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Link to published version (if available):
10.1161/ATVBAHA.120.315639

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Per particle triglyceride-rich lipoproteins imply higher myocardial infarction risk than low-density lipoproteins: Copenhagen General Population Study

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Keywords
Epidemiology; Fibrate; Lipids; Metabolomics; Statin; Stroke

Subject terms
Lipids and Cholesterol, Cardiovascular Disease, Myocardial Infarction, Atherosclerosis

Word count
Main text : 7,461 (max 7,500)
Abstract : 245 (max 250)
References : 46 (No max)
Figures and Tables : 6 (max 6)

TOC category
Clinical

TOC subcategory
Atherosclerosis/Lipoproteins
Abstract

**Objective:** Apolipoprotein B (apoB)-containing triglyceride-rich lipoproteins and low-density lipoproteins (LDL) are each causal for myocardial infarction and atherosclerotic cardiovascular disease; however, the relative importance is unknown. We tested the hypothesis that for the same number of apoB-containing particles from smaller LDL through to larger triglyceride-rich lipoproteins, the risk of myocardial infarction is similar.

**Approach and Results:** We included 29,039 individuals with no history of myocardial infarction nested within 109,751 individuals from the Copenhagen General Population Study. Particle number of apoB-containing lipoprotein subfractions were measured using nuclear magnetic resonance spectroscopy. During a mean follow-up of 10 years, 2,309 individuals developed myocardial infarction. Multivariable adjusted hazard ratios for myocardial infarction per $1 \cdot 10^{15}$ more particles were higher with larger size and more triglyceride content of apoB-containing lipoproteins using ten different subfractions, ranging from 11 (95% confidence interval, 5.6-22) for extra extra large very low-density lipoproteins (VLDL), to 1.06 (1.05-1.07) for intermediate-density lipoproteins (IDL), through to 1.01 (1.01-1.01) for small LDL. When combining the particle number of six VLDL subfractions and combining IDL and three LDL subfractions, hazard ratios for myocardial infarction per $1 \cdot 10^{17}$ more particles were 3.5 (2.7-4.5) for VLDL and 1.3 (1.2-1.4) for IDL and LDL combined.

**Conclusions:** For the same number of apoB-containing particles ($1 \cdot 10^{17}$ particles/L), the hazard ratio for myocardial infarction was 3.5-fold for VLDL and 1.3-fold for IDL and LDL combined. These findings challenge plasma apoB and non-high-density lipoprotein cholesterol as summary indices of all apoB-containing lipoproteins.
Abbreviations

ApoB: Apolipoprotein B
LDL: Low-density lipoprotein
Non-HDL: Non-high-density lipoprotein
VLDL: Very low-density lipoprotein
IDL: Intermediate-density lipoprotein
CRP: C-reactive protein
ICD: International Classification of Diseases
NMR: Nuclear magnetic resonance
BMI: Body mass index
Introduction

Accumulation of cholesterol from apolipoprotein B (apoB)-containing lipoproteins in the arterial intima is well-established as a key component in the pathogenesis of atherosclerosis, myocardial infarction, and atherosclerotic cardiovascular disease. While current clinical practice primarily targets the correction and monitoring of low-density lipoprotein (LDL) cholesterol for risk prevention, guidelines from the US and Europe now recommend additional assessment of levels of triglycerides, non-high-density lipoprotein (non-HDL) cholesterol, and apoB\(^1,2\), addressing the fact that a substantial residual risk of atherosclerotic cardiovascular disease still remains after reducing LDL cholesterol to recommended values\(^3,4\). Residual risk may be explained partly by remaining apoB-containing triglyceride-rich lipoprotein particles\(^5-16\), particles that carry both triglycerides and cholesterol\(^17\).

Triglyceride-rich very low-density lipoproteins (VLDL), intermediate-density lipoproteins (IDL) and LDL are each causal risk factors for myocardial infarction and atherosclerotic cardiovascular disease\(^18-28\). From a biological point of view, it seems plausible that for the same number of particles, large triglyceride-rich VLDLs may be more important than LDL for risk of myocardial infarction\(^29,30\). Observations supporting this idea include the accelerated atherosclerosis seen in patients with remnant hyperlipidaemia\(^31\), that larger triglyceride-rich VLDLs per particle carry more cholesterol than the smaller LDL particles\(^37\), and that larger triglyceride-rich VLDLs compared to LDL may get trapped more easily in the intima of the arterial wall\(^29,30,32,33\). Therefore, larger versus smaller particles have the potential to deposit the most cholesterol in the arterial wall. Further, as elevated triglycerides, but not elevated LDL cholesterol are associated with systemic elevated C-reactive protein (CRP) as a marker of low-grade inflammation\(^21,34\), triglyceride-rich VLDLs may promote intimal inflammation increasing the likelihood of plaque rupture and thus myocardial infarction\(^29,30\). However, it is presently unknown whether the same increase in number of apoB-containing triglyceride-rich lipoproteins versus LDL associate similarly or differently with risk of myocardial infarction.

