accurately estimate the true value of RC, it does correlate well with both measured RC and CVD incidence (55-58). Unlike non-HDL-C and total apoB, RC does not provide information on LDL or LDL-C. This is important because RC and LDL-C do not necessarily correlate well. Thus, RC may provide additional information on residual risk, particularly in individuals who might have normal LDL-C but with known and/or progressive CVD (56-58) (Table 1.2).

1.9 Epidemiology of diabetes mellitus and relationship to cardiovascular disease

1.9.1 Global and national diabetes mellitus burden

The prevalence of type 1 and type 2 diabetes mellitus (T1DM and T2DM) has been increasing worldwide and in 2019 reached 460 million individuals (59-61). Of this, 438 million were classified as T2DM and 22 million were classified as T1DM (60, 61). Despite minimal increases in incidence rate, both the prevalence and age-standardized prevalence rate of DM increased steadily between 2007 and 2017 in Canada (2). DM is among the 10 greatest causes of death globally and in Canada (3, 4, 59).

1.9.2 Pathophysiology of type 1 and type 2 diabetes mellitus phenotypes

T1DM is characterized by the absence of insulin production resulting in glucose dysregulation, due to destruction of the pancreatic beta cells (62, 63). In contrast, dysregulation of glucose in T2DM is the product of reduced insulin production and sensitivity, often caused by a variety of unfavorable lifestyle factors such as obesity (26, 63). Both forms of DM are often associated with dyslipidemia. Individuals with T2DM generally present with elevated non-fasting remnant lipoproteins, TG, small dense LDL, low HDL-C and normal LDL-C (51, 52).

Dyslipidemia in individuals with T1DM is often characterized by elevated non-fasting remnant lipoproteins, TG, small dense LDL, but can also include higher LDL-C (64-66).

1.9.3 Pathological modification of lipid metabolism pathways in diabetes mellitus

Individuals with DM experience elevated risk of CVD compared to individuals without DM (67, 68). Lipid-related mechanisms for this are complex but insulin dysregulation is a central contributing factor (51). Among other actions, normal functions of insulin provide negative feedback for VLDL secretion and hormone sensitive lipase (HSL) as well as upregulating LPL and LDL-R (51, 52, 64, 69). Thus, DM can contribute to increased intestinal and hepatic lipoprotein secretion, reduced lipolysis of lipoprotein TG content, and reduced lipoprotein uptake by the liver (51, 52, 64, 70). Ultimately, there is a prolonged circulation of greater concentrations of remnant lipoproteins, and increased transfer of TG to HDL and LDL (leading to reduced efficacy of reverse cholesterol transport, and small dense LDL formation) (51, 52). Increased exposure of the arterial wall to atherogenic lipoproteins may contribute to greater arterial entrapment. In addition, clinical situations of increased circulating concentrations of glucose can exacerbate glycation of arterial ECM proteoglycans (and/or advanced glycation end product formation) resulting in greater retention of lipids within the arterial wall (18, 51, 65, 71) (Figure 1.3).

1.9.4 Diabetes-specific pharmacological management of cardiovascular disease

The CCS recommends that all individuals with DM are screened for dyslipidemia and CVD risk. In conjunction with changes to diet and lifestyle, statins are prescribed for the majority of individuals regardless of lipid levels. If elevated lipids do not reach recommended targets with statin use, subsequent addition of ezetimibe to the treatment plan is recommended. Furthermore, the addition of PCSK9 inhibitors and/or IPE is recommend for those with DM who

have known atherosclerotic CVD (ASCVD) and/or persistent dyslipidemia (30). According to the Diabetes Canada Clinical Practice Guidelines, individuals who require better glucose control and/or have existing ASCVD (or CVD risk factors in older adults) may also be prescribed additional therapies. These can include certain glucagon-like peptide-1 receptor agonists (GLP1-RA) and/or sodium-glucose cotransporter 2 inhibitors (SGLT2i) which target glucose levels in addition to modulating CVD risk (Table 1.1) (72, 73). It is important to note that the primary recommended pharmacotherapy for controlling blood glucose levels in T2DM is metformin (73), and while not prescribed for CVD protection specifically, it may also have lipid lowering effects in the liver and intestine (74).

1.10 Population-based prospective cohort studies on the use of non-fasting remnant cholesterol for cardiovascular disease risk prediction

1.10.1 Remnant cholesterol and cardiovascular disease risk prediction in general populations

Key data regarding the relationship between non-fasting RC and CVD risk comes from large prospective cohorts established in Copenhagen: The Copenhagen City Heart Study (CCHS), the Copenhagen General Population Study (CGPS) and the Copenhagen Ischemic Heart Disease Study (CIHDS). Several papers have observationally determined the association between calculated non-fasting RC with various CVD diagnoses including MI, IHD, ischemic stroke, peripheral artery disease and CVD-related death (56, 57, 75-78). Non-fasting RC was associated with increasing CVD risk in all cases. For instance, non-fasting RC >1.5 mmol/L was associated with 1.8-2.4 times the risk of IHD, 3.4-4.2 times the risk of MI and 1.99 times the risk of ischemic stroke compared to those with non-fasting RC <0.5mmol/L (56, 75, 77). Patterns of increasing risk of MI were also shown to persist regardless of body mass index (BMI) (57). Mendelian randomization studies within these cohorts have strengthened this evidence by determining significant positive causal relationships between non-fasting RC, and MI, IHD and

CVD-related death (58, 79-81). Among some of the most important findings from these papers are a 2.8 times greater causal risk of IHD per 1 mmol/L increase in non-fasting RC (compared to only 1.5 times greater causal risk per 1 mmol/L increase in LDL-C) (58). Similarly, Jorgensen et al. (2013) found a 2.2 times greater causal risk of MI for a 2-fold genetic increase of non-fasting RC (79).

1.10.2 Remnant cholesterol and cardiovascular disease risk prediction in diabetes mellitus

Several studies have analyzed the prospective relationship between RC (calculated and measured in the fasting state) and CVD in various populations with DM in which participants often had other comorbidities such as existing CVD or chronic kidney disease. Observational findings from these all showed significant positive relationships between RC and a variety of CVD outcomes, including IHD, major adverse cardiovascular events (MACE) and CVD-related death, especially in those with DM that was poorly controlled (82-89). For example, Yu et al. (2021) found that individuals with DM and diabetic nephropathy had approximately 2 times the risk of CVD death if fasting RC was ≥0.77 mmol/L compared to <0.27 mmol/L (84). Additionally, Cao et al. (2020) found that among patients with existing coronary artery disease, individuals with DM and high fasting RC had approximately 1.6 times the risk of MACE compared to individuals without DM and low RC. Moreover, groups with either; high fasting RC but controlled DM or high fasting RC and uncontrolled DM, had 1.4 and 2.2 times the risk of MACE compared to those with low RC and controlled DM (86). One study using *non-fasting* RC did not find a significantly increased risk of CVD outcomes in participants with DM and existing angina (hazard ratio (HR) 1.05; 95% CI 0.46-2.37) (90). Interestingly, a sub-analysis from the CGPS and CCHS cohorts found a significant positive observational, but null causal relationship between non-fasting RC and IHD in those with DM (80).

1.10.3 Research questions remaining

There is a lack of Canadian epidemiological data on non-fasting RC and CVD in both the general population, as well as those with DM. Many of the studies that do exist analyzing the RC-CVD relationship in patients with DM only assessed fasting RC. In contrast, *non-fasting* RC is of particular importance as it more accurately reflects accumulating concentrations of cholesterol from a greater number of remnant lipoproteins. We propose that the latter is more likely representative of CVD risk as most people are in the non-fasted state for the majority of the day (9). More population-specific evidence is needed to support the determination of RC predictive power and normal reference ranges for possible use in CVD screening in Canadian clinical settings.

1.11 Objectives, Hypotheses and Methods

1.11.1 First objective and hypothesis

The first objective was to, in a Canadian general population, determine non-fasting RC and LDL-C concentrations, compare the levels of RC and LDL-C in those with and without CVD incidence, and determine the relationship between non-fasting RC (compared to LDL-C) and incident CVD. It was hypothesized that non-fasting RC and LDL-C would both be strong predictors of incident CVD risk.

1.11.2 Second objective and hypothesis

The second objective was to determine the relationship of non-fasting RC (compared to LDL-C, HDL-C and TG) with incident CVD in Canadian participants with and without DM. It was hypothesized that non-fasting RC would be a stronger predictor of CVD risk in those with DM compared to those without DM.

1.11.3 Overview of methods

Alberta's Tomorrow Project (ATP) is a Canadian cohort with n=52, 810 participants from Alberta (91). Non-fasting blood samples were obtained from a subset of patients which were then used to determine non-fasting TC, HDL-C, TG, RC and LDL-C (91, 92). Linkage to Alberta Health databases (including Physician Claims, Discharge Abstract Data, Pharmaceutical Information Network, and Alberta Blue Cross datasets) was possible through Personal Health Numbers provided by participants and allowed for individual-level determination of CVD outcomes and other covariates such as DM diagnosis (91, 93).

Similar analyses were employed to accomplish each objective: Descriptive statistics were used to compare lipid levels between groups based on CVD diagnosis, followed by multivariate logistic regression to determine the relationship between non-fasting lipids as continuous and categorical variables, and CVD as a binary outcome variable, while adjusting for comorbidities, age, sex, statin use and other lipids. Prior to the analysis for the second objective, participants were stratified by prevalent DM diagnosis and groups were analyzed separately.

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1.13 Tables

Table 1.1 Summary of pharmacotherapies used in Canada for cardiovascular-disease risk reduction (30, 73).

	Pharmaceutical agent	For use under the following conditions:
Lipid-targeting agents	Statins	• First-line pharmacotherapy for anyone with elevated CVD risk • Includes elevated LDL-C/non-HDL-C/apoB; existing ASCVD; majority of individuals with DM; other statin-indicated conditions
	Ezetimibe	• Statin-treated **&** LDL-C/non-HDL-C/apoB not at target
	PCSK9i	• Statin-treated **&** LDL-C/non-HDL-C/apoB not at target **&** have genetic hyperlipidemia **OR** • Statin-treated **&** LDL-C/non-HDL-C/apoB not at target **&** has DM (or other indicated conditions) **&** has ASCVD
	IPE	• Statin-treated **&** has elevated TG **&** has DM (or other indicated conditions) **&** has ASCVD **OR** • Statin-treated **&** has elevated TG **&** has DM **&** at least 50 years of age **&** has other CVD risk factor(s)
Glucose- and CVD-targeting agents	GLP-1RA	• Has DM **&** glucose not at target with primary therapy **&** at least 60 years of age **&** >1 CVD risk factor **OR** • Has DM **&** has existing ASCVD
	SGLT2i	• Has DM **&** glucose not at target with primary therapy **&** at least 60 years of age **&** >1 CVD risk factor **OR** • Has DM **&** has existing ASCVD or HF

ApoB, apolipoprotein B; ASCVD, atherosclerotic cardiovascular disease; CVD, cardiovascular disease; DM, diabetes mellitus; GLP-1RA, glucagon-like peptide-1 receptor agonist; HF, heart failure; IPE, icosapent ethyl; LDL-C, low-density lipoprotein cholesterol; non-HDL-C, non-high-density lipoprotein cholesterol; PCSK9i, proprotein convertase subtilisin/kexin type 9 inhibitor; SGLT2i, sodium-glucose cotransporter-2 inhibitor.

Table 1.2 Summary of current and novel lipid biomarkers for cardiovascular disease risk monitoring in Canada (9, 30, 53, 54).

	LDL-C	Non-HDL-C	Total apoB	Non-fasting RC
How is it measured?	Calculation: TC – HDL-C – (TG/5)	Calculation: TC – HDL-C	Direct measurement	Calculation: TC – HDL-C – LDL-C
What does it measure?	[Cholesterol] in LDL	[Cholesterol] in all atherogenic lipoproteins: CMr (if non-fasted), VLDL, IDL & LDL	Atherogenic [lipoprotein particle]: apoB48 (if non-fasted) & apoB100	[Cholesterol] in CMr, VLDL & IDL
Cost effective?	Yes	Yes	No	Yes
Included in standard lipid profile?	Yes	Yes	No	No
Included in lipid screening practices?	Yes; fasting or non-fasting	Yes; fasting or non-fasting	Yes; fasting or non-fasting	No

ApoB, apolipoprotein B; HDL-C, high-density lipoprotein cholesterol; LDL, low-density lipoprotein; LDL-C, low-density lipoprotein cholesterol; Non-HDL-C, non-high-density lipoprotein cholesterol; RC, remnant cholesterol; TC, total cholesterol; TG, triglycerides.

1.14 Figures

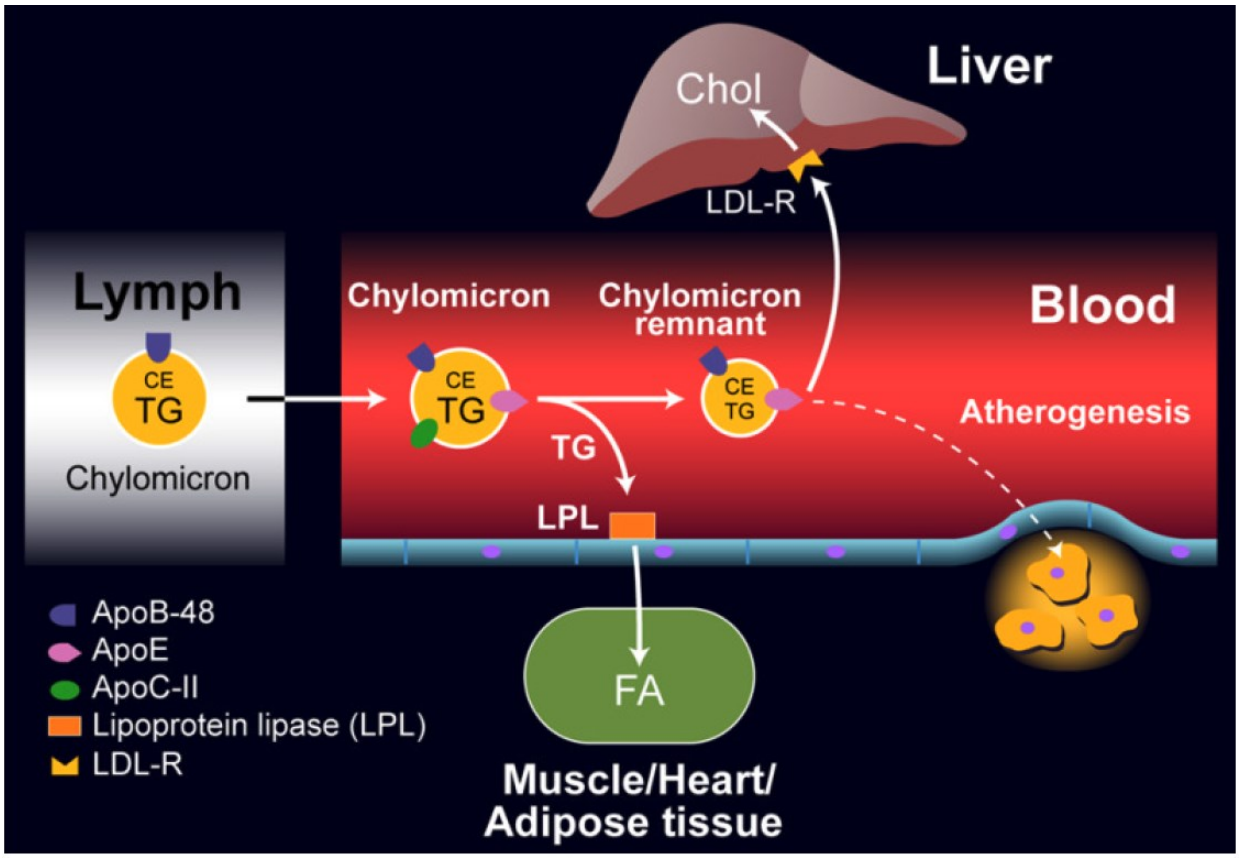

Figure 1.1 Summary of intestinally-derived lipid transport pathway. Apo; apolipoprotein; CE, cholesteryl ester; Chol, cholesterol; FA, fatty acid; LDL-R, low-density lipoprotein receptor; LPL, lipoprotein lipase; TG, triglycerides. Reprinted from Figure 5 from Feingold KR. Introduction to Lipids and Lipoproteins. In: Feingold KR, Anawalt B, Boyce A, et al., eds. Endotext. South Dartmouth (MA): MDText.com, Inc.; January 19, 2021. Copyright 2000-2022, MDText.com, Inc. Used under CC-BY-NC-ND license.

