Apolipoprotein A-V is a potential target for treating coronary artery disease: evidence from genetic and metabolomic analyses

Dorina Ibi, Manon Boot, Martijn E. T. Dollé, J. Wouter Jukema, Frits R. Rosendaal, Constantinos Christodoulides, Matt J. Neville, Robert Koivula, Patrick C. N. Rensen, Fredrik Karpe, Raymond Noordam, and Ko Willems van Dijk

1Department of Human Genetics, Leiden University Medical Center, Leiden, The Netherlands; 2Department of Public Health and Primary Care, National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands; 3Department of Cardiology, Leiden University Medical Center, Leiden, The Netherlands; 4Netherlands Heart Institute, Utrecht, The Netherlands; 5Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, The Netherlands; 6Oxford Centre for Diabetes, Endocrinology and Metabolism, Radcliffe Department of Medicine, University of Oxford, Oxford, United Kingdom; 7NIHR Oxford Biomedical Research Centre, Oxford University Hospitals Foundation Trust, Oxford, United Kingdom; 8Division Endocrinology, Department of Internal Medicine, Einthoven Laboratory for Experimental Vascular Medicine, and 9Division of Gerontology and Geriatrics, Department of Internal Medicine, Leiden University Medical Center, Leiden, The Netherlands

Abstract Triglyceride (TG)-lowering LPL variants in combination with genetic LDL-C-lowering variants are associated with reduced risk of coronary artery disease (CAD). Genetic variation in the APOA5 gene encoding apolipoprotein A-V also strongly affects TG levels, but the potential clinical impact and underlying mechanisms are yet to be resolved. Here, we aimed to study the effects of APOA5 genetic variation on CAD risk and plasma lipoproteins through factorial genetic association analyses. Using data from 309,780 European-ancestry participants from the UK Biobank, we evaluated the effects of lower TG levels as a result of genetic variation in APOA5 and/or LPL on CAD risk with or without a background of reduced LDL-C. Next, we compared lower TG levels via APOA5 and LPL variation with over 100 lipoprotein measurements in a combined sample from the Netherlands Epidemiology of Obesity study (N = 4,838) and the Oxford Biobank (N = 6,999). We found that lower TG levels due to combined APOA5 and LPL variation and genetically-influenced lower LDL-C levels afforded the largest reduction in CAD risk (odds ratio: 0.78 (0.73–0.82)). Compared to patients with genetically-influenced lower TG via LPL, genetically-influenced lower TG via APOA5 had similar and independent, but notably larger, effects on the lipoprotein profile. Our results suggest that lower TG levels as a result of APOA5 variation have strong beneficial effects on CAD risk and the lipoprotein profile, which suggest apo A-V may be a potential novel therapeutic target for CAD prevention.

Supplementary Key words cardiovascular disease • lipoproteins • triglycerides • lipid-lowering therapy • hyperlipidemia • LDL • genetic variation • lipoprotein lipase • factorial analysis • clinical data

INTRODUCTION

Current guidelines for coronary artery disease (CAD) prevention focus on statins as the first-line treatment aimed at reducing LDL-C. However, statins reduce cardiovascular risk by only approximately 20%–30% (1, 2). In addition to LDL-C, elevated levels of triglycerides (TG) and TG-rich lipoproteins (TRLs) have emerged as independent and causal risk factors for CAD (3–5).

Numerous genes have been linked to TG metabolism, among which LPL, which encodes LPL, has been shown to play a major role (6, 7). In addition to LPL, APOA5, encoding apolipoprotein A-V (apo A-V), is an important determinant of plasma TG levels (8–10). Apo A-V is mainly expressed in the liver and is present on and exchanged between TRLs and HDL-C (11, 12). Despite its low plasma concentration (≈150 ng/ml) compared with other apolipoproteins, apo A-V appears to be a potent regulator of circulating TG levels (13). In-vivo experiments found that mice overexpressing human APOA5 had 66% lower plasma TG levels than controls, primarily due to a lower TG content in VLDL particles (14, 15). Reciprocally, APOA5 knockout mice had a fourfold increase in plasma TG levels (14) and resembled apo A-V-deficient patients exhibiting type V familial hyperlipoproteinemia (10, 11). Furthermore, genome-wide association studies have identified rare and common variants in the APOA5 locus to be associated with...
TG levels (8, 9, 16). Despite playing a crucial role in TG metabolism, the precise mechanism(s) through which apo A-V regulates TG levels remain under debate. Most evidence suggests that apo A-V enhances LPL-dependent TG lipolysis, either directly or indirectly (17, 18). Other hypotheses suggest that apo A-V regulates hepatic VLDL production (18) or facilitates the recognition of VLDL particles by members of the LDL receptor family and heparan sulfate proteoglycans (19, 20), thereby enhancing the clearance of these particles from the circulation.

