Genetics and life course epidemiology of cardiometabolic disease: towards personalized medicine

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PART I: GENETICS OF LIPID METABOLISM

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Genome-Wide Association Study of the Postprandial Triglyceride Response Yields Common Genetic Variation in Hepatic Lipase (*LIPC*)

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Circulation: Genomic and Precision Medicine 2020; 13:e002693

ABSTRACT

Background: The increase in serum triglyceride (TG) concentrations in response to a meal is considered a risk factor for cardiovascular disease. We aimed to elucidate the genetics of the postprandial TG response through genome-wide association studies (GWAS).

Methods: Participants of the Netherlands Epidemiology of Obesity (NEO) study (n=5,630) consumed a liquid mixed meal after an overnight fast. GWAS of fasting and postprandial serum TG at 150 minutes were performed. To identify genetic variation of postprandial TG independent of fasting TG, we calculated the TG response at 150 min by the residuals of a nonlinear regression that predicted TG at 150 min as a function of fasting TG. Association analyses were adjusted for age, sex and principal components in a linear regression model. Next, using as rs7350789-A as a determinant, we performed linear regression analyses on the residuals of the postprandial response of 149 nuclear magnetic resonance (NMR) based metabolite measures.

Results: GWAS of fasting TG and postprandial serum TG at 150 min resulted in completely overlapping loci, replicating previous GWAS. From GWAS of the TG response, we identified rs7350789-A (allele frequency=0.36), mapping to hepatic lipase (*LIPC*), to be associated with a smaller increase in TG concentrations at 150 min (beta= -0.11; p-value=$5.1x10^{-8}$). GWAS of fasting and postprandial serum TG at 150 minutes were performed. Rs7350789-A was associated with responses of 33 metabolite measures (p-value<$1.4x10^{-3}$), mainly smaller increases of the TG component in almost all high-density lipoprotein (HDL) sub-particles (HDL-TG), a smaller decrease of HDL diameter and smaller increases of most components of very low density lipoproteins (VLDL) sub-particles.

Conclusions: GWAS of the TG response identified a variant near *LIPC* as a main contributor to postprandial TG metabolism independent of fasting TG concentrations, resulting in smaller increases of HDL-TG and VLDL sub-particles.

INTRODUCTION

It is becoming increasingly clear that both fasting and postprandial (nonfasting) serum triglyceride (TG) concentrations are independent risk factors for developing cardiovascular disease (CVD).[1-4] While clinical TG measures are commonly assessed after an overnight fast, it should be acknowledged that individuals spend most of the day in a postprandial state due to frequent food intake.[5] The typical eating pattern in Western society consists of three relatively regularly scheduled meals per day interspersed with more variable eating events. This eating pattern prevents serum TG to reach fasting levels for most of the day.[6] Therefore, postprandial serum TG concentrations may provide additional insight in CVD risk and the metabolic capacity of an individual to deal with dietary stimuli.[7-9]

The postprandial state is a dynamic, non-steady-state condition that is characterized by increased serum levels of TG and TG-rich lipoproteins (TRLs), which mainly include chylomicrons (CM), but also very low-density lipoproteins (VLDL), and their remnants. Cell culture studies and epidemiological data indicate that TRLs and particularly their remnants exert adverse effects on endothelial cells and may penetrate into the sub-endothelial space to initiate atherosclerotic plaques.[6,10] Large prospective studies have shown that delayed clearance of TRLs and their remnants from the blood stream is associated with atherosclerosis progression and therefore is a risk factor for CVD. [1-3,11] For example, the Copenhagen City Heart Study reported a significant association between higher nonfasting TG concentrations and higher myocardial infarction risk over 31 years of follow-up.[12]

In cohort studies, the postprandial TG response has been shown to be highly variable.[5,6] This inter-individual variability is at least partly genetic, although dietary pattern, physical activity, alcohol consumption, age, gender and insulin sensitivity are more than likely involved.[6,13-14] One cross-sectional study reported that healthy sons of men with established coronary artery disease (CHD) have higher postprandial serum TG concentrations after consumption of a high-fat meal compared with sons of control subjects without CVD,[14] which suggested that prolonged postprandial hypertriglyceridemia is associated with familial risk for CVD. Multiple genetic variants in the *APOA1/C3/A4/A5* gene cluster have been associated with postprandial lipemia in relatively small studies.[15-17] Thus far, a limited number of genome-wide association studies (GWAS) on postprandial TG concentrations have been performed. These include the Heredity and Phenotype Intervention (HAPI) Heart study in Old Order Amish individuals (n=809), identifying a rare null mutation in *APOC3*, likely due to a founder effect.[18] In addition, a study embedded in the Genetics of Lipid Lowering

Drugs and Diet Network (GOLDN), consisting of 1,715 participants, yielded evidence for association of a variant (rs964184) in the vicinity of the APOA1/C3/A4/A5 gene cluster. However, this association was attenuated upon additional statistical adjustment for fasting TG.[19]

In the present study, we set out to elucidate the genetics and underlying biology of the postprandial TG response to a liquid mixed meal. To this end, we performed a GWAS and a subsequent in-depth assessment of nuclear magnetic resonance (NMR) derived metabolite responses of the identified genomic loci in 5,630 middle-aged individuals of the Netherlands Epidemiology of Obesity (NEO) study.

METHODS

The methods are now available as supplemental data. The NEO study design was approved by the medical ethics committee of the Leiden University Medical Center (LUMC), and all participants gave their written informed consent.

RESULTS

Population characteristics

Characteristics of the NEO study population as well as for the separate Leiden and Leiderdorp subcohorts are summarized in Table 1. The mean age in the total population was 56 years and 48.5% of the population were men. Compared with the Leiderdorp subcohort, participants in the Leiden subcohort were more frequently men (49.9% versus 44.3%), had a higher mean body mass index (BMI) (31.3 kg/m^2 versus 26.3 kg/m^2), used more frequently lipid-lowering drugs (17.6% versus 10.4%), had higher fasting and postprandial serum TG concentrations (1.34 mmol/l versus 1.00 mmol/l, respectively, for fasting TG; and 2.01 mmol/l versus 1.63 mmol/l, respectively, for TG at 150 min). Compared to the Leiderdorp subcohort, the median untransformed TG response at 150 min was somewhat lower in the Leiden subcohort (0.06 [IQR: -0.18, 0.30] and -0.03 [IQR: -0.26, 0.22] mmol/l, respectively, as calculated using the nonlinear prediction model between fasting and postprandial TG levels), but showed overlapping and wide interquartile ranges.

Genome-Wide Association Analysis

The results for the fasting and postprandial TG GWAS analyses in the total cohort are shown in Figure 1. Genetic variants mapping to the known TG loci near *APOA1,*

LPL, APOE, GCKR and *CILP2* showed genome-wide significant signals in both fasting and postprandial TG concentrations. Interestingly, the signals in the postprandial analyses completely mirrored the signals of the fasting analyses, although there is some variation in the –log10 p-values that were reached in the separate analyses. Locus *MLXIPL* on chromosome 7 was borderline significant in the fasting TG GWAS, but achieved a p-value of $1.2x10^{-10}$ in the postprandial TG analyses. However, both effect sizes for rs71556736-T (the top hit in *MLXIPL* locus) were similar (fasting, beta (SE) = -0.14 (0.03) SD and postprandial, beta (SE) = -0.17 (0.03) SD). The full list with the lead hits for both fasting and postprandial TG is given in Supplementary table 1.