We tested the hypothesis that for the same number of more apoB-containing particles from smaller LDL through to larger triglyceride-rich lipoproteins, the risk of myocardial infarction is similar. For this purpose, we measured number of apoB-containing particles using nuclear magnetic resonance spectroscopy in 29,039 individuals nested within 109,751 individuals from the Copenhagen General Population Study, and followed them during a mean of 10 years during which time 2,309 developed myocardial infarction. We studied myocardial infarction as this is the hardest and best diagnosed endpoint within atherosclerotic cardiovascular disease in the Danish registries.
Methods

This study was approved by Herlev and Gentofte Hospital as well as a Danish Ethics committee (H-KF-01-144/01), and was conducted according to the Declaration of Helsinki. Written informed consent was given by all participants. All were whites of Danish descent. No individuals were lost to follow-up due to the complete Danish registries. The Danish Data Protection Agency does not allow open access to our data; however, upon reasonable request the corresponding author can allow joint analyses with other researchers.

Participants

The Copenhagen General Population Study is a prospective cohort study of 109,751 Danish adults recruited in 2003-2015, selected to represent the general population. Randomly invited individuals aged 20-100 filled in a questionnaire, had a physical examination, and had blood samples drawn for biochemical analyses. We studied 2,309 individuals with and 26,730 individuals without myocardial infarction during follow-up. All were free of myocardial infarction at baseline.

Myocardial infarction

Information on baseline and incident myocardial infarction were defined according to World Health Organization International Classification of Diseases (ICD) codes ICD-8 410 and ICD-10 I21-22 and were collected from April 1977 until December 2018 using data from hospital admissions and diagnoses in the national Danish Patient Registry; after recruitment death from myocardial infarction were also ascertained from the national Danish Causes of Death Registry. Deaths were assessed using the national Danish Civil Registration System. All individuals living in Denmark are assigned a unique Civil Person Registration number, and with use of this, exact dates of emigration (n=83) or deaths (n=9,223) can be obtained through the Danish Civil Registration System.

Laboratory analyses

High-throughput nuclear magnetic resonance (NMR) spectroscopy was used to measure particle number and cholesterol content of chylomicrons and extra extra large VLDL (XXL VLDL), extra large (XL) VLDL, large (L) VLDL, medium (M) VLDL, small (S) VLDL, extra small (XS) VLDL, IDL, large (L) LDL, medium (M) LDL, and small (S) LDL in serum samples collected nonfasting at baseline from individuals participating in the Copenhagen General Population Study. Until NMR analysis, serum samples were stored at -80 degrees to maintain lipoprotein composition during long-term storage. The NMR analysis took place at the Metabolomics Core Facility at the University of Bristol using the Nightingale assay. Standard hospital assays were used to measure nonfasting plasma levels of total cholesterol, glucose, and high-sensitive CRP.

Other covariates at baseline

Weight, height, and waist circumference were measured at the date of examination. Body mass index (BMI) was calculated using measured weight (kg) divided by measured height squared (m²). Systolic blood pressure was measured using an automated blood pressure device. Current smoking status, time since last meal (hours), alcohol intake (g/week), use of lipid-lowering therapy, and low education defined as less than 3 years of education following mandatory primary school were self-reported. Low physical activity in leisure time was also
self-reported and was defined as less than 4 hours of low-intensity physical activity or less than 2 hours of high-intensity physical activity per week. Type 2 diabetes mellitus was defined using diagnostic codes obtained from the national Danish Patient Registry (ICD-8 250 and ICD-10 E11, E13, and E14), as self-reported diabetes, use of anti-diabetic medication, or by a nonfasting plasma glucose above 11 mmol/L (198 mg/dL).

**Statistical analysis**

We used STATA/SE 13.1. Missing information on systolic blood pressure, alcohol intake, waist circumference, physical activity, educational level, time since last meal, CRP, and BMI were imputed based on sex and age using multivariate imputation with chained equations (0.8% of all covariate information). If only individuals with complete information were included, results were similar to those reported.

To facilitate easy interpretation when comparing risk estimates between the different subfractions of apoB-containing lipoproteins including both apoB-48 and apoB-100, values of each 10 subfraction were scaled to $1 \times 10^{15}/L$ more particles equivalent to the number of LDL particles corresponding to 0.003 mmol/L (0.12 mg/dL) LDL cholesterol; this scaling was chosen to make the values realistic for all lipoprotein fractions as the highest number of the rare XXL VLDL in any individual was $1.3 \times 10^{15}$ particles/L (Supplementary Methods). When comparing risk estimates between VLDL and IDL+LDL combined, values were scaled to $1 \times 10^{17}/L$ more particles equivalent to the number of LDL particles corresponding to 0.3 mmol/L (12 mg/dL) LDL cholesterol; this scaling was chosen to make the values clinically meaningful for all lipoprotein fractions as the highest number of total VLDL in any individual was $3 \times 10^{17}$ particles/L. Importantly however, this paper compares number of particles between different apoB-containing lipoproteins, and not cholesterol content in these particles.