Previously, factorial Mendelian Randomization analyses showed that genetically-influenced lower plasma TG levels via LPL have additional beneficial effects on reducing CAD risk on top of genetically-influenced lower LDL-C (21). As an important TG regulator, apo A-V could therefore be an interesting additional therapeutic target for CAD prevention. In the present study, we aimed to study APOA5 genetic variation in relation to CAD, as well as the detailed lipoprotein profile, separately and in combination with variation in LDL-C and LDL-C-lowering through factorial genetic analyses in multiple cohorts.

MATERIALS AND METHODS

Study design and population

In this study, we aimed to: (1) assess the clinical relevance of genetically-influenced lower TG levels via APOA5 and/or LPL variants on top of genetically-influenced lower LDL-C on CAD risk and (2) investigate the mechanisms of apo A-V relative to LPL by estimating the individual and combined associations with metabolomic measures of genetically-influenced lower TG via APOA5 and genetically-influenced lower TG via LPL.

For the first aim, we performed single instrument and factorial genetic association analyses (supplemental Fig. S1–S3) using individual-level data from 309,780 CAD cases and controls in the UK Biobank. The UK Biobank cohort is a prospective general population cohort of 502,628 participants aged 40–70 years from across the United Kingdom. For the present study, we restricted the analyses to the UK Biobank participants who reported to be of European ethnicity, were present in the full release imputed genotyped datasets (N = 309,780).

In NEO, OBB, and UK Biobank, we calculated weighted genetic scores for both APOA5 and LPL using TG-lowering alleles. For the APOA5 genetic score, we used two variants (rs662799 and rs3135506; Extended Methods, supplemental Table S2) that comprise most of the variation in the APOA5 locus, are in linkage equilibrium (R^2=0.003), and are strongly associated with TG levels (22). For the APOA5 genetic score, we used two variants (rs662799 and rs3135506; Extended Methods, supplemental Table S2) that comprise most of the variation in the APOA5 locus, are in linkage equilibrium (R^2=0.003), and are strongly associated with TG levels (22). For the APOA5 genetic score, we used two variants (rs662799 and rs3135506; Extended Methods, supplemental Table S2) that comprise most of the variation in the APOA5 locus, are in linkage equilibrium (R^2=0.003), and are strongly associated with TG levels (22).

Using the beta estimates of the independent lead variants, we calculated weighted LDL-C genetic risk scores (GRS) per participant. To limit bias by pleiotropy, we did not allow overlap in independent lead variants between LDL-C and the other lipid traits (notably HDL-C and TG) based on a p-value cut-off of 5 x 10^{-8}. Next, based on the weighted GRS of LDL-C, LPL, and APOA5, we stratified the study population into different groups based on the median values of the three GRS (supplemental Fig. S3).

Study outcomes

Cardiovascular disease outcomes. In UK Biobank, the clinical outcome was CAD. Information on incident CAD was collected through information from the data provided by the NHS record systems. Diagnoses were coded according to the International Classification of Diseases (24). CAD was defined as: angina pectoris (I20), myocardial infarction (I21 and I22), and acute and chronic ischemic heart disease (I24 and I25).
NMR-based metabolomic profile. In NEO and OBB, the primary outcomes were the fasting NMR-based metabolomic measurements. In both cohorts, a high-throughput proton NMR-metabolomics platform (25) (Nightingale Health Ltd, Helsinki, Finland) was used to measure 159 metabolic measures (excluding ratios) at the Medical Research Council Integrative Epidemiology Unit at the University of Bristol, Bristol, United Kingdom, which were quantified by Nightingale library. This method provides lipoprotein subclass profiling with lipid concentrations within 14 lipoprotein subclasses. Details of the experimentation and applications of the NMR-metabolomics platform have been described previously (25), as well as representative coefficients of variations for the metabolic biomarkers (26).

In this study, we excluded all ratios, resulting in a final number of 145 NMR-derived metabolic measures present in both NEO and OBB cohort. Values below the detection limit were treated as missing. For all analyses, metabolic measures were inverse rank transformed to obtain normal distributions.