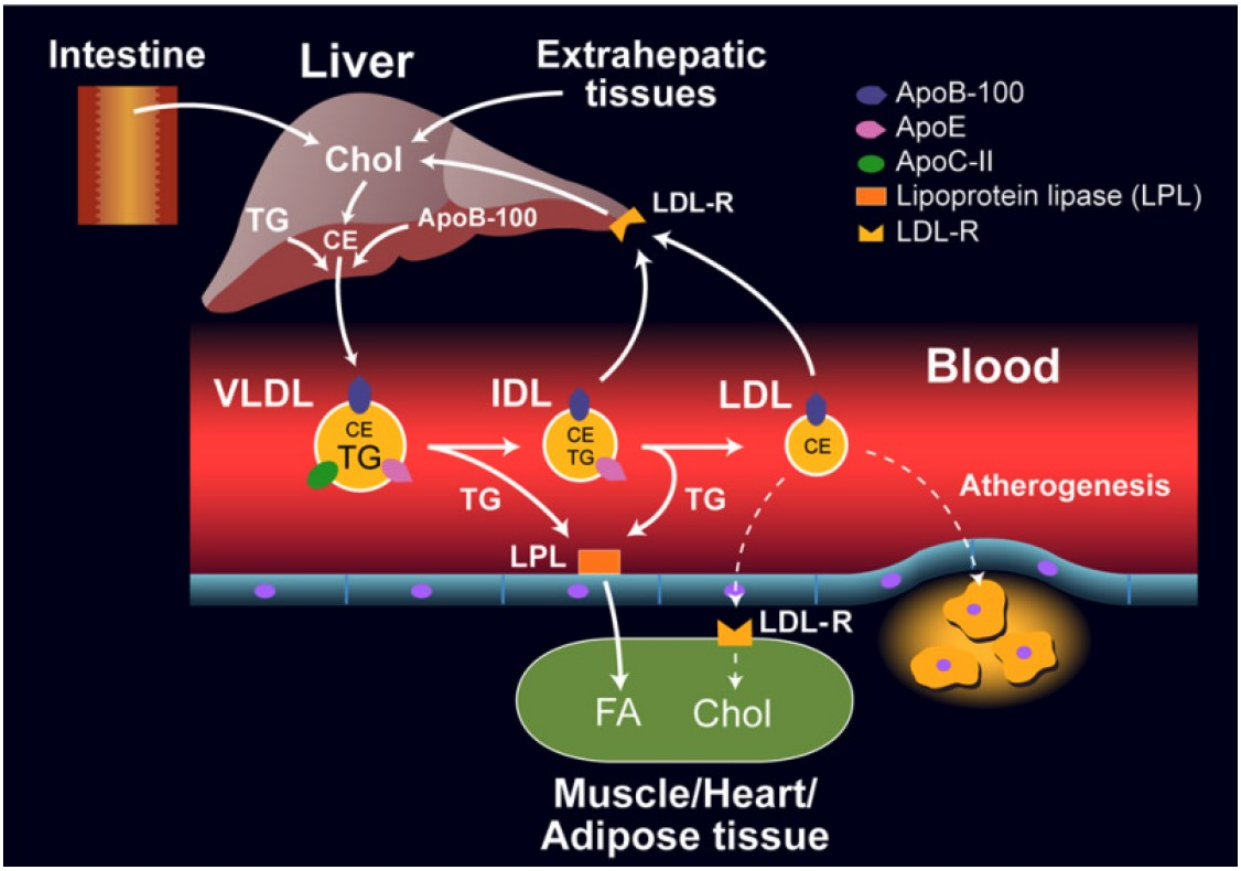

Figure 1.2 Summary of hepatically-derived lipid transport pathway. Apo; apolipoprotein; CE, cholesteryl ester; Chol, cholesterol; FA, fatty acid; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; LDL-R, low-density lipoprotein receptor; LPL, lipoprotein lipase; TG, triglycerides; VLDL, very low-density lipoprotein. Reprinted from Figure 7 from Feingold KR. Introduction to Lipids and Lipoproteins. In: Feingold KR, Anawalt B, Boyce A, et al., eds. Endotext. South Dartmouth (MA): MDText.com, Inc.; January 19, 2021. Copyright 2000-2022, MDText.com, Inc. Used under CC-BY-NC-ND license.

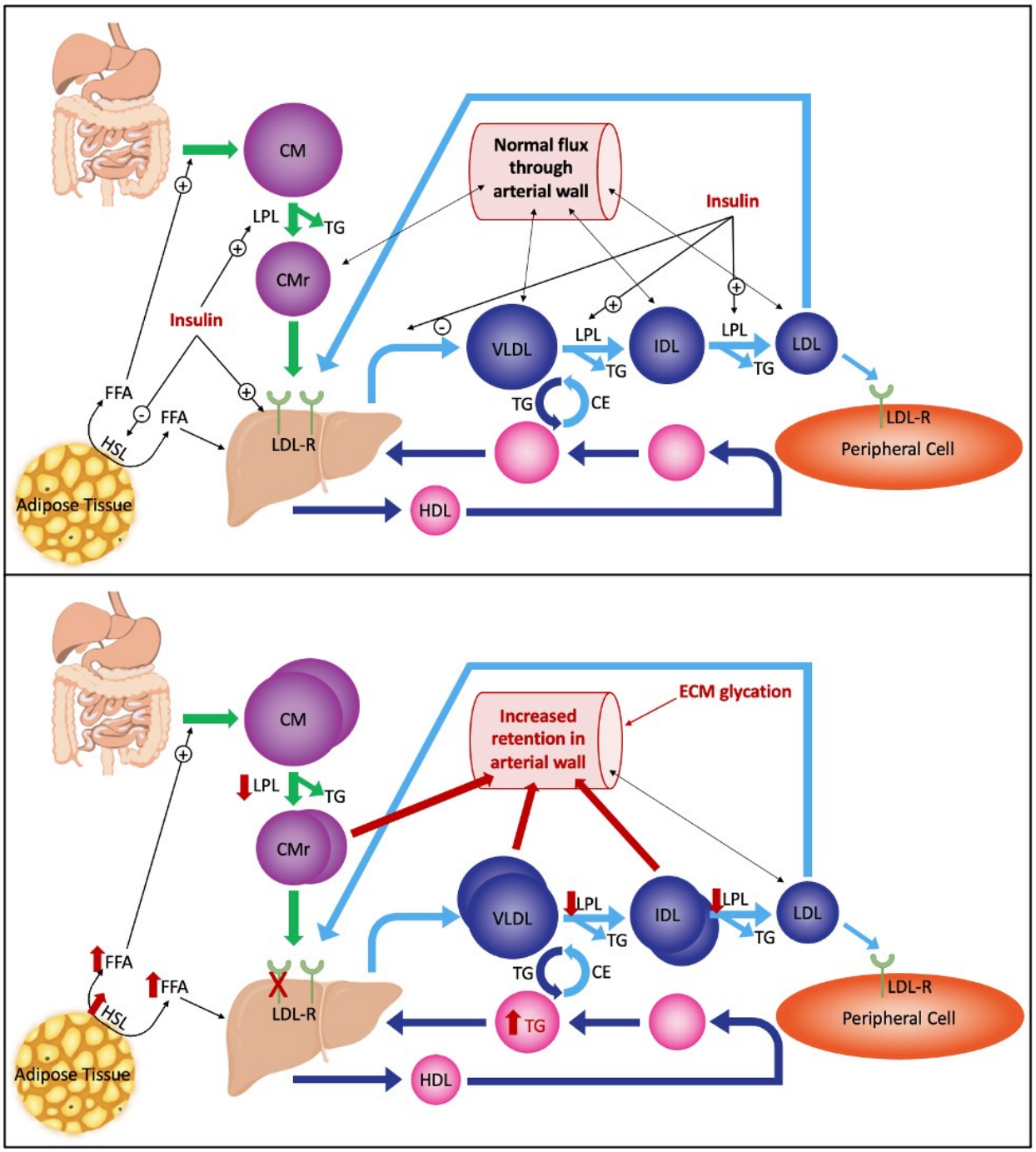

Figure 1.3 Summary of lipid dysregulation mechanisms in individuals with diabetes. Top panel: Normal lipid metabolism; Bottom panel: Lipid dysregulation. CE, cholesteryl ester; CM, chylomicron CMr, chylomicron remnant; ECM, extracellular matrix; FFA, free fatty acid; HDL, high-density lipoprotein; HSL, hormone sensitive lipase; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; LDL-R, low-density lipoprotein receptor; LPL, lipoprotein lipase;

TG, triglycerides; VLDL, very low-density lipoprotein. Adapted from Fig. 1 and Fig. 3 from Vergès B. Pathophysiology of diabetic dyslipidaemia: where are we? Diabetologia. 2015;58(5):886-99). Doi: 10.1007/s00125-015-3525-8. Copyright Bruno Vergès 2015. Used under CC-BY license.

Chapter 2: Non-fasting remnant cholesterol and implications for CVD risk reduction in Alberta's Tomorrow Project, a prospective cohort study.

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2.1 Introduction

In Canada, the primary lipid-screening tool for cardiovascular disease (CVD) is fasting low-density lipoprotein cholesterol (LDL-C) (1). Despite fasting LDL-C being a central screening and treatment target for dyslipidemia, substantial proportions of the population (including those with insulin resistance and diabetes) retain significant residual CVD risk (2). Recently, non-fasting remnant cholesterol (RC) (a calculation of remnant lipoprotein cholesterol using lipid measurements taken during the non-fasted state) has emerged as a novel CVD risk marker (3). Non-fasting RC originates from both the liver (very-low density lipoprotein remnant lipoproteins) and intestine (chylomicron remnant lipoproteins) and can be readily calculated using existing lipid panel criteria from samples drawn during the non-fasting state: RC = total cholesterol (TC) – [LDL-C + high-density lipoprotein cholesterol (HDL-C)] (4). Longitudinal data from Europe has demonstrated that a non-fasting lipid profile induces only a small (clinically irrelevant) lipid variation compared to a fasting lipid profile and that circulating non-fasting RC can independently predict CVD risk and has been shown to be a better predictor of CVD risk than LDL-C (4, 5). Indeed, Varbo et al. (2013) documented that risk of ischemic heart disease (IHD) was 2.8 times greater for every 1 mmol/L increase in non-fasting RC (4). Non-fasting lipid assessment has been added to lipid screening guidelines in Europe and more recently, to Canadian and American guidelines (1, 6, 7). However, while key data on non-fasting RC has been published in Europe, a significant void exists in North America. In 2012, Sidhu and

Naugler determined non-fasting lipid values using community lab data in a cohort in Alberta to characterize the effect of fasting time on plasma lipid subclass (8). More recently, Lawler et al. (2020) used an Ontario-based population with atherosclerotic CVD (ASCVD) to report on the association of hypertriglyceridemia with ASCVD(9). This study calculated RC values however was not able to verify either fasting or non-fasting status of the subjects (9). As a result, no Canadian cohort to our knowledge has determined non-fasting RC, its normative range in the population, nor assessed its association with CVD. These voids create challenges in understanding the normal distribution ranges and/or proposing reference ranges for clinical utility in Canada.

To conduct analysis on non-fasting lipids in Canada we utilized Alberta's Tomorrow Project (ATP), a longitudinal chronic disease cohort study (10). ATP began in October 2000 in Alberta, Canada (Phase I) and has been in partnership with the expanded Canadian Partnership for Tomorrow's Health (CanPath) since 2008 (phase II) (11-13). Participants have been followed-up through data linkage to health care databases (11-13). The purpose of this study was to measure and generate non-fasting RC and other lipid risk indices from the ATP cohort and to compare the levels of RC and LDL-C in those with and without CVD incidence. We hypothesized that both non-fasting RC and LDL-C would yield strong and positive relationships with the incidence of CVD.

2.2 Methods

2.2.1 Study population

The present study is a retrospective analysis of ATP and Canadian Partnership for Tomorrow's Health (CanPath). Recruitment and enrollment for Phase 1 of ATP occurred between 2000-2008 (n=29, 878), and Phase 2 occurred between 2009-2015 (n=22, 932) when the

ATP merged with CanPath, for a total ATP cohort of n=52, 810 (12). Recruitment and enrollment data for ATP is described in further detail elsewhere (10, 11, 13).

The subset of ATP participants used for the present analysis included those who had blood taken, consented to follow-up through data linkage and provided their Personal Health Number (PHN) (n=16, 700). The analysis further focused on those who had complete lipid-panel biochemical data, calculated RC and LDL-C greater than 0, triglycerides (TG) <4.5mmol/L, and no prevalent CVD (n=13,988) (Figure 2.1).

2.2.2 Biochemistry and metabolism

Blood sample collection for ATP/CanPath began in 2008 and ended in 2015. Participants (n=27, 910) provided around 50mL of blood in the postprandial state, which was separated into plasma and serum for lipid analysis and stored at -80°C (11, 12). Plasma HDL-C, TG, and TC were measured. LDL-C, non-high-density lipoprotein cholesterol (non-HDL-C) and non-fasting RC were calculated as [LDL-C=TC – HDL-C – (TG/2.2) (Friedwald formula); non-HDL-C=TC – HDL-C; and RC=TC – (LDL-C + HDL-C)].

2.2.3 Cardiovascular disease

Individual level information on incident CVD (and related medical procedures) were obtained from ATP personal linked healthcare data. Incident CVD and related procedures were defined as occurring in those without CVD diagnosis or procedures prior to or within 6 months of enrollment to ATP, or within 1 year of linkage to Alberta Health data. Incident (as opposed to prevalent) CVD was used as the primary outcome to maintain a temporal relationship between lipid profile measurement and CVD occurrence. CVD diagnosis included IHD, myocardial infarction (MI), angina, heart failure (HF), transient ischemic attack (TIA), and acute ischemic stroke (AIS). Procedures included percutaneous coronary intervention (PCI) and coronary artery

bypass graft (CABG). These were aggregated into a 'CVD composite' variable which was our primary outcome of interest. Where participant numbers allowed, components of the composite CVD outcome either individually or aggregated by similar diagnosis were considered as secondary outcomes. For example, MI and angina were aggregated as 'acute coronary syndrome' (ACS), TIA and AIS as 'stroke', and PCI and CABG as 'procedures'.

2.2.4 Statin use

For the present study, statin use was taken from both Alberta Blue Cross data, and Alberta's Pharmaceutical Information Network which captures all statin use irrespective of age or formulary status and linked to ATP study ID. Statin users were defined as participants in ATP that had been prescribed a statin prior to their CVD diagnoses and related procedures.

2.2.5 Elixhauser comorbidity index

The Elixhauser Comorbidity Index identifies 30 different comorbidities that can be used to generate a score for individuals based on their identified comorbid conditions within the physician claims and hospital discharge datasets (14).

2.2.6 Statistical analysis

Data was analysed using Stata/SE version 16.1 (StataCorp, LLC, College Station, Texas). Means and standard deviation (SD) were calculated for descriptive statistics of continuous variables. Baseline means for participants with and without CVD were compared using t-tests. Univariate logistic regression was used to determine the unadjusted odds ratios (OR) and 95% confidence intervals (CI) for the association between non-fasting lipids (RC, LDL-C) as both continuous and categorical (quartile) explanatory variables, and incident CVD. To further explore this relationship, a multivariate logistic regression was used to determine the adjusted odds ratios (aOR) and 95% CIs for the relationships between both the continuous and categorical

RC and LDL-C variables and CVD outcomes, while adjusting for age, sex, statin use prior to CVD diagnosis, Elixhauser comorbidity index, and either LDL-C or RC respectively. Adjusted analyses were additionally stratified by both sex and statin use. Cox proportional hazard regressions were additionally performed to determine the relationship between RC, LDL-C and the primary CVD outcome while adjusting for follow-up time. A p-value <0.05 was considered statistically significant.

In an exploratory analysis, we further assessed the relationship between non-fasting lipids and *prevalent* CVD. For these analyses, any patient was deemed a prevalent case if they had any of the predefined CVD diagnoses or procedures prior to their blood draw or within ±6 months of ATP enrollment. Analyses were similar to those completed for incident CVD.

2.2.7 Ethics approval

The former Alberta Cancer Board Research Ethics Committee and the University of Calgary Conjoint Health Research Ethics Board approved the recruitment and data collection for ATP (13). Further details to access ATP data is available from [www.myATP.ca] or by emailing [ATP.Research@ahs.ca]. The present analysis of non-fasting lipids in the ATP cohort was approved by the University of Alberta Research Ethics Board (Pro00073641).

2.3 Results

2.3.1 Cohort characteristics and non-fasting lipid profile

The final cohort subset of ATP contained n=13,988 individuals with a mean age of 61.8 years and was approximately 69.4% female (Table 2.1, Figure 2.1). Individuals with incident CVD were significantly older but had a lower Elixhauser comorbidity score and proportion of females compared to those without incident CVD. Around 18.9% of the total ATP subset were statin users, with a significantly greater proportion of individuals with CVD being statin users

compared to those without (Table 2.1). By lipid quartile, statin use increased with increasing quartiles of non-fasting RC, whereas statin use was highest in the lowest quartile of non-fasting LDL-C (Figure 2.2).

Individuals with incident CVD had significantly higher mean non-fasting RC and TG but lower LDL-C, HDL-C, TC and non-HDL-C compared to those without CVD (Table 2.1). Similar results for the primary lipids of interest were seen for *prevalent* CVD (Table 2.4).

2.3.2 Composite CVD incidence per mmol/L increase of non-fasting RC and LDL-C

Table 2.2 and Figure 2.3 present results from the univariate and multivariate logistic regressions performed, which included RC and LDL-C as continuous explanatory variables. Unadjusted analyses showed that composite CVD incidence was significantly and positively related to non-fasting RC (OR 1.81, CI 1.56-2.09). Conversely, it was significantly and *inversely* associated with non-fasting LDL-C (OR 0.76, CI 0.71-0.82). These results remained significant in the adjusted analysis: Per mmol/L increase in non-fasting RC, participants had 1.48 (CI 1.27-1.73) times the odds of having incident composite CVD. In contrast, patients had only 0.73 (CI 0.68-0.79) times the odds of having incident composite CVD per mmol/L increase in LDL-C. Results from the Cox regression were similar (Table 2.5).

In particular, increasing non-fasting RC was significantly associated with increased odds of composite CVD incidence in females (aOR 1.77, CI 1.44-2.19) but not males (aOR 1.10, 0.87-1.40), and in statin non-users (aOR 1.52, CI 1.26-1.82) but not in statin users (aOR 1.32, CI 0.98-1.79), although the point estimates were similar regardless of statin use. Increasing LDL-C remained significantly associated with reduced odds of incident composite CVD in both males (aOR 0.65, CI 0.57-0.74) and females (aOR 0.77, CI 0.70-0.85), and in both statin users (aOR

0.83, CI 0.73-0.94) and non-users (aOR 0.68, CI 0.62-0.74). Tables 2.6 and 2.7 summarize these results from the stratified analysis.

Univariate and multivariate logistic regression examining the odds of *prevalent* composite CVD per mmol/L increase in non-fasting RC and LDL-C yielded generally similar results (both unstratified and stratified by sex and statin use) (Tables 2.8-2.10).

