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Triglyceride content increases while cholesterol content decreases in HDL and LDL+IDL fractions following normal meals: The Copenhagen General Population Study of 25,656 individuals

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ABSTRACT

Background and aims: During fat tolerance tests, plasma triglycerides increase while high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, and intermediate-density lipoprotein (IDL) cholesterol decrease. However, it is unknown whether triglyceride content increases and cholesterol content decreases in HDL and LDL + IDL fractions following normal meals in the general population. Therefore, we tested the hypothesis that triglyceride content increases while cholesterol content decreases in HDL and LDL + IDL fractions following normal meals.

Methods: In this cross-sectional study, we included 25,656 individuals aged 20–100 years, all without lipid-lowering therapy at examination and selected for metabolomic profiling from the Copenhagen General Population Study. Triglyceride and cholesterol content of 14 lipoprotein fractions weas measured using nuclear magnetic resonance (NMR) spectroscopy. Time since last meal was recorded by the examiner immediately before blood sampling.

Results: Following normal meals in age and sex-adjusted analyses and when compared with fasting levels, plasma triglycerides were higher for up to 5–6 h, and triglyceride content was higher for up to 6–7 h in HDL fractions, for up to 6–7 h in LDL + IDL fractions, and for up to 5–6 h in very-low-density lipoprotein (VLDL) fractions. Further, plasma cholesterol was lower for up to 2–3 h, and cholesterol content was lower for up to 0–1 h in HDL fractions and for up to 4–5 h in LDL + IDL fractions, while cholesterol content was higher for up to 4–5 h in VLDL fractions.

Conclusions: Following normal meals, triglyceride content increases while cholesterol content decreases in HDL and LDL + IDL fractions.

1. Introduction

Previously, lipid profiles were recommended to be measured in the fasting state, although in most individuals, the postprandial period predominates over the fasting state [1]. However, emerging evidence demonstrates only minimal changes in plasma lipids, lipoproteins, and apolipoproteins following intake of normal meals in the general population [2–4]. In addition, nonfasting plasma triglycerides have been shown to better predict cardiovascular risk than fasting plasma triglycerides [5–7]. Accordingly, recent international lipid guidelines now

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endorse use of non-fasting lipid profiles for cardiovascular risk prediction [8–10].

Traditionally, fasting lipid profiles were recommended to limit postprandial increase in plasma triglycerides as observed following oral fat tolerance tests during which individuals typically ingest 1 g fat per kilogram bodyweight [11], the latter typically performed in smaller groups of selected individuals during 6–12 h. In this design, the postprandial increase in plasma triglycerides was often accompanied by a decrease in low-density lipoproteins (LDL) cholesterol and high-density lipoproteins (HDL) cholesterol [12,13]. While most of such studies on postprandial lipid profiles evaluate traditional lipid measures including plasma triglycerides, HDL cholesterol, and LDL cholesterol, some studies have additionally reported large postprandial changes in triglyceride and cholesterol content of lipoprotein fractions [12–17].

It is an established fact that elevated LDL cholesterol causes atherosclerotic cardiovascular disease (ASCVD) [18]. Less well studied are LDL triglycerides and HDL triglycerides; however, a recent study found that elevated LDL triglycerides were a strong marker of ASCVD [19] whereas the clinical importance of HDL triglycerides is unclear. Notably, in long-term, population-based studies, low HDL cholesterol has been suggested to be a marker of elevated plasma triglycerides and triglyceride-rich remnant lipoproteins [20]. Importantly, it is unknown whether triglyceride content increases while cholesterol content decreases in HDL and LDL + intermediate-density lipoprotein (IDL) fractions following normal meals in individuals in the general population.

We tested the hypothesis that triglyceride content increases while cholesterol content decreases in HDL and LDL + IDL fractions following normal meals. To test our hypothesis, we cross-sectionally studied 25,656 individuals aged 20–100 years, all without lipid-lowering therapy at examination and selected for metabolomic profiling from the Copenhagen General Population Study. All included individuals had detailed lipid and lipoprotein profiles measured by nuclear magnetic resonance (NMR) spectroscopy of 14 different lipoprotein fractions within HDL, LDL, IDL and very low-density lipoprotein (VLDL).

2. Patients and methods

This study was conducted in accordance with the Declaration of Helsinki and was approved by the Danish Data Protection Agency and the Ethics Committee of the Capital Region of Denmark (H-KF-01-144/01). All participants provided written informed consent.

2.1. The Copenhagen General populations study

The Copenhagen General Population Study is an ongoing prospective cohort study of 109,751 Danish adults aged 20–100 years, recruited in 2003–2015, and with a participation rate of 43% of those invited. Invited individuals were randomly selected from the national Danish Civil Registration System to reflect the adult white Danish population. At examination, participants filled in a questionnaire, had a physical examination, and had blood samples collected for biochemical analyses. A subgroup of 30,335 individuals were selected for metabolomic profiling using NMR spectroscopy, and among these, we included 25,656 individuals not taking lipid-lowering therapy at examination. Time since last meal (hours) was obtained by the examiner immediately before blood sampling.