The association between more apoB-containing lipoproteins and myocardial infarction was estimated using Cox proportional hazards regression with 95% confidence intervals with age as underlying timescale (=age adjusted). Entry was at study examination (=left truncation) and individuals were followed until date of event, emigration, death, or end of follow-up December 2018. Multivariable adjustment included age (underlying timescale), date of birth, sex, smoking, systolic blood pressure, physical activity in leisure time, educational level, time since last meal, alcohol consumption, and use of lipid-lowering therapy. The covariates chosen for adjustment were based on standard risk factors for myocardial infarction and on factors known to influence blood lipid measurements. On purpose, we did not adjust for diabetes mellitus, BMI, waist circumference, and CRP in the main analysis: while the three former will partly explain elevated triglyceride-rich lipoproteins, inflammation is a consequence of elevated triglyceride-rich lipoproteins\(^\text{21}\); however, these covariates were included in sensitivity analyses. Additional sensitivity analyses were made using data only on 13,546 individuals recruited consecutively from November 2003 to May 2005 without using a nested design and with an alternative model where adjustment for LDL particle number was carried out when using VLDL subfractions as exposure.

Hazard ratios and confidence intervals were corrected for within person variability, that is, regression dilution bias, using a non-parametric method\(^\text{35}\). Using measurements on 496 individuals from the Copenhagen General Population Study, who had blood samples drawn 10.8 years apart and who did not use lipid-lowering therapy at either time point, regression dilution ratios were calculated ranging between 0.59 and 0.79 (Table I in the online-only Data Supplement).
Results

From 109,751 individuals from the Copenhagen General Population Study, 29,039 without myocardial infarction at baseline were selected for measurement of number of apoB-containing lipoproteins within 10 subfractions ranging from the smallest LDL to the largest triglyceride-rich VLDL. When compared to 26,730 individuals not developing myocardial infarction during a mean 10 years of follow-up, the 2,309 individuals who did develop myocardial infarction were more likely to be older (67 vs 62 years), have diabetes (10% vs 6.2%), have higher systolic blood pressure (150 mmHg vs 140 mmHg), have higher alcohol intake per week (120 vs 108), have larger waist circumference (96 vs 92), and have higher CRP (1.9 vs 1.6), and were less likely to be female (38% vs 53%) (Table 1). Number of particles and cholesterol content in the different subfractions of apoB-containing lipoproteins are shown in Table 2. Intercorrelation for number of particles and cholesterol content among the different subfractions of apoB-containing lipoproteins are shown in Table 3.

Ten subfractions of apolipoprotein B-containing lipoproteins

Multivariable adjusted hazard ratios for myocardial infarction per $1 \times 10^{15}$ more particles were higher with larger size and more triglyceride content of apoB-containing lipoproteins, ranging from 11 (95% confidence interval, 5.6-22) for extra extra large VLDL, to 1.06 (1.05-1.07) for extra small VLDL, to 1.02 (1.01-1.02) for IDL, through to 1.01 (1.01-1.01) for small LDL (Figure 1, top). When combining the particle number of six VLDL subfractions into two groups (extra extra large, extra large and large in one group and medium, small and extra small in a second group) and the particle number of three LDL subfractions into one group similar results were observed, with hazard ratios ranging from 1.06 (1.05-1.08) for the largest group of VLDL to 1.00 (1.00-1.00) for LDL (Figure 1, bottom). The explanation for the smaller hazard ratios for myocardial infarction when groups of lipoprotein fractions are combined compared to each of the subfractions separately, likely is the fact that the reference group with the lowest concentrations typically in combined groups will have higher levels of apoB-containing lipoproteins than in each of the subfractions separately.

The number of particles/L for each of the 10 apoB-containing lipoprotein subfractions differ substantially between different individuals, as some individuals are dominated by triglyceride-rich lipoproteins while others are dominated by LDL. On average for all 29,039 individuals studied, 1% of all particles were in the largest VLDL (L+XL+XXL), 13% in the smallest VLDL (XS+S+M), 16% in IDL, and 70% in LDL (Figure 1, bottom). Therefore, since LDL is the most abundant atherogenic lipoprotein in plasma in most individuals, LDL particles will be driving the majority of their lipid-related risk, even though the atherogenic potential per particle is higher for VLDL particles than for LDL particles.

Very low-density lipoproteins versus intermediate-density and low-density lipoproteins combined

The multivariable adjusted hazard ratio for myocardial infarction per $1 \times 10^{17}$ more particles was higher for VLDL than for IDL and LDL combined, but not for a 0.3 mmol/L (12 mg/dL) higher cholesterol content in the two fractions (Figure 2); this difference in particle number versus cholesterol content is likely explained by the fact that VLDL per particle due to larger size contain more cholesterol than IDL and LDL particles. The hazard ratios per $1 \times 10^{17}$ more particles were 3.5 (95% confidence interval, 2.7-4.5) for VLDL and 1.3 (1.2-1.4) for IDL and LDL combined. Per 0.3 mmol/L higher cholesterol content, the corresponding hazard ratios were 1.10 (1.08-1.12) for VLDL and 1.11 (1.07-1.15) for IDL and LDL combined.