2.3.3 Composite CVD incidence by quartile of non-fasting RC and LDL-C

The number of incident cases of composite CVD increased parallel to increasing quartiles of non-fasting RC. Conversely, cases tended to decline with increasing quartiles of non-fasting LDL-C (Figure 2.2). This is further demonstrated in the results of both the unadjusted and adjusted logistic regressions analyzing the association between CVD incidence and RC and LDL-C as categorical (quartile) explanatory variables (Table 2.3). The adjusted analysis showed that quartiles 3 and 4 of non-fasting RC were significantly associated with increased odds of incident composite CVD in comparison to quartile 1 (aOR 1.39 (CI 1.15-1.68) and aOR 1.46 (CI 1.22-1.76) respectively). Individuals with non-fasting LDL-C in quartiles 2, 3 and 4 had aOR 0.64 (CI 0.54-0.76), 0.53 (CI 0.44-0.63) and 0.53 (CI 0.44-0.63) (respectively) of incident composite CVD compared to those in quartile 1. Results from the Cox regression were similar (Table 2.11).

When adjusted results were stratified by sex (Table 2.12), results by quartile of RC remained significant for females only. Females with RC levels in quartiles 3 and 4 had, respectively, an aOR of 1.40 (CI 1.10-1.79) and 1.70 (1.34-2.16) for incident composite CVD compared to those in quartile 1. LDL-C concentrations in all quartiles compared to quartile 1 indicated significantly reduced odds of composite CVD incidence in both males (aOR 0.65, CI 0.51-0.85; aOR 0.46, CI 0.35-0.61 and aOR 0.47 CI 0.35-0.63 for quartiles 2, 3 and 4

respectively) and females (aOR 0.61, CI 0.48-0.77; aOR 0.56, CI 0.44-0.70 and aOR 0.54, CI 0.43-0.68 for quartiles 2, 3 and 4 respectively).

In statin non-users, the top two quartiles of RC had significantly increased adjusted odds of incident composite CVD compared to those in the first quartile (aOR 1.44, CI 1.17-1.78 and aOR 1.52, CI 1.23-1.87 respectively). Further, all quartiles of LDL-C showed significantly decreased adjusted odds of incident composite CVD compared to the reference quartile (aOR 0.52, CI 0.43-0.64; aOR 0.45, CI 0.37-0.55 and aOR 0.47, CI 0.38-0.57 respectively for quartiles 2, 3 and 4). No significant differences in incident composite CVD between quartiles of RC or LDL-C were seen in those prescribed statins, except for a significant protective effect seen in quartile 4 of LDL-C compared to quartile 1 (aOR 0.64, CI 0.45-0.92). Table 2.13 presents adjusted results stratified by statin use.

In the analysis of *prevalent* composite CVD, all quartiles of RC and LDL-C were significantly associated with increased odds and reduced odds of CVD, respectively. Males with RC levels in quartiles 2-4 had significantly greater odds of CVD compared to those in quartile 1 while similar trends were seen in females. A significantly protective effect against prevalent CVD for all quartiles of LDL-C was seen in both males and females. Stratification by statin use yielded generally similar results as for the analysis of incident CVD. See Tables 2.14-2.17.

2.3.4 Association between non-fasting RC, LDL-C and components of 'CVD composite'

Secondary outcomes generally followed the same trends as the composite CVD variable (Table 2.2). Prior to adjustment, the odds of IHD, ACS, HF and procedures were significantly increased per mmol/L increase of RC. After adjustment, only the odds of IHD remained significantly increased per mmol/L increase of RC. The odds of all diagnoses and procedures were significantly reduced per mmol/L increase in LDL-C prior to and after adjustment. Smaller

patient numbers for each secondary outcome variable likely drove the loss of significance due to reduced statistical power.

After stratification of adjusted analyses (see Tables 2.6 and 2.7), females had significantly increased adjusted odds of IHD and ACS per mmol/L increase of RC, whereas no significant associations were seen in males for any of the secondary outcomes. Per mmol/L increase in LDL-C, males and females both had significantly lower adjusted odds of IHD and ACS. Males also had significantly lower adjusted odds of stroke and procedures, whereas females also had significantly lower adjusted odds of HF. Adjusted odds of IHD, ACS and HF were significantly increased per mmol/L increase of RC in statin non-users, however no significant associations were seen in statin users. LDL-C maintained a significant protective effect against all secondary outcomes in statin non-users, but was only significantly protective against IHD in statin users, after adjustment.

In general, results from the analysis of LDL-C and *prevalent* CVD components followed similar patterns to *incident* CVD components, however non-fasting RC and *prevalent* components showed less discernable patterns (Tables 2.8-2.10).

2.4 Interpretation

The dataset represented in this paper is the first to our knowledge to establish the relationship of non-fasting RC with CVD in a large Canadian population. Only RC (not LDL-C) was associated with increasing odds of incidence for CVD, particularly in females. Intriguingly, the frequency of statin users increased with RC quartiles and did not appear to influence CVD diagnoses. Conversely, the highest number of statin users were observed in the lowest quartile of LDL-C, while still representing the highest number of CVD diagnoses. These results appear to align with studies in humans and rodent models with dyslipidemia showing that statins, which

reduce cholesterol synthesis in the liver, may also induce an upregulation of absorption and secretion of cholesterol in the intestine (15, 16).

A recent population study from Ontario, Canada revealed that hypertriglyceridemia was positively associated with CVD events under conditions of pre-existing ASCVD (possibly, as noted by the authors, due to cholesterol content of TG-rich lipoproteins) (9). The authors were unable to verify fasting or non-fasting status of the subjects and did not contrast the ASCVD risk relationship with RC values. The median RC value for those with ASCVD was 0.6mmol/L [0.4-0.8 mmol/L] which is lower than what was observed for ATP (mean 0.78±0.38 mmol/L). One explanation for this could be a higher proportion of sampling in the fasted state relative to sampling in the non-fasted state for ATP.

The combined Copenhagen Heart Study (cCHS) is a large prospective European cohort composed of the Copenhagen General Population Study, Copenhagen City Heart Study and Copenhagen Ischemic Heart Disease Study that previously demonstrated a causal relationship between non-fasting RC and CVD and is described in further detail elsewhere (4, 17). In comparison, the mean non-fasting RC values in ATP (0.78±0.38 mmol/L) were indeed comparable to RC values from the cCHS (ranging from median 0.6 (0.4-0.9) mmol/L to 0.7 (0.5-1.0) mmol/L) (4). However, the cCHS also observed a corresponding positive relationship of LDL-C with CVD risk (albeit not as strong as RC in some analyses), contrary to the current ATP analysis. Possibly, these incongruent findings could be due to lower mean LDL-C in the ATP (2.86±0.85 mmol/L) compared to a range of median 3.2 mmol/L to 3.7 mmol/L in the cCHS. We also note the time frame of blood sampling in the cCHS was 1991-2009 and for ATP was 2008-2015, which could impact this relationship. In a post-hoc analysis stratifying patients by LDL-C, ATP participants with LDL-C >3.4 mmol/L (the threshold for initiating pharmacotherapy in

Canada) had similar results to a recent study by Castaner et al. (2020) which found no relationship between LDL-C (mean 3.34±0.82 mmol/L) and CVD (1, 18).

In the ATP, statin use was linear with increasing quartiles of non-fasting RC but highest in the lowest quartile of LDL-C. The lowest quartile of LDL-C also had the greatest incident cases of CVD. These findings may suggest that despite lowering LDL-C through statin use, residual CVD risk in the lowest quartile of LDL-C is inherent in a subpopulation of individuals. For instance, it has been reported that certain high-risk populations such as those with diabetes, are often at elevated CVD-risk despite lowering LDL-C (19).

We also acknowledge that postprandial TG has a much stronger positive association with CVD compared to fasting TG (20). Currently, there are few available standard pharmacological therapies to target non-fasting RC and/or hypertriglyceridemia. Statins tend to have mixed or null effect on TG and/or TG-rich lipoproteins and therefore may not influence non-fasting RC per se (21). Certainly, the demographic and outcomes of the Reduction of Cardiovascular Events with Icosapent Ethyl-Intervention Trial (REDUCE-IT) trial would suggest additional benefit of TG lowering to those subjects already well controlled for LDL-C (22). Alternatively, in statin-treated individuals with residually high LDL-C, Ezetimibe is recommended to further reduce CVD risk (1, 23). For those who are statin intolerant, other drugs such as bempedoic acid are being investigated (24). We postulate that adding non-fasting RC to the management of ASCVD risk may further benefit these populations.

2.5 Limitations and Future Directions

For this study, a calculated measure of RC that closely correlates with TG was used (21). Though recent studies have shown that calculated RC values can be adopted in a clinical setting for prognostic, predictive and therapeutic purposes (21, 25), RC is an evolving field. In future

there may be more reliable, cost-effective options available for direct measurement of RC such as using nuclear magnetic resonance (NMR) (26). In the meantime, a calculated measurement can be used with a non-fasting/ambulatory sample, which may provide additional options for clinicians at point of care. Indeed, new lipid guidelines are taking this approach. Additionally, while the ATP cohort provides some unique insights into the relationship of RC and CVD, the demographic may not be representative of all Canadians (e.g. age, sex).

Future work will need to delineate the predictive power of non-fasting RC, explore its utility as a novel and/or adjunct CVD risk marker in Canada, and validate the RC/CVD relationship in other sample Canadian populations. It will be important to determine normative reference values of non-fasting RC that can be used by physicians in Canada. Given similarities between the distribution range of RC in both Canada and Europe, it may be possible to consider European reference values to inform their utility for practice in Canada (5).

2.6 Conclusion

The predominantly female ATP cohort represents an opportunity to particularly assess the impact of RC on CVD incidence in women. Indeed, the data from this cohort suggests that non-fasting RC is significantly associated with CVD risk, especially in females and may be a useful adjunct target, especially in the context of well-controlled LDL-C and high statin use.

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Alberta Health. The interpretation and conclusions contained herein are those of the researchers and do not necessarily represent the views of the Government of Alberta. Neither the Government nor Alberta Health express any opinion in relation to this study.

2.8 Disclaimers

The views expressed herein represent the views of the author(s) and not of Alberta's Tomorrow Project or any of its funders. The interpretation and conclusions contained herein are those of the researchers and do not necessarily represent the views of the Government of Alberta. Neither the Government nor Alberta Health express any opinion in relation to this study.

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2.10 Tables

Table 2.1 Baseline cohort characteristics and non-fasting lipid panel.

	Total (n= 13, 988)	**Without incident CVD** (n=12, 832)	**With incident CVD** (n=1, 156)	**p-value** (with vs without CVD)
Age (yrs)	61.8±9.7	61.2±9.6	68.1±8.6	**<0.0001**
Females n (%)	9, 710 (69.4)	9,040 (70.5)	670 (58.0)	**<0.0001**
Statin use n (%)	2, 639 (18.9)	2, 342 (18.3)	297 (25.7)	**<0.0001**
Elixhauser score	2.80±2.19	2.83±2.19	2.52±2.17	**<0.0001**
LDL-C (mmol/L)	2.86±0.85	2.88±0.84	2.69±0.93	**<0.0001**
HDL-C (mmol/L)	1.51±0.44	1.52±0.45	1.39±0.42	**<0.0001**
TC (mmol/L)	5.15±0.96	5.17±0.95	4.95±1.06	**<0.0001**
Non-HDL-C (mmol/L)	3.65±0.95	3.66±0.94	3.56±1.01	**0.002**
RC (mmol/L)	0.78±0.38	0.78±0.38	0.87±0.40	**<0.0001**
TG (mmol/L)	1.73±0.84	1.71±0.84	1.92±0.88	**<0.0001**

Data presented as mean ± SD or n (%). CVD, Cardiovascular disease; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Non-HDL-C, non-high density lipoprotein cholesterol; RC, remnant cholesterol; TC, total cholesterol; TG, triglycerides.

Table 2.2 Unadjusted and adjusted odds ratios of CVD incidence per mmol/L increase of non-fasting RC and LDL-C.

		Unadjusted		**Adjusted**[1]	
RC	**CVD** n (%)	**OR**	**p-value**	**OR**	**p-value**
CVD composite	1,156 (8.3)	1.81 (1.56-2.09)	**<0.001**	1.48 (1.27-1.73)	**<0.001**
IHD	1,056 (7.6)	1.83 (1.57-2.13)	**<0.001**	1.49 (1.27-1.75)	**<0.001**
ACS[2]	153 (1.1)	2.18 (1.51-3.15)	**<0.001**	1.42 (0.97-2.08)	0.07
HF	168 (1.2)	1.95 (1.36-2.79)	**<0.001**	1.47 (1.01-2.14)	0.05
Stroke[3]	53 (0.38)	1.75 (0.92-3.33)	0.09	1.12 (0.58-2.17)	0.73
Procedures[4]	127 (0.91)	2.08 (1.39-3.13)	**<0.001**	1.18 (0.77-1.80)	0.44
LDL-C	**CVD** n (%)	**OR**	**p-value**	**OR**	**p-value**
CVD composite	1,156 (8.3)	0.76 (0.71-0.82)	**<0.001**	0.73 (0.68-0.79)	**<0.001**
IHD	1,056 (7.6)	0.77 (0.71-0.83)	**<0.001**	0.74 (0.69-0.81)	**<0.001**
ACS[2]	153 (1.1)	0.51 (0.41-0.62)	**<0.001**	0.55 (0.44-0.68)	**<0.001**
HF	168 (1.2)	0.64 (0.53-0.77)	**<0.001**	0.64 (0.53-0.78)	**<0.001**
Stroke[3]	53 (0.38)	0.46 (0.33-0.65)	**<0.001**	0.47 (0.33-0.69)	**<0.001**
Procedures[4]	127 (0.91)	0.41 (0.33-0.52)	**<0.001**	0.49 (0.39-0.62)	**<0.001**

Data presented as unadjusted and adjusted (by multivariable logistic regression) OR (CI). ACS, acute coronary syndrome; CVD, cardiovascular disease; HF, heart failure; IHD, ischemic heart disease; LDL-C, low-density lipoprotein cholesterol; OR, odds ratio; RC, remnant cholesterol.

[1]RC model adjusted for age, sex, statin use, Elixhauser comorbidity index and LDL-C. LDL-C model adjusted for age, sex, statin use, Elixhauser comorbidity index and RC.

[2]Aggregate of myocardial infarction and angina.

[3]Aggregate of transient ischemic attack and acute ischemic stroke.

[4]Aggregate of percutaneous coronary intervention and coronary artery bypass graft.

Table 2.3 Unadjusted and adjusted odds ratios of composite CVD incidence by quartile of non-fasting RC and LDL-C.

	Unadjusted		**Adjusted**[1]	
RC Quartile (range)	**OR**	**p-value**	**OR**	**p-value**
1 (0.06-0.49)	1	(reference)	1	(reference)
2 (0.50-0.70)	1.13 (0.93-1.36)	0.21	0.98 (0.80-1.19)	0.82
3 (0.71-0.99)	1.62 (1.35-1.94)	**<0.001**	1.39 (1.15-1.68)	**0.001**
4 (1.00-2.04)	1.86 (1.56-2.22)	**<0.001**	1.46 (1.22-1.76)	**<0.001**
LDL-C Quartile (range)	**OR**	**p-value**	**OR**	**p-value**
1 (0.26-2.28)	1	(reference)	1	(reference)
2 (2.29-2.82)	0.65 (0.55-0.77)	**<0.001**	0.64 (0.54-0.76)	**<0.001**
3 (2.83-3.40)	0.57 (0.49-0.68)	**<0.001**	0.53 (0.44-0.63)	**<0.001**
4 (3.41-8.83)	0.62 (0.52-0.73)	**<0.001**	0.53 (0.44-0.63)	**<0.001**

Data presented as unadjusted and adjusted (by multivariable logistic regression) OR (CI) with quartile 1 as the reference group. LDL-C, low-density lipoprotein cholesterol; OR, odds ratio; RC, remnant cholesterol.

[1]RC model adjusted for age, sex, statin use, Elixhauser comorbidity index and LDL-C. LDL-C model adjusted for age, sex, statin use, Elixhauser comorbidity index and RC.

Table 2.4 Mean non-fasting RC and LDL-C for those with and without prevalence of composite CVD.

Lipid	**Total** (n=15, 764)	**Without prevalent CVD** (n=13, 988)	**With prevalent CVD** (n=1, 776)	**p-value** (with vs without)
RC	0.79±0.38	0.78±0.38	0.86±0.39	**<0.0001**
LDL-C	2.83±0.87	2.86±0.85	2.59±0.96	**<0.0001**

Data presented as mean ± standard deviation. CVD, cardiovascular disease; LDL-C, low-density lipoprotein cholesterol; RC, remnant cholesterol.

Table 2.5 Unadjusted and adjusted hazard ratios for composite CVD incidence per mmol/L increase of non-fasting RC and LDL-C.

Lipid	CVD n (%)	Unadjusted		Adjusted[1]	
		HR	**p-value**	**HR**	**p-value**
RC	1156 (8.3)	1.63 (1.42-1.87)	**<0.001**	1.34 (1.16-1.55)	**<0.001**
LDL-C		0.75 (0.70-0.81)	**<0.001**	0.75 (0.70-0.81)	**<0.001**

Data presented as unadjusted and adjusted (by Cox proportional hazard regression) HR (95% confidence interval). CVD, cardiovascular disease; HR, hazard ratio; LDL-C, low-density lipoprotein cholesterol; RC, remnant cholesterol.

[1]RC models adjusted for age, sex, statin use, Elixhauser comorbidity index and LDL-C. LDL-C models adjusted for age, sex, statin use, Elixhauser comorbidity index and RC.

Table 2.6 Adjusted odds ratios of CVD incidence per mmol/L increase of non-fasting RC and LDL-C, stratified by sex.