2.2. Triglycerides and cholesterol in lipoprotein fractions

High-throughput NMR spectroscopy [21,22] was used to measure triglyceride content, cholesterol content, and particle number of 14 lipoprotein fractions comprising four fractions of HDL including small (S) HDL, medium (M) HDL, large (L) HDL, and extra-large (XL) HDL; three fractions of LDL including S LDL, M LDL, and L LDL; one fraction of intermediate-density lipoprotein (IDL); and six fractions of VLDL including extra small (XS) VLDL, S VLDL, M VLDL, L VLDL, XL VLDL, and chylomicrons and extra extra-large (XXL) VLDL. To preserve lipoprotein composition during long-term storage, serum samples were stored at −80 °C until NMR analysis. The NMR analyses were conducted using the Nightingale assay at the Metabolomic Core Facility at the University of Bristol. The lipids measured by the Nightingale method has been validated against gel permeation high-performance liquid chromatography, ultracentrifugation (only for VLDL cholesterol), and standard biochemical assays with correlation coefficients on 0.88–0.95 [21,23]. This is in line with findings from the Copenhagen General Population Study (Supplementary Fig. 1).

2.3. Covariates

Weight (kg) and height (m) were measured by the examiner on the day of examination, and body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2). Plasma cholesterol, plasma triglycerides, and plasma albumin were measured on fresh blood samples at the day of examination using validated standard biochemical assays at the Department of Clinical Biochemistry at Copenhagen University Hospital in Herlev.

2.4. Statistical analyses

Stata/SE 17.0 was used to perform all statistical analyses. Differences across groups of time since last meal were tested using Kruskal-Wallis test for continuous variables and chi-squared test for categorical variables. Missing information (0.49% of all covariate information) was imputed based on age and sex using single imputation; results were similar without imputation. As it is a standard procedure in ultracentrifugation [24] and was previously done using NMR spectroscopy [25–27], triglyceride and cholesterol content of lipoprotein fractions were corrected for recovery relative to plasma triglycerides and plasma cholesterol measured on fresh blood samples at the day of examination to obtain clinically relatable lipid levels. Distribution of plasma triglycerides, triglyceride content in all lipoprotein fractions, cholesterol content in VLDL fractions, and VLDL particle number were skewed and logarithmically transformed in statistical analyses to approach normal distribution.

General linear regression models were used to adjust for age and sex; for age; and for age, sex, and plasma albumin. Wilcoxon Signed Rank tests compared between groups plasma triglycerides, plasma cholesterol, triglyceride content in lipoprotein fractions, cholesterol content in lipoprotein fractions, and particle number according to time since last meal ranging from fasting (≥8 h), 0–1 h, 1–2 h, 2–3 h, 3–4 h, 4–5 h, 5–6 h, 6–7 h, or 7–8 h with fasting (≥8 h) as the reference. For sensitivity, we added analyses stratified according to BMI and sex, and analyses additionally adjusted for plasma albumin to account for haemodilution following intake of fluid. In analyses stratified according to BMI, individuals were divided into two groups based on the World Health Organization (WHO) classification of overweight (BMI≥25 kg/m^2); one group with BMI <25 kg/m^2 and another group with BMI≥25 kg/m^2. Further, we performed a sensitivity analysis including individuals on lipid-lowering therapy to avoid the potential collider bias that can be induced by excluding statin users [28]. Finally, we performed a sensitivity analysis additionally adjusted for current smoking status, hypertension, type 2 diabetes, level of education, physical activity in leisure time, and weekly alcohol consumption. To account for multiple comparisons, we used a Bonferroni corrected p-value $<0.05/8 = 0.00625$.

3. Results

Among 30,335 individuals selected for metabolomic profiling nested within 109,751 individuals from the Copenhagen General Population Study, we studied 25,656 individuals aged 20–100 years and without lipid-lowering therapy at examination (Supplementary Fig. 2). Baseline characteristics according to time since last meal are shown in Table 1.

Table 1
Baseline characteristics of 25,656 individuals in the Copenhagen General Population Study according to time since the last meal.

		Time since last meal, hours							
	All	Fasting	0–1	1–2	2–3	3–4	4–5	5–6	6–7	7–8	*p*-values
	n = 25,656	n = 377	n = 3113	n = 4742	n = 6771	n = 5544	n = 3051	n = 1398	n = 432	n = 228	
Main covariates											
Women	13,543 (53%)	148 (39%)	1831 (59%)	2599 (55%)	3743 (55%)	2938 (53%)	1418 (46%)	575 (41%)	190 (44%)	101 (44%)	<0.001
Age, years	61 (50–72)	58 (49–68)	55 (47–65)	59 (49–69)	63 (53–73)	64 (53–74)	59 (50–71)	57 (48–67)	60 (50–72)	64 (52–74)	<0.001
Albumin, μmol/L	605 (566–645)	628 (588–664)	603 (566–643)	599 (562–638)	598 (560–636)	602 (564–643)	618 (578–659)	631 (590–671)	620 (586–666)	617 (579–659)	<0.001
BMI, kg/m^2	26 (23–29)	26 (24–29)	26 (23–28)	26 (23–29)	26 (23–29)	26 (23–29)	26 (24–29)	26 (23–29)	26 (23–29)	27 (24–30)	<0.001
Other covariates used for adjustment in sensitivity analysis											
Current smoker	6207 (24%)	169 (45%)	760 (24%)	1076 (23%)	1540 (23%)	1278 (23%)	770 (25%)	395 (28%)	133 (31%)	86 (38%)	<0.001
Hypertension	14,162 (55%)	197 (52%)	1372 (44%)	2446 (52%)	3883 (57%)	3320 (60%)	1797 (59%)	767 (59%)	251 (58%)	129 (57%)	<0.001
Diabetes	1006 (3.9%)	19 (5.0%)	108 (3.5%)	184 (3.9%)	293 (4.3%)	201 (3.6%)	125 (4.1%)	49 (3.5%)	16 (3.7%)	11 (4.8%)	0.37
Short education	16,159 (63%)	277 (73%)	1780 (57%)	2831 (60%)	4318 (64%)	3642 (66%)	1940 (64%)	887 (63%)	315 (73%)	169 (74%)	<0.001
Low physical activity	11,345 (45%)	130 (35%)	1431 (46%)	2209 (47%)	3072 (46%)	2371 (43%)	1326 (44%)	586 (42%)	147 (35%)	73 (33%)	<0.001
Alcohol above recommendations	10,212 (43%)	171 (50%)	1053 (36%)	1826 (41%)	2733 (43%)	2247 (43%)	1271 (45%)	615 (46%)	186 (46%)	110 (54%)	<0.001