Table 1. Characteristics of the Total, Leiden and Leiderdorp cohorts from the NEO study

Characteristics	Total cohort (N=5630)	Leiden subcohort (N=4192)	Leiderdorp subcohort (N=1438)
Number of participants	5630	4192	1438
Age (year)	56.0(5.94)	55.9(5.92)	56.1(6.0)
Men	48.5%	49.9%	44.3%
BMI (kg/m2)	30.0(4.83)	31.2(4.20)	26.2(4.46)
Lipid-lowering drug users	15.8%	17.7%	10.4%
Fasting TG (mmol/l)	1.25(0.88, 1.77)	1.34(0.95, 1.87)	1.00(0.71, 1.45)
Postprandial TG at 30 min (mmol/l)	1.43(1.04, 1.95)	1.51(1.12, 2.06)	1.20(0.89, 1.67)
Postprandial TG at 150min (mmol/l)	1.91(1.37, 2.64)	2.01(1.47, 2.72)	1.63(1.14, 2.30)
TG response (mmol/l)	-0.0078 (-0.24, 0.24)	-0.031 (-0.26, 0.22)	0.058 (-0.18, 0.30)

Results are presented as median (inter quartile range) for not normally distributed data, mean (SD), or number (percentage). TG response: residuals at 150 min. BMI indicates body mass index; NEO, Netherlands Epidemiology of Obesity; and TG, triglyceride.

Figure 2 displays the Manhattan plot for the TG response residuals at 150 min in the total NEO cohort. On chromosome 15, rs7350789 reached a suggestive p-value of $5.1x10^{-8}$. In addition to rs7350789, there were six independent variants with a suggestive p-value ($p<5.0x10^{-6}$, Supplementary table 2), but here we pursued the variant with the strongest evidence for association, based on the high number of associated variants in strong linkage disequilibrium (LD). None of the loci identified for either fasting and postprandial TG levels showed evidence for association (p-values > 0.05; Supplementary table 3). The regional association plot is shown in Figure 3A, showing that rs7350789 is 23 kb upstream of the *LIPC*, which encodes hepatic lipase (HL). Rs7350789-A had a per-allele decrease (SE) in TG response residual of -0.11 (0.020) SD (Table 2, Figure 3B). Because the two subcohorts from the NEO study had different study characteristics at fasting, analyses on the rs7350789 variant were stratified accordingly. Given the differences in the median postprandial TG response residuals (Table 1), rs7350789-A in the *LIPC* locus still showed an effect

in the same direction, although the magnitude of the effect was somewhat smaller in the Leiden subcohort compared to the Leiderdorp subcohort (beta (SE) = -0.09 (0.02) SD and beta (SE) = -0.15 (0.04) SD, respectively). Adjustment for BMI and lipid-lowering medication did not change the results (beta= -0.11 (0.019) SD and beta= -0.11 (0.020) SD), respectively, (Supplementary table 4). Furthermore, effect sizes were similar in men and women (beta=-0.11 (0.030) SD and beta= -0.10 (0.026) SD, respectively). Rs7350789-A, mapping to *LIPC*, was not associated with either fasting TG levels (beta (SE)= 0.031 (0.019) SD, p-value=0.1) or postprandial TG levels (beta (SE)= -0.010 (0.019) SD, p-value=0.6).

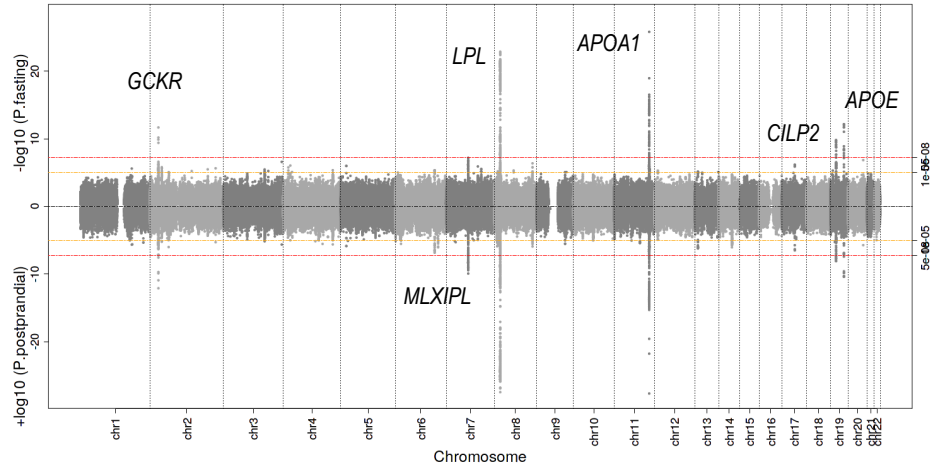


Figure 1. Miami plot showing P-values of the single-nucleotide polymorphism (SNP) associations with fasting TG and postprandial TG.

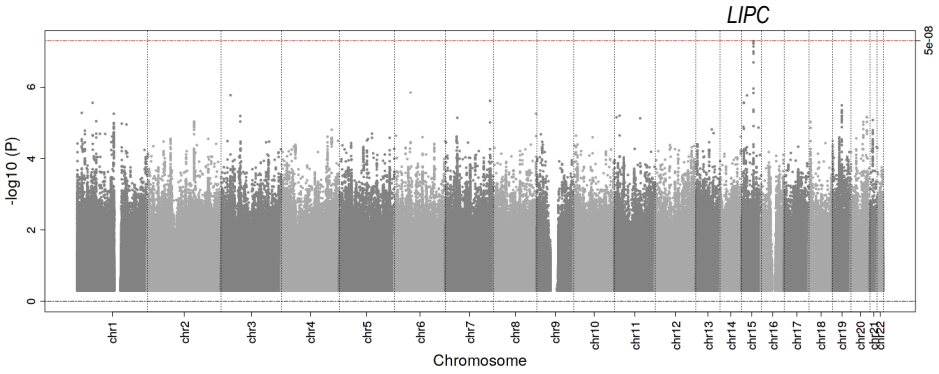


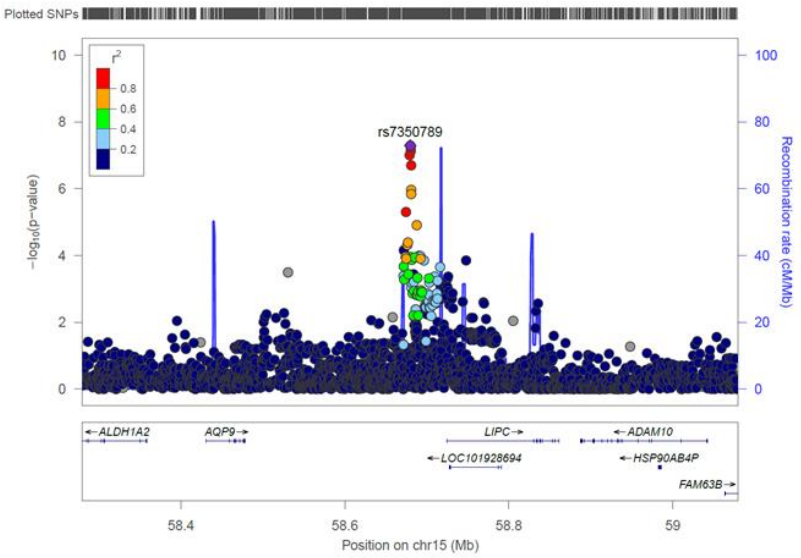
Figure 2. Manhattan plot of the GWAS of postprandial TG residuals at 150 min in the NEO Study. x axis shows chromosomal positions. y axis shows -log10 P-values. The red line indicates the genome-wide significance threshold ($P=5.0 \times 10^{-8}$).

Table 2. Summary statistics of the association of the suggestive (borderline) *LIPC* SNP and TG response in response to a mixed-meal in NEO

							Total cohort		Leiden cohort		Leiderdorp cohort	
CHR	**SNP**	**Position**	**Gene**	**EA/NEA**	**EAF**	**Imputation Quality**	**Beta*(SE)**	**Pvalue**	**Beta*(SE)**	**Pvalue**	**Beta*(SE)**	**Pvalue**
Suggestive SNP												
15	rs7350789	58679668	*LIPC*	A/G	0.36	1	-0.11(.02)	$5.1x10^{-8}$	-0.09(0.02)	$9.2x10^{-5}$	-0.15(0.04)	$5.2x10^{-5}$

Threshold for genome-wide significance is $5x10^{-8}$. Threshold for the suggestive signals is $1x10^{-6}$.
CHR indicates chomosome; EAF, effect allele frequency; (N)EA, (non) effect allele; and SNP, single nucleotide polymorphism.
* Beta coefficient expressed in SD units.