	Males			**Females**		
RC[1]	**CVD** n (%)	**OR**	**p-value**	**CVD** n (%)	**OR**	**p-value**
CVD composite	486 (11.4)	1.10 (0.87-1.40)	0.42	670 (6.9)	1.77 (1.44-2.19)	**<0.001**
IHD	447 (10.5)	1.09 (0.86-1.40)	0.47	609 (6.3)	1.80 (1.45-2.23)	**<0.001**
ACS[3]	101 (2.4)	1.15 (0.72-1.84)	0.56	52 (0.54)	2.01 (1.03-3.91)	**0.04**
HF	67 (1.6)	1.32 (0.74-2.36)	0.35	101 (1.0)	1.57 (0.95-2.60)	0.08
Stroke[4]	32 (0.75)	0.94 (0.40-2.17)	0.88	n/a[6]	n/a	n/a
Procedure[5]	99 (2.3)	1.00 (0.62-1.62)	1.00	n/a[6]	n/a	n/a
LDL-C[2]	**CVD** n (%)	**OR**	**p-value**	**CVD** n (%)	**OR**	**p-value**
CVD composite	486 (11.4)	0.65 (0.57-0.74)	**<0.001**	670 (6.9)	0.77 (0.70-0.85)	**<0.001**
IHD	447 (10.5)	0.65 (0.57-0.74)	**<0.001**	609 (6.3)	0.79 (0.71-0.87)	**<0.001**
ACS[3]	101 (2.4)	0.56 (0.43-0.73)	**<0.001**	52 (0.54)	0.50 (0.35-0.71)	**<0.001**
HF	67 (1.6)	0.73 (0.53-1.00)	0.05	101 (1.0)	0.58 (0.45-0.75)	**<0.001**
Stroke[4]	32 (0.75)	0.50 (0.31-0.80)	**0.004**	n/a[6]	n/a	n/a
Procedure[5]	99 (2.3)	0.51 (0.39-0.67)	**<0.001**	n/a[6]	n/a	n/a

Data presented as adjusted (by multivariable logistic regression) OR (95% confidence interval), stratified by sex. ACS, acute coronary syndrome; CVD, cardiovascular disease; HF, heart failure; IHD, ischemic heart disease; LDL-C, low-density lipoprotein cholesterol; OR, odds ratio; RC, remnant cholesterol.

[1]RC models adjusted for age, sex, statin use, Elixhauser comorbidity index and LDL-C.

[2]LDL-C models adjusted for age, sex, statin use, Elixhauser comorbidity index and RC.

[3]Aggregate of myocardial infarction and angina.

[4]Aggregate of transient ischemic attack and acute ischemic stroke.

[5]Aggregate of percutaneous coronary intervention and coronary artery bypass graft.

[6]n/a indicates too few case numbers to report.

Table 2.7 Adjusted odds ratios of CVD incidence per mmol/L increase of non-fasting RC and LDL-C, stratified by statin use.

	Statin non-users			**Statin users**		
RC[1]	**CVD** n (%)	**OR**	**p-value**	**CVD** n (%)	**OR**	**p-value**
CVD composite	859 (7.6)	1.52 (1.26-1.82)	**<0.001**	297 (11.3)	1.32 (0.98-1.79)	0.07
IHD	773 (6.8)	1.52 (1.25-1.83)	**<0.001**	283 (10.6)	1.35 (1.00-1.84)	0.05
ACS[3]	100 (0.90)	1.97 (1.25-3.10)	**0.004**	53 (1.9)	0.72 (0.35-1.48)	0.38
HF	107 (0.97)	1.70 (1.07-2.72)	**0.03**	61 (2.1)	1.11 (0.59-2.10)	0.74
Stroke[4]	34 (0.31)	0.79 (0.33-1.93)	0.61	n/a[6]	n/a	n/a
Procedure[5]	72 (0.65)	1.59 (0.93-2.70)	0.09	55 (1.9)	0.73 (0.36-1.46)	0.37
LDL-C[2]	**CVD** n (%)	**OR**	**p-value**	**CVD** n (%)	**OR**	**p-value**
CVD composite	859 (7.6)	0.68 (0.62-0.74)	**<0.001**	297 (11.3)	0.83 (0.73-0.94)	**0.004**
IHD	773 (6.8)	0.69 (0.62-0.76)	**<0.001**	283 (10.6)	0.84 (0.73-0.96)	**0.009**
ACS[3]	100 (0.90)	0.36 (0.27-0.47)	**<0.001**	53 (1.9)	0.98 (0.73-1.31)	0.87
HF	107 (0.97)	0.51 (0.39-0.67)	**<0.001**	61 (2.1)	0.81 (0.61-1.07)	0.14
Stroke[4]	34 (0.31)	0.41 (0.26-0.67)	**<0.001**	n/a[6]	n/a	n/a
Procedure[5]	72 (0.65)	0.27 (0.19-0.38)	**<0.001**	55 (1.9)	0.86 (0.64-1.16)	0.32

Data presented as adjusted (by multivariable logistic regression) OR (95% confidence interval), stratified by statin use. ACS, acute coronary syndrome; CVD, cardiovascular disease; HF, heart failure; IHD, ischemic heart disease; LDL-C, low-density lipoprotein cholesterol; OR, odds ratio; RC, remnant cholesterol.

[1]RC models adjusted for age, sex, statin use, Elixhauser comorbidity index and LDL-C.

[2]LDL-C models adjusted for age, sex, statin use, Elixhauser comorbidity index and RC.

[3]Aggregate of myocardial infarction and angina.

[4]Aggregate of transient ischemic attach and acute ischemic stroke.

[5]Aggregate of percutaneous coronary intervention and coronary artery bypass graft.

[6]n/a indicates too few case numbers to report.

Table 2.8 Adjusted and unadjusted odds ratios of CVD prevalence per mmol/L increase of non-fasting RC and LDL-C.

		Unadjusted		Adjusted[1]	
RC	**CVD** n (%)	**OR**	**p-value**	**OR**	**p-value**
CVD composite	1,766 (11.3)	1.65 (1.46-1.87)	**<0.001**	1.40 (1.21-1.62)	**<0.001**
IHD	1,657 (10.5)	1.68 (1.48-1.90)	**<0.001**	1.41 (1.22-1.63)	**<0.001**
ACS[2]	303 (1.9)	1.46 (1.11-1.93)	**0.008**	0.93 (0.69-1.26)	0.64
HF	289 (1.8)	1.72 (1.30-2.27)	**<0.001**	1.30 (0.95-1.78)	0.10
Stroke[3]	67 (0.43)	1.36 (0.75-2.45)	0.32	0.95 (0.51-1.77)	0.87
Procedure[4]	185 (1.2)	1.34 (0.93-1.91)	0.11	0.73 (0.49-1.08)	0.12
LDL-C	**CVD** n (%)	**OR**	**p-value**	**OR**	**p-value**
CVD composite	1,766 (11.3)	0.68 (0.64-0.72)	**<0.001**	0.72 (0.67-0.77)	**<0.001**
IHD	1,657 (10.5)	0.67 (0.63-0.71)	**<0.001**	0.71 (0.66-0.76)	**<0.001**
ACS[2]	303 (1.9)	0.35 (0.30-0.40)	**<0.001**	0.37 (0.31-0.44)	**<0.001**
HF	289 (1.8)	0.64 (0.55-0.73)	**<0.001**	0.73 (0.62-0.85)	**<0.001**
Stroke[3]	67 (0.43)	0.43 (0.32-0.58)	**<0.001**	0.48 (0.34-0.66)	**<0.001**
Procedure[4]	185 (1.2)	0.21 (0.17-0.26)	**<0.001**	0.20 (0.16-0.26)	**<0.001**

Data presented as unadjusted and adjusted (by multivariable logistic regression) OR (95% confidence interval). ACS, acute coronary syndrome; CVD, cardiovascular disease; HF, heart failure; IHD, ischemic heart disease; LDL-C, low-density lipoprotein cholesterol; OR, odds ratio; RC, remnant cholesterol.

[1]RC models adjusted for age, sex, statin use, Elixhauser comorbidity index and LDL-C. LDL-C models adjusted for age, sex, statin use, Elixhauser comorbidity index and RC.

[2]Aggregate of myocardial infarction and angina.

[3]Aggregate of transient ischemic attack and acute ischemic stroke.

[4]Aggregate of percutaneous coronary intervention and coronary artery bypass graft.

Table 2.9 Adjusted odds ratios for CVD prevalence per mmol/L increase of non-fasting RC and LDL-C, stratified by sex.

RC[1]	**Males**			**Females**		
	CVD n (%)	**OR**	**p-value**	**CVD** n (%)	**OR**	**p-value**
CVD composite	848 (16.5)	1.27 (1.03-1.57)	**0.03**	928 (8.7)	1.44 (1.18-1.75)	**<0.001**
IHD	809 (15.8)	1.28 (1.04-1.59)	**0.02**	848 (8.0)	1.43 (1.17-1.75)	**0.001**
ACS[3]	219 (4.3)	0.82 (0.57-1.17)	0.28	84 (0.79)	1.12 (0.62-2.02)	0.71
HF	146 (2.9)	1.23 (0.80-1.91)	0.35	143 (1.3)	1.39 (0.88-2.19)	0.16
Stroke[4]	32 (0.62)	0.74 (0.30-1.82)	0.52	35 (0.33)	1.23 (0.52-2.92)	0.64
Procedure[5]	158 (3.1)	0.65 (0.42-1.01)	0.05	n/a[6]	n/a	n/a
LDL-C[2]	**CVD** n (%)	**OR**	**p-value**	**CVD** n (%)	**OR**	**p-value**
CVD composite	848 (16.5)	0.60 (0.54-0.67)	**<0.001**	928 (8.7)	0.81 (0.74-0.89)	**<0.001**
IHD	809 (15.8)	0.59 (0.52-0.65)	**<0.001**	848 (8.0)	0.81 (0.74-0.89)	**<0.001**
ACS[3]	219 (4.3)	0.30 (0.24-0.37)	**<0.001**	84 (0.79)	0.57 (0.43-0.76)	**<0.001**
HF	146 (2.9)	0.67 (0.53-0.84)	**0.001**	143 (1.3)	0.80 (0.64-0.99)	**0.04**
Stroke[4]	32 (0.62)	0.44 (0.27-0.73)	**0.001**	35 (0.33)	0.51 (0.32-0.80)	**0.003**
Procedure[5]	158 (3.1)	0.18 (0.13-0.23)	**<0.001**	n/a[6]	n/a	n/a

Data presented as adjusted (by multivariable logistic regression) OR (95% confidence interval), stratified by sex. ACS, acute coronary syndrome; CVD, cardiovascular disease; HF, heart failure; IHD, ischemic heart disease; LDL-C, low-density lipoprotein cholesterol; OR, odds ratio; RC, remnant cholesterol.

[1]RC models adjusted for age, sex, statin use, Elixhauser comorbidity index and LDL-C.

[2]LDL-C models adjusted for age, sex, statin use, Elixhauser comorbidity index and RC.

[3]Aggregate of myocardial infarction and angina.

[4]Aggregate of transient ischemic attack and acute ischemic stroke.

[5]Aggregate of percutaneous coronary intervention and coronary artery bypass graft.

[6]n/a indicates too few case numbers to report.

Table 2.10 Adjusted odds ratios of CVD prevalence per mmol/L increase of non-fasting RC and LDL-C, stratified by statin use.

RC[1]	**Statin non-user**			**Statin user**		
	CVD n (%)	**OR**	**p-value**	**CVD** n (%)	**OR**	**p-value**
CVD composite	1610 (12.4)	1.41 (1.21-1.64)	**<0.001**	166 (5.9)	1.30 (0.86-1.97)	0.22
IHD	1494 (11.6)	1.42 (1.22-1.66)	**<0.001**	163 (5.7)	1.26 (0.83-1.90)	0.28
ACS[3]	241 (2.0)	0.97 (0.68-1.38)	0.87	62 (1.7)	0.92 (0.48-1.78)	0.82
HF	221 (1.8)	1.26 (0.87-1.83)	0.21	68 (1.8)	1.51 (0.82-2.77)	0.18
Stroke[4]	46 (0.39)	0.90 (0.42-1.91)	0.77	n/a[6]	n/a	n/a
Procedure[5]	156 (1.3)	0.75 (0.48-1.18)	0.22	n/a[6]	n/a	n/a
LDL-C[2]	**CVD** n (%)	**OR**	**p-value**	**CVD** n (%)	**OR**	**p-value**
CVD composite	1610 (12.4)	0.70 (0.65-0.75)	**<0.001**	166 (5.9)	0.88 (0.73-1.05)	0.15
IHD	1494 (11.6)	0.68 (0.63-0.73)	**<0.001**	163 (5.7)	0.90 (0.75-1.08)	0.25
ACS[3]	241 (2.0)	0.27 (0.22-0.33)	**<0.001**	62 (1.7)	0.92 (0.69-1.23)	0.56
HF	221 (1.8)	0.71 (0.59-0.85)	**<0.001**	68 (1.8)	0.80 (0.59-1.07)	0.13
Stroke[4]	46 (0.39)	0.36 (0.24-0.54)	**<0.001**	n/a[6]	n/a	n/a
Procedure[5]	156 (1.3)	0.14 (0.10-0.18)	**<0.001**	n/a[6]	n/a	n/a

Data presented as adjusted (by multivariable logistic regression) OR (95% confidence interval), stratified by statin use. ACS, acute coronary syndrome; CVD, cardiovascular disease; HF, heart failure; IHD, ischemic heart disease; LDL-C, low-density lipoprotein cholesterol; OR, odds ratio; RC, remnant cholesterol.

[1]RC adjusted for age, sex, statin use, Elixhauser comorbidity index and LDL-C.

[2]LDL-C adjusted for age, sex, statin use, Elixhauser comorbidity index and RC.

[3]Aggregate of myocardial infarction and angina.

[4]Aggregate of transient ischemic attack and acute ischemic stroke.

[5]Aggregate of percutaneous coronary intervention and coronary artery bypass graft.

[6]n/a indicates too few case numbers to report.

Table 2.11 Unadjusted and adjusted hazard ratios for composite CVD incidence by quartile of non-fasting RC and LDL-C.

		Unadjusted		Adjusted[1]	
Lipid	**Quartile**	**HR**	**p-value**	**HR**	**p-value**
RC	1	1	(reference)	1	(reference)
	2	1.08 (0.90-1.30)	0.42	0.97 (0.80-1.16)	0.71
	3	1.48 (1.24-1.75)	**<0.001**	1.28 (1.07-1.52)	**0.007**
	4	1.67 (1.41-1.98)	**<0.001**	1.33 (1.12-1.58)	**0.001**
LDL-C	1	1	(reference)	1	(reference)
	2	0.65 (0.56-0.76)	**<0.001**	0.66 (0.56-0.78)	**<0.001**
	3	0.57 (0.49-0.67)	**<0.001**	0.56 (0.47-0.66)	**<0.001**
	4	0.60 (0.52-0.71)	**<0.001**	0.56 (0.48-0.66)	**<0.001**

Data presented as unadjusted and adjusted (by Cox proportional hazard regression) HR (95% confidence interval). HR, hazard ratio; LDL-C, low-density lipoprotein cholesterol; RC, remnant cholesterol.

[1]RC models adjusted for age, sex, statin use, Elixhauser comorbidity index and LDL-C. LDL-C models adjusted for age, sex, statin use, Elixhauser comorbidity index and RC.

Table 2.12 Adjusted odds ratios of composite CVD incidence by quartile of non-fasting RC and LDL-C, stratified by sex.

	Males		Females	
RC Quartile[1]	**OR**	**p-value**	**OR**	**p-value**
1	1	(reference)	1	(reference)
2	0.86 (0.62-1.19)	0.37	1.03 (0.81-1.33)	0.79
3	1.29 (0.95-1.74)	0.10	1.40 (1.10-1.79)	**0.006**
4	1.09 (0.82-1.46)	0.55	1.70 (1.34-2.16)	**<0.001**
LDL-C Quartile[2]	**OR**	**p-value**	**OR**	**p-value**
1	1	(reference)	1	(reference)
2	0.65 (0.51-0.85)	**0.001**	0.61 (0.48-0.77)	**<0.001**
3	0.46 (0.35-0.61)	**<0.001**	0.56 (0.44-0.70)	**<0.001**
4	0.47 (0.35-0.63)	**<0.001**	0.54 (0.43-0.68)	**<0.001**

Data presented as adjusted (by multivariable logistic regression) OR (95% confidence interval), stratified by sex. LDL-C, low-density lipoprotein cholesterol; OR, odds ratio; RC, remnant cholesterol.

[1]RC models adjusted for age, sex, statin use, Elixhauser comorbidity index and LDL-C.

[2]LDL-C models adjusted for age, sex, statin use, Elixhauser comorbidity index and RC.

Table 2.13 Adjusted odds ratios of composite CVD incidence by quartiles of non-fasting RC and LDL-C, stratified by statin use.

	Statin non-user		Statin user	
RC Quartile[1]	**OR**	**p-value**	**OR**	**p-value**
1	1	(reference)	1	(reference)
2	0.96 (0.77-1.20)	0.73	1.04 (0.66-1.64)	0.88
3	1.44 (1.17-1.78)	**0.001**	1.24 (0.81-1.91)	0.33
4	1.52 (1.23-1.87)	**<0.001**	1.25 (0.83-1.90)	0.28
LDL-C Quartile[2]	**OR**	**p-value**	**OR**	**p-value**
1	1	(reference)	1	(reference)
2	0.52 (0.43-0.64)	**<0.001**	0.98 (0.72-1.35)	0.92
3	0.45 (0.37-0.55)	**<0.001**	0.81 (0.55-1.18)	0.27
4	0.47 (0.38-0.57)	**<0.001**	0.64 (0.45-0.92)	**0.02**

Data presented as adjusted (by multivariable logistic regression) OR (95% confidence interval), stratified by statin use. LDL-C, low-density lipoprotein cholesterol; OR, odds ratio; RC, remnant cholesterol.

[1]RC models adjusted for age, sex, statin use, Elixhauser comorbidity index and LDL-C.
[2]LDL-C models adjusted for age, sex, statin use, Elixhauser comorbidity index and RC.

Table 2.14 Number of prevalent cases of composite CVD, by quartile of non-fasting RC and LDL-C.

	RC (mmol/L)					LDL-C (mmol/L)			
	Q1 n=4015	**Q2** n=3976	**Q3** n=3907	**Q4** n=3866		**Q1** n=3994	**Q2** n=3940	**Q3** n=3894	**Q4** n=3936
CVD composite n (%)	325 (8.1)	444 (11.2)	466 (11.9)	541 (14.0)		696 (17.4)	367 (9.3)	353 (9.1)	360 (9.2)

CVD, cardiovascular disease; LDL-C, low-density lipoprotein cholesterol; RC, remnant cholesterol; Q, quartile.