Data are n (%) for categorical variables and median (interquartile range) for continuous variables. Baseline characteristics were obtained at the day of examination. Time since last meal (hours) was recorded by the examiner immediately before blood sampling. Time since last meal was obtained once for each participant. Hypertension was systolic blood pressure >140 mmHg. Short education was <3 years following the mandatory primary school. Low physical activity in leisure time was <4 h of low-intensity physical activity or <2 h of high-intensity physical activity per week. Alcohol above recommendations was >10 units (120 g) per week. BMI = body mass index.

3.1. Triglycerides and cholesterol in plasma and main lipoprotein fractions

In age and sex-adjusted analyses and when compared with fasting levels, HDL triglycerides were higher for up to 6–7 h, LDL + IDL triglycerides were higher for up to 6–7 h, VLDL triglycerides were higher for up to 5–6 h, and plasma triglycerides were higher for up to 5–6 h (Fig. 1, upper panel). Further, HDL cholesterol was lower for up to 0–1 h, LDL + IDL cholesterol was lower for up to 4–5 h, and plasma cholesterol was lower for up to 2–3 h, while no changes in VLDL cholesterol were observed (Fig. 1, middle panel). When compared with fasting levels, VLDL particle number was higher for up to 4–5 h (Fig. 1, bottom panel). When compared to fasting levels, 0–1 h after last meal VLDL triglycerides were 0.20 mmol/L higher, LDL + IDL triglycerides were 0.04 mmol/L higher, and HDL triglycerides were 0.03 mmol/L higher. Also, when compared to fasting levels, 0–1 h after last meal VLDL cholesterol were 0.01 mmol/L higher, LDL + IDL cholesterol were 0.17 mmol/L lower, and HDL cholesterol were 0.07 mmol/L lower. Results were similar in women and men, although most pronounced in men (Supplementary Fig. 3). When including individuals on lipid-lowering therapy, analyses provided similar findings to those in Fig. 1 (Supplementary Fig. 4). Further, as a meal is often accompanied by fluid intake resulting in haemodilution, we added analyses additionally adjusted for plasma albumin (Supplementary Fig. 5). In these analyses, plasma cholesterol, HDL cholesterol, and LDL + IDL cholesterol did no longer differ according to time since last meal, while the observed higher plasma triglycerides, triglyceride content of lipoprotein fractions, and VLDL particle number remained. Finally, results were similar in analyses additionally adjusted for current smoking status, hypertension, type 2 diabetes, level of education, physical activity in leisure time, and weekly alcohol consumption.

3.2. Triglycerides in lipoprotein fractions

In age and sex-adjusted analyses and when compared with fasting levels, triglyceride content was higher for up to 6–7 h in both the smallest and largest HDL fractions, for up to 6–7 h in S LDL, M LDL, L LDL, and IDL, and for up to 5–6 h after the last meal in all VLDL fractions (Fig. 2). When compared to fasting levels, 0–1 h after last meal triglyceride content was 0.02 mmol/L higher in the smallest HDL fractions, 0.01 higher in the largest HDL fractions, 0.005 mmol/L higher in S LDL, 0.006 mmol/L higher in M LDL, 0.01 mmol/L higher in L LDL, 0.02 mmol/L higher in IDL, 0.06 mmol/L higher in smallest VLDL fractions, 0.12 mmol/L in middle VLDL fractions, and 0.02 mmol/L higher in largest VLDL fractions.

3.3. Cholesterol in main lipoprotein fractions according to plasma triglycerides

In age, sex, and plasma albumin-adjusted analyses, the observed higher plasma triglycerides for up to 5–6 h following the last meal were accompanied by lower cholesterol content in the smallest HDL fractions for up to 4–5 h and in LDL + IDL fractions for 0–1 h, and higher cholesterol content in the two largest subgroups of HDL for up to 6–7 h and in the four largest fractions of VLDL for up to 4–5 h after last meal (Fig. 3). In contrast, cholesterol content in the two smallest subgroups of VLDL was similar according to time since last meal.

3.4. Stratification by body mass index

In analyses stratified according to BMI, individuals were divided into two groups based on the WHO classification of overweight (BMI≥25 kg/m^2); one group with BMI <25 kg/m^2 and another group with BMI≥25 kg/m^2 (Fig. 4). Lipid responses following meals were similar in the two BMI groups; however, in general, individuals with BMI≥25 kg/m^2 had higher levels of plasma triglycerides, VLDL triglycerides, VLDL