Statistical analyses

Factorial genetic association analyses with CAD risk in the UK Biobank cohort. We performed three types of genetic analyses on CAD cases and controls in the UK Biobank: 1, single instrument genetic analyses, where each dichotomized genetic score (LDL-C, LPL, and APOA5 GRS) was associated with CAD outcomes, assuming that the other alleles were randomly distributed in the other groups (supplemental Fig. S1); 2, 2 × 2 factorial genetic analyses resulting from three different combinations (LDL-C-lowering and lower TG via LPL alleles, LDL-C-lowering and lower TG via APOA5 alleles, and lower TG via both LPL and APOA5 alleles) (supplemental Fig. S2); 3, 2 × 2 factorial genetic analyses with the combination of the three genetic scores to assess the clinical relevance of lower TG via APOA5 and LPL variants on top of genetically-influenced lower LDL-C (supplemental Fig. S3).

Analyses in UK Biobank were performed in R (Version 3.6.1, the R Project, https://www.r-project.org/) using logistic regression adjusted for age, sex, and the first 10 principal components in unrelated individuals.

Factorial genetic association analyses with NMR-metabolomics. Using four “naturally randomized” subgroups based on LPL and APOA5 GRS, we performed linear regression analyses to estimate the associations with NMR-based metabolomic measures between groups using a 2 × 2 factorial design in NEO and OBB separately. These association analyses were adjusted for age, sex, and the first four genomic principal components to correct for possible population stratification within the separate study samples. In addition, we included in the regression model an additive interaction term by using a product term between the continuous LPL and APOA5 genetic scores to test whether they had additive effects on the NMR-based metabolomics measures. Finally, these analyses were also performed for replication purposes using nonfasting NMR-based metabolomics measures in the UK Biobank cohort.

All analyses in the NEO and OBB cohort were adjusted for multiple testing, dividing the alpha by 37, as this was the number of independent metabolic measures in our study. The number of independent biomarkers was determined using the method by Li and Ji (27). Associations were considered to be statistically significant in case the p value was below 1.35 × 10^-3 (i.e., 0.05/37). All results for the NEO cohort were based on analyses weighted toward the reference BMI distribution of the general Dutch population and, therefore, apply to a population-based study without oversampling of individuals with overweight or obesity. A more detailed description of the weighting can be found elsewhere (28).

Finally, the separate results from the NEO and the OBB cohorts were meta-analyzed using the fixed-effect model of rmeta package in R. Linear regression analyses were carried out using STATA Statistical Software version 12.0 (Statacorp, College Station, Texas, USA) and R version 3.6.1 (The R Project, https://www.r-project.org/). The circular plots were designed using Python version 2.7.6 (Python Software Foundation, https://python.org/).
TABLE 2. Characteristics of the NEO and the OBB cohort, as well as their combination

| Characteristics | NEO\a | OBB | Total\b |
|-----------------|-------|-----|---------|
| Number of participants | 4,838 | 6,999 | 11,837 |
| Age (years) | 55.5 (6.0) | 41.6 (5.9) | 47.3 (5.9) |
| Men (%) | 42 | 44 | 43 |
| BMI (kg/m²) | 26.0 (4.3) | 25.8 (4.6) | 25.9 (4.5) |
| Fasting serum concentrations (mmol/L) | | | |
| TG (median (IQR)) | 0.99 (0.71) | 0.93 (0.65) | 0.95 (0.67) |
| Total cholesterol | 5.80 (1.01) | 5.18 (1.01) | 5.43 (1.01) |
| LDL-cholesterol | 3.66 (0.94) | 3.22 (1.26) | 3.40 (1.13) |
| HDL-cholesterol | 1.60 (0.47) | 1.38 (0.42) | 1.47 (0.44) |
| APOA5 GRS (median (IQR)) | 0.86 (0.00) | 0.86 (0.00) | 0.86 (0.00) |
| LPL GRS (median (IQR)) | 0.45 (0.24) | 0.45 (0.24) | 0.45 (0.24) |

Values are mean (SD), unless otherwise specified. GRS unit is in SD.
\aIn NEO, the results are based on analyses weighted toward the reference BMI distribution of the general Dutch population.
\bThe total represents averaged results from the individual analyses in NEO and OBB cohort.