(A)



(B)

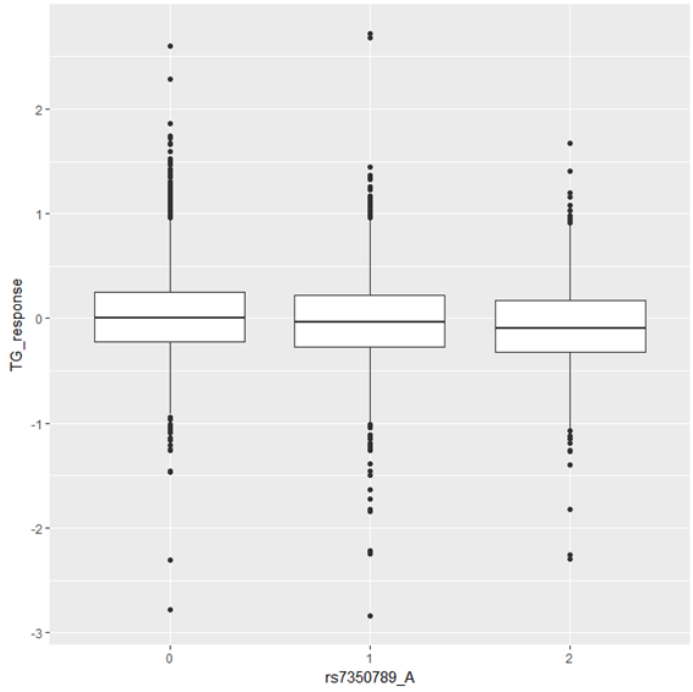


Figure 3. *LIPC* and TG response. **A**, Regional association plot for LIPC on chromosome 15. The purple diamond represents the lead SNP for the locus. **B**, Rs7350789-A genotype specific effects on TG response measured as residuals at 150 min.

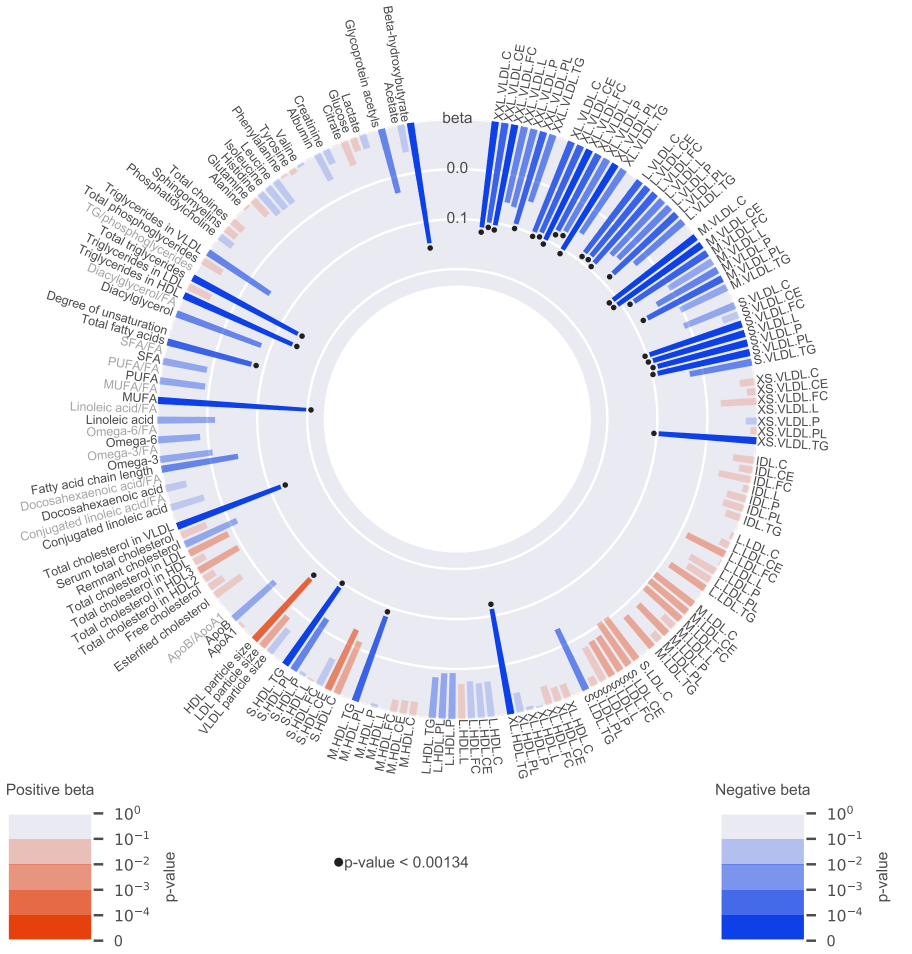


Figure 4. The associations of rs7350789-A and residuals of postprandial NMR-based metabolite measures. Bar heights represent the magnitude of the beta coefficient from linear regression, which is expressed in SD (SD) units. Red bars indicate positive betas and blue bars indicate negative betas. The transparency of the bars indicates the level of statistical significance. A P-value <0.00134 is regarded statistical significant, as represented by the black dots. Full names for the NMR-based metabolite measures are reported in Blauw et al.20.

NMR-based metabolomics

Based on the single signal of the postprandial residual TG GWAS in the full cohort, we conducted association analyses between rs7350789-A in *LIPC* and residuals of the postprandial response of 149 NMR-based metabolite measures at T=150 (Figure 4, Supplementary table 5). Rs7350789-A was significantly associated with postprandial responses of 33 residuals of metabolite measures (p-value < $1.4x10^{-3}$). Overall, the rs7350789-A allele was associated with a smaller increase of the TG-component

in almost all high-density lipoprotein (HDL) sub-particles (largest effect on HDL-TG, p-value=4.5x10^{-7}), a smaller decrease in HDL diameter (p-value=2.8x10^{-4}) and a smaller increase of most components of VLDL sub-particles (largest effect on VLDL-C, p-value=2.7x10^{-6}). In addition, this variation in the *LIPC* locus was significantly associated with a smaller increase in plasma monounsaturated fatty acids (MUFA) and total fatty acids (TotFA) and a larger decrease of beta-hydroxybutyrate (bOHBut).

To gain additional insight in the role of rs7350789-A, we performed association analyses of this SNP with fasting and postprandial NMR-based metabolite levels (Supplementary figure 2A and 2B, Supplementary table 6). In the fasting state, rs7350789-A was associated with higher metabolite measures of many components of the larger HDL, low-density lipoprotein (LDL), intermediate-density lipoprotein (IDL) and very small VLDL particles. The strongest beta's were observed for the TG-component of these particles and this is reflected by the compound measures TG in LDL and TG in HDL, which were both significantly increased (p-value=3.39x10^{-57} and p-value=1.35x10^{-67}, respectively). In addition, both HDL and LDL particle size were significantly larger (p-value=3.21x10^{-36} and p-value=1.59x10^{-26}, respectively), whereas VLDL particle size was significantly smaller (p-value=9.49x10^{-09}). Association analyses of rs7350789-A with postprandial NMR-based metabolite levels were remarkably similar to the associations of rs7350789-A with fasting levels.