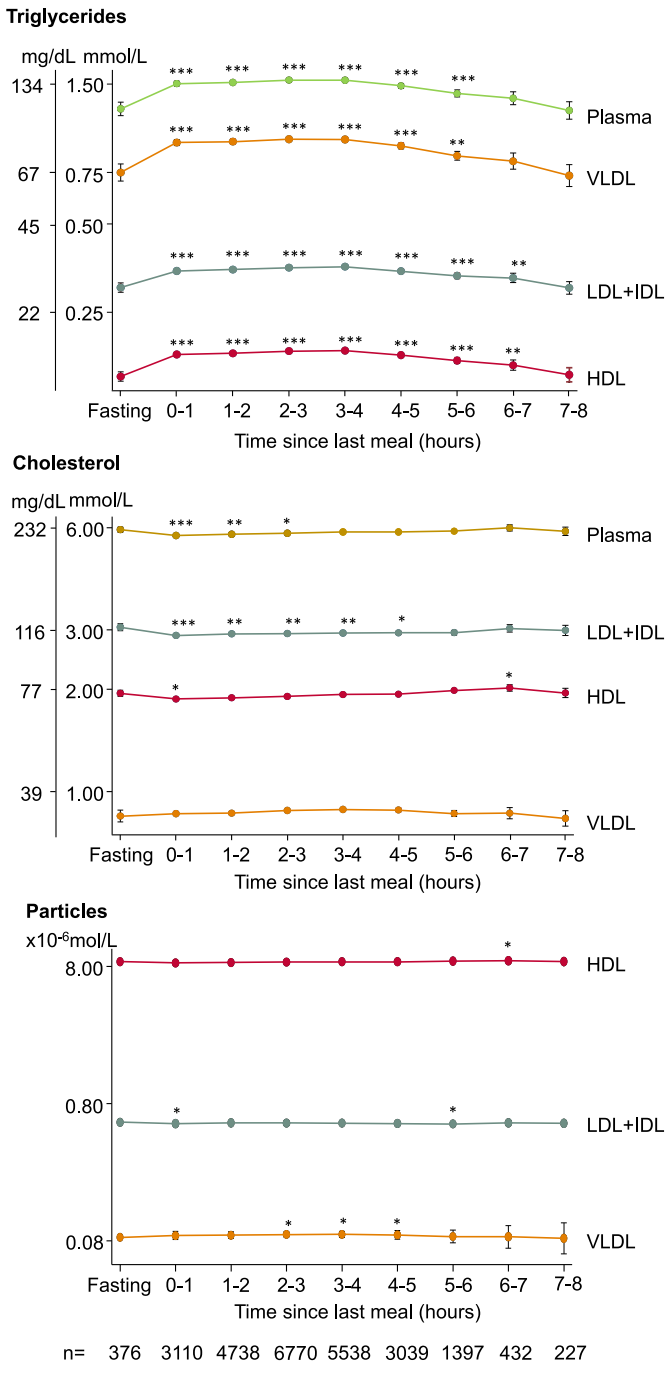

Fig. 1. Triglycerides, cholesterol, and lipoprotein particle number in plasma and in main lipoproteins fractions according to time since the last meal.
Dots represent quadratic means adjusted for age and sex and error bars represent 95% confidence intervals. Plasma triglycerides and plasma cholesterol were measured in fresh blood samples using routine hospital assays. Triglyceride and cholesterol content in lipoprotein fractions was measured using nuclear magnetic resonance spectroscopy and corrected for recovery. The y-axes are on a logarithmic scale. Wilcoxon Signed Rank tests were used to calculate *p*-values based on 8 parallel tests *versus* fasting levels (≥8 h) and are as follows: $*p < 0.05$; $**p < 0.01$; $***p < 0.001$. HDL: high-density lipoproteins. IDL: intermediate-density lipoproteins. LDL: low-density lipoproteins. VLDL: very low-density lipoproteins.

cholesterol, and VLDL particle number, and lower level of HDL cholesterol. When comparing individuals with BMI≥25 kg/m^2 to individuals with BMI<25 kg/m^2, other parameters than BMI could to some extent influence lipid levels (Supplementary Tables 1–2). As an example, when