Factorial genetic association analyses with CAD risk

The characteristics of the UK Biobank cohort stratified by genotype group based on the LPL, APOA5, and LDL-C GRS are shown in supplemental Table S3. Results from factorial genetic analyses with CAD in the UK Biobank are presented in Fig. 1. The group with lower TG via APOA5 and groups with lower TG via LPL had a similar reduced odds ratio for CAD risk (OR (95% CI): 0.95 (0.92;0.97) vs. 0.94 (0.91;0.97), respectively. In addition, the effects of the genetic scores on CAD were also additive based on the comparison between the sum of the individual effects (LPL: OR=0.94; APOA5: OR=0.95) and the effect of both scores combined (both LPL and APOA5: OR=0.89). Based on an approximation of the OR with the risk ratio when the outcome incidence is <10%, the sum of the risk reduction of the individual LPL and APOA5 scores translated into 9%, which was similar to the risk reduction in the group with both genetic exposures (11%). When combined with genetically-influenced lower LDL-C levels, genetically-influenced lower TG via APOA5 were associated with the same CAD risk as the genetically lower TG via LPL (OR (95% CI):0.83 (0.79;0.86) vs. 0.83 (0.80;0.86), respectively). The most beneficial effect on CAD risk was observed when genetically-influenced lower TG via both LPL and APOA5 were combined with genetically-lower LDL-C (OR (95% CI): (0.78 (0.73;0.82)).

2 × 2 factorial analyses with NMR-based metabolomic measures

The characteristics of the combined population of NEO and OBB cohorts stratified by the dichotomized LPL and APOA5 GRS are shown in supplemental Table S4. Compared with the reference group (genetically-influenced higher TG via both LPL and APOA5), lower genetically-influenced TG levels via LPL only were associated with altered levels of eight metabolomic measures (particularly higher levels of medium-sized HDL subparticles; Figure 2 and supplemental Table S5) and lower genetically-influenced TG levels via APOA5 only were associated with changed levels of 80 metabolomic measures (particularly lower levels of all sizes of VLDL subparticles; Figure 3 and supplemental Table S5). Despite these observed differences, in general, the effects of the APOA5 and LPL genetic scores on the metabolomic measures showed a moderate overlap $R^2 = 0.68$ (supplemental Fig. S4).

Compared to the same reference group, lower genetically-influenced TG levels via both LPL and

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**Fig. 1.** Associations of genotype group with Coronary Artery Disease in the UK Biobank cohort. Values are mean (SD) for LDL-C levels and median (IQR) for TG levels. GRS unit is in SD, CI, Confidence interval; OR, odds ratio; GRS, genetic risk scores.
APOA5 were associated with altered levels of 86 metabolomic measures (Fig. 4 and supplemental Table S5). Overall, the effects of these associations showed an additive pattern of the individual associations of genetically-influenced lower TG levels via APOA5 and genetically-influenced lower TG levels via LPL but no evidence for an interaction between these scores ($p$ for interaction $> 1.35 \times 10^{-3}$). More specifically, the group with genetically-influenced lower TG levels via both APOA5 and LPL was associated with lower levels of all VLDL subparticles and most LDL subparticles, as well as a lower average VLDL particle size (VLDLD: beta (SE) = $-0.30 \ (0.03)$, $p = 2.3 \times 10^{-22}$). In line with these results, levels of apolipoprotein B (apoB), total serum cholesterol, cholesterol in VLDL (VLDL-C), and cholesterol in LDL (LDL-C) were also lower (apoB: beta (SE) = $-0.28 \ (0.03)$, $p = 3.6 \times 10^{-10}$), whereas most HDL subparticles, HDL-C, and ApoA1 were higher (ApoA1: beta (SE) = $0.12 \ (0.03)$, $p = 2.2 \times 10^{-18}$). In addition, genetically-influenced lower TG levels via both LPL and APOA5 were associated with lower levels of total FAs (beta (SE) = $-0.27 \ (0.06)$, $p = 9.4 \times 10^{-17}$) and several free FAs (omega-3, omega-6, monounsaturated FAs, polyunsaturated FAs, and short-chain FAs) and with a higher degree of unsaturation. Replication analyses in the UK Biobank cohort confirmed these observations, despite the fact that the metabolomics measurements were done irrespective of fasting status, which likely increased the variability of the measurements (supplemental Fig. S5).

**DISCUSSION**

In this study, exposure to genetically-influenced lower TG levels via APOA5 had additional beneficial effects on CAD risk on top of genetically-influenced lower TG levels via LPL and genetically-influenced lower LDL-C levels. This was further supported by the independent and additive beneficial effects on the lipoprotein profile, of the genetically-influenced lower TG via APOA5 on top of genetically-influenced lower TG via LPL. Therefore, our data suggests that pharmacological TG-lowering therapy via APOA5 may have additional beneficial effects on the lipoprotein profile...
and CAD risk on top of LPL-enhancement therapy as well as LDL-C-lowering therapy.