Table 2.15 Adjusted odds ratios of composite CVD prevalence by quartile of non-fasting RC and LDL-C.

	Unadjusted		Adjusted	
RC Quartile[1]	**OR**	**p-value**	**OR**	**p-value**
1	1	(reference)	1	(reference)
2	1.43 (1.23-1.66)	**<0.001**	1.33 (1.12-1.57)	**0.001**
3	1.54 (1.32-1.78)	**<0.001**	1.31 (1.11-1.55)	**0.002**
4	1.85 (1.60-2.14)	**<0.001**	1.50 (1.27-1.77)	**<0.001**
LDL-C Quartile[2]	**OR**	**p-value**	**OR**	**p-value**
1	1	(reference)	1	(reference)
2	0.49 (0.43-0.56)	**<0.001**	0.50 (0.42-0.58)	**<0.001**
3	0.47 (0.41-0.54)	**<0.001**	0.46 (0.39-0.54)	**<0.001**
4	0.48 (0.42-0.55)	**<0.001**	0.48 (0.41-0.56)	**<0.001**

Data presented as unadjusted and adjusted (by multivariable logistic regression) OR (95% confidence interval). LDL-C, low-density lipoprotein cholesterol; OR, odds ratio; RC, remnant cholesterol.

[1]RC models adjusted for age, sex, statin use, Elixhauser comorbidity index and LDL-C.

[2]LDL-C models adjusted for age, sex, statin use, Elixhauser comorbidity index and RC.

Table 2.16 Adjusted odds ratios for composite CVD prevalence by quartile of non-fasting RC and LDL-C, stratified by sex.

RC Quartile[1]	**Males**		**Females**	
	OR	**p-value**	**OR**	**p-value**
1	1	(reference)	1	(reference)
2	1.44 (1.09-1.90)	**0.01**	1.25 (1.01-1.54)	**0.04**
3	1.36 (1.03-1.79)	**0.03**	1.24 (1.00-1.55)	0.05
4	1.51 (1.16-1.96)	**0.002**	1.44 (1.15-1.79)	**0.001**
LDL-C Quartile[2]	**OR**	**p-value**	**OR**	**p-value**
1	1	(reference)	1	(reference)
2	0.44 (0.35-0.55)	**<0.001**	0.56 (0.45-0.70)	**<0.001**
3	0.36 (0.28-0.46)	**<0.001**	0.55 (0.44-0.68)	**<0.001**
4	0.37 (0.29-0.48)	**<0.001**	0.57 (0.47-0.70)	**<0.001**

Data presented as unadjusted and adjusted (by multivariable logistic regression) OR (95% confidence interval), stratified by sex. LDL-C, low-density lipoprotein cholesterol; OR, odds ratio; RC, remnant cholesterol.

[1]RC models adjusted for age, sex, statin use, Elixhauser comorbidity index and LDL-C.

[2]LDL-C models adjusted for age, sex, statin use, Elixhauser comorbidity index and RC.

Table 2.17 Adjusted odds ratios for composite CVD prevalence by quartile of non-fasting RC and LDL-C, stratified by statin use.

RC Quartile[1]	**Statin non-user**		**Statin user**	
	OR	**p-value**	**OR**	**p-value**
1	1	(reference)	1	(reference)
2	1.33 (1.11-1.58)	**0.002**	1.32 (0.71-2.46)	0.38
3	1.32 (1.10-1.57)	**0.002**	1.23 (0.68-2.23)	0.49
4	1.53 (1.29-1.82)	**<0.001**	1.23 (0.70-2.19)	0.47
LDL-C Quartile[2]	**OR**	**p-value**	**OR**	**p-value**
1	1	(reference)	1	(reference)
2	0.48 (0.41-0.56)	**<0.001**	0.61 (0.38-0.97)	**0.04**
3	0.44 (0.38-0.52)	**<0.001**	0.55 (0.31-0.97)	**0.04**
4	0.46 (0.39-0.55)	**<0.001**	0.63 (0.38-1.04)	0.07

Data presented as unadjusted and adjusted (by multivariable logistic regression) OR (95% confidence interval), stratified by statin use. LDL-C, low-density lipoprotein cholesterol; OR, odds ratio; RC, remnant cholesterol.

[1]RC models adjusted for age, sex, statin use, Elixhauser comorbidity index and LDL-C.

[2]LDL-C models adjusted for age, sex, statin use, Elixhauser comorbidity index and RC.

2.11 Figures

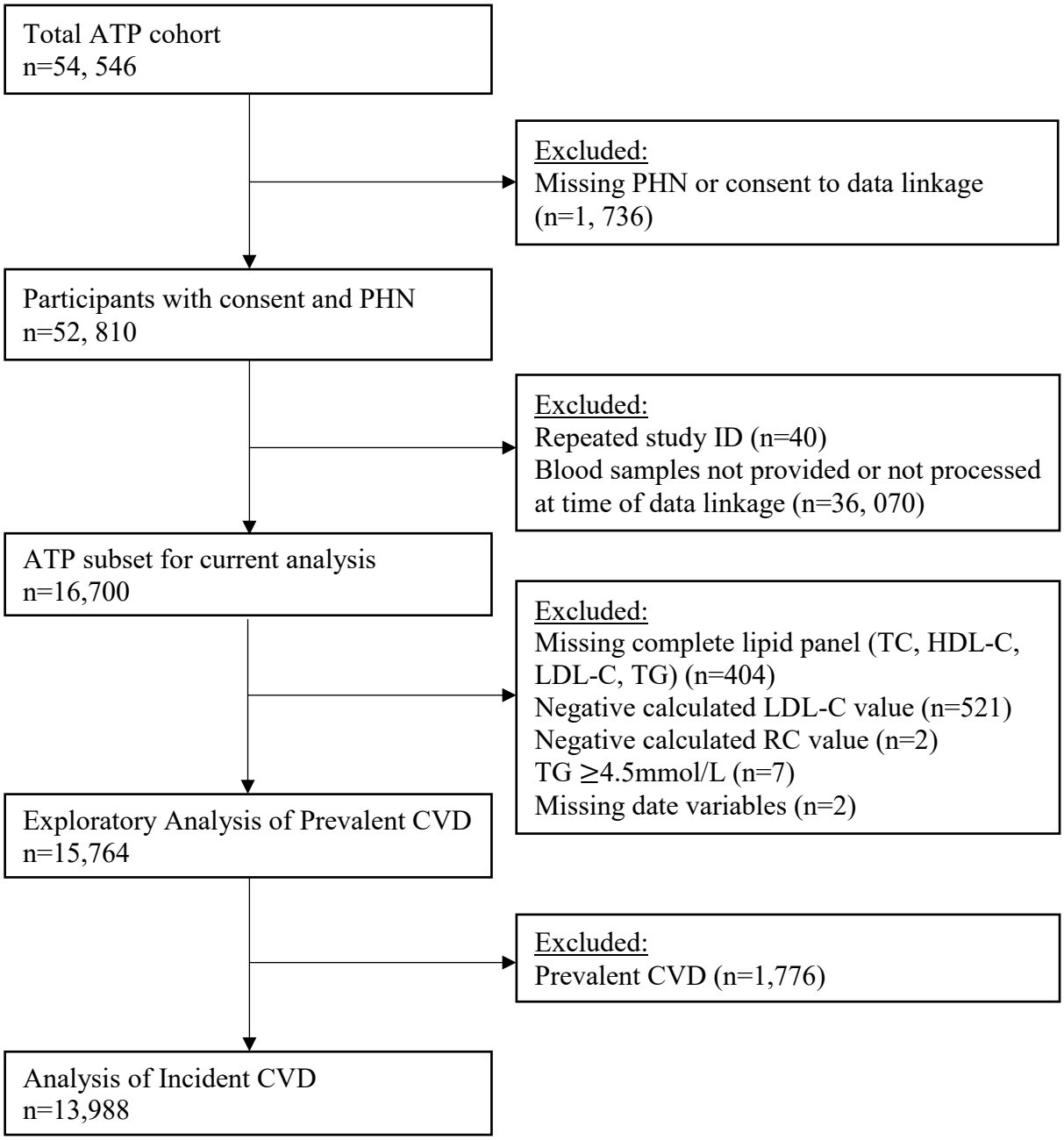

Figure 2.1 Flow diagram of ATP sample selection. ATP, Alberta's Tomorrow Project; CVD, cardiovascular disease; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; PHN; personal health number; RC, remnant cholesterol TC, total cholesterol; TG, triglycerides.

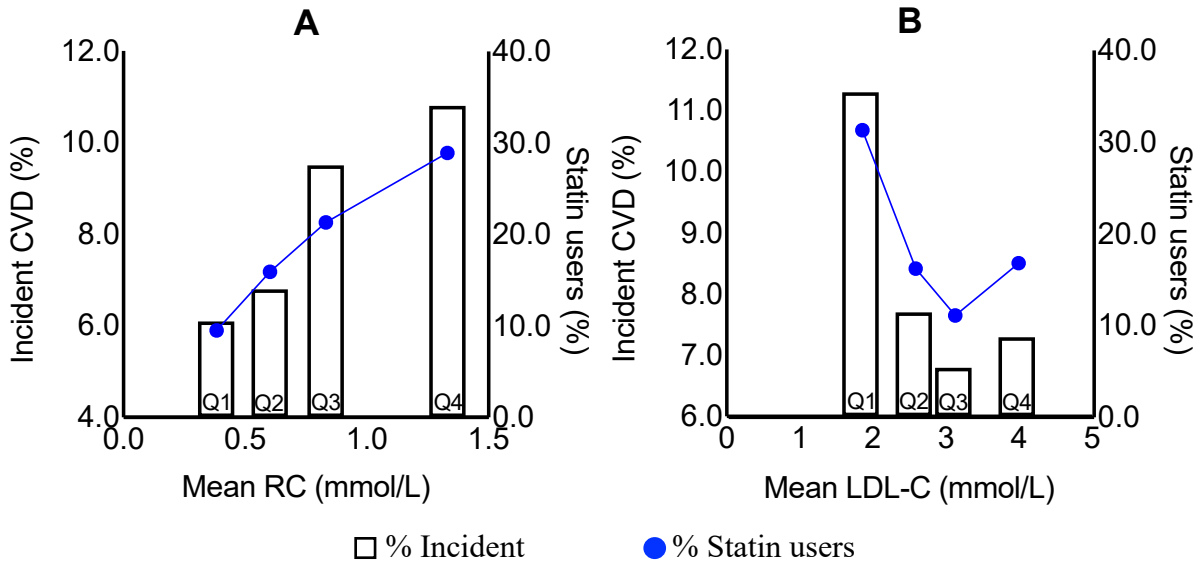

Figure 2.2 Percentage of incident composite CVD diagnoses and statin users by quartile of non-fasting RC (A) and LDL-C (B). Categorical lipid variables were used in the regressions, however the figure shows quartiles plotted at the mean lipid value for each quartile. CVD, cardiovascular disease; LDL-C, low-density lipoprotein cholesterol; Q, quartile; RC, remnant cholesterol. Figure created using GraphPad Prism Version 9.3.1.

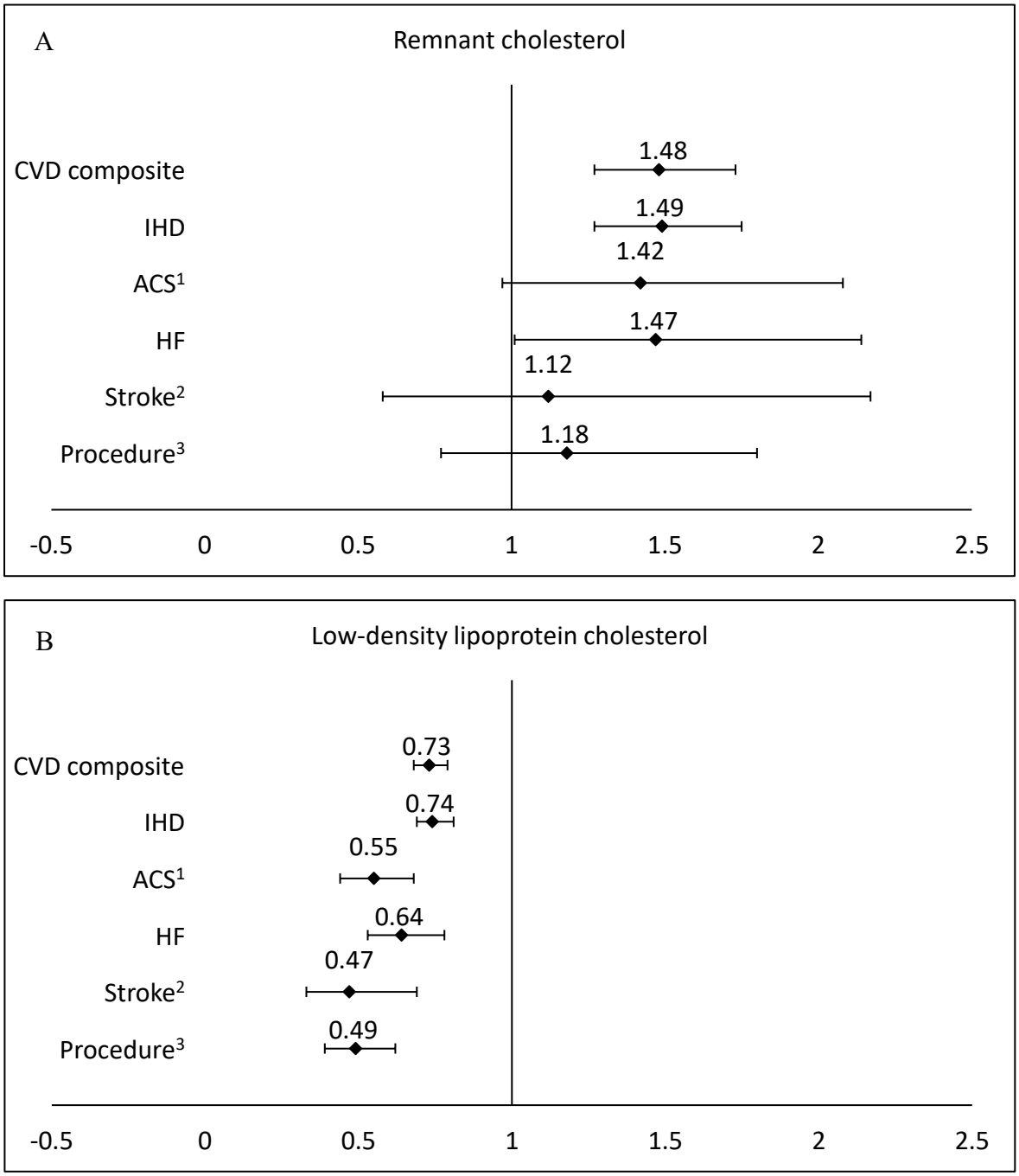

Figure 2.3 Odds ratios and 95% confidence intervals for CVD incidence per mmol/L increase of non-fasting RC (A) and LDL-C (B). **A** is adjusted for sex, age, Elixhauser comorbidity index, statin use and LDL-C. **B** is adjusted for sex, age, Elixhauser comorbidity index, statin use and remnant cholesterol. ACS, acute coronary syndrome; CVD, cardiovascular disease; HF, heart failure; IHD, ischemic heart disease.

[1]Aggregate of myocardial infarction and angina.

[2]Aggregate of acute ischemic stroke and transient ischemic attack.

[3]Aggregate of percutaneous coronary intervention and coronary artery bypass graft.

Chapter 3: Non-fasting lipids and cardiovascular disease in those with and without diabetes in Alberta's Tomorrow Project: A prospective cohort Study.

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3.1 Introduction

The prevalence and incidence of both diabetes mellitus (DM) and cardiovascular diseases (CVD) including atherosclerotic cardiovascular diseases (ASCVD), have increased globally in recent years. In 2019, 460 million and 523 million individuals worldwide had existing DM and CVD respectively and mortality from both of these chronic diseases continues to rise (1, 2). Moreover, individuals with either Type 1 DM (T1DM) or Type 2 DM (T2DM) have a significantly greater risk of CVD compared to those without (3, 4).

T2DM is a product of many factors including an inadequate production of, and response to, insulin and is often associated with obesity and dyslipidemia (5). Dyslipidemia in those with T2DM often includes elevated circulating concentrations of small dense low-density lipoprotein cholesterol (LDL-C), triglyceride (TG) and non-fasting remnant lipoproteins, together with lower high-density lipoprotein cholesterol (HDL-C) (6, 7). In contrast, T1DM is an autoimmune disease in which insulin is no longer produced after the destruction of beta cells in the pancreas (8). Interestingly, both fasting and non-fasting remnant lipoprotein levels have been shown to be increased in individuals with T1DM despite an otherwise normal lipid profile (9, 10).

The relationship of non-fasting remnant cholesterol (RC) (total cholesterol (TC) – HDL-C – LDL-C) with (ASCVD) has been studied extensively in Europe (11). RC is considered a heterogenous population of lipoprotein particles which include the cholesterol in chylomicron remnants (CMr), very low-density and intermediate-density lipoproteins (VLDL and IDL) (11).

By capturing post-prandial dyslipidemia and quotidian atherogenic lipid levels, non-fasting as opposed to fasting lipid measurements have been shown to be similarly or more representative of CVD risk (12-15). In the Danish general population, non-fasting RC appears to be more strongly associated with CVD risk compared to LDL-C (11). Despite the greater risk of CVD in those with DM and the importance of non-fasting lipid measurements, non-fasting RC has not been well studied in individuals with DM (16-18).

Alberta's Tomorrow Project (ATP) presents a unique opportunity to analyze this relationship in a Canadian longitudinal cohort. The project was initiated in 2000 and merged with the Canadian Partnership for Tomorrow's Health (CanPath) in 2008 at which time non-fasting lipid levels of participants were measured. Individual-level determination of DM and CVD diagnosis is possible via linkage to Alberta Health databases (19-22).