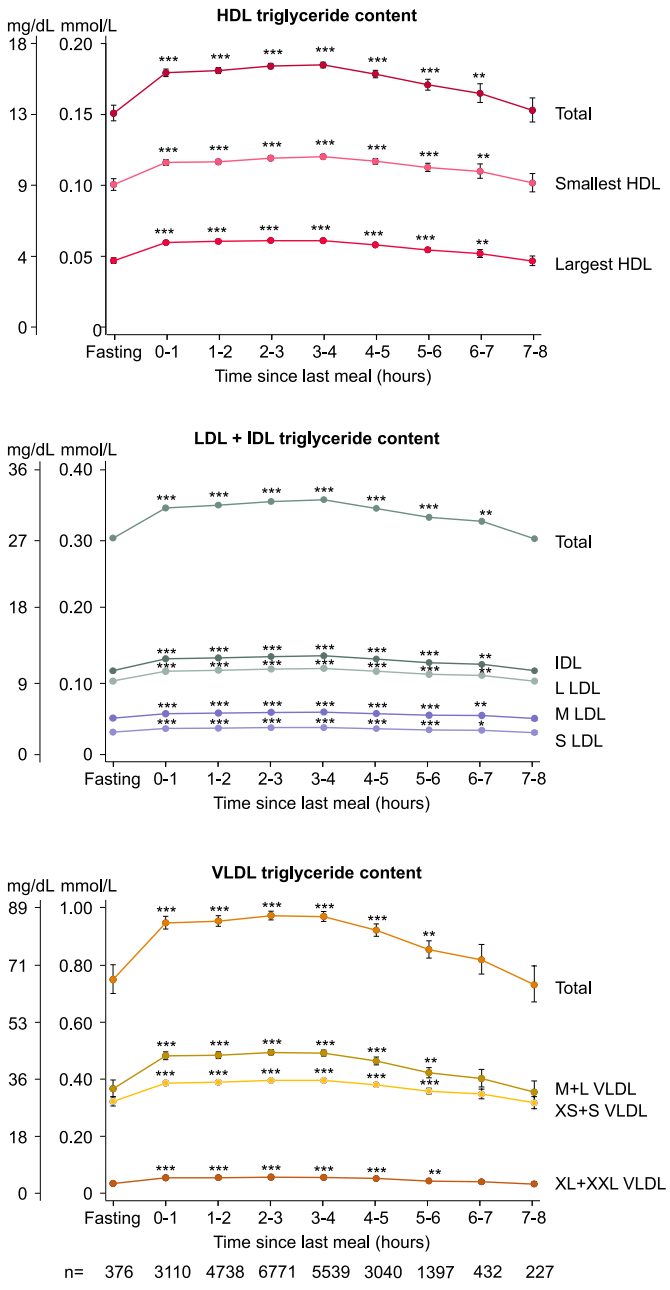

Fig. 2. Concentrations of triglycerides in lipoproteins fractions according to time since last meal.
Dots represent quadratic means adjusted for age and sex and error bars represent 95% confidence intervals. Triglyceride content in lipoprotein fractions was measured using nuclear magnetic resonance spectroscopy and was corrected for recovery. Wilcoxon Signed Rank tests were used to calculate *p*-values based on 8 parallel tests *versus* fasting levels (≥8 h) and are as follows: $*p < 0.05$; $**p < 0.01$; $***p < 0.001$. Smallest HDL includes S + M HDL. Largest HDL includes L + XL HDL. HDL: high-density lipoproteins. IDL: intermediate-density lipoproteins. L: large. LDL: low-density lipoproteins. M: medium. S: small. VLDL: very low-density lipoproteins. XL: extra large. XS: extra small. XXL: extra extra large.

compared to individuals with BMI<25 kg/m^2, individuals with BMI≥25 kg/m^2 were less likely to be women (46% *versus* 62%), less likely to be current smokers (22% *versus* 28%), more likely to have hypertension (62% *versus* 46%), more likely to have diabetes (5.2% *versus* 2.2%), and more likely to have low level of physical activity (42% *versus* 49%).

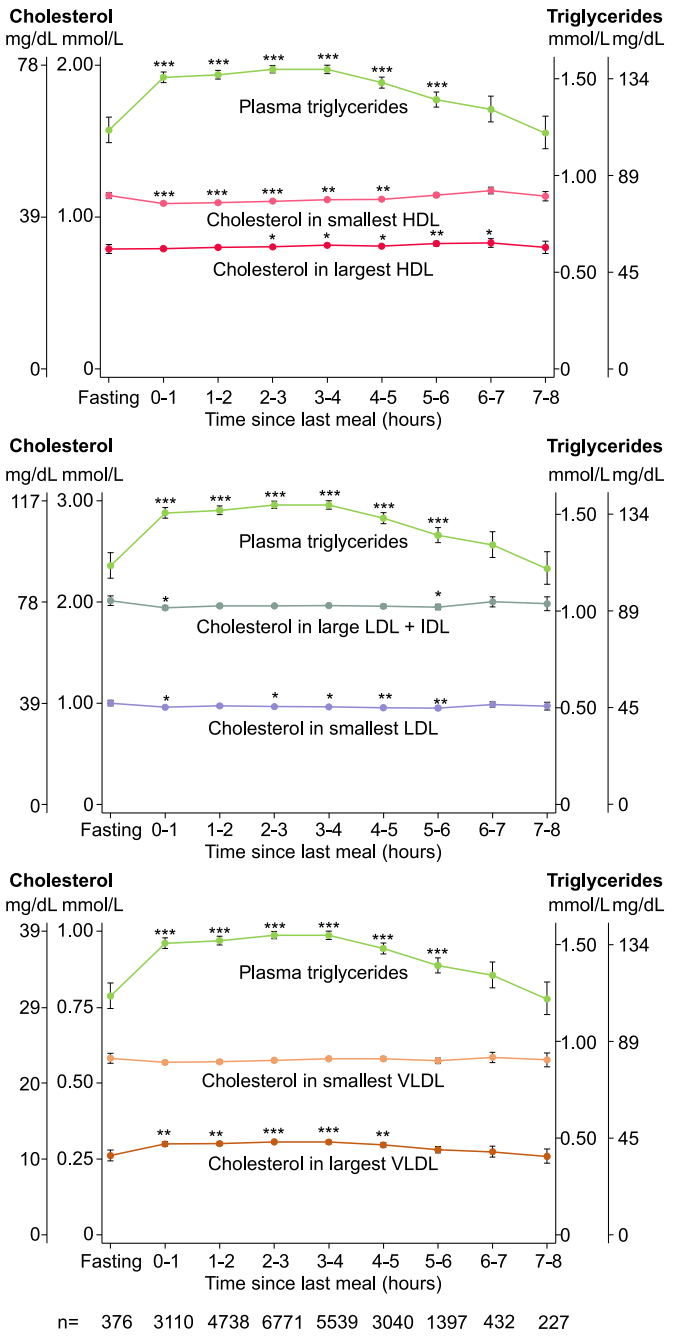

Fig. 3. Cholesterol in main lipoprotein fractions along with plasma triglycerides according to time since last meal.
Dots represent quadratic means adjusted for age, sex, and albumin and error bars represent 95% confidence intervals. Plasma triglycerides were measured on fresh blood samples using routine hospital assays. Cholesterol content in lipoprotein fractions was measured using nuclear magnetic resonance spectroscopy and was corrected for recovery. Left y-axis shows lipoprotein cholesterol concentration; right y-axis shows plasma triglyceride concentration. Wilcoxon Signed Rank tests were used to calculate *p*-values based on 8 parallel tests *versus* fasting levels (≥8 h) and are as follows: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. Smallest HDL includes S + M HDL. Largest HDL includes L + XL HDL. Smallest LDL includes S + M LDL. Smallest VLDL includes XS + S VLDL. Largest VLDL includes M + L + XL + XXL VLDL. HDL: high-density lipoproteins. IDL: intermediate-density lipoproteins. L: large. LDL: low-density lipoproteins. M: medium. S: small. VLDL: very low-density lipoproteins. XL: extra large. XS: extra small. XXL: extra extra large.