When VLDL and IDL were combined, the multivariable adjusted hazard ratio for myocardial infarction per $1 \times 10^{17}$ more particles were 2.9 (95% confidence interval, 2.3-3.6)
for VLDL and IDL combined and 1.4 (1.3-1.5) for LDL (Figure 2). The corresponding values per 0.3 mmol/L higher cholesterol content were 1.29 (1.22-1.36) and 1.05 (1.03-1.07), respectively.

**Sensitivity analyses**

In sensitivity analyses adjusting additionally for BMI, waist circumference, CRP, diabetes, and for all four combined, the observed associations for increased particle number and cholesterol content on risk of myocardial infarction were only slightly attenuated (Figure I in the online-only Data Supplement). An additional sensitivity analysis was made using data only on 13,546 individuals recruited consecutively from November 2003 to May 2005, with similar risk estimates as for the nested design (compare Figure II in the online-only Data Supplement with Figure 1). Also, when risk estimates for VLDL subfractions per $1 \cdot 10^{15}$ more particles were adjusted for LDL particle number, hazard ratios were only slightly attenuated (compare Figure III in the online-only Data Supplement with Figure 1).

**Comparison with previous studies**

To enable easy comparison of our data with previous studies, we calculated risk estimates per standard deviation increase in subfractions and compared them with results in two previous studies. Risk estimates were similar. However, as LDL in most individuals is much more abundant than IDL and VLDL, the number of particles per one standard deviation in VLDL is much lower than the number of particles per one standard deviation in LDL. This explain our current finding, that if the different lipoprotein subfractions are compared for the same number of more apoB-containing lipoproteins, the risk of myocardial infarction is higher for VLDL than for LDL.
Discussion

In this nested cohort study of 29,039 individuals selected within 109,751 individuals from the general population, we observed higher risk of myocardial infarction with larger sized triglyceride-rich apoB-containing lipoproteins compared with LDL (Graphic Abstract). For the same number of more apoB-containing particles (1·10^{17} particles/L), the hazard ratio for myocardial infarction was 3.5-fold for VLDL and 1.3-fold for IDL and LDL combined. These novel findings challenge the use of plasma apoB and non-HDL cholesterol as summary indices of all apoB-containing lipoproteins.

A unique feature of the present study is the fact that we were able to study low versus very high levels of the same ten subfractions of apoB-containing lipoproteins using individual participant data. This is important as some individuals mainly have elevated LDL in plasma while others mainly have elevated triglyceride-rich VLDL, and yet others have various combinations of elevated VLDL, IDL and LDL. In our study on average for all individuals, LDL and IDL accounted for 86% of all apoB-containing particles carrying 77% of cholesterol, leaving 23% of cholesterol carried by apoB-containing lipoproteins in the remaining 14% of particles consisting of triglyceride-rich VLDL. The relative differences in particle number of VLDL, IDL and LDL are important when thinking about the overall risk contribution from triglyceride-rich VLDL versus IDL and LDL. For most individuals, because the average number of LDL particles is 5 times higher than IDL, 6 times higher than the smallest VLDLs, and 100 times higher than the largest VLDL particles, despite differences in the hazard ratio, LDL particles are still driving the majority of their lipid-related risk. However, the finding that individuals with a large number of the very largest triglyceride-rich VLDL have a much higher risk of myocardial infarction than individuals with mainly elevated LDL could be perceived as a surprising finding; however, to us this observation seems mechanistically plausible.

A straight forward and plausible explanation for our findings is the idea that per particle, large triglyceride-rich lipoproteins have higher atherogenic potential compared with LDL. This is due to the facts that the larger particles carry more cholesterol per particle and are trapped more easily in the arterial intima compared to smaller particles. While circulating in the bloodstream, a small fraction of apoB-containing lipoproteins will penetrate into and get trapped in the arterial intima, promoting foam cell formation and atherosclerotic plaques, ultimately leading to myocardial infarction and atherosclerotic cardiovascular disease. Although large versus small lipoprotein size reduces the penetration into the arterial wall, once entrance into the arterial intima has occurred the largest sized triglyceride-rich particles seems to be trapped preferentially compared to the smaller LDL within the arterial intima. Further, as elevated triglyceride-rich lipoproteins in contrast to elevated LDL is associated with systemic elevated CRP, it is also likely that triglycerides in triglyceride-rich lipoproteins are hydrolysed within the intima leading to local inflammation, plaque rupture and thus myocardial infarction.