DISCUSSION

Our study aimed to identify genes independent of fasting TG concentrations that influence the postprandial TG response. To this end, we performed a GWAS of residuals of the TG response after taking into account the strong nonlinear correlation between fasting and postprandial TG concentrations. We identified rs7350789, mapping to *LIPC* that encodes for HL, to be associated with the TG response residuals. Association analyses of postprandial metabolite response residuals and rs73509789-A showed that this variation in the *LIPC* locus is significantly associated with 33 metabolite measures: mainly with a smaller increase of the TG-component in almost all HDL sub-particles, a smaller decrease of HDL diameter and a smaller increase of most components of VLDL sub-particles at 150 min after the meal.

The GWAS of fasting and postprandial TG concentrations in the NEO study revealed overlapping genetic loci, including the previously identified loci near *APOA1, LPL, APOE, GCKR and CILP2.* However, the signals found in the postprandial TG level GWAS resulted in somewhat different –log10 p-values for the various loci as compared

to the fasting TG GWAS. Locus *MLXIPL* on chromosome 7 was borderline significant in the fasting TG GWAS, but achieved a p-value of $1.2x10^{-10}$ in the postprandial TG GWAS. Given the similarity of the effect sizes, the difference in p-values is likely due to power. In conclusion, the results indicate that the major loci that affect postprandial TG concentrations are the same as those that play a major role in determining fasting TG concentrations.

In previous large-scale GWAS meta-analyses performed by the Global Lipids Genetics Consortium (GLGC), rs1532085 near the *LIPC* locus was associated with TG concentrations.[20] Interestingly, this SNP is 3.7 kb upstream from the residual TG response SNP rs7350789 and the LD of rs1532085 and rs7350789 is high (R^2=0.81). However, in our GWAS of fasting and postprandial TG, this *LIPC* SNP was not a significant hit. This is likely due to power problems, because the GLGC meta-analysis consisted of many tens of thousands of individuals. The effect size of rs7350789-A in the total NEO cohort was -0.11 SD per additional risk allele, indicating a smaller increase of plasma TG after a meal in carriers of this allele. Sensitivity analyses stratified by NEO subcohort (Leiden and Leiderdorp) and additional adjustment for BMI and lipid lowering medication did not materially change this result. Furthermore, although studies have shown differences in activity of HL in men and women,[21,22] we did not observe differences in the effect size of rs7359789-A on TG response residuals in men and women. These data indicate that although the –log10 p-value did not reach the genome wide threshold of $5.0x10^{-}$, the association of rs7350789 with the TG response residuals is robust.

Since rs7350789 is not in the coding region of a gene, we assessed whether this SNP was in an expression quantitative trait locus (eQTL) for liver gene expression levels. A recent mega-analysis of liver eQTLs identified rs11853674-G as an eQTL for *LIPC* with a negative beta (beta(SE)= -0.51(0.11)).[23] Rs7350789-A is in moderate LD with rs11853674-G (R^2=0.46) and thus associated with decreased expression of *LIPC* and likely reduced hepatic production of HL. In addition, recent genetic association analyses of HL activity showed that rs10468017-T near the *LIPC* gene is associated with decreased HL activity.[24] Rs10468017-T is in strong LD with rs7350789-A (R2=0.74) and this further corroborates that rs7350789-A reduces hepatic production of HL.

The *LIPC* gene is expressed in the liver and the majority of the secreted HL is associated with cell surface proteoglycans, which keep HL in an enzymatically inactive form. Cell surface bound HL is exchanged with circulating HDL and under postprandial conditions the HDL-associated HL is presumably activated by changes in the composition of the HDL. The activated HL is thought to hydrolyse TG from chylo-

micron remnants, IDL and HDL.[25,26] The results from the NMR-analyses showed that in the fasting state, rs7350789-A was not associated with total TG levels, but with the redistribution of TG over the different lipoprotein fractions, mainly from VLDL particles to the larger HDL, LDL, IDL and very small VLDL particles (Supplementary figure 2A) which is concordant with a previous report.[27] This redistribution is nearly identical in the postprandial state (Supplementary figure 2B) and this likely reflects the lagging effect of the associations in the fasting state. These data indicate that, apparently also in the fasting state, lower *LIPC* gene expression is associated with higher TG content of both HDL and LDL sized particles. Moreover, these data indicate that HL is functional in the fasting state.

Since rs7350789-A is also associated with higher plasma levels of HDL particles, this HDL could function as an increased circulating reservoir of liver derived HL. This increased reservoir of HDL-associated HL is activated after the meal and could explain the smaller than expected increase of TG in HDL particles and smaller than expected increase of the larger VLDL particles. Our results from the NMR-analysis support this role of HL in hydrolysing TG in HDL particles after a meal, since rs7350789-A is associated with a smaller increase of the postprandial TG concentration of all HDL sub-particles. The meal thus specifically seems to activate the HDL-TG hydrolysis activity of HDL-associated HL.

In addition, rs7350789-A is associated with a smaller increase of most VLDL sub-particles, but not specifically VLDL-TG or IDL-TG. Moreover, no effect was found in the responses of any of the LDL sub-particles. These data indicate that in addition to a role for HL in decreasing HDL-TG, HL plays a role in lipoprotein metabolism that is independent of its lipolytic function. *In vitro* as well *in vivo* experiments have suggested that HL can act as a bridge between lipoproteins and the cell surface, promoting cellular uptake of lipoproteins and HDL.[28,29] Our NMR data indicate that, independent of lipolytic activity, HL indeed seems to plays a role in the clearance of VLDL sub-particles irrespective of the particle size. The meal thus seems to uncover a role for HL in whole particle VLDL clearance. Our data indicate that HL has different roles in lipid and lipoprotein metabolism in the fasting versus the postprandial state.

In parallel, rs7350789-A was associated with a smaller than expected decrease of the HDL diameter. Although this effect is very small, apparently, TG content of HDL particles plays a role in their size distribution. Whether this is a direct effect due to changes in HL activity or an indirect effect due to changes in substrate specificity of the HDL particles themselves, remains to be determined.

Surprisingly, the association of rs7350789-A with NMR metabolite residuals showed a larger decrease of the postprandial bOHBut concentration. This ketone body is connected to the oxidation of fatty acids.[30] Possibly, either as a result of the smaller increase of serum HDL-TG or the increased clearance of VLDL-particles, fatty acid oxidation is decreased. The pathophysiological mechanism underlying the association of variation in the *LIPC* locus and bOHBut remains to be investigated. Other findings of interest for follow-up studies are the associations between the *LIPC* locus and the smaller increase of postprandial MUFA and TotFA levels.

Our results show that carriers of the rs7350789-A allele near *LIPC* have a smaller than expected increase of TG after a meal. However, in the fasting state, the same variant is associated with redistribution of TG from VLDL to HDL and LDL. Since these associations represent both pro- and anti-atherogenic effects, it is difficult to predict the CVD risk in carriers of the rs7350789-A genetic variation. A lookup of rs7350789-A in relation to CHD in publicly available data of the CARDIoGRAMplusC4D consortium [31] did not reveal evidence for an association (beta (SE)= -0.015 (0.009), p-value=0.12). Given the small absolute effect of rs7350789-A on the postprandial TG response, this may not be surprising. Even though our variant has not been associated with CVD risk, recent Mendelian Randomization analyses showed that the LDL TG raising alleles rs1800588-T and rs10468017-T near *LIPC* were associated with lower HL activity and increased CVD risk in CARDIoGRAMplusC4D.[24] As indicated above, rs10468017 is in LD with our *LIPC* variant rs7350789. Decomposing the Mendelian Randomization analyses revealed that the major contribution of the *LIPC* locus to CHD risk was contributed by rs1800588, which was not in LD with rs7350789 and thus explains the lack of association of rs7350789 with CHD (rs10468017-T: beta (SE)= -0.015 (0.011), p-value=0.15; rs1800588-T: beta (SE)= -0.038 (0.011), p-value=0.0005).