4. Discussion

In this cross-sectional study of 25,656 individuals from the Copenhagen General Population Study selected for metabolomic profiling, we found that triglyceride content increased while cholesterol content decreased in HDL and LDL + IDL fractions following normal meals in individuals in the general population (Fig. 5). The population-based approach to addressing these questions is novel.

Mechanistically, these finding can be explained in a simple and straightforward manner. Our results are likely related to the close interaction between HDL, LDL, and triglyceride-rich remnant lipoproteins in their metabolic pathways, which is in part regulated by the action of the cholesteryl ester transfer protein (CETP) [29,30]. This enzyme facilitates the bidirectional transfer of cholesteryl esters for triglycerides from HDL and cholesterol-rich LDL to triglyceride-rich remnant lipoproteins and triglyceride-rich LDL [30–33]. Accordingly, even though triglycerides are mainly carried by triglyceride-rich remnant lipoproteins, postprandially, HDL and LDL appear to be enriched with triglycerides likely due to the action of CETP.

Food intake is important when evaluating high plasma triglycerides, and previous epidemiological studies have reported that post-prandial hypertriglyceridemia is an independent risk factor for cardiovascular events [5–7]. Nevertheless, to our knowledge, this is the first population-based study to investigate the triglyceride and cholesterol content of lipoprotein fractions following normal meals in individuals in the general population. In support of our findings, studies on post-prandial lipid response to oral fat tolerance tests in short-term studies of selected groups of individuals or patients have likewise found that triglyceride content increased while cholesterol content decreased in HDL and LDL + IDL fractions [14–16]. In contrast, one study [13] reported decreased LDL triglycerides and another study [34] reported decreased triglyceride content and increased cholesterol content in HDL and LDL fractions following oral fat tolerance tests; however, only 22 and 8 healthy individuals were included in the two studies, and therefore, their findings may be different from the overall pattern in the general population. Also, in a long-term population-based study, low high-density lipoprotein (HDL) cholesterol was suggested as a stable marker of elevated plasma triglycerides and triglyceride-rich remnant lipoproteins, like high HbA1c is a stable marker of elevated plasma glucose [20]. Statistically, this could lead to HDL cholesterol appearing to be protective in observational studies, when in reality it was serving as stable marker of fluctuating aetiological factors [35], a hypothesis supported by both randomised controlled trials and Mendelian randomization studies over the last 15 years [36]. This is in line with the small postprandial changes in HDL cholesterol suggested by the present study.

In a standard fat load test (conducted under stricter conditions than the present study), plasma triglyceride level typically rises from 2 to 6 h with a peak at 3–4 h in most subjects [11,37,38]. Our data indicate a flatter response with an immediate increase in plasma triglyceride 0–1 h after a meal and at most a slight increase thereafter. The most likely explanation for this difference is that individuals in the general population eat much less fat in an average meal than during a fat tolerance test of typically 70 g fat, whereby the smaller amount of fat is absorbed much faster than when a 70 g fat load is absorbed. This could possibly be due to saturation of the triglyceride metabolic pathway in the intestine, including intestinal triglyceride degradation, absorption of free fatty acids and monoacylglycerol into enterocytes, and rebuilding of triglycerides before secretion of chylomicrons at the apical site of enterocytes.

We observed an increase in plasma volume after normal meals in individuals in the general population most likely due to intake of fluid, as indicated by lower plasma albumin after a meal. Therefore, it could be speculated that a corresponding decrease in particle concentration of IDL and LDL should also have occurred, since these larger blood constituents would be diluted as well. However, intake of fluid will occur

