Universiteit Leiden
The Netherlands

# Novel insights into blood markers and cardiovascular disease: Results of the Netherlands Epidemiology of Obesity study

Christen, T.

**Citation**
Christen, T. (2020, October 29). *Novel insights into blood markers and cardiovascular disease: Results of the Netherlands Epidemiology of Obesity study*. Retrieved from https://hdl.handle.net/1887/137989

| | |
|---|---|
| Version: | Publisher's Version |
| License: | Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden |
| Downloaded from: | https://hdl.handle.net/1887/137989 |

**Note:** To cite this publication please use the final published version (if applicable).

Cover Page

Universiteit Leiden


The handle http://hdl.handle.net/1887/137989 holds various files of this Leiden University dissertation.

**Author**: Christen, T.
**Title**: Novel insights into blood markers and cardiovascular disease: Results of the Netherlands Epidemiology of Obesity study
**Issue Date**: 2020-10-29

# CHAPTER 7

# THE ROLE OF TRIGLYCERIDES IN THE ASSOCIATION BETWEEN HDL-CHOLESTEROL AND CORONARY ARTERY DISEASE: A TWO-SAMPLE MULTIVARIABLE MENDELIAN RANDOMIZATION STUDY

T Christen, G Davey Smith, R de Mutsert, S Trompet, RAJ Smit KH Wade, NJ Timpson, DO Mook-Kanamori, K Willems van Dijk, PCN Rensen, EJP de Koning, FR Rosendaal, JW Jukema

In preparation

# Abstract

## Background

Whilst lower levels of high-density lipoprotein cholesterol (HDL-c) strongly predict cardiovascular disease, this relationship is unlikely to be causal. The extent to which the association between HDL-c and coronary artery disease is explained by other correlated cardiometabolic factors such as fasting triglyceride (TG), postprandial TG response and low-density lipoprotein cholesterol (LDL-c) concentrations is unclear.

## Aim

To investigate whether the association between HDL-c and coronary artery disease is explained by the residual postprandial TG in addition to other plasma lipoproteins, we performed a multivariable Mendelian Randomisation study.

## Methods

HDL-c, LDL- c, fasting TG and postprandial TG concentrations were determined in 5,490 participants of the Netherlands Epidemiology of Obesity (NEO) study. We performed a two-sample multivariable Mendelian randomization analysis, using genetic variants reported to be associated with plasma lipoproteins by the Global Lipids Genetics Consortium (GLGC). Firstly, we estimated the association between these genetic variants and HDL-c, fasting TG concentrations, postprandial TG response and LDL-c in the NEO study. Then, we obtained the estimates of association between the same genetic variants and coronary artery disease from the publicly available summary statistics from the CARDIoGRAMplusC4D consortium. These estimates were combined using two-sample and multivariable Mendelian randomization methodology, including genetically influenced concentrations of all other plasma lipoproteins in the multivariable Mendelian randomization analyses.

## Results

We observed a 19% risk reduction (95% confidence interval (CI): 5 – 30%) in the association between HDL-c and coronary artery disease, which attenuated to 10% (95% CI: -8 – 24 %) after inclusion of genetically-influenced fasting TG, and to 2% (95% CI: -16 – 18%) after additional inclusion of genetically-influenced LDL-c. Additional inclusion of the genetically-influenced residual postprandial TG response, neither in addition to other lipids, nor in its own, did not change the risk reduction.

## Discussion

Our results confirm that the association between HDL-c and coronary artery disease is almost totally explained by LDL-c and fasting TG. We could not distinguish an effect of the residual postprandial TG response.