Previously, it was reported that genetically-influenced lower TG levels through LPL have an additive lowering effect on CAD risk on top of genetically-influenced lower LDL-C (21, 29), which were confirmed by the beneficial effects of this combination on the lipoprotein profile recently shown by our group (30). The results from the current study extend these findings by suggesting that genetically-influenced lower TG via APOA5 have similar beneficial effects on CAD risk and the lipoprotein profile as genetically-influenced lower TG via LPL. Collectively, genetically-influenced lower TG through APOA5 and genetically-influenced lower TG through LPL were associated with an additively improved lipoprotein profile and CAD risk. More importantly, exposure to genetically-influenced lower TG levels via APOA5 gave an additional reduction in primary CAD risk on top of exposure to genetically-influenced lower TG via LPL and genetically-influenced lower LDL-C levels. Data from other MR studies have shown that particularly, apoB may be the key trait accounting for the relationship between lipoproteins and CAD (29, 31). Since in our study both the APOA5 and LPL genetic scores were associated with lower levels of VLDL subparticles and the LDL-C genetic score with lower levels of LDL subparticles, these all translated to lower levels of apoB. Thus, the observed reduction in CAD might be explained by lower levels of apoB, which was indeed the lowest in the group with the three genetic exposures. Altogether, these data suggest that apo A-V might be an attractive therapeutic target for additional treatment to reduce CAD risk. This opens up a novel avenue for the development of potentially effective drugs in CAD prevention, which is of high importance given the residual risk that remains in patients already on statin therapy (1, 2). One feasible approach, given the small size of the apo A-V (39 Kda), may be an APOA5 expression construct targeted to muscle or liver.

Previously, association studies of APOA5 variants with lipoprotein subparticles have been performed, although mostly with a less extensive metabolomics panel and limited cohort size. These studies showed the strongest associations of APOA5 variants with chylomicrons and large VLDLs (32–35), which is in line with the strong associations of lower TG via APOA5 observed in our study. Guardiola et al. showed that the rare TG-increasing alleles the APOA5 variants used in our study, notably rs3135506 and rs662799, were associated with an atherogenic lipoprotein profile (34). Similarly, in our study, we showed that the TG-lowering alleles of rs3135506 and rs662799 had a lowering effect on the atherogenic TRLs, including mostly VLDL subparticles. In addition, lower TG levels via APOA5 were associated with lower levels of glycoprotein acetyls, a biomarker for inflammation (36), suggesting that APOA5 may also play a role in atherogenesis by affecting inflammation. Sarwar et al. (33) reported no effect of APOA5 on LDL, which is partially in concordance with our study, where we showed lower levels of only some of the LDL subparticles.

To our knowledge, the present study is the first showing the effects of lower TG via APOA5 on an extensive NMR-metabolomic panel and its comparison with lower TG via LPL. Overall, the effect sizes of the associations of the APOA5 alleles were stronger than those of the LPL alleles. Nevertheless, the directionality and pattern of these effects largely overlapped. In general, genetically-influenced lower TG levels via APOA5 were predominantly associated with lower levels of VLDL subparticles and a smaller VLDL particle size and a lower number of particles, as indicated by apoB levels. Total cholesterol and total TG levels were lower in both, as well as total FAs. These associations could be due to enhanced TG hydrolysis, which is further confirmed by the higher levels of HLD subparticles and HDL particle size that result due to increased availability of surface components of TG-rich particles (37). However, these increasing effects on HDL subparticles...
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were higher in the group with genetically-influenced lower TG via \textit{LPL} than the group with genetically-influenced lower TG via \textit{APOA5}. Except for the HDL subparticles, overall, the effect sizes of the associations with \textit{APOA5} were larger than the effect sizes of the associations with \textit{LPL}. Whether these effects are additional to \textit{LPL}-dependent TG hydrolysis via other mechanisms, we cannot conclude based on the present findings. In addition to \textit{LPL}-dependent TG hydrolysis, a role for apo A-V in hepatic VLDL production has been suggested by previous studies in mice (18). In addition to \textit{LPL}-dependent TG hydrolysis and hepatic VLDL production, studies have shown that apo A-V also facilitates the recognition of TG-rich VLDL particles by the LDL receptor and heparan sulfate proteoglycans, thereby enhancing clearance of these particles (20). These potential other functions of apo A-V, we could not identify nor exclude with our present study design and need to be investigated in future studies. Nevertheless, from these results, we can conclude that \textit{LPL} and \textit{APOA5} are most likely associated with clinical outcomes via the same intermediates.