Consistent with previously found causal associations of both elevated LDL and triglyceride-rich lipoproteins with increased risk of myocardial infarction and atherosclerotic cardiovascular disease, we here observed that elevation of all subfractions of apoB-containing lipoproteins were associated with increased risk of myocardial infarction. In support of these findings, previous studies in 4,662 and 13,441 individuals found risk of cardiovascular events increased similarly per standard deviation increase of all apoB-containing lipoproteins, exactly as in the present study (Figure 3). Interestingly, Holmes et al. found that per standard deviation increase with 85 fold more cholesterol in LDL than in XXL VLDL, the odds ratios for myocardial infarction were 1.16 for LDL and 1.21 for XXL VLDL. Such observations are in agreement with the present findings that for the same increase in number of particles in the different subfractions, triglyceride-rich lipoproteins generate greater risk of myocardial infarction than LDL.
It could be argued that observations from randomized double-blind trials may seem somewhat contradictory to our findings. Many would argue that fibrates reduce triglycerides and VLDL particle number substantially, yet many fibrate trials were negative\textsuperscript{29}. However, none of the double-blind fibrate trials recruited patients with elevated triglycerides and VLDL, but rather recruited patients with elevated LDL cholesterol, and every single fibrate trial showed reduced atherosclerotic cardiovascular disease (ASCVD) events in the subgroup with baseline elevated triglycerides. Also, when all randomized trials with some triglyceride reduction using fibrates, niacin, omega-3 fatty acids, and statins were meta-analyzed, baseline median plasma triglyceride was only 1.7 mmol/L (151 mg/dL) while mean LDL cholesterol was 3.3 mmol/L (127 mg/dL)\textsuperscript{41}. This is a reflection of the fact that essentially all trials recruited patients due to elevated LDL cholesterol with the only exception being the REDUCE-IT trial that recruited patients due to elevated triglycerides\textsuperscript{42}. At total plasma triglycerides of 1.7 mmol/L (151 mg/dL), more than a third of triglycerides is carried of high-density lipoprotein (HDL), LDL and IDL\textsuperscript{17}, suggesting that at such low levels, plasma triglycerides is not the optimal marker for triglyceride-rich lipoproteins. Despite of this, a 1 mmol/L (88 mg/dL) reduction in plasma triglycerides conferred a relative risk of ASCVD of 0.84 (95% confidence interval: 0.75-0.94; p=0.0026)\textsuperscript{41}; apparent discrepancy between the individual studies could be largely driven by sampling variation in patient populations, baseline lipid levels, treatment doses or formulations, and cotherapies (eg, statins). The REDUCE-IT trial with median baseline triglycerides of 2.5 mmol/L (216 mg/dL) observed a relative risk for ASCVD of 0.75 (0.68-0.83) after a triglyceride reduction of 0.44 mmol/L (39 mg/dL)\textsuperscript{42}; however, it is possible that the effect of icosapent ethyl 4 g/day on reduced ASCVD is not explained solely on reduced triglyceride-rich lipoproteins\textsuperscript{41,43}. Taken together, as most previous lipid-lowering trials did not recruit patients with elevated triglycerides, we need more large randomized trials of potent triglyceride-lowering drugs used in patients with elevated triglycerides to evaluate if reduction of number of VLDL particles will reduce risk of ASCVD.

Some may also argue that recent Mendelian randomization studies observing that all apoB-containing particles appeared to have similar association on risk of cardiovascular disease per particle\textsuperscript{44,45} may contrast our findings. However, these studies essentially only examined people with low levels of triglyceride-rich lipoproteins (median triglycerides 1.3 mmol/L; 118 mg/dL) and therefore mainly studied elevated LDL particles (mean LDL cholesterol 3.4 mmol/L; 131 mg/dL). In other words, lower levels of apoB through lipoprotein lipase (LPL) and LDL receptor (LDLR) genetic variants in both cases will mainly be driven by lower levels of LDL, and therefore it is not surprising that the lower risk of coronary heart disease is similar for a similar reduction in apoB due to the two different types of genetic variants. We answer a different question, as we directly study triglyceride-rich lipoproteins and LDL within each individual using individual participant data and come to the conclusion that per particle, VLDL is more atherogenic than LDL. When future studies possible can identify genetic instruments for apoB-containing lipoprotein particle number, Mendelian randomization studies may substantiate such findings even further.

Strengths of our study include the large statistical power through a nested design within 109,751 individuals from the general population, accumulating 2,309 hard endpoints of myocardial infarction during 10 years of follow-up. Also, in contrast to previous studies we were able to measure number of apoB-containing lipoproteins in ten subfractions in all individuals. Finally, we did not lose track of any individual during follow-up due to the complete Danish health registries.

Possible limitations concern the validation of the nuclear magnetic resonance spectroscopy measurement, which due to its novelty has not yet entered into standard laboratory practice and therefore is unstandardized. Therefore, the absolute values of particle number of the different subfractions of apoB-containing lipoproteins are not necessarily accurate and should be interpreted cautiously; however, comparison between the ten different lipoprotein subfractions is valid as all were measured directly using the
same nuclear magnetic resonance spectroscopy measurement. Another limitation relates to the use of samples stored at -80°C which potentially could affect the measurements; however, comparison with measurements done on 496 fresh samples indicated that concentrations of the various apoB-containing lipoprotein subfractions were not disturbed to any major degree by freezing. Another potential limitation concerns the availability and completeness of the diagnostic information; however, myocardial infarction diagnoses were drawn from Danish health registers which is 99.5% correct when validated. Further, intercorrelation among ten different subfractions of apoB-containing lipoproteins needs to be considered; however, strong intercorrelation were mainly observed among the different VLDL subclasses and among IDL and the different LDL subclasses, either as particle number or cholesterol content (Table 3). Also, as we studied white individuals of Danish descent only, our results may not necessarily apply to other ethnic groups. That said, we are not aware of data to suggest that the present results should not apply to all ethnicities. Finally, it is a limitation that we were not able to measure the contribution from lipoprotein(a) to the ten different subfractions of apoB-containing lipoproteins; however, as lipoprotein(a) is mainly found within the LDL fractions, high lipoprotein(a) cannot explain the higher risk of myocardial infarction associated with high levels of triglyceride-rich lipoproteins versus LDL.