## Introduction

Imbalances in circulating lipids have extensively been described as risk factors for atherosclerosis and coronary artery disease (CAD), which is a major cause of death. [1, 207, 208] Notably, high concentrations of high density lipoprotein cholesterol (HDL-c) are strongly associated with a lower cardiovascular risk in observational studies and in naïve Mendelian randomisation studies [45]. However, multivariable Mendelian randomisation studies have indicated that HDL-c itself is unlikely to exert a causal role in the development of CAD but this association is rather explained by LDL-c, TG and apolipoprotein B [58, 209], which supports clinical trials of therapeutic raising of HDL-c showing a likely non-causal role of HDL-c in CAD risk. [48, 199] High concentrations of low density lipoprotein cholesterol (LDL-c), very low density lipoprotein cholesterol (VLDL-c) and remnant particles are considered likely causal factors in the development of atherosclerosis and CAD. [210, 211] A potential explanation for the apparent observational association between HDL-cholesterol and CAD is that a high HDL-cholesterol concentration is a marker of a favourable flux of catabolism components of triglyceride-rich lipoproteins by lipoprotein lipase (LPL). [212-214] This hypothesis implies that HDL-c concentrations are a proxy for the physiological capacity to clear dietary triglycerides from the circulation; therefore the strong observational association between HDL-c and CAD may be explained by postprandial TG concentrations (ppTG). However, previous work showed that postprandial TG concentrations have the same genetic background as fasting TG concentrations. [215] Therefore, the residuals of an orthogonal nonlinear least squares regression of postprandial and fasting TG concentrations were calculated. In this study, we used these residuals to study the effects of the postprandial TG response independent of fasting TG concentrations.

We aimed to explore the role of residual postprandial TG response in addition to LDL-c and fasting TG concentration in the association between HDL-c concentration and CAD, using a two-sample multivariable Mendelian randomization analysis, as visualised in the directed acyclic graph in Figure 1.

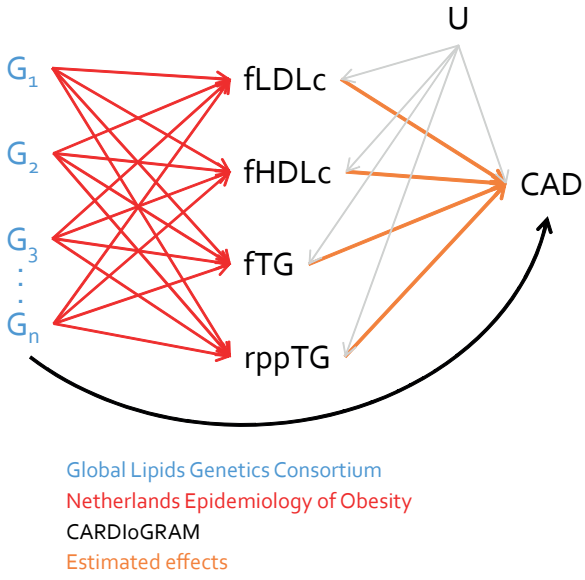


*Figure 1 – Directed acyclic graph of the data and analyses used to investigate the assumed causal pathways between genetic variants, blood lipids and CAD. CAD, coronary artery disease; CARDIoGRAM, Coronary ARtery DIsease Genome wide Replication and Meta-analysis (CARDIoGRAM) plus The Coronary Artery Disease (C4D) Genetics; fHDL, fasting high-density lipoprotein; fLDL, fasting low-density lipoprotein; fTG, fasting triglycerides; G, genetic variant; rppTGr, residual postprandial triglyceride response.*

# Methods

## Study design and study population

The Netherlands Epidemiology of Obesity (NEO) study is a population-based, prospective cohort study of 6,671 men and women aged between 45 and 65 years. The study design and population are described in detail elsewhere. [59] Briefly, all inhabitants with a self-reported body mass index (BMI) of 27 kg/m$^2$ or higher and living in the greater area of Leiden, the Netherlands were eligible to participate in the NEO study. In addition, all inhabitants aged between 45 and 65 years from one adjacent municipality (Leiderdorp, the Netherlands) were invited to participate irrespective of their BMI, allowing for a reference distribution of BMI. Participants visited the NEO study centre for extensive baseline measurements, including blood sampling. Research nurses recorded current medication use by means of a medication inventory. Prior to the study visit, participants completed questionnaires at home with respect to demographic, lifestyle, and clinical information.

The Medical Ethical Committee of the Leiden University Medical Centre (LUMC) approved the protocol. All participants gave their written informed consent.