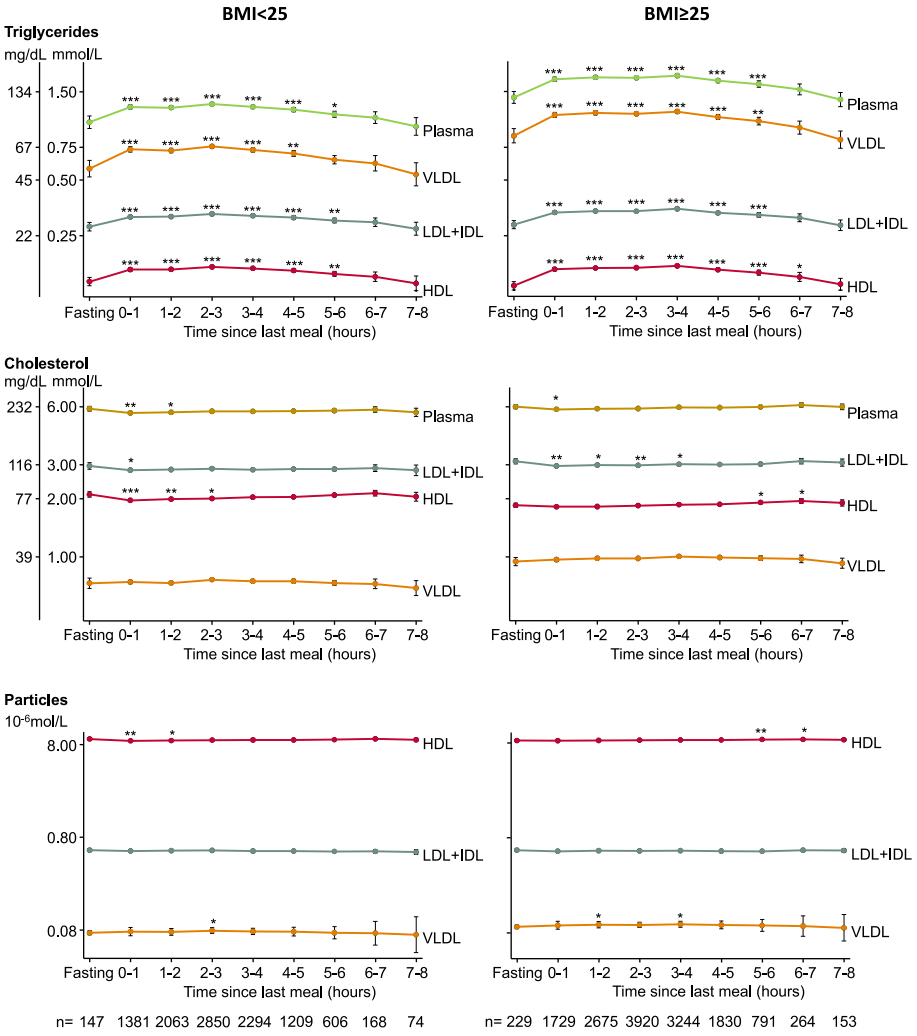

Fig. 4. Triglycerides, cholesterol, and lipoprotein particle number in plasma and in main lipoproteins fractions according to time since last meal in individuals with normal weight and overweight/obesity, separately.

Dots represent quadratic means adjusted for age and sex and error bars represent 95% confidence intervals. Plasma triglycerides and plasma cholesterol were measured in fresh blood samples using routine hospital assays. Triglyceride and cholesterol content in lipoprotein fractions were measured using nuclear magnetic resonance spectroscopy and corrected for recovery. The y-axes are on a logarithmic scale. Wilcoxon Signed Rank tests were used to calculate *p*-values based on 8 parallel tests *versus* fasting levels (≥8 h) and are as follows: $*p < 0.05$; $**p < 0.01$; $***p < 0.001$. HDL: high-density lipoproteins. IDL: intermediate-density lipoproteins. LDL: low-density lipoproteins. VLDL: very low-density lipoproteins.

simultaneously with intake of fat whereby the number of IDL and LDL particles will be continuously supplemented due to conversion of new VLDL particles into IDL and LDL.

Triglycerides appear to increase in VLDL more strongly than in LDL + IDL. This might be because fat from a meal is incorporated into chylomicrons turning into remnants after the action of lipoprotein lipase. These remnants are taken up by the liver and part of their cholesterol and triglyceride content is incorporated in VLDL particles finally giving rise to IDL and LDL particles after hydrolysis of triglycerides by the enzyme lipoprotein lipase. Also, triglycerides are exchanged from VLDL particles to IDL + LDL and HDL facilitated by cholesteryl ester transfer protein. In other words, triglycerides at first are incorporated into chylomicrons and VLDLs, whereas triglyceride content of IDL, LDL, and HDL reflects a downstream process that takes some time to occur.

Comparing responses in women *versus* men, men had a slightly higher increase in plasma triglycerides and a corresponding larger decrease in cholesterol content in IDL + LDL. A possible explanation for this gender difference could be that men compared to women eat more fat in an average meal and drink more fluid during food intake; however, we do not have data to document this.

It may seem somewhat surprising that a very low VLDL triglyceride content was observed within the largest XL + XXL VLDL fraction that contains chylomicron as compared to smaller VLDL fractions. Likely explanations for this include i) that chylomicrons and very large VLDLs have a very short half-life in plasma compared to smaller VLDL fractions [39,40], and ii) that the total intake of fat for the average meal in individuals in the general population is modest compared to the 70 g fat intake during a typical fat tolerance test [11]. Although not completely clear, similar statements could explain the higher triglyceride and cholesterol content in the largest HDL compared to the smallest HDL.

4.1. *Strengths and limitations*

Strengths of the present study include the large sample size representing the Danish adult general population with direct assessment of lipids including triglyceride and cholesterol content of 14 lipoprotein fractions available. Another strength is the fact that time since last meal was recorded by the examiner immediately before blood sampling, simply by a direct question to the participant at this time.