The objective of this analysis was to determine the relationship of non-fasting RC compared to other traditional lipid parameters with incident CVD in individuals with and without DM. We hypothesized that RC would be a strong predictor of incident CVD diagnosis in participants with DM.

3.2 Methods

3.2.1 Study design and participants

Phase 1 of ATP began in 2000 (n=29,878) and collected data on a variety of different factors associated with chronic disease (19, 21, 22). In 2008 it merged with CanPath (Phase 2) at which time an additional n=22,932 participants were recruited, and non-fasted blood samples were collected from consenting participants (19, 20). The ATP cohort is described in further detail elsewhere (19, 21, 22). ATP participants were included in the present analysis if they provided their Personal Health Number (PHN), consent to data linkage, and had a non-fasting

blood sample (n=13,631). Participants with missing or implausible lipid values (e.g. negative values), TG 4.5mmol/L or greater (which is known to affect lipid calculations) (12, 23), incident DM diagnosis or prevalent CVD were excluded.

3.2.2 Outcome variables

The primary outcome variable in this analysis was incident CVD diagnosis, a composite variable comprised of diagnosis of ischemic heart disease (IHD), myocardial infarction, angina, heart failure, transient ischemic attack acute ischemic stroke, percutaneous coronary intervention or coronary artery bypass graft. Individual-level diagnosis was determined via linkage to Alberta Health Discharge Abstract Data (DAD) and Physician Claims datasets. A washout period was implemented to avoid prevalent user bias; incidence was therefore defined as CVD diagnosis 6 months post-enrollment to ATP and 1-year post-linkage to Alberta Health data to ensure events were truly incident cases.

3.2.3 Explanatory variables

Non-fasting RC was the novel explanatory variable of interest in this analysis, while LDL-C, TG, and HDL-C were of interest as traditional CVD-related lipid predictors. Non-fasting blood draws (~50 mL) were performed when consenting participants joined Phase 2 of the ATP (19, 20). Samples were stored at -80°C (20) and TG, HDL-C and TC were measured from plasma, while RC and LDL-C were calculated by [TC – LDL-C – HDL-C] and the Friedewald equation, respectively.

3.2.4 Covariates

Several individual-level covariates were determined by linking ATP study ID to various Alberta Health databases. Diagnosis of DM was determined based on Physician Claims, DAD, Pharmaceutical Information Network (PIN), and Alberta Blue Cross (ABC) datasets. DM was

determined using the well validated National Diabetes Surveillance System definition which included 1 DM-related hospitalization or 2 DM-related physician claims in 2 years, or 1 DM-related physician claims plus the use of any diabetes medication (24). ABC and PIN datasets were used to identify statin use and a 3-tier categorical statin variable was created based on time of statin prescription (never, pre-ATP blood draw and post-ATP blood draw but prior to incident CVD). The Elixhauser comorbidity index, which assigns a score to participants based on 30 different comorbidities (25), minus DM for this analysis, was used to control for potentially confounding comorbid conditions. Comorbidities were identified using Physician Claims, Emergency Department and DAD datasets.

3.2.5 Statistical analysis

Cohort characteristics and non-fasting lipid panels were summarized using standard descriptive statistics (mean and standard deviation (SD) for continuous variables, and proportion (%) for categorical variables). T-tests and two-sample tests of proportions were used to compare characteristics between individuals with and without DM.

Univariate and multivariate logistic regression were used to assess the association between non-fasting RC (and other lipids) and incident CVD, stratified by DM status. The odds of incident CVD per unit increase of non-fasting lipids, as well as by quartile of non-fasting lipids was evaluated after adjustment for age, sex, statin use and Elixhauser comorbidity index. These relationships were further explored through additional stratification of the cohort by statin use and sex, where participant numbers allowed. Additionally, due to an inverse association observed between LDL-C and CVD, a subset of data from participants who had routine fasting blood draws within approximately two months of the ATP blood draw were used in a post-hoc analysis (n=1,646) to compare the odds of incident CVD based on fasting and non-fasting LDL-

C using multivariate logistic regression. P-values <0.05 were considered significant. Analyses were performed with Stata/SE 16.1 (StataCorp, LLC, College Station, Texas).

3.2.6 Ethics

The ATP was approved by the Alberta Cancer Board Research Ethics Committee and the University of Calgary Conjoint Health Research Ethics Board (21, 22), and the current analysis was approved by the University of Alberta Research Ethics Board (Pro00073641).

3.3 Results

3.3.1 Cohort characteristics

After exclusions, the final cohort contained n=881 individuals with and n=12,750 individuals without DM, for a total of n=13,631 participants (Figure 3.1). On average, individuals with DM were significantly older, had a greater number of comorbidities, a greater proportion of statin users and incident CVD diagnoses, as well as significantly elevated RC and TG compared to individuals without DM. In contrast, TC, LDL-C, non-high-density lipoprotein cholesterol (Non-HDL-C), HDL-C and the proportion of females were significantly lower in those with DM compared to those without (Table 3.1).

3.3.2 Association between RC and CVD

Mean non-fasting RC concentrations were higher in individuals with incident CVD compared to those without, although this was only significant in individuals without DM (Figure 3.2, panel A). Non-fasting RC was associated with increased incident CVD in individuals with DM ((n=107 (12.2%), adjusted odds ratio (aOR) 1.32, 95% confidence interval (CI) 0.79-2.22) and without (n=947 (7.4%), (aOR 1.39, 95% CI 1.16-1.65) but did not reach significance in those with DM likely due to small sample size and number of events) (Tables 3.2 and 3.3, Figure 3.3).

Exploratory analyses revealed a positive relationship for statin use with CVD and non-fasting RC in individuals without DM, but not in individuals with DM (Figure 3.4, panel A). Despite this, aOR's for incident CVD remained non-significant in statin-users of both groups and no consistent trends were observed, likely owing to reduced sample sizes and event rates within each stratum (Tables 3.4 and 3.5). Stratification by sex also did not yield consistent patterns except in females without DM where adjusted odds of CVD were significantly increased by 69% per unit increase in RC, and males with DM which showed similar trends that persisted by quartile (Tables 3.6 and 3.7).

3.3.3 Association between LDL-C and CVD

LDL-C was significantly lower in individuals with compared to without CVD, regardless of DM diagnosis (Figure 3.2, panel B). Consequently, inverse relationships between LDL-C and CVD were observed (Tables 3.2 and 3.3, Figure 3.3). In individuals with and without DM, statin users had lower levels of LDL-C but high incidence of CVD (Figure 3.4, panel B).

Trends of reduced incident CVD with increasing LDL-C were generally present in statin non-users and post-blood draw statin users without DM but were less clear in other groups (Tables 3.4 and 3.5). Both males and females irrespective of DM had reduced odds of CVD per unit increase of LDL-C, although significance was not achieved in males with DM (Tables 3.6 and 3.7).

3.3.4 Association between HDL-C, TG and CVD

HDL-C was significantly lower in all individuals with CVD compared to those without (Figure 3.2, panel C). Accordingly, in those with and without DM, adjusted odds of CVD were significantly reduced per unit increase in HDL-C and by quartile (Tables 3.2 and 3.3, Figure 3.3).

Statin use tended to be inversely associated with HDL-C and positively associated with CVD regardless of DM diagnosis, (Figure 3.4, panel C) although few differences in odds of CVD between strata of statin use were noted. Odds of CVD were also reduced in males and females, although significance was only achieved in females (Tables 3.4-3.7).

TG was higher in individuals with CVD compared to those without, however this difference was not significant in individuals with DM (Figure 3.2, panel D). Accordingly, while trends were similar between groups, elevated odds of incident CVD with increasing TG only reached significance in those without DM (Tables 3.2 and 3.3, Figure 3.3).

Statin use tended to be positively associated with CVD and TG in individuals without DM only (Figure 3.4). Despite this, no consistent trends were observed for TG by statin use. By sex, TG also tended to be associated with increased CVD, but this was only significant in females without DM. These trends were less consistent by quartile of TG (Tables 3.4-3.7).

3.3.5 Post-hoc analysis

Post-hoc analysis of fasting and non-fasting LDL-C indicated that odds of CVD were not significantly reduced per unit increase in LDL-C in this subset, despite trends for this in the subgroup with DM (Tables 3.8-3.10).

3.4 Discussion

We report here that non-fasting RC tended to be associated with increased risk of CVD in individuals with DM, while this relationship was statistically significant in those without DM. HDL-C and TG displayed typical associations with CVD risk, while LDL-C showed an inverse relationship with CVD. One potential explanation for this observation is the likely association with statin use. Unlike for the other lipids (RC, TG and HDL-C) where higher statin use was generally associated with less favorable lipid levels, the highest number of statin users and CVD

events were observed in the lowest quartiles of LDL-C. This is also suggestive of the residual CVD risk experienced by some (usually high-risk) individuals (26, 27). For example, while individuals with DM do not necessarily present clinically with elevated levels of LDL-C, production and circulation of non-fasting remnant lipoproteins such as CMr and VLDL is often increased, due in part to lipoprotein lipase and insulin dysregulation. This likely contributes to the elevated CVD risk experienced by these individuals (6, 7).

Several studies have analyzed the relationship between *fasting* RC and incidence of CVD. For instance, Yu et al. (2021) reported that in Chinese participants with T2DM, for every 10mg/dL increase in fasting RC, odds of CVD death were increased by 11.5% (17). Limited data has been published on *non-fasting* RC and CVD in populations with DM, however Varbo et al. (2013) found in a subset of the Danish general population with DM that for every 1 mmol/L increase in non-fasting RC the odds of IHD were increased by 50% (28). Our data is supportive of the findings from both of these studies, as we observed (for every 1 mmol/L increase of non-fasting RC) a 32% increased risk of our composite CVD endpoint, but lacked statistical power to achieve significance in our DM subgroup.

Alternatively, it is possible that residual confounding factors contributed to a lack of significant findings in the ATP cohort with DM. As noted by Varbo et al. (2013) in finding that the *causal* relative risk for the relationship between RC and IHD (based on mendelian randomization in the aforementioned study) was 1.0 (CI 0.5-1.9), it is possible that the relationship between non-fasting RC and CVD is not so easily discerned due to confounding with other CVD risk factors in this high-risk population (28). For instance, another study by Cao et al. (2020) found that fasting RC was a stronger predictor of major adverse cardiovascular events in those with coronary artery disease who had poorly controlled DM compared to well

controlled or no DM (18). While a distinction between well vs poorly controlled DM could not be made in the present analysis, it is possible that differences in this respect may have modified the associations observed between RC and CVD in ATP participants.

The strengths of this study include the large population-based sample size, long duration of follow-up, and appropriate lipid testing of all ATP participants. However, this study is not without limitations. Most notably, plasma RC was not directly measured in this cohort. Calculated RC is an indirect estimation and may not capture RC concentration accurately (29). However, calculated RC is shown to be highly associated with both measured RC and CVD risk in European general populations (30, 31) and if shown to have sufficient CVD predictive power in Canadian populations with DM, presents a possible additional cost-effective CVD biomarker (12) to incorporate into clinical practice. Second, the primary pharmacological intervention for T2DM in Canada includes metformin (32) which may alter lipid metabolism (33). The present analysis did not account for a possible effect of metformin on the relationship between lipids and CVD risk prediction in individuals with DM and may be a possible future avenue of investigation. Third, International Classification of Diseases (ICD) coding from the administrative databases were used to identify all incident CVD events. Although there is no reason to believe coding was different based on RC data (which was not available to the clinicians at the time of the diagnosis), it is possible misclassification of events may have occurred. Importantly, there is no reason to believe that misclassification of events occurred differentially based on a participant's lipid profile. Last, the DM subgroup was relatively small and events were limited. This was further compounded by the addition of other subgroups (sex, statin use) within the DM group. As a result, many of the strata specific analyses were likely

under powered to find differences and reliance of statistical significance only should be viewed with caution.

3.5 Conclusion

This study is the first to our knowledge to provide an analysis of non-fasting RC compared to traditional lipid biomarkers in relation to CVD risk prediction in Canadians with DM. Overall, higher non-fasting RC (along with elevated TG and reduced HDL-C) tended to be associated with increased CVD in individuals with DM, whereas LDL-C did not. This suggests that non-fasting RC may be of beneficial use for predicting CVD risk in clinical settings. However, also highlighted by this study is the need to further explore the RC-CVD relationship in larger high-risk Canadian populations and to determine non-fasting RC reference ranges for implementation in clinical practice.

3.6 Acknowledgements

Alberta's Tomorrow Project is only possible due to the commitment of its research participants, its staff and its funders: Alberta Health, Alberta Cancer Foundation, Canadian Partnership Against Cancer and substantial in-kind funding from Alberta Health Services. The views expressed herein represent the views of the author(s) and not of Alberta's Tomorrow Project or any of its funders. This study is based in part on data provided by Alberta Health. The interpretation and conclusions contained herein are those of the researchers and do not necessarily represent the views of the Government of Alberta. Neither the Government nor Alberta Health express any opinion in relation to this study.

3.7 Disclaimers

The views expressed herein represent the views of the author(s) and not of Alberta's Tomorrow Project or any of its funders. The interpretation and conclusions contained herein are

those of the researchers and do not necessarily represent the views of the Government of Alberta. Neither the Government nor Alberta Health express any opinion in relation to this study.

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3.9 Tables

Table 3.1 Non-fasting lipid panel and ATP cohort characteristics stratified by diabetes status.

	Total (n=13, 631)	**Without diabetes** (n=12,750)	**With diabetes** (n=881)	**p-value** (With vs without diabetes)
RC (mmol/L)	0.78±0.38	0.77±0.38	0.94±0.41	**<0.0001**
TC (mmol/L)	5.17±0.96	5.21±0.93	4.45±1.00	**<0.0001**
LDL-C (mmol/L)	2.87±0.84	2.92±0.82	2.22±0.90	**<0.0001**
Non-HDL-C (mmol/L)	3.65±0.94	3.68±0.93	3.16±0.96	**<0.0001**
HDL-C (mmol/L)	1.51±0.44	1.53±0.44	1.29±0.40	**<0.0001**
TG (mmol/L)	1.72±0.84	1.69±0.83	2.07±0.90	**<0.0001**
Females n (%)	9, 520 (69.8)	9, 012 (70.7)	508 (57.7)	**<0.0001**
Age (yrs)	61.6±9.7	61.5±9.7	64.2±9.1	**<0.0001**
Elixhauser score	2.75±2.14	2.67±2.09	4.01±2.47	**<0.0001**
Statin use n (%) Pre-blood/pre-CVD	1,129 (8.3)	809 (6.4)	320 (36.3)	**<0.0001**
Statin use n (%) Post-blood/pre-CVD	1,328 (9.7)	1,115 (8.8)	213 (24.2)	**<0.0001**
Incident CVD n (%)	1,054 (7.7)	947 (7.4)	107 (12.2)	**<0.0001**

Data presented as mean +/- standard deviation or n (%). ATP, Alberta's Tomorrow Project; CVD, cardiovascular disease; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Non-HDL-C, non-high-density lipoprotein cholesterol; RC, remnant cholesterol; TC, total cholesterol; TG, triglycerides.

Table 3.2 Crude and adjusted odds ratios for the relationship between mmol/L increases in non-fasting lipids and incident CVD.

	Without diabetes n=12,750			**With diabetes** n=881		
	CVD n (%)	**Crude OR**	**p-value**	**CVD** n (%)	**Crude OR**	**p-value**
RC[1]	947 (7.4)	1.69 (1.43-1.99)	**<0.001**	107 (12.2)	1.52 (0.94-2.47)	0.09
LDL-C[2]		0.85 (0.79-0.93)	**<0.001**		0.66 (0.51-0.85)	**0.001**
HDL-C[3]		0.56 (0.48-0.66)	**<0.001**		0.49 (0.28-0.87)	**0.02**
TG[3]		1.27 (1.18-1.37)	**<0.001**		1.24 (0.99-1.54)	0.06
	CVD n (%)	**Adjusted OR**	**p-value**	**CVD** n (%)	**Adjusted OR**	**p-value**
RC[1]	947 (7.4)	1.39 (1.16-1.65)	**<0.001**	107 (12.2)	1.32 (0.79-2.22)	0.29
LDL-C[2]		0.79 (0.72-0.86)	**<0.001**		0.58 (0.44-0.78)	**<0.001**
HDL-C[3]		0.61 (0.51-0.72)	**<0.001**		0.41 (0.22-0.75)	**0.004**
TG[3]		1.15 (1.06-1.25)	**0.001**		1.22 (0.97-1.55)	0.09

Data presented as crude and adjusted (by multivariable logistic regression) odds ratios (95% confidence intervals). CVD, cardiovascular disease; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; OR, odds ratio; RC, remnant cholesterol; TG, triglycerides.

[1]RC model adjusted for age, sex, statin use, Elixhauser comorbidity index, and LDL-C.

[2]LDL-C model adjusted for age, sex, statin use, Elixhauser comorbidity index, and RC.

[3]HDL-C, TG models adjusted for age, sex, statin use and Elixhauser comorbidity index.

Table 3.3 Adjusted odds ratios for the relationship between non-fasting lipid quartiles and incident CVD.