Several assumptions and limitations of the genetic approach used in this study should be considered when interpreting the results of our study. Mendelian randomization assumes that genetic variants are associated with the outcome only through the exposure of interest so that the results cannot be violated by (directional) pleiotropy. To take this assumption into account, we chose \textit{APOA5} variants that are located within the \textit{APOA5} gene: rs3135506 in the second exon and rs662799 located 2kb upstream of the \textit{APOA5} gene. In addition, it has been previously found that rs3135506, also known as S19W, is a functional SNP that leads to an amino acid change, which subsequently leads to a 50% decrease in secretion, due to diminished translocation of apo A-V across the ER (38). Even though the effect of rs662799 on protein and functional level is less clear, rs662799 is in LD with rs2266788 ($R^2=0.77$), which has been associated with \textit{APOA5} gene expression (39). Although these data support our assumption that the observed effect on CAD via the \textit{APOA5} genetic score occurs through apo A-V, we cannot formally exclude the possibility that alternative variants in linkage with variants in our \textit{APOA5} GRS are the actual causative variants. Although the potential for such an alternative causative variant seems high given that \textit{APOA5} is part of the \textit{APOA1-C3A4-A5} gene locus, such a variant remains to be identified. In addition, from the multitude of associations of the \textit{APOA5} genetic score with the NMR profile (Fig. 3), we cannot conclude that the effect on CAD is mediated through the effect of apo A-V on plasma TG. As such, this analysis is not a proper Mendelian randomization analysis testing the causative effect of TG on CAD. Similarly, the \textit{LPL} genetic score comprised variants that were in or within 10 kb of the \textit{LPL} gene itself and were either coding variants associated with \textit{LPL} function or significant expression quantitative trait loci (40, 41). This makes it likely that the genetically-influenced lower TG via the \textit{LPL} genetic score truly resulted through \textit{LPL}. But similar to \textit{APOA5}, the \textit{LPL} GRS is associated with a multitude of metabolites in the NMR profile (Fig. 2). Furthermore, we attempted to minimize possible pleiotropic effects of the LDL-C genetic score by including variants associated with LDL-C only, hence without associations to other lipid traits. Another potential limitation of our study is the inclusion of only two variants in the \textit{APOA5} score, which in combination with a lower allele frequency could potentially lead to an underestimated effect estimate. Finally, our data are pertinent only to European populations, given that all the analyses in the NEO, OBB, and UK Biobank were performed in participants of European decent.

In summary, our study showed that genetically-influenced lower TG via \textit{APOA5} have additional beneficial effects on CAD risk and lipoprotein profile, which were independent from and comparable to the effects of genetically-influenced lower TG via \textit{LPL} alleles. Altogether, these results indicate that apo A-V is a potential novel therapeutic target for CAD prevention to be explored in detail in future studies.

Data availability

Processed data for every figure described in the article are contained within the article and the supplementary materials. Because of consent issues, we cannot make the individual data of study participants available to other researchers for purposes of reproducing the results or replicating the procedure. 

Supplemental data

This article contains supplemental data (21–24, 28, 38, 42–48).

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Author contributions

D. I., M. B., R. N., and K. W. v. D. conceptualization; D. I., M. B., R. N., and K. W. v. D. methodology; R. N. validation; D. I.
and M. B. formal analysis; D. I. data curation; D. I. writing—original draft; D. I. visualization; K. W. v. D. supervision; K. W. v. D. project administration; D. I., M. B., M. E. T., D., J. W., J., F. R. R., C. C., M. J. N., R. K., P. C. N. R., F. K., R. N., and K. W. v. D. writing—review and editing.

**Author ORCIDs**

Dorina Ibi [https://orcid.org/0000-0003-0908-4039](https://orcid.org/0000-0003-0908-4039)
Raymond Noordam [https://orcid.org/0000-0001-7801-809X](https://orcid.org/0000-0001-7801-809X)
Ko Willems van Dijk [https://orcid.org/0000-0002-2172-7394](https://orcid.org/0000-0002-2172-7394)

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**Conflict of interest**

The authors declare that they have no conflicts of interest with the contents of this article.

**Abbreviations**

apo A-V, apolipoprotein A-V; apo B, apolipoprotein B; GRS, genetic risk scores; NEO, Netherlands Epidemiology of Obesity; OBB, Oxford Biobank.

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