A key limitation includes comparability of lipid values across time

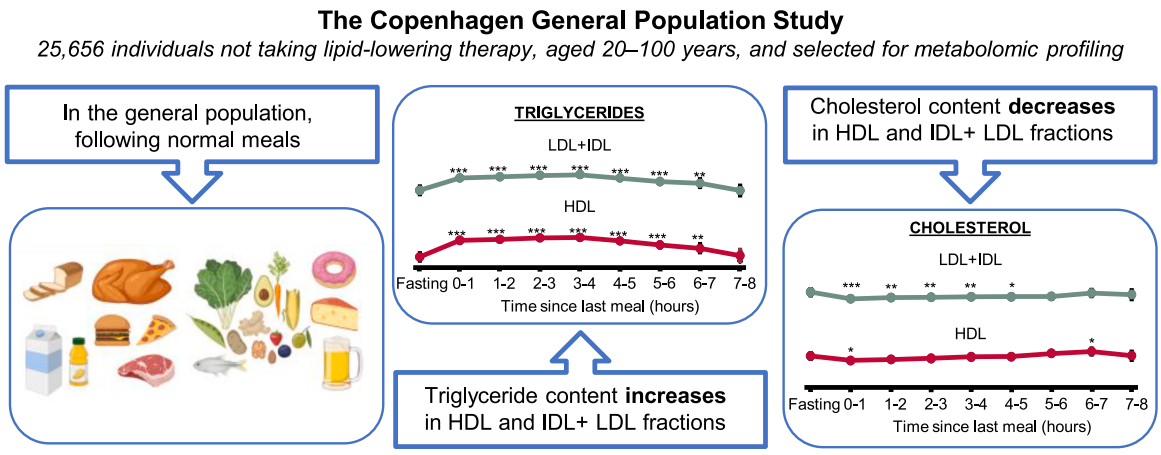

Fig. 5. Graphical abstract.
Dots represent quadratic means adjusted for age and sex and error bars represent 95% confidence intervals. Triglyceride and cholesterol content in lipoprotein fractions was measured using nuclear magnetic resonance spectroscopy and corrected for recovery. Wilcoxon Signed Rank tests were used to calculate *p*-values based on 8 parallel tests *versus* fasting levels (≥8 h) and are as follows: $*p < 0.05$; $**p < 0.01$; $***p < 0.001$. HDL: high-density lipoproteins. IDL: intermediate-density lipoproteins. LDL: low-density lipoproteins. VLDL: very low-density lipoproteins. Parts of the figure were created using Biorender.com.

points after a meal. In our cross-sectional analysis, the validity of the results depends on comparing subjects who attended at different times after eating. The inherent assumption is that this was a random process, that is, subjects were as likely to attend 2–3 h after a meal as to attend 7–8 h after a meal. However, this may not be the case in that certain types of individuals may have preferentially attended having eaten *versus* not having eaten for a while.; e.g. those who attended at longer times after a meal may have been shift workers or had other demographic, medical, social, or logistic reasons not to have eaten recently. Thus, there may be unknown differences in the type of subjects attending at various times and these could be confounding factors in generation of key comparisons. This phenomenon may not be accounted for by the statistical correction for age, sex and BMI and therefore could bias the results. However, results were similar in analyses additionally adjusted for current smoking status, hypertension, type 2 diabetes, level of education, physical activity in leisure time, and weekly alcohol consumption. Another limitation considers that the number of individuals who attended not having eaten for >8 h (classed as 'fasting'), was ten times less than the groups attending at 0–1 to 3–4 h. These differences simply reflect the choice of the individual participant prior to attending the examination in the afternoon between 3 and 7 pm.

Furthermore, a limitation is that time since last meal was self-reported and not directly evaluated. That said, this information was gathered by the examiner exactly at the time of blood sampling by directly asking the participant about when the person ate the last meal. Another limitation is that groups organized according to time since last meal were not matched for age and sex; however, all analyses were adjusted for age and sex.

Other possible limitations include that total plasma triglycerides and plasma cholesterol measured by NMR spectroscopy were lower than reported by standard hospital assays. To account for this, cholesterol and triglyceride content in the various lipoprotein fractions was corrected for recovery relative to total plasma cholesterol and plasma triglycerides measured by standardized biochemical assays, as done in previous studies [25–27]. This means that the values presented in the present study are directly relatable to values from normal lipid profiles, which could be perceived as a strength. Another potential limitation is that samples were stored at −80 °C before NMR measurements, which could have influenced the composition of lipoprotein fractions; however, we are not aware of any data suggesting this is an issue. Finally, all participants were white people of Danish descent, and accordingly, our findings may not generalize to other ethnic groups, although we are not aware of any data to suggest that these results may not apply to other ethnicities.

Clinically, our findings suggest that triglyceride content increases while cholesterol content decreases in HDL and IDL + LDL fractions following normal meals, and thus indirectly endorses the use of non-fasting lipid measurements for risk prediction in the general population as non-fasting measurements better capture the average lipid levels in a person, since the non-fasting state dominates during most of a 24-h cycle [1]. Further, non-fasting lipid profile sampling is practically easier for patients, laboratories, and doctors alike [1,4]. Interestingly, elevated LDL triglycerides are robustly associated with increased risk of atherosclerotic cardiovascular disease [19], implying that masking of elevated LDL triglycerides using fasting lipid profiles makes it more difficult for the physician to evaluate the accurate risk profile in a patient.

In conclusion, in this study of 25,656 individuals from the general population, we observed that triglyceride content increased while cholesterol content decreased in HDL and LDL + IDL fractions following normal meals. Compared to previous results from oral fat tolerance tests, the added value of the present study is the finding that this occurs in individuals in the general population eating regular meals.

Declaration of interest

BGN report consultancies and talks sponsored by AstraZeneca, Sanofi, Regeneron, Akcea, Amgen, Kowa, Denka Seiken, Amarin, Novartis, Novo Nordisk, Esperion, Abbott, Ultragenix, and Silence Therapeutics. GDS reports Scientific Advisory Board Membership for Relation Theraputics and insitro. There are no financial or other conflicts of interest for MØJ, JMV, or MB.

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CRediT authorship contribution statement

Mia Ø. Johansen: Conceptualization, Methodology, Software,

Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Supervision, Visualization, Funding acquisition. **Juan Moreno-Vedia:** Conceptualization, Methodology, Software, Formal analysis, Writing – original draft, Visualization. **Mie Balling:** Conceptualization, Methodology, Investigation, Writing – review & editing, Supervision, Visualization. **George Davey Smith:** Conceptualization, Methodology, Investigation, Resources, Writing – review & editing, Supervision, Funding acquisition. **Børge G. Nordestgaard:** Conceptualization, Methodology, Investigation, Resources, Writing – review & editing, Supervision, Visualization, Project administration, Funding acquisition.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.atherosclerosis.2023.117316.

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