	Without diabetes n=12,750		**With diabetes** n=881	
Quartile (RC)[1]	**OR**	**p-value**	**OR**	**p-value**
1	1 (reference)	n/a	1 (reference)	n/a
2	0.90 (0.73-1.11)	0.33	1.13 (0.59-2.16)	0.72
3	1.33 (1.09-1.62)	**0.005**	1.67 (0.90-3.09)	0.10
4	1.30 (1.07-1.59)	**0.008**	1.28 (0.68-2.40)	0.44
Quartile (LDL-C)[2]	**OR**	**p-value**	**OR**	**p-value**
1	1 (reference)	n/a	1 (reference)	n/a
2	0.68 (0.56-0.82)	**<0.001**	0.68 (0.39-1.18)	0.17
3	0.64 (0.52-0.77)	**<0.001**	0.41 (0.22-0.75)	**0.004**
4	0.61 (0.50-0.73)	**<0.001**	0.30 (0.15-0.58)	**<0.001**
Quartile (HDL-C)[3]	**OR**	**p-value**	**OR**	**p-value**
1	1 (reference)	n/a	1 (reference)	n/a
2	0.83 (0.69-0.99)	**0.04**	0.56 (0.32-0.98)	**0.04**
3	0.73 (0.60-0.89)	**0.002**	0.48 (0.27-0.88)	**0.02**
4	0.53 (0.43-0.66)	**<0.001**	0.30 (0.16-0.58)	**<0.001**
Quartile (TG)[3]	**OR**	**p-value**	**OR**	**p-value**
1	1 (reference)	n/a	1 (reference)	n/a
2	0.84 (0.68-1.04)	0.10	1.04 (0.54-2.00)	0.90
3	1.18 (0.97-1.44)	0.10	1.69 (0.92-3.14)	0.09
4	1.23 (1.01-1.50)	**0.04**	1.47 (0.79-2.75)	0.22

Data presented as adjusted (by multivariable logistic regression) odds ratios (95% confidence intervals) using quartile 1 as the reference group. CVD, cardiovascular disease; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; OR, odds ratio; RC, remnant cholesterol; TG, triglycerides.

[1]RC model adjusted for age, sex, statin use, Elixhauser comorbidity index, and LDL-C.

[2]LDL-C model adjusted for age, sex, statin use, Elixhauser comorbidity index, and RC.

[3]HDL-C, TG models adjusted for age, sex, statin use and Elixhauser comorbidity index.

Table 3.4 Adjusted odds ratios for the relationship between mmol/L increases in non-fasting lipids and incident CVD, after stratification by statin use.

Never statin users						
	Without diabetes n=10, 826			**With diabetes** n=348		
	CVD n (%)	**OR**	**p-value**	**CVD** n (%)	**OR**	**p-value**
RC[1]	748 (6.9)	1.37 (1.12-1.67)	**0.002**	48 (13.8)	1.70 (0.72-3.98)	0.22
LDL-C[2]		0.77 (0.70-0.85)	**<0.001**		0.24 (0.14-0.40)	**<0.001**
HDL-C[3]		0.61 (0.50-0.74)	**<0.001**		0.36 (0.14-0.94)	**0.04**
TG[3]		1.14 (1.05-1.25)	**0.004**		1.36 (0.94-1.97)	0.10
Statin users pre-blood draw						
	Without diabetes n=809			**With diabetes** n=320		
	CVD n (%)	**OR**	**p-value**	**CVD** n (%)	**OR**	**p-value**
RC[1]	73 (9.0)	0.96 (0.50-1.83)	0.89	n/a[4]	n/a	n/a
LDL-C[2]		0.94 (0.72-1.23)	0.66		n/a	n/a
HDL-C[3]		0.67 (0.34-1.31)	0.24		n/a	n/a
TG[3]		0.98 (0.73-1.31)	0.88		n/a	n/a
Statin users post-blood blood draw						
	Without diabetes n=1,115			**With diabetes** n=213		
	CVD n (%)	**OR**	**p-value**	**CVD** n (%)	**OR**	**p-value**
RC[1]	126 (11.3)	1.56 (0.98-2.46)	0.06	34 (16.0)	0.64 (0.24-1.68)	0.36
LDL-C[2]		0.76 (0.62-0.93)	**0.008**		0.94 (0.59-1.47)	0.78
HDL-C[3]		0.65 (0.38-1.11)	0.12		0.50 (0.16-1.53)	0.23
TG[3]		1.22 (0.99-1.51)	0.06		0.82 (0.53-1.27)	0.37

Data presented as adjusted (by multivariable logistic regression) odds ratios (95% confidence intervals), after stratification by statin use. CVD, cardiovascular disease; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; OR, odds ratio; RC, remnant cholesterol; TG, triglycerides.

[1]RC model adjusted for age, sex, Elixhauser comorbidity index, and LDL-C.

[2]LDL-C model adjusted for age, sex, Elixhauser comorbidity index, and RC.

[3]HDL-C, TG models adjusted for age, sex and Elixhauser comorbidity index.

[4]Too few observations to report.

Table 3.5 Adjusted odds ratios for the relationship between non-fasting lipid quartiles and incident CVD, after stratification by statin use.

Never statin user				
	Without diabetes n=10,826		**With diabetes** n=348	
Quartile (RC)[1]	**OR**	**p-value**	**OR**	**p-value**
1	1 (reference)	n/a	1 (reference)	n/a
2	0.90 (0.71-1.13)	0.35	2.35 (0.76-7.28)	0.14
3	1.32 (1.06-1.64)	**0.01**	3.39 (1.21-9.51)	**0.02**
4	1.30 (1.05-1.62)	**0.02**	2.38 (0.80-7.04)	0.12
Quartile (LDL-C)[2]	**OR**	**p-value**	**OR**	**p-value**
1	1 (reference)	n/a	1 (reference)	n/a
2	0.58 (0.47-0.72)	**<0.001**	0.16 (0.06-0.45)	**0.001**
3	0.57 (0.46-0.71)	**<0.001**	0.07 (0.02-0.20)	**<0.001**
4	0.57 (0.46-0.70)	**<0.001**	0.04 (0.01-0.13)	**<0.001**
Quartile (HDL-C)[3]	**OR**	**p-value**	**OR**	**p-value**
1	1 (reference)	n/a	1 (reference)	n/a
2	0.76 (0.61-0.93)	**0.008**	0.28 (0.10-0.75)	**0.01**
3	0.73 (0.58-0.91)	**0.004**	0.32 (0.12-0.80)	**0.02**
4	0.53 (0.41-0.67)	**<0.001**	0.26 (0.10-0.66)	**0.005**
Quartile (TG)[3]	**OR**	**p-value**	**OR**	**p-value**
1	1 (reference)	n/a	1 (reference)	n/a
2	0.84 (0.67-1.06)	0.14	1.35 (0.48-3.82)	0.57
3	1.17 (0.94-1.45)	0.16	2.95 (1.13-7.67)	**0.03**
4	1.23 (0.99-1.53)	0.07	2.07 (0.77-5.60)	0.15
Statin use pre-blood draw				
	Without diabetes n=809		**With diabetes** n=320	
Quartile (RC)[1]	**OR**	**p-value**	**OR**	**p-value**
1	1 (reference)	n/a	1 (reference)	n/a
2	1.19 (0.49-2.89)	0.70	0.22 (0.04-1.20)	0.08
3	1.40 (0.63-3.15)	0.41	0.96 (0.28-3.27)	0.95
4	0.86 (0.37-2.01)	0.73	1.52 (0.50-4.63)	0.46
Quartile (LDL-C)[2]	**OR**	**p-value**	**OR**	**p-value**
1	1 (reference)	n/a	1 (reference)	n/a
2	1.26 (0.66-2.39)	0.48	0.77 (0.24-2.44)	0.66
3	1.31 (0.62-2.77)	0.48	1.24 (0.41-3.73)	0.70
4	0.95 (0.47-1.95)	0.90	0.99 (0.24-4.12)	0.99
Quartile (HDL-C)[3]	**OR**	**p-value**	**OR**	**p-value**
1	1 (reference)	n/a	1 (reference)	n/a
2	1.11 (0.59-2.08)	0.76	0.56 (0.17-1.79)	0.32
3	0.92 (0.46-1.84)	0.81	0.51 (0.16-1.64)	0.26
4	0.53 (0.22-1.26)	0.15	0.20 (0.05-0.87)	**0.03**

Quartile (TG)[3]	**OR**	**p-value**	**OR**	**p-value**
1	1 (reference)	n/a	1 (reference)	n/a
2	1.00 (0.41-2.45)	1.00	0.26 (0.05-1.50)	0.13
3	1.35 (0.60-3.01)	0.47	1.16 (0.32-4.19)	0.82
4	0.80 (0.34-1.85)	0.60	1.97 (0.62-6.28)	0.25
Statin use post-blood draw				
	Without diabetes n=1,115		**With diabetes** n=213	
Quartile (RC)[1]	**OR**	**p-value**	**OR**	**p-value**
1	1 (reference)	n/a	1 (reference)	n/a
2	0.77 (0.38-1.59)	0.48	1.23 (0.42-3.60)	0.70
3	1.23 (0.64-2.35)	0.53	0.95 (0.32-2.88)	0.93
4	1.41 (0.76-2.61)	0.28	0.56 (0.17-1.84)	0.34
Quartile (LDL-C)[2]	**OR**	**p-value**	**OR**	**p-value**
1	1 (reference)	n/a	1 (reference)	n/a
2	1.04 (0.64-1.69)	0.88	1.36 (0.53-3.49)	0.52
3	0.69 (0.39-1.23)	0.21	0.72 (0.24-2.17)	0.57
4	0.53 (0.32-0.89)	**0.02**	1.02 (0.34-3.08)	0.97
Quartile (HDL-C)[3]	**OR**	**p-value**	**OR**	**p-value**
1	1 (reference)	n/a	1 (reference)	n/a
2	1.15 (0.74-1.80)	0.53	0.91 (0.37-2.21)	0.83
3	0.61 (0.34-1.10)	0.10	0.45 (0.14-1.43)	0.17
4	0.61 (0.31-1.18)	0.14	0.36 (0.10-1.24)	0.11
Quartile (TG)[3]	**OR**	**p-value**	**OR**	**p-value**
1	1 (reference)	n/a	1 (reference)	n/a
2	0.70 (0.34-1.43)	0.32	1.16 (0.40-3.34)	0.78
3	1.01 (0.53-1.93)	0.98	0.93 (0.31-2.77)	0.89
4	1.30 (0.70-2.41)	0.40	0.57 (0.17-1.86)	0.35

Data presented as adjusted (by multivariable logistic regression) odds ratios (95% confidence intervals) using quartile 1 as the reference group, after stratification by statin use. CVD, cardiovascular disease; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; OR, odds ratio; RC, remnant cholesterol; TG, triglycerides.

[1]RC model adjusted for age, sex, Elixhauser comorbidity index, and LDL-C.

[2]LDL-C model adjusted for age, sex, Elixhauser comorbidity index, and RC.

[3]HDL-C, TG models adjusted for age, sex and Elixhauser comorbidity index.

Table 3.6 Adjusted odds ratios for the relationship between mmol/L increases in non-fasting lipids and incident composite CVD, after stratification by sex.

Males						
	Without diabetes n=3,738			**With diabetes** n=373		
	CVD n (%)	**OR**	**p-value**	**CVD** n (%)	**OR**	**p-value**
RC[1]	380 (10.2)	1.00 (0.77-1.31)	0.98	49 (13.1)	1.59 (0.75-3.38)	0.22
LDL-C[2]		0.68 (0.59-0.79)	**<0.001**		0.68 (0.44-1.05)	0.08
HDL-C[3]		0.82 (0.60-1.12)	0.20		0.32 (0.11-0.95)	0.04
TG[3]		1.01 (0.90-1.15)	0.82		1.37 (0.97-1.93)	0.07
Females						
	Without diabetes n=9,012			**With diabetes** n=508		
	CVD n (%)	**OR**	**p-value**	**CVD** n (%)	**OR**	**p-value**
RC[1]	567 (6.3)	1.69 (1.34-2.13)	**<0.001**	58 (11.4)	1.11 (0.53-2.30)	0.78
LDL-C[2]		0.84 (0.75-0.93)	**0.001**		0.51 (0.34-0.76)	**0.001**
HDL-C[3]		0.53 (0.43-0.66)	**<0.001**		0.46 (0.21-0.98)	**0.04**
TG[3]		1.26 (1.13-1.40)	**<0.001**		1.10 (0.79-1.53)	0.56

Data presented as adjusted (by multivariable logistic regression) odds ratios (95% confidence intervals), after stratification by sex. CVD, cardiovascular disease; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; OR, odds ratio; RC, remnant cholesterol; TG, triglycerides.

[1]RC model adjusted for age, statin use, Elixhauser comorbidity index, and LDL-C.

[2]LDL-C model adjusted for age, statin use, Elixhauser comorbidity index, and RC.

[3]HDL-C, TG models adjusted for age, statin use and Elixhauser comorbidity index.

Table 3.7 Adjusted odds ratios for the relationship between non-fasting lipid quartiles and incident composite CVD, after stratification by sex.

Males				
	Without diabetes n=3,738		**With diabetes** n=373	
Quartile (RC)[1]	**OR**	**p-value**	**OR**	**p-value**
1	1 (reference)	n/a	1 (reference)	n/a
2	0.80 (0.56-1.14)	0.21	1.25 (0.46-3.40)	0.66
3	1.21 (0.87-1.68)	0.25	1.14 (0.41-3.18)	0.81
4	0.98 (0.71-1.34)	0.88	1.57 (0.62-3.98)	0.34
Quartile (LDL-C)[2]	**OR**	**p-value**	**OR**	**p-value**
1	1 (reference)	n/a	1 (reference)	n/a
2	0.76 (0.57-1.01)	0.06	0.70 (0.30-1.61)	0.40
3	0.57 (0.42-0.79)	**0.001**	0.51 (0.21-1.24)	0.14
4	0.54 (0.39-0.74)	**<0.001**	0.42 (0.16-1.16)	0.10
Quartile (HDL-C)[3]	**OR**	**p-value**	**OR**	**p-value**
1	1 (reference)	n/a	1 (reference)	n/a
2	1.00 (0.78-1.29)	1.00	0.59 (0.28-1.27)	0.18
3	0.85 (0.61-1.18)	0.33	0.39 (0.15-1.05)	0.06
4	0.75 (0.50-1.15)	0.19	0.49 (0.17-1.41)	0.19
Quartile (TG)[3]	**OR**	**p-value**	**OR**	**p-value**
1	1 (reference)	n/a	1 (reference)	n/a
2	0.68 (0.47-0.97)	**0.04**	1.19 (0.43-3.29)	0.74
3	1.03 (0.74-1.42)	0.87	1.16 (0.41-3.33)	0.78
4	0.92 (0.67-1.26)	0.60	1.95 (0.75-5.05)	0.17
Females				
	Without diabetes n=9,012		**With diabetes** n=508	
Quartile (RC)[1]	**OR**	**p-value**	**OR**	**p-value**
1	1 (reference)	n/a	1 (reference)	n/a
2	0.95 (0.73-1.24)	0.71	0.95 (0.39-2.30)	0.91
3	1.37 (1.07-1.76)	**0.01**	1.95 (0.90-4.25)	0.09
4	1.52 (1.18-1.96)	**0.001**	0.92 (0.38-2.24)	0.85
Quartile (LDL-C)[2]	**OR**	**p-value**	**OR**	**p-value**
1	1 (reference)	n/a	1 (reference)	n/a
2	0.60 (0.47-0.78)	**<0.001**	0.57 (0.27-1.21)	0.14
3	0.64 (0.50-0.82)	**<0.001**	0.30 (0.13-0.71)	**0.006**
4	0.62 (0.49-0.79)	**<0.001**	0.21 (0.08-0.53)	**0.001**
Quartile (HDL-C)[3]	**OR**	**p-value**	**OR**	**p-value**
1	1 (reference)	n/a	1 (reference)	n/a
2	0.66 (0.52-0.86)	**0.002**	0.53 (0.22-1.25)	0.15
3	0.61 (0.48-0.79)	**<0.001**	0.53 (0.24-1.20)	0.13
4	0.44 (0.34-0.57)	**<0.001**	0.25 (0.11-0.59)	**0.002**

Quartile (TG)[3]	OR	p-value	OR	p-value
1	1 (reference)	n/a	1 (reference)	n/a
2	0.93 (0.71-1.21)	0.57	0.87 (0.36-2.10)	0.76
3	1.24 (0.97-1.60)	0.09	2.09 (0.97-4.50)	0.06
4	1.45 (0.12-1.87)	**0.004**	0.96 (0.40-2.32)	0.94

Data presented as adjusted (by multivariable logistic regression) odds ratios (95% confidence intervals) using quartile 1 as the reference group, after stratification by sex. CVD, cardiovascular disease; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; OR, odds ratio; RC, remnant cholesterol; TG, triglycerides.

[1]RC model adjusted for age, statin use, Elixhauser comorbidity index, and LDL-C.

[2]LDL-C model adjusted for age, statin use, Elixhauser comorbidity index, and RC.

[3]HDL-C, TG models adjusted for age, statin use and Elixhauser comorbidity index.

Table 3.8 Mean non-fasting and fasting LDL-C in total ATP cohort and registry ATP subset, stratified by diabetes and incident CVD status.

	Without diabetes n=12,750		**With diabetes** n=881	
	Without CVD	**With CVD**	**Without CVD**	**With CVD**
Total ATP non-fasting LDL-C (mmol/L)	2.92±0.81[a]	2.82±0.90[b]	2.26±0.91[c]	1.95±0.74[d]
	Without diabetes n=1,425		**With diabetes** n=221	
Registry ATP non-fasting LDL-C (mmol/L)	2.88±0.81[a]	2.99±1.00[a]	2.14±0.95[b]	1.92±0.64[b]
Registry ATP Fasting LDL-C (mmol/L)	2.91±0.81[a]	3.06±0.98[a]	2.22±0.81[b]	1.99±0.75[b]

Data analyzed with one-way ANOVA for multiple comparisons and presented as mean +/- standard deviation. Different letters indicate statistically significant differences ($p<0.05$). ATP, Alberta's Tomorrow Project; CVD, cardiovascular disease; LDL-C, low-density lipoprotein cholesterol.

Table 3.9 Adjusted odds ratios for the relationship between mmol/L increases in non-fasting and fasting LDL-C and incident CVD in the total ATP cohort and registry ATP subset.

	Without diabetes n=12,750		**With diabetes** n=881	
	OR	**p-value**	**OR**	**p-value**
Total ATP Non-fasting LDL-C[1]	0.79 (0.72-0.86)	**<0.001**	0.58 (0.44-0.78)	**<0.001**
	Without diabetes n=1,425		**With diabetes** n=221	
Registry ATP Non-fasting LDL-C[1]	1.21 (0.87-1.68)	0.25	0.72 (0.28-1.83)	0.49
Registry ATP Fasting LDL-C[2]	1.13 (0.81-1.59)	0.48	0.64 (0.20-2.03)	0.45

Data presented as adjusted (by multivariable logistic regression) odds ratios (95% confidence intervals). ATP, Alberta's Tomorrow Project; LDL-C, low-density lipoprotein cholesterol; OR, odds ratio.