One of the strengths of the NEO study is the mixed meal-response design that allows for standardized assessment of postprandial meal responses. Many studies that include nonfasting measures have no information on the timing and composition of the last meal before the blood draw. It should be noted however, that a limitation of the study is that the last postprandial measurement was performed 150 min after the meal intake, which may not be the peak of the postprandial TG excursion. Previous research indicated that serum TG may peak up to 3-4 hours after meal ingestion.[32,33]

In conclusion, we performed a GWAS to identify loci involved in the TG response following a mixed-meal and to provide insight into postprandial lipoprotein metabolism by a metabolomics analysis, using the identified loci. We identified a variant near

LIPC as a contributor to postprandial TG and lipoprotein metabolism independent of fasting TG concentrations, mainly affecting HDL and VLDL sub-particles. In addition, we showed that the same loci that affect fasting TG concentrations also play a major role in determining postprandial TG concentrations.

ACKNOWLEDGMENTS

We express our gratitude to all individuals who participate in the study. We are grateful to all participating general practitioners for inviting eligible participants. We furthermore thank P. van Beelen and all research nurses for collecting the data and P. Noordijk and her team for sample handling and storage and I. de Jonge, MSc for data management of the NEO study. In addition, we thank Alexander Blauw for writing the Python script to design the circular figure.

The NEO study is supported by the participating Departments, the Division and the Board of Directors of the Leiden University Medical Centre, and by the Leiden University, Research Profile Area 'Vascular and Regenerative Medicine'. DvH was supported by the European Commission funded project HUMAN (Health-2013-INNOVATION-1-602757). The authors acknowledge the support from The Netherlands Cardiovascular Research Initiative: an initiative with support of the Dutch Heart Foundation (CVON2014-02 ENERGISE).

REFERENCES

1. Nordestgaard BG, Benn M, Schnohr P, Tybjærg-Hansen A. Nonfasting Triglycerides and Risk of Myocardial Infarction, Ischemic Heart Disease, and Death in Men and Women. *JAMA*. 2007; 298:299-308.
2. Bansal S, Buring JE, Rifai N, Mora S, Sacks FM, Ridker PM. Fasting Compared With Nonfasting Triglycerides and Risk of Cardiovascular Events in Women. *JAMA*. 2007; 298:309-316.
3. Pirillo A, Norata GD, Catapano AL. Postprandial lipemia as a cardiometabolic risk factor. *Curr Med Res Opin*. 2014;30:1489–1503.
4. Alcala-Diaz JF, Delgado-Lista J, Perez-Martinez P, Garcia-Rios A, Marin C, Quintana-Navarro GM, Gomez-Luna P, Camargo A, Almaden Y, Caballero J, et al. Hypertriglyceridemia influences the degree of postprandial lipemic response in patients with metabolic syndrome and coronary artery disease: From the cordioprev study. *PLoS One*. 2014;9:1–9.
5. Rosenson RS, Davidson MH, Hirsh BJ, Kathiresan S, Gaudet D. Genetics and Causality of Triglyceride-Rich Lipoproteins in Atherosclerotic Cardiovascular Disease. *J Am Coll Cardiol*. 2014; 64:2525–2540.
6. Lopez-Miranda J, Williams C, Lairon D. Dietary, physiological, genetic and pathological influences on postprandial lipid metabolism. *Br J Nutr*. 2007;98:458–473.
7. Nakamura A, Monma Y, Kajitani S, Noda K, Nakajima S, Endo H, Takahashi T, Nozaki E Effect of glycemic state on postprandial hyperlipidemia and hyperinsulinemia in patients with coronary artery disease. *Heart Vessels*. 2016;31:1446–14455.
8. Boquist S, Ruotolo G, Tang R, Björkegren J, Bond MG, de Faire U, Karpe F, Hamsten A. Alimentary lipemia, postprandial triglyceride-rich lipoproteins, and common carotid intima-media thickness in healthy, middle-aged men. *Circulation*. 1999;100:723–728.
9. Sharrett AR, Chambless LE, Heiss G, Paton CC, Patsch W. Association of postprandial triglyceride and retinyl palmitate responses with asymptomatic carotid artery atherosclerosis in middle-aged men and women. The Atherosclerosis Risk in Communities (ARIC) Study. *Arterioscler Thromb Vasc Biol*. 1995;15:2122–2129.
10. Hyson D, Rutledge JC, Berglund L. Postprandial lipemia and cardiovascular disease. *Curr Atheroscler Rep*. 2003;56:437–444.
11. Stampfer MJ, Krauss RM, Ma J, Blanche PJ, Holl LG, Sacks FM, Hennekens CH. A Prospective Study of Triglyceride Level, Low-Density Lipoprotein Particle Diameter, and Risk of Myocardial Infarction. *JAMA J Am Med Assoc*. 1996;276:882-888.
12. Langsted A, Freiberg JJ, Tybjaerg-Hansen A, Schnohr P, Jensen GB, Nordestgaard BG. Nonfasting cholesterol and triglycerides and association with risk of myocardial infarction and total mortality: the Copenhagen City Heart Study with 31 years of follow-up. *J Intern Med*. 2011; 270:65–75.
13. Tiret L, Gerdes C, Murphy MJ, Dallongeville J, Nicaud V, O'Reilly DS, Beisiegel U, De Backer G. Postprandial response to a fat tolerance test in young adults with a paternal history of premature coronary heart disease - the EARS II study. *Eur J Clin Invest*. 2000;30:578–585.
14. Uiterwaal CS, Grobbee DE, Witteman JC, van Stiphout WA, Krauss XH, Havekes LM, de Bruijn AM, van Tol A, Hofman A. Postprandial Triglyceride Response in Young Adult Men and Familial Risk for Coronary Atherosclerosis. *Ann Intern Med*. 1994;121:576-583.

15. Ordovas JM, Cassidy DK, Civeira F, Bisgaier CL, Schaefer EJ. Familial apolipoprotein A-I, C-III, and A-IV deficiency and premature atherosclerosis due to deletion of a gene complex on chromosome 11. *J Biol Chem*. 1989;264:16339–16342.
16. Marín C, López-Miranda J, Gómez P, Paz E, Pérez-Martínez P, Fuentes F, Jiménez-Perepérez JA, Ordovás JM, Pérez-Jiménez F. Effects of the human apolipoprotein A-I promoter G-A mutation on postprandial lipoprotein metabolism. *Am J Clin Nutr*. 2002;76:319–325.
17. Calabresi L, Cassinotti M, Gianfranceschi G, Safa O, Murakami T, Sirtori CR, Franceschini G. Increased postprandial lipemia in Apo A-IMilano carriers. *Arterioscler Thromb a J Vasc Biol*. 1993; 13:521–528.
18. Pollin TI, Damcott CM, Shen H, Ott SH, Shelton J, Horenstein RB, Post W, McLenithan JC, Bielak LF, Peyser PA, Mitchell BD, Miller M, O'Connell JR, Shuldiner AR. A null mutation in human APOC3 confers a favorable plasma lipid profile and apparent cardioprotection. *Science*. 2008; 322:1702–1705.
19. Wojczynski MK, Parnell LD, Pollin TI, Lai CQ, Feitosa MF, O'Connell JR, Frazier-Wood AC, Gibson Q, Aslibekyan S, Ryan KA, et al. Genome-wide association study of triglyceride response to a high-fat meal among participants of the NHLBI Genetics of Lipid Lowering Drugs and Diet Network (GOLDN). *Metabolism*. 2015;64:1359–1371.
20. Willer CJ, Schmidt EM, Sengupta S, Peloso GM, Gustafsson S, Kanoni S, Ganna A, Chen J, Buchkovich ML, Mora S, et al. Discovery and Refinement of Loci Associated with Lipid Levels. *Nat Genet*. 2013;45:1–24.
21. SORVA R, KUUSI T, DUNKEL L, TASKINEN M-R. Effects of Endogenous Sex Steroids on Serum Lipoproteins and Postheparin Plasma Lipolytic Enzymes. *J Clin Endocrinol Metab*. 1988;66:408–13.
22. Feitosa MF, Myers RH, Pankow JS, Province MA, Borecki IB. LIPC variants in the promoter and intron 1 modify HDL-C levels in a sex-specific fashion. *Atherosclerosis*. 2009; 204:171–177.
23. Strunz T, Grassmann F, Gayán J, Nahkuri S, Souza-Costa D, Maugeais C, Fauser S, Nogoceke E, Weber BHF. A mega-analysis of expression quantitative trait loci (eQTL) provides insight into the regulatory architecture of gene expression variation in liver. *Sci Rep*. 2018;8.
24. Silbernagel G, Scharnagl H, Kleber ME, Delgado G, Stojakovic T, Laaksonen R, Erdmann J, Rankinen T, Bouchard C, Landmesser U, at al. LDL triglycerides, hepatic lipase activity, and coronary artery disease: An epidemiologic and Mendelian randomization study. *Atherosclerosis*. 2019; 282:37–44.
25. Deeb SS, Zambon A, Carr MC, Ayyobi AF, Brunzell JD. HEPATIC LIPASE AND DYSLIPIDEMIA: INTERACTIONS BETWEEN GENETIC VARIANTS, OBESITY, GENDER AND DIET Running Title: Hepatic Lipase Regulation and Dyslipidemia. *JLR Papers In Press*. 2003;44:1279-1286.
26. Santamarina-Fojo S, Gonza□lez-Navarro H, Freeman L, Wagner E, Nong Z. Hepatic Lipase, Lipoprotein Metabolism, and Atherogenesis. *Arterioscler Thromb Vasc Biol*. 2004;24:1750–1754.
27. Kettunen J, Demirkan A, Würtz P, Draisma HH, Haller T, Rawal R, Vaarhorst A, Kangas AJ, Lyytikäinen LP, Pirinen M, et al. Genome-wide study for circulating metabolites identifies 62 loci and reveals novel systemic effects of LPA. *Nat Commun*. 2016;7:11122.