**Conclusion**

For the same number of more apoB-containing particles (1·10^{17} particles/L), the hazard ratio for myocardial infarction was 3.5-fold for VLDL and 1.3-fold for IDL and LDL combined. These novel findings support further focus on triglyceride-rich lipoproteins for prevention of myocardial infarction and atherosclerotic cardiovascular disease. These findings of differential atherogenicity for different apoB-containing particles challenge the use of plasma apoB and non-HDL cholesterol as summary indices of all apoB-containing lipoproteins.
Acknowledgments

The authors thank staff and participants of the Copenhagen General Population Study for their important contribution.

Sources of Funding

This work was supported by Innovation Fund Denmark [grantno:9039-00360B to BGN], and Overlæge Johan Boserup og Lise Boserups legat [grantno: 20795-24 to MOJ]. George Davey Smith was supported by the Medical Research Council Integrative Epidemiology Unit at the University of Bristol MC_UU_00011/1. The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the paper.

Disclosures

Børge G. Nordestgaard report consultancies and talks sponsored by AstraZeneca, Sanofi, Regeneron, Akcea, Amgen, Kowa, Denka Seiken, Amarin, Novartis, Novo Nordisk, and Silence Therapeutics. There are no financial or other conflicts of interest for MOJ, SVK, SFN, SA or GDS.
Supplemental Materials
Expanded Methods
Online-only Figure I – III
Online-only Table I
Major Resources Table
Online-only Reference
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Highlights

- Per particle triglyceride-rich lipoproteins imply higher myocardial infarction risk than low-density lipoproteins

- In most individuals, low-density lipoproteins are quantitatively the dominant atherogenic lipoproteins

- In individuals with elevated triglyceride-rich lipoproteins, these particles may be quantitatively the dominant atherogenic lipoproteins

- These findings challenge plasma apoB and non-high-density lipoprotein cholesterol as summary indices of all apoB-containing lipoproteins.
Figure legends

Figure 1: Associations of subfractions of apolipoprotein B-containing lipoprotein particle number with risk of myocardial infarction.

A total of 29,039 individuals nested within 109,751 individuals from the Copenhagen General Population Study were selected for metabolomic profiling and included in the analyses. During a mean follow-up of 10 years (ranging from 0 to 15 years), 2,309 individuals developed incident myocardial infarction. To facilitate easy interpretation when comparing risk estimates between the different subfractions of apolipoprotein B-containing lipoproteins, values of each subfraction were scaled to $1 \cdot 10^{15}$/L more particles. Hazard ratios were multivariable adjusted for age, year of birth, sex, current smoking status, physical activity level in leisure time, level of education after primary school, time since last meal, alcohol intake, and use of lipid-lowering therapy, all at baseline. Hazard ratios were corrected for regression dilution bias. Abbreviations: VLDL = Very low-density lipoproteins. IDL = intermediate-density lipoproteins. LDL = low-density lipoproteins. XXL = extra extra large. XL = extra large. L = large. M = medium. S = small. XS = extra small. HR = hazard ratio. CI = confidence interval.

Figure 2: Associations of VLDL, IDL, and LDL particle number and cholesterol content with risk of myocardial infarction.

A total of 29,039 individuals nested within 109,751 individuals from the Copenhagen General Population Study were selected for metabolomic profiling and included in the analyses. Hazard ratios for myocardial infarction according to $1 \cdot 10^{17}$/L more particles (left panel) and to 0.3 mmol/L (12 mg/dL) cholesterol content increase (right panel). Hazard ratios were multivariable adjusted for age at examination, year of birth, sex, current smoking status, physical activity level in leisure time, level of education after primary school, time since last meal, alcohol intake, and use of lipid-lowering therapy, all at baseline. Hazard ratios were corrected for regression dilution bias. Abbreviations: VLDL = Very low-density lipoproteins. IDL = intermediate-density lipoproteins. LDL = low-density lipoproteins. HR = hazard ratio. CI = confidence interval.

Figure 3: Associations of subfractions of apolipoprotein B-containing lipoproteins with risk of myocardial infarction per one standard deviation higher number of particles.