[1]Non-fasting models adjusted for age, sex, statin use, Elixhauser comorbidity index and non-fasting RC

[2]Fasting model adjusted for age, sex, statin use, Elixhauser comorbidity index and fasting RC

Table 3.10 Adjusted odds ratios for the relationship between non-fasting and fasting LDL-C quartiles and incident CVD in the total ATP cohort and registry ATP subset.

	Without diabetes n=12,750		**With diabetes** n=881	
Total ATP Quartile (Non-fasting LDL-C)[1]	**OR**	**p-value**	**OR**	**p-value**
1	1 (reference)	n/a	1 (reference)	n/a
2	0.68 (0.56-0.82)	**<0.001**	0.68 (0.39-1.18)	0.17
3	0.64 (0.52-0.77)	**<0.001**	0.41 (0.22-0.75)	**0.004**
4	0.61 (0.50-0.73)	**<0.001**	0.30 (0.15-0.58)	**<0.001**
	Without diabetes n=1,425		**With diabetes** n=221	
Registry ATP Quartile (Non-fasting LDL-C)[1]	**OR**	**p-value**	**OR**	**p-value**
1	1 (reference)	n/a	1 (reference)	n/a
2	1.39 (0.62-3.13)	0.42	1.18 (0.14-10.10)	0.88
3	1.06 (0.44-2.53)	0.90	1.45 (0.20-10.34)	0.71
4	1.38 (0.61-3.11)	0.44	0.56 (0.04-7.57)	0.66
Registry ATP Quartile (Fasting LDL-C)[2]	**OR**	**p-value**	**OR**	**p-value**
1	1 (reference)	n/a	1 (reference)	n/a
2	1.11 (0.47-2.61)	0.82	0.63 (0.09-4.52)	0.64
3	1.18 (0.50-2.79)	0.70	0.71 (0.09-5.69)	0.74
4	1.28 (0.55-2.96)	0.57	0.23 (0.02-3.38)	0.29

Data presented as adjusted (by multivariable logistic regression) odds ratios (95% confidence intervals) using quartile 1 as the reference group. ATP, Alberta's Tomorrow Project; LDL-C, low-density lipoprotein cholesterol; OR, odds ratio.

[1]Non-fasting models adjusted for age, sex, statin use, Elixhauser comorbidity index and non-fasting RC

[2]Fasting model adjusted for age, sex, statin use, Elixhauser comorbidity index and fasting RC

3.10 Figures

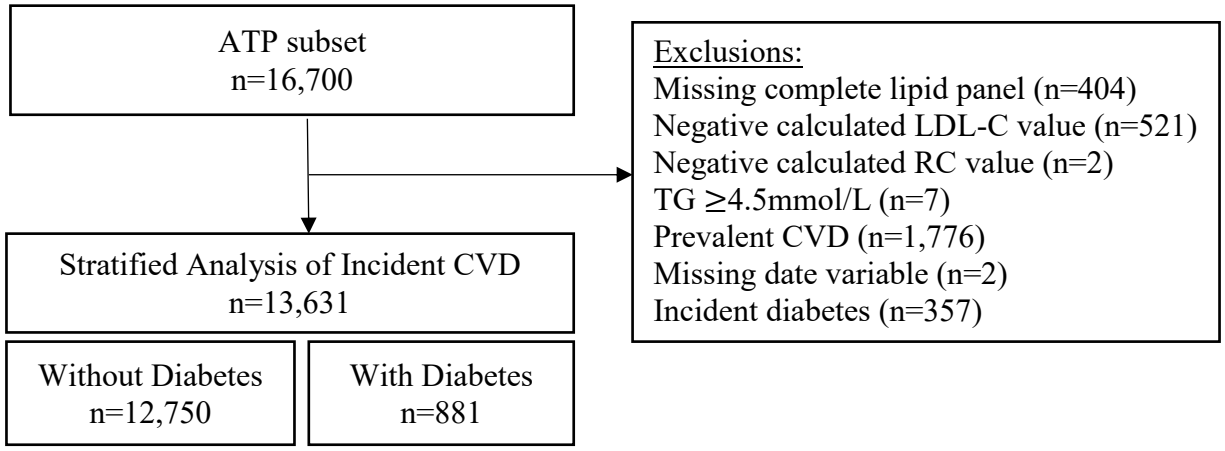

Figure 3.1 ATP exclusion criteria and sample stratification. ATP, Alberta's Tomorrow Project; CVD, cardiovascular disease; LDL-C, low-density lipoprotein cholesterol; RC, remnant cholesterol; TG, triglycerides

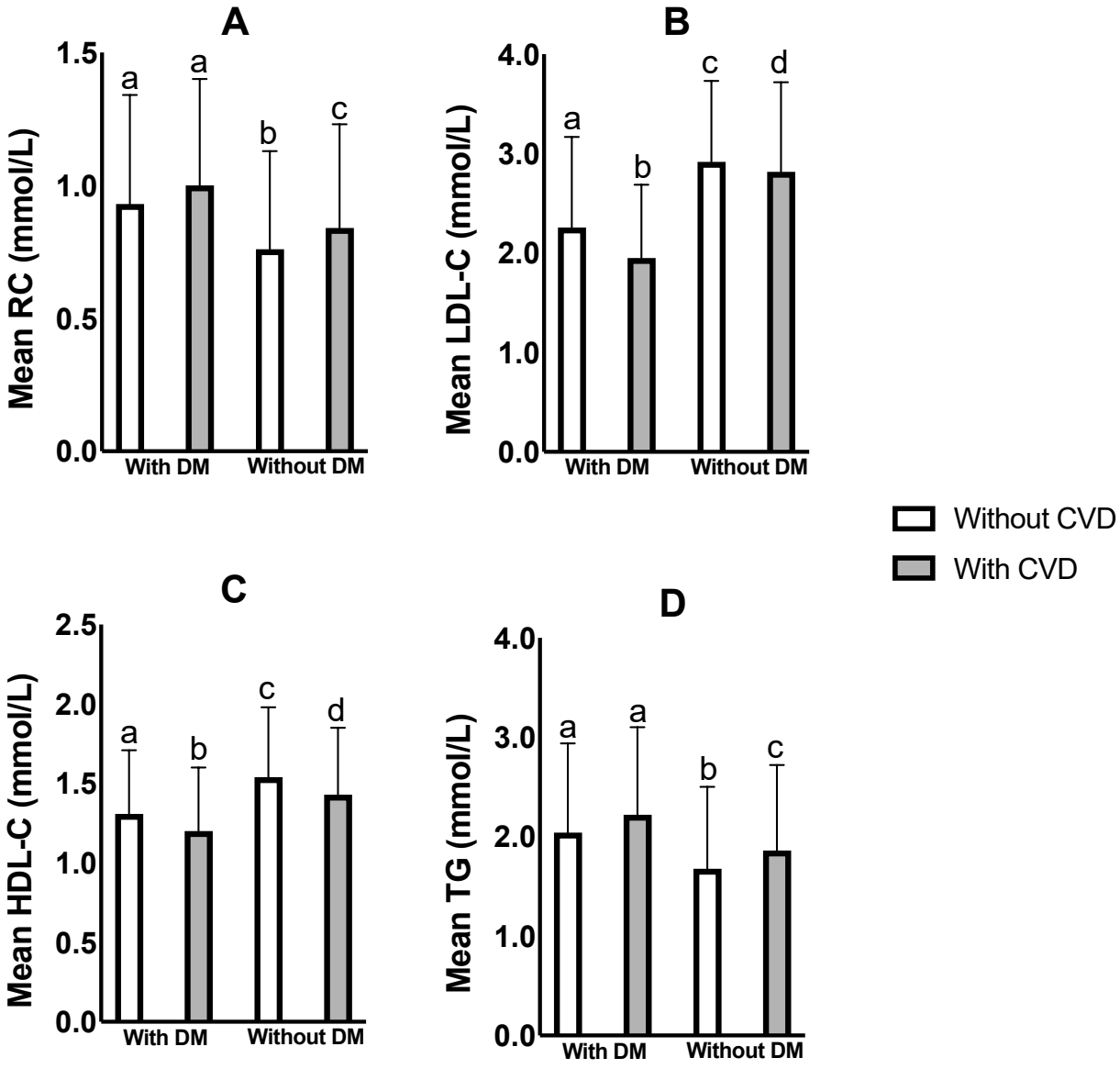

Figure 3.2 Mean non-fasting lipids stratified by CVD and diabetes diagnoses. Data analyzed with one-way ANOVA for multiple comparisons and presented as mean +/- standard deviation. Different letters indicate statistically significant differences ($p<0.05$). CVD, cardiovascular disease; DM, diabetes mellitus; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; RC, remnant cholesterol; TG, triglycerides. Figure created using GraphPad Prism Version 9.3.1.

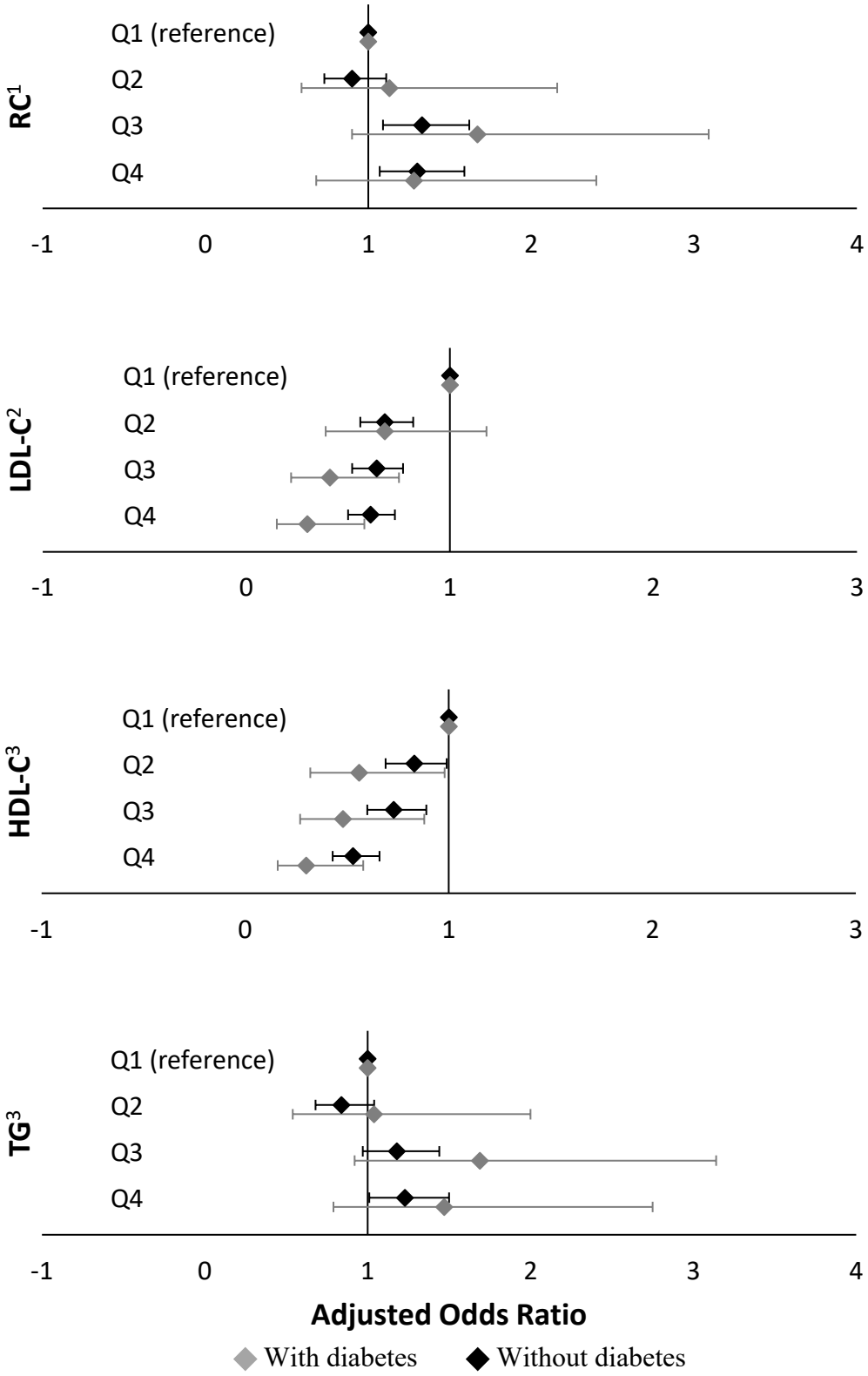
RC1
Q1 (reference)
Q2
Q3
Q4
-1
0
1
2
3
4
LDL-C2
Q1 (reference)
Q2
Q3
Q4
-1
0
1
2
3
HDL-C3
Q1 (reference)
Q2
Q3
Q4
-1
0
1
2
3
TG3
Q1 (reference)
Q2
Q3
Q4
-1
0
1
2
3
4
Adjusted Odds Ratio
With diabetes
Without diabetes

Figure 3.3 Adjusted odds ratios for the relationship between non-fasting lipid quartiles and incident CVD. Data presented as adjusted (by multivariable logistic regression) odds ratios with 95% confidence intervals. CVD, cardiovascular disease; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Q, quartile; RC, remnant cholesterol; TG, triglycerides.

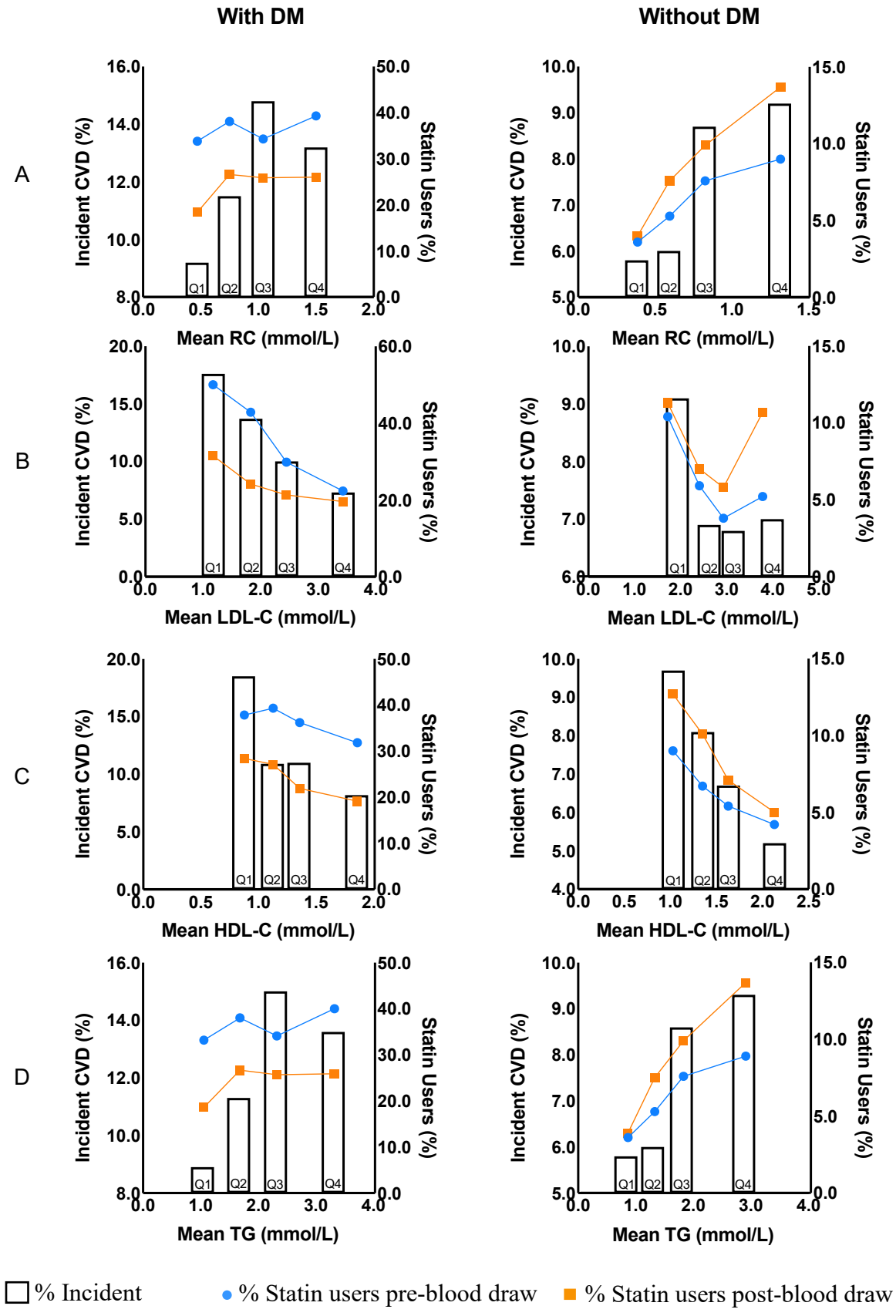
With DM
Without DM
A
B
C
D
Incident CVD (%)
Statin Users (%)
Mean RC (mmol/L)
Mean LDL-C (mmol/L)
Mean HDL-C (mmol/L)
Mean TG (mmol/L)
Q1
Q2
Q3
Q4
% Incident
% Statin users pre-blood draw
% Statin users post-blood draw

Figure 3.4 Proportion of statin users and incident CVD diagnoses by non-fasting lipid quartile. CVD, cardiovascular disease; DM, diabetes mellitus; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Q, quartile; RC, remnant cholesterol; TG, triglycerides. Figure Created using GraphPad Prism Version 9.3.1.

[1]RC model adjusted for age, sex, statin use, Elixhauser comorbidity index, and LDL-C.
[2]LDL-C model adjusted for age, sex, statin use, Elixhauser comorbidity index, and RC.
[3]HDL-C, TG models adjusted for age, sex, statin use and Elixhauser comorbidity index.

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