28. Zambon A, Deeb SS, Bensadoun A, Foster KE, Brunzell JD. In vivo evidence of a role for hepatic lipase in human apoB-containing lipoprotein metabolism, independent of its lipolytic activity. *J Lipid Res*. 2000;41:2094–2099.
29. Freeman L, Amar MJ, Shamburek R, Paigen B, Brewer HB Jr, Santamarina-Fojo S, González-Navarro H. Lipolytic and ligand-binding functions of hepatic lipase protect against atherosclerosis in LDL receptor-deficient mice. *J Lipid Res*. 2007;48:104–113.
30. Benjamin B, Wada Y, Grundy SM, Szuszkiewicz-Garcia M, Vega GL. Fatty acid oxidation in normotriglyceridemic men. *J Clin Lipidol*. 2016;10:283–288.
31. Nikpay M, Goel A, Won HH, Hall LM, Willenborg C, Kanoni S, Saleheen D, Kyriakou T, Nelson CP, Hopewell JC, et al. A comprehensive 1000 Genomes–based genome-wide association meta-analysis of coronary artery disease. *Nat Genet*. 2015;47:1121–1130.
32. Borén J, Matikainen N, Adiels M, Taskinen M-R. Postprandial hypertriglyceridemia as a coronary risk factor. *Clin Chim Acta*. 2014;431:131–142.
33. Pirillo A, Norata GD, Catapano AL. Postprandial lipemia as a cardiometabolic risk factor. *Curr Med Res Opin*. 2014;30:1489–503.
34. Blauw LL, Noordam R, Soidinsalo S, Blauw CA, Li-Gao R, de Mutsert R, Berbée JFP, Wang Y, van Heemst D, Rosendaal FR, et al. Mendelian randomization reveals unexpected effects of CETP on the lipoprotein profile. *Eur J Hum Genet*. 2019;27:422–31.

SUPPLEMENTAL MATERIAL

1 Methods

1.1 Study design and population

The Netherlands Epidemiology of Obesity (NEO) study is a population-based prospective cohort study of men and women aged between 45 and 65 years. From the greater area of Leiden, The Netherlands, all inhabitants with a self-reported body mass index (BMI) of 27 kg/m^2 or higher were eligible to participate. In addition, inhabitants from one nearby municipality (Leiderdorp, The Netherlands) in the same age group were invited to participate regardless of their BMI, forming a reference population for BMI distribution. In total, 6,671 participants were included from September 2008 until September 2012. Participants visited the NEO study center for extensive physical examination. After an overnight fast of at least 10 hours, fasting blood samples were taken at the study center. Within 5 min after the first blood sample was taken, participants drank a liquid mixed meal (400 mL) of 600 kcal, with 16% of energy derived from protein, 50% from carbohydrates and 34% from fat. Postprandial blood samples were taken 30 and 150 min after ingestion. 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 NEO study design was approved by the medical ethics committee of the Leiden University Medical Center (LUMC), and all participants gave their written informed consent. Detailed information about the study design and data collection has been described elsewhere.[1]

For our study, we excluded participants lacking genetic data (N = 927), as described in detail below and elsewhere.[2] Furthermore, we excluded participants with missing triglyceride (TG) data (either fasting or postprandial; N = 113) and one participant with very high fasting TG (TG at fasting >20 mmol/l). There were no individuals with missing covariates resulting in a final study population of 5,630 individuals.

1.2 Genotyping and imputation

DNA was isolated from venous blood samples. Genotyping was performed using the Illumina HumanCoreExome-24 BeadChip (Illumina Inc., San Diego, California, United States of America). Quality control steps on the genotyped dataset and generation of the principal components (PCs) were performed using PLINK (v1.07).[2] Participants were excluded in the process of quality control when 1) the sample call rate was <98%, 2) there was a sex mismatch, 3) heterozygosity rate was not within ±3 SD of mean heterozygosity rate, 4) participants widely diverged based on the first two PCs (±3.5 SD), 5) samples were duplicates, and 6) concordance with another DNA sample was >0.25 (related individuals). Genetic variants were excluded when 1) genotype call rate was <98%, and 2) variants were not in Hardy-Weinberg equilibrium (p-value $<1x10^{-6}$). Detailed quality control steps have been described elsewhere.[3] Subsequently, genotypes were imputed to the 1000 Genome Project reference panel [4] (v3 2011) using IMPUTE (v2.2) software.[5] For this current study we did not use a newer and larger reference panels such as Haplotype Reference Consortium (HRC) because as Given the size of our NEO sample population (5744 participants after quality control), we did not have sufficient power to detect associations of rare variants with outcomes. Therefore, we chose to continue using the 1000G imputations over a larger reference panel that specifically focuses in improving the imputation accuracy of rare variants such as the Haplotype Reference Consortium (HRC).

1.3 Serum TG concentration and the definition of TG response

After centrifugation, aliquots of plasma and serum were stored at -80°C. Serum TG concentrations (fasting and postprandial) were measured with enzymatic calorimetric assays (Roche Modular P800 Analyzer, Roche Diagnostics, Mannheim, Germany; CV <3%).

In addition to fasting and postprandial TG concentrations at 150 min, we assessed the TG response at 150 min as our main outcome. The postprandial TG concentrations are highly correlated with fasting TG, in a non-linear relationship (Figure S1D). Therefore, in order to identify genetic variants that modulate postprandial metabolite levels independently from fasting TG, the postprandial TG response

at 150 min was defined as the residuals of the nonlinear errors-in-variables model

$$TG_{150} = \beta_0 + \beta_1 \frac{TG_0^*}{\beta_2 TG_0^* + 1} + \varepsilon$$

$$TG_0 = TG_0^* + \delta$$

with β_0, β_1, β_2 the regression coefficients, δ and ε the measurement noise on the fasting and postprandial TG concentrations respectively, TG_0^* the actual fasting TG concentrations without measurement noise, and the postprandial residuals *e* defined as

$$e = TG_{150} - \left(\beta_0 + \beta_1 \frac{TG_0}{\beta_2 TG_0 + 1}\right)$$

Rank-based inverse normal transformation (INT) was used to obtain normally distributed residuals for further analyses.