Hazard ratios or odds ratios for coronary heart disease or myocardial infarction are per one standard deviation higher number of particles. Hazard ratios and odds ratios from Holmes et al[36] and Würtz et al[37] including 95% confidence intervals were visual reads from figures when estimates were not given. Abbreviations: VLDL = Very low-density lipoproteins. IDL = intermediate-density lipoproteins. LDL = low-density lipoproteins. XXL = extra extra large. XL = extra large. L = large. M = medium. S = small. XS = extra small. HR = hazard ratio. CI = confidence interval.
**Table 1.** Baseline characteristics of individuals in the Copenhagen General Population Study according to myocardial infarction during follow-up.

<table>
<thead>
<tr>
<th>Potential confounders</th>
<th>All (n = 29,039)</th>
<th>Myocardial infarction (n = 2,309)</th>
<th>Without myocardial infarction (n = 26,730)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>62 (51 - 72)</td>
<td>67 (58 - 75)</td>
<td>62 (51 - 72)</td>
</tr>
<tr>
<td>Women</td>
<td>15,097 (52%)</td>
<td>870 (38%)</td>
<td>14,227 (53%)</td>
</tr>
<tr>
<td>Current smoker</td>
<td>6,863 (24%)</td>
<td>597 (26%)</td>
<td>6,266 (23%)</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>140 (129 - 157)</td>
<td>150 (135 - 163)</td>
<td>140 (128 - 156)</td>
</tr>
<tr>
<td>Low level of education</td>
<td>18,450 (64%)</td>
<td>1,567 (68%)</td>
<td>16,883 (63%)</td>
</tr>
<tr>
<td>Low physical activity</td>
<td>15,978 (56%)</td>
<td>1,299 (57%)</td>
<td>14,679 (56%)</td>
</tr>
<tr>
<td>Lipid-lowering therapy</td>
<td>3,480 (12%)</td>
<td>490 (21%)</td>
<td>2,990 (11%)</td>
</tr>
<tr>
<td>Alcohol intake, g/week</td>
<td>108 (48 - 192)</td>
<td>120 (48 - 204)</td>
<td>108 (48 - 192)</td>
</tr>
<tr>
<td>Other covariates</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>5.6 (4.9 - 6.4)</td>
<td>5.8 (4.9 - 6.6)</td>
<td>5.6 (4.9 - 6.4)</td>
</tr>
<tr>
<td>mg/dL</td>
<td>218 (191 - 250)</td>
<td>226 (191 - 257)</td>
<td>218 (191 - 250)</td>
</tr>
<tr>
<td>Time since last meal, hours</td>
<td>2.5 (1.5 - 3.5)</td>
<td>2.5 (1.5 - 3.5)</td>
<td>2.5 (1.5 - 3.5)</td>
</tr>
<tr>
<td>Within biological pathway*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>26 (23 - 29)</td>
<td>27 (24 - 30)</td>
<td>26 (23 - 29)</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>92 (82 - 101)</td>
<td>96 (88 - 104)</td>
<td>91 (82 - 100)</td>
</tr>
<tr>
<td>C-reactive protein, mg/L</td>
<td>1.6 (0.97 - 3.0)</td>
<td>1.9 (1.2 - 3.4)</td>
<td>1.6 (0.95 - 3.0)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>1,885 (6.5%)</td>
<td>224 (10%)</td>
<td>1,661 (6.2%)</td>
</tr>
</tbody>
</table>

Data are n (%) for categorical values and median (interquartile range) for continuous values. Baseline characteristics were at the date of examination. Low education was less than 3 years following the mandatory primary school. Low physical activity in leisure time was less than 4 hours of low intensity physical activity or less than 2 hours of high intensity physical activity per week. Type 2 diabetes mellitus was defined using diagnostic codes obtained from the national Danish Patient Registry (ICD-8 250 and ICD-10 E11, E13, and E14), as self-reported disease, use of anti-diabetic medication, or by a nonfasting plasma glucose above 11 mmol/L (198 mg/dL).

*While elevated body mass index, elevated waist circumference and diabetes mellitus lead to elevated levels of triglyceride-rich lipoproteins, elevated C-reactive protein is a consequence of elevated triglyceride-rich lipoproteins.*

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Table 2. Number of particles and cholesterol content in different apolipoprotein B-containing lipoproteins in individuals in the Copenhagen General Population Study.