## Blood sampling

### *Serum lipids*

After 5 minutes rest, fasting blood samples were collected from the antecubital vein after a >10 hour overnight fast. All participants consumed the same liquid mixed meal of 400 mL, that contained 600 kcal of which 16 percent (En%) was derived from protein, 50 En% from carbohydrates and 34 En% from fat. In total, the 400 mL meal contained 75.0 g of carbohydrates and 2.4 g of saturated, 13.7 g of monounsaturated and 6.8 g of polyunsaturated fat. Participants were instructed to drink the mixed meal within 5 minutes. Blood was sampled 30 and 150 minutes after the meal ingestion. Triglyceride concentrations were determined using an enzymatic colorimetric assay using a TG GPO-PAP kit (11730711216, Roche) on on an automated analyser (Roche Modular P800, Roche Diagnostics, Almere, The Netherlands). The postprandial TG response was calculated by using the residuals of the TG concentration distribution of 150 minutes adjusted for fasting TG concentrations. The specific method has been described elsewhere. [215] Fasting serum total cholesterol concentrations were measured with enzymatic colorimetric assays (Roche Modular P800 Analyzer, Roche Diagnostics, Mannheim, Germany) and fasting serum HDL-c concentrations with third generation homogenous HDL-c methods (Roche Modular P800 Analyzer,

Roche Diagnostics, Mannheim, Germany). LDL cholesterol concentrations were calculated using the Friedewald equation. [128]

Furthermore, aliquots of plasma and serum were stored after centrifugation at -80°C. DNA was extracted and genotyping was performed by the Centre National de Génotypage (Evry Cedex, France), using the Illumina HumanCore-Exome-24 BeadChip (Illumina Inc., San Diego, California, United States of America). Subsequently, genotypes were imputed to the 1000 Genome Project reference panel (v3 2011) using IMPUTE (v2.2) software. [104, 105] Known genetic variants for HDL-c, LDL-c, fasting TG concentration and TG response reported by Global Lipids Genetics Consortium study (n=171) were extracted using a p-value threshold of $p<5*10^{-8}$ and a linkage disequilibrium threshold of $r^2<0.001$. [67, 215, 216]

## CARDIoGRAMplusC4D

Outcome measures were obtained from publicly-available data from the CARDIoGRAMplusC4D consortium. The CARDIoGRAMplusC4D consortium included 60,801 CAD cases and 123,504 controls of European ancestry. [61] In general, CAD was defined as fatal or nonfatal coronary artery disease, percutaneous coronary intervention, coronary artery bypass graft surgery, stenosis >50%, or angina. Specific definitions have been described previously. [217]

The NEO study did not overlap with the GLGC and CARDIoGRAMplusC4D consortia, but there was an overlap of 10.3% between GLGC and CARDIoGRAMplusC4D.

## Statistical analysis

We performed a two-sample multivariable Mendelian randomization study, using the baseline measurements of the NEO study as the exposure sample and the publicly available summary statistics of the CARDIoGRAMplucC4D consortium as outcome sample.

For the present analyses, we excluded participants of the NEO study with poor genotyping quality, using criteria described elsewhere. [109] We also excluded participants with missing HDL-c, LDL-c and fasting and postprandial TG measurements. Furthermore, participants with meal irregularities (who arrived at study site not fasting, did not or not completely drink meal, ate between postprandial blood samples) were excluded. Baseline characteristics were summarized as mean (SD; normally distributed data only), median ($25^{th}$, $75^{th}$ percentiles; non-normally distributed data only), or as percentage (categorical data). We as-

sumed all genetic effects to be additive. Four principal components of ancestry were calculated to be able to correct for population stratification.

## Two-sample multivariable Mendelian randomization

We estimated the effects of HDL-c, fasting TG concentrations, TG response, and LDL-c with CAD using two-sample multivariable Mendelian randomization analysis. [58]

First, we created composite instruments for the four lipid measurements in the NEO study: fasting LDL-c, HDL-c, and TG concentrations, and postprandial TG response at 150 minutes. These were adjusted for four principal components of ancestry, and weighted towards a BMI distribution of the reference cohort.

Second, we constructed a single composite instrument CAD from CARDIoGRAMplusC4D. We then used two-sample Mendelian randomization methodology to estimate the causal effect of each of the plasma lipoproteins individually on CAD. Therefore we used inverse variance weighted meta-analysis in which the inverse of the variance of the effect of the variant on the outcome was used to weight the causal estimates of the variant.