1.4 Genome-wide association study (Statistical analyses)

The GWAS was conducted for all autosomal chromosomes. Additive (per-allele) linear regression analyses were performed using the ProbABEL (version 0.4.5) statistical package,[6] adjusted for age, sex and the first four PCs. Genetic variants with an imputation quality below 0.4 and a minor allele frequency below 1% (rare variants) were excluded and not considered in the results.

To identify independent SNPs, we used Functional Mapping and Annotation (FUMA) [7] an integrative web-based platform used to analyze GWAS studies. Genome-wide statistical significance was considered at a p-value of $<5.0\text{x}10^{-8}$, whereas a p-value of $<5.0\text{x}10^{-6}$ was considered a suggestive signal.

The packages "ggplot2" [8] and "EASYSTRATA" [9] in R statistical software were used to post-process the GWAS results and to prepare the figures of the data presented. The metabolomics data were visualized using a custom made package in Python.

Based on the independent signals from the overall GWAS, we performed a number of sensitivity analyses. First, we stratified the study population based on the 2 different subcohorts in the NEO cohort (notably, the Leiden population with a BMI $\geq$ 27 kg/m^2 and the Leiderdorp population, a smaller

subcohort with a normal distribution of BMI, representative of the overall Dutch population). Secondly, we performed sensitivity analyses in which the linear regression analyses were additionally adjusted for BMI, lipid-lowering medication and were stratified by sex.

1.5 NMR-based metabolite biomarker profiling

A high-throughput proton nuclear magnetic resonance (NMR) metabolomics platform [10] (Nightingale Health Ltd., Helsinki, Finland) was used to measure 159 metabolite measures (excluding ratios) at the Medical Research Council Integrative Epidemiology Unit (MRC IEU) 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. The 14 subclass sizes were defined as follows: extremely large very low-density lipoproteins (VLDL) with particle diameters from 75 nm upwards and a possible contribution of chylomicrons, five VLDL subclasses (average particle diameters of 64.0 nm, 53.6 nm, 44.5 nm, 36.8 nm, and 31.3 nm), intermediate-density lipoprotein (IDL) (28.6 nm), three low-density lipoprotein (LDL) subclasses (25.5 nm, 23.0 nm, and 18.7 nm), and four high-density lipoprotein (HDL) subclasses (14.3 nm, 12.1 nm, 10.9 nm, and 8.7 nm). Within the lipoprotein subclasses, the following components were quantified: total cholesterol, total lipids, phospholipids, free cholesterol, cholesteryl esters, and triglycerides. The mean size for VLDL, LDL and HDL particles was calculated by weighting the corresponding subclass diameters with their particle concentrations. Furthermore, 58 metabolite measures were determined that belong to classes of apolipoproteins, cholesterol, fatty acids, glycerides, phospholipids, amino acids, fluid balance, glycolysis-related metabolites, inflammation, and ketone bodies. Details of the experimentation and applications of the NMR metabolomics platform have been described previously [9], as well as representative coefficients of variations for the metabolite biomarkers.[11] A full list of the measured biomarkers is provided elsewhere.[12]

In this study, we excluded all ratios, resulting in a final number of 149 NMR-derived metabolite measures. In order to assess the postprandial metabolite responses (150 min after meal intake) as

independent measures from fasting, metabolite concentrations residuals of non-linear regressions were used for each metabolite, similar to the TG response residuals. A detailed description is given in the supplementary materials. The analyses were performed on ranked-based INT residuals.

Using the identified variants from the GWAS analyses as determinants, we additionally performed linear regression analyses on the residuals of the postprandial response of 149 metabolite measures. These association analyses were adjusted for age, sex and the first four PCs. In order to adjust for multiple testing, we divided the alpha by the 37, which was the number of the independent metabolite measures in our study. The number of independent biomarkers in our study was determined by using the method as described by Li and Ji.[13] Therefore, associations with a p-value<1.4×10^{-3} were considered statistically significant.

In order to determine the response direction of the significantly associated metabolite measures, we performed a paired t-test of the fasting and the corresponding postprandial levels. To determine the significance threshold, alpha was divided by the number of the independent metabolite measures, similar to the step above.

2 Expanded methods: A nonlinear errors-in-variables model of post-prandial response

Here we describe a statistical method to determine a measure of the response of triglyceride and other metabolite levels to a standardized meal that is independent of fasting levels. The most straightforward approach to correct for fasting levels is to subtract the fasting levels from the postprandial levels. Using this approach one implicitly assumes that the meal induced change in metabolite levels is independent from fasting levels – i.e. one assumes that postprandial levels depend on fasting levels with a slope of one and a variable intercept. Although this approximation is valid for some metabolites, for many others the response is proportional to the fasting levels or shows a nonlinear dependency (Fig. S1).

A more general approach is to construct a nonlinear prediction model from fasting levels and subsequently take the residuals as a measure of the postprandial response. Since typically both fasting and postprandial levels are measured with considerable amount of measurement noise, orthogonal regression must be used instead of ordinary regression in order to obtain an unbiased estimate of the regression parameters. In the remainder of this section an orthogonal nonlinear least squares (OrNLS)[14] regression model is proposed that provides a measure of postprandial response that is statistically independent from fasting levels.

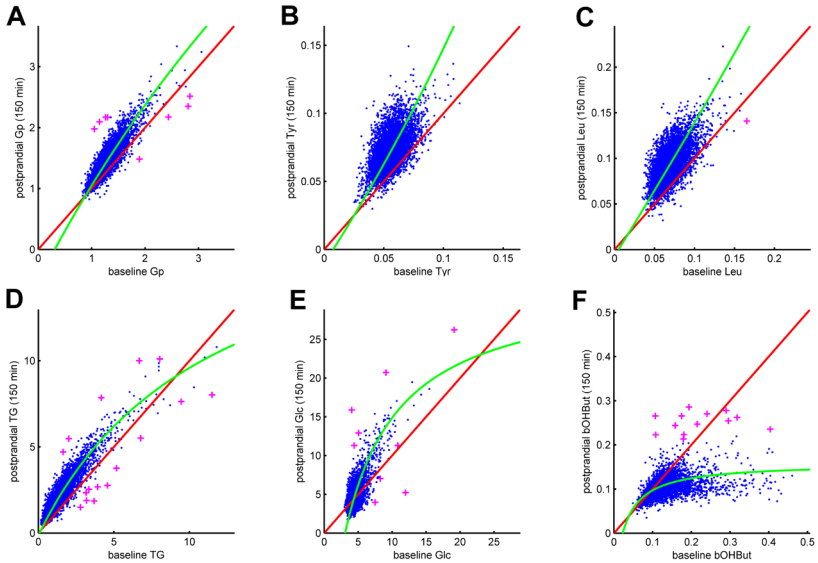


Figure S1: Scatterplots of postprandial versus fasting levels of glycoprotein acetyls (A), tyrosine (B), leucine (C), triglycerides (D), glucose (E) and β-hydroxybutyrate (F). The size of the postprandial responses of glycoprotein acetyls, tyrosine and leucine is proportional to the fasting levels, showing that for some metabolites the regression coefficient β_1 is larger than one. The postprandial responses of triglycerides, glucose and β-hydroxybutyrate show pronounced nonlinear effects. The red line in the figure is the $y = x$ line, the green line is the OrNLS regression fit, and the magenta data points are the SD outliers.

[1]Here, orthogonal least squares regression is distinguished from ordinary least squares regression in abbreviations by using 'Or' for orthogonal and 'O' for ordinary.