<table>
<thead>
<tr>
<th>Lipoprotein particle</th>
<th>Number of particles</th>
<th>Cholesterol content</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median (IQR)</td>
<td>Range (min-max)</td>
</tr>
<tr>
<td></td>
<td>(min-max)</td>
<td></td>
</tr>
<tr>
<td>XXL VLDL</td>
<td>8·10^{13}</td>
<td>0.0-1.3·10^{15}</td>
</tr>
<tr>
<td></td>
<td>(4·10^{13}-1·10^{14})</td>
<td></td>
</tr>
<tr>
<td>XL VLDL</td>
<td>4·10^{14}</td>
<td>0.0-7·10^{15}</td>
</tr>
<tr>
<td></td>
<td>(2·10^{14}-7·10^{14})</td>
<td></td>
</tr>
<tr>
<td>L VLDL</td>
<td>3·10^{15}</td>
<td>0.0-4·10^{16}</td>
</tr>
<tr>
<td></td>
<td>(1·10^{15}-5·10^{15})</td>
<td></td>
</tr>
<tr>
<td>M VLDL</td>
<td>1·10^{16}</td>
<td>0.0-9·10^{16}</td>
</tr>
<tr>
<td></td>
<td>(6·10^{15}-1·10^{16})</td>
<td></td>
</tr>
<tr>
<td>S VLDL</td>
<td>2·10^{16}</td>
<td>0.0-8·10^{16}</td>
</tr>
<tr>
<td></td>
<td>(1·10^{16}-2·10^{16})</td>
<td></td>
</tr>
<tr>
<td>XS VLDL</td>
<td>2·10^{16}</td>
<td>0.0-8·10^{16}</td>
</tr>
<tr>
<td></td>
<td>(2·10^{16}-3·10^{16})</td>
<td></td>
</tr>
<tr>
<td>IDL</td>
<td>6·10^{16}</td>
<td>5·10^{15}-2·10^{17}</td>
</tr>
<tr>
<td></td>
<td>(5·10^{16}-7·10^{16})</td>
<td></td>
</tr>
<tr>
<td>L LDL</td>
<td>1·10^{17}</td>
<td>0.0-3·10^{17}</td>
</tr>
<tr>
<td></td>
<td>(8·10^{16}-1·10^{17})</td>
<td></td>
</tr>
<tr>
<td>M LDL</td>
<td>8·10^{16}</td>
<td>0.0-2·10^{17}</td>
</tr>
<tr>
<td></td>
<td>(7·10^{16}-9·10^{16})</td>
<td></td>
</tr>
<tr>
<td>S LDL</td>
<td>9·10^{16}</td>
<td>0.0-3·10^{17}</td>
</tr>
<tr>
<td></td>
<td>(8·10^{16}-1·10^{17})</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Intercorrelation between particle numbers and cholesterol content of different apolipoprotein B-containing lipoprotein subfractions shown as $R^2$-values.

<table>
<thead>
<tr>
<th>Cholesterol content</th>
<th>XXL VLDL</th>
<th>XL VLDL</th>
<th>L VLDL</th>
<th>M VLDL</th>
<th>S VLDL</th>
<th>XS VLDL</th>
<th>IDL</th>
<th>L LDL</th>
<th>M LDL</th>
<th>S LDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>XXL VLDL</td>
<td>1.00</td>
<td>0.98</td>
<td>0.98</td>
<td>0.82</td>
<td>0.41</td>
<td>0.26</td>
<td>0.19</td>
<td>0.15</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td>XL VLDL</td>
<td>0.99</td>
<td>1.00</td>
<td>0.98</td>
<td>0.80</td>
<td>0.34</td>
<td>0.19</td>
<td>0.13</td>
<td>0.09</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td>L VLDL</td>
<td>0.98</td>
<td>1.00</td>
<td>0.99</td>
<td>0.80</td>
<td>0.32</td>
<td>0.15</td>
<td>0.10</td>
<td>0.06</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>M VLDL</td>
<td>0.98</td>
<td>0.99</td>
<td>1.00</td>
<td>0.89</td>
<td>0.46</td>
<td>0.30</td>
<td>0.24</td>
<td>0.21</td>
<td>0.17</td>
<td></td>
</tr>
<tr>
<td>S VLDL</td>
<td>0.94</td>
<td>0.95</td>
<td>0.96</td>
<td>0.98</td>
<td>0.79</td>
<td>0.63</td>
<td>0.59</td>
<td>0.56</td>
<td>0.52</td>
<td></td>
</tr>
<tr>
<td>XS VLDL</td>
<td>0.60</td>
<td>0.56</td>
<td>0.57</td>
<td>0.62</td>
<td>0.74</td>
<td>0.92</td>
<td>0.88</td>
<td>0.84</td>
<td>0.80</td>
<td></td>
</tr>
<tr>
<td>IDL</td>
<td>0.29</td>
<td>0.22</td>
<td>0.21</td>
<td>0.25</td>
<td>0.39</td>
<td>0.88</td>
<td>0.99</td>
<td>0.96</td>
<td>0.95</td>
<td></td>
</tr>
<tr>
<td>L LDL</td>
<td>0.22</td>
<td>0.15</td>
<td>0.14</td>
<td>0.19</td>
<td>0.33</td>
<td>0.82</td>
<td>0.98</td>
<td>0.99</td>
<td>0.99</td>
<td>1.00</td>
</tr>
<tr>
<td>M LDL</td>
<td>0.22</td>
<td>0.15</td>
<td>0.15</td>
<td>0.19</td>
<td>0.33</td>
<td>0.80</td>
<td>0.97</td>
<td>0.99</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>S LDL</td>
<td>0.24</td>
<td>0.18</td>
<td>0.17</td>
<td>0.21</td>
<td>0.34</td>
<td>0.78</td>
<td>0.95</td>
<td>0.98</td>
<td>1.00</td>
<td></td>
</tr>
</tbody>
</table>