In addition, we estimated adjusted beta coefficients between HDL-c and CAD by including genetically influenced fTG, postprandial TG response and LDL-c in the multivariable Mendelian randomization analyses using regression-based methods. We report odds ratios (ORs) and 95% confidence intervals (CIs) for the effects of HDL-c on CAD, and after inclusion of combinations of the other plasma lipids. The genetic variants that were used may influence other phenotypes, i.e. horizontal pleiotropy. To investigate the potential effects of horizontal pleiotropy, and to adjust for these effects, we performed multivariable MR-Egger as a sensitivity analysis using the R-packages *MendelianRandomization* and *TwoSampleMR,* and the code from the seminal multivariable MR-Egger paper. [218]

# Results

## Baseline characteristics

In total, 6,671 participants were included in the NEO study. Consecutive exclusion of related participants, participants of non-European descent, or with insufficient genotyping quality (n=927), participants with missing HDL-c, fasting TG measurements or TG response (n= 148), participants with meal irregularities (not fasting, did not or not completely drink meal, or ate between blood samples, n= 33), resulted in a final study population of 5,490 participants.

Participants had a mean age of 56 years, 45% were men and 16% were current smokers. Participants had a mean BMI of 26 kg/m$^2$ (SD: 4) and HDL-c of 1.6 mmol/L (SD: 0.5). Further baseline characteristics are presented in Table 1.

*Table 1 – Baseline characteristics of the participants of The Netherlands Epidemiology of Obesity study, men and women aged between 45 and 65 years of whom the triglyceride response was assessed after a mixed meal challenge (n = 5,490).*

| | |
|---|---|
| Age (y) | 56 (6) |
| Sex (% men) | 45 |
| BMI (kg/m$^2$) | 26 (4) |
| Alcohol intake (g/d) | 10 (3-22) |
| Physical activity (MET-h/week) | 30 (16-50) |
| Education level (% high*) | 47 |
| | |
| Total cholesterol (mmol/L) | 5.7 (1.1) |
| HDL-c (mmol/L) | 1.6 (0.5) |
| LDL-c (mmol/L) | 3.6 (1.0) |
| Fasting TG (mmol/L) | 1.0 (0.7-1.5) |

Results are based on analyses weighted toward the BMI distribution of the general population (n = 5,490). Data are shown as mean (standard deviation), median (interquartile range), or weighted percentage.

BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MET-h, metabolic equivalents of task; TG, triglycerides

*****Low education: none, primary school, or lower vocational education as highest level of education.

## Mendelian randomization analyses

In univariate Mendelian randomization analyses, each 1 mmol/L higher level of HDL-c decreased the risk of CAD by 19% (95% CI: 5-30%). Inclusion of fasting TG in the multivariable Mendelian randomization almost halved the risk reduction to 10% (95% CI: -7-24%), which was similar to the risk reduction after additional inclusion of genetically influenced LDL-c concentration (risk reduction: 8%; 95% CI: -7-20%). Simultaneous inclusion of genetically influenced fTG and LDL-c concentrations completely attenuated the risk reduction to 2% (95% CI: -15–18%). Additional inclusion of the residual postprandial TG response in the multivariable Mendelian randomization did not change these results individually or in combination with the other plasma lipoproteins (results not shown).

*Table 2 – Two-sample multivariable Mendelian randomization analysis of the odds ratio of HDLc on the risk of coronary artery disease. Odds ratios are based on the effects of genetic variants on lipid concentrations in The Netherlands Epidemiology of Obesity study (n=5,490) and their effects on coronary artery disease in the CARDIoGRAMplusC4D consortium (60,801 CAD cases and 123,504 controls).*

| | Odds ratio per 1 mmol/L (95%CI) | Additionally included in the multivariable MR model |
|---|---|---|
| HDLc | 0.81 (0.70, 0.95) | none |
| | 0.92 (0.80, 1.07) | LDLc |
| | 0.90 (0.76, 1.07) | fTG |
| | 0.80 (0.68, 0.93) | rppTGr |
| | 0.98 (0.83, 1.15) | LDLc, fTG |
| | 0.90 (0.77, 1.05) | LDLc, rppTGr |
| | 0.88 (0.74, 1.05) | fTG, rppTGr |
| | 0.96 (0.81, 1.14) | LDLc, fTG, rppTGr |

Results are based on weighted analyses of the genetic effects on lipid concentrations in the NEO-study (n=5,490). Data are shown as odds ratios of coronary artery disease per 1 mmol/L increase in HDL-c concentration and their 95% confidence intervals.

fTG, fasting triglycerides; HDLc, high-density lipoprotein cholesterol; LDLc, low-density lipoprotein cholesterol; MR, Mendelian randomization; rppTGr, residual postprandial triglyceride response.

## Discussion

This study is a proof of principle of a two-sample multivariable Mendelian randomization strategy to improve understanding of the causal link between HDL-c and CAD. We confirmed that fasting TG and LDL-c combined almost totally explain the association between low HDL-c and increased risk of CAD. In addition, our results suggested that fasting TG and LDL-c have a similar magnitude of effect on the effect of HDL-c on CAD. The genetic instrument for residual postprandial TG response was not strong enough to estimate its role in the relation between HDL-c and CAD.

Our analyses provided strong evidence for the absence of a causal effect of HDL-c on CAD, after inclusion of LDL-c and fTG in the multivariable analyses. [58] As previous studies in small samples have shown that HDL-c is correlated with the postprandial TG response, [212] we hypothesized that postprandial TG response would partly explain the underlying mechanism of the strong observational associations between HDL-c and CAD. The biological explanation for this may be that ingested lipids are first taken up by VLDL-c or chylomicrons, which are a major determinant of measured serum TG concentrations. Its lipolyzed products are transferred to HDL-c, while leaving atherogenic remnant particles. [210, 219, 220] As a consequence, HDL-c concentration is a marker of the capacity to clear alimentary fat. Both fasting and postprandial TG concentrations are determined by the capacity to clear TGs from the serum by LPL, CETP, and PLTP, and therefore the genetic determinants are similar. [212, 221]

Because fasting TG concentrations and postprandial TG response have a strongly similar biological and genetic background, we aimed to investigate isolated effects of the postprandial TG response by using a measure of the response that excluded effects of fasting TG. [212, 215, 222, 223] In recent work we discovered one genetic variant that determined postprandial TG response independent from fasting TG concentration, of which a proxy that was used for the genetic instrument in this study. [215] The genetic instrument for postprandial TG response appeared not to be strong enough to distinguish from fasting TG concentration and to estimate its causal effects. [215]

Two possible pathways are hypothesized for the likely causal effect of fTG and LDL-c CAD risk, via remnant particles and via lipolytic toxins. [224, 225] Triglyceride-rich lipoproteins metabolised by LPL, leading to an abundance of remnant particles, which in turn affect monocyte activation [226] and endothelial inflammation [227]. The lipolytic toxins hypothesis suggests that oxidized free fatty acids that result from lipolysis contribute to the susceptibility of the vascular wall to macrophage and LDL-c infiltration. [228-230] In combination with the

role of other TG-mediated pathways, this may be an explanation for the strong observational association between HDL-c concentrations as a marker of TG and its clearance, and CAD risk [231-233]

This study uniquely uses a two-sample multivariable Mendelian randomization analysis to investigate the relation between a phenotype that is subject to genetic pleiotropy and is expensive or difficult to measure and a rare outcome. the present study are the large number of participants, who were well phenotyped in a fasting and postprandial state.

This study also has some limitations that need to be considered. First, the mixed meal that was used, was not standardized towards body size or composition, and contained 34 energy-percent of fat. This may not fully represent the usual daily feeding behaviour of the participants. Second, using a two-sample Mendelian randomisation analysis requires additional assumptions: the causal relationships should be identical in both samples, the covariance matrix should be the same in both samples and the error variances should be known. Also the exposure should be measured without error. However, heterogeneity tests require more power than available in the present study, while measurement error can not be ruled out. Therefore the possibility remains that there is residual bias in these results.

In conclusion, this study confirms that in the general population, genetically-determined fasting TG and LDL-c almost totally explain the relationship between HDL-c and CAD. Further studies are needed to investigate the determinants of the capacity to increase and to clear serum lipids, and which metabolites of this clearance influence the risk of developing atherosclerosis or cardiovascular disease.