General workflow

The workflow shown in Fig. S2 was used to compute the residuals for each metabolite. First, extreme outliers were removed from the dataset if they were further away than twice the distance between the 5-th and 95-th percentile (resp. Q_5 and Q_{95}) in logarithmic space

$$\begin{aligned} x &< Q_5 - 2 \cdot (Q_{95} - Q_5) \quad \text{or} \\ x &> Q_{95} + 2 \cdot (Q_{95} - Q_5) \end{aligned}$$

Using this criterion both measurements with abnormally high values and with values that were very close to zero were eliminated. To make the OrNLS fit robust to outliers, during the least squares minimization procedure datapoints were removed iteratively when they deviated more than 5 standard deviations from the mean (step 2-5). Since for some metabolites the correlation between postprandial and fasting levels was very low, for metabolites with an R^2 less than 0.2 the linear model regression model was used to prevent overfitting. If the slope of the linear model was not significantly different from 1 with a p-value < 0.01, the slope was set to 1 and only the intercept was fitted. For the final computation of the residuals all datapoints were taken into account except the extreme outliers that were removed in the first step (step 6).

1. `Eliminate extreme outliers from the dataset`
2. `Define the SD-outliers as an empty set`
3. `if (`$R^2 \geq 0.2$`), fit the OrNLS regression model on the data except SD-outliers`
   `if (`$R^2 < 0.2$`), fit the OrLS regression model on the data except SD-outliers`
4. `Define the SD-outliers as the data points that have orthogonal residuals larger than 5 times the standard deviation`
5. `If the set of SD-outliers has changed in the last step, go to step 3 and reperform the OrNLS regression`
6. `If the set of SD-outliers has not changed, calculate the vertical residuals of all data points except the extreme outliers`

Figure S2: Workflow for computing the postprandial response of a metabolite.

Errors-in-variables model

To determine the proper statistical model to investigate genetic or environmental determinants that are specific for the postprandial response, we first assume an errors-in-variables model in which the postprandial levels y_i are linearly proportional to the fasting levels x_i

$$y_i = \beta_0 + \xi_i \beta_1 + g_i + \varepsilon_i \tag{1}$$

$$x_i = \xi_i + \delta_i \tag{2}$$

In equation (1) and (2), ξ_i are the actual fasting levels without measurement noise, g_i the meal response specific genetic or environmental contribution to variation in postprandial levels, β_0 and β_1 the intercept and fasting reponse coefficient, and δ_i and ε_i the measurement noise on the fasting and postprandial data respectively.[2]

[2]In this discussion, variables with subscript i are used to denote actual data whereas the same variable without subscript is used to denote the random variable. That is, y_i refers to the postprandial level of subject i, whereas y refers to the abstract random process that generated the values y_i and that is composed of the processes ξ, g and ε.

In the remainder of this discussion Genome-Wide Association Studies (GWAS) will be taken as example. The factor g_i is then assumed to be composed of a linear combination of genetic variants v_{ij} with effect sizes γ_j

$$g_i = v_{i0}\gamma_0 + v_{i1}\gamma_1 + v_{i2}\gamma_2 + \cdots$$

For simplification, it will be assumed that δ and ε in (1) and (2) are uncorrelated with g and ξ and that δ and ε are normally distributed with the same variance $\mathcal{N}(0, \sigma^2)$. Genetic variants that only affect the fasting levels are assumed to be incorporated in the term ξ_i and are assumed to affect y_i in the same proportion β_1 as the non-genetically determined fasting levels. As a consequence, fasting variants that affect postprandial levels with an effect size that is different from β_1 are assumed to affect fasting and postprandial levels independently, and their effect will therefore be separated into a pure fasting and response portion.

For the purpose of regression it is important to realize that the actual fasting levels ξ_i cannot be observed, and that performing ordinary least-squares (OLS) regression of y_i on x_i gives biased estimates of the regression coefficients due to the measurement error δ

$$\begin{aligned} E\left(\hat{\beta}_1^{\mathrm{OLS}}\right) &= \frac{E(x \cdot y)}{E(x^2)} \\ &= \beta_1 \frac{E(\xi^2)}{E(\xi^2) + \sigma^2} + \frac{E(\xi \cdot g)}{E(\xi^2) + \sigma^2} \end{aligned}$$

where we simplified notation by assuming that, without loss of generality, all intercept terms are set to zero (i.e. ξ and g have zero mean and $\beta_0 = 0$). The term $E(\xi \cdot g)$ refers to the expected association between fasting levels and the genetic component that has independent effects on fasting and postprandial levels. This association will not be exactly zero but can be assumed to be negligable with respect to the total variance in fasting levels $E(x^2)$ – thus not contributing to the OLS estimate of β_1.

A consequence of the biased OLS estimate is that the residuals are correlated with the actual fasting levels ξ_i and therefore they do not give a clean measure of the contribution of meal-specific genetic effects g_i since now also the effects of genetic variants affecting ξ_i are included

$$\begin{aligned} e_i^{\mathrm{OLS}} &= y_i - x_i \cdot E\left(\hat{\beta}_1^{\mathrm{OLS}}\right) \\ &= g_i + \beta_1 \frac{\sigma^2}{E(\xi^2) + \sigma^2}\xi_i + \varepsilon_i - \beta_1 \frac{E(\xi^2)}{E(\xi^2) + \sigma^2}\delta_i \end{aligned}$$

where again we assumed zero intercepts and $E(\xi \cdot g) \ll E(x^2)$.

The correct method to perform regression on variables with equal amount of measurement error is orthogonal least-squares (OrLS), which is a special case of Deming regression [13]. In contrast to OLS, which minimizes the vertical distance of each data point to the regression line, OrLS minimizes the perpendicular distance of each data point to the regression line – i.e. the distance to the closest point on the regression line – which gives an unbiased estimate of the regression coefficients in case of uncorrelated measurement noise with equal variances. Due to the genetic term in (1), the OrLS estimate contains a minor bias:

$$E\left(\hat{\beta}_1^{\mathrm{OrLS}}\right) = \frac{E(y^2) - E(x^2)}{2E(x \cdot y)} + \sqrt{\left(\frac{E(y^2) - E(x^2)}{2E(x \cdot y)}\right)^2 + 1}$$

with

$$\frac{E(y^2) - E(x^2)}{2E(x \cdot y)} = \frac{1}{2\beta_1}\left(\beta_1^2 - 1 + \frac{E(g^2)}{E(\xi^2)}\right)$$

where again we assumed zero intercepts and $E(\xi \cdot g) \ll E(x^2)$. Refactoring shows that the bias can be approximated as

$$\begin{aligned} E\left(\hat{\beta}_1^{\mathrm{OrLS}}\right) &= \frac{\beta_1}{2}\left(1-\beta_1^{-2}+B+\beta_1^{-2}B\right)+\frac{\beta_1}{2}\sqrt{\left(1+\beta_1^{-2}+B-\beta_1^{-2}B\right)^2+4\beta_1^{-2}B^2} \\ &\approx \beta_1(1+B) \end{aligned}$$

assuming that $\left(1+\beta_1^{-2}+B-\beta_1^{-2}B\right)^2 \gg 4\beta_1^{-2}B^2$, with B the bias in the OrLS estimate

$$B = \frac{E(g^2)}{E(\xi^2)+E((\beta_1\xi)^2)}$$

That is, the OrLS estimate is biased by the ratio of the genetic variance and the sum of the fasting variance and fasting related variance in postprandial levels $\frac{E(g^2)}{E(\xi^2)+E((\beta_1\xi)^2)}$. Importantly, the OrLS bias will typically be much smaller than the bias $\frac{\sigma^2}{E(\xi^2)+\sigma^2}$ in the OLS estimate, which depends on the variance of the measurement noise. The vertical OrLS residuals now become

$$\begin{aligned} e_i^{\mathrm{OrLS}} &= y_i - x_i \cdot E\left(\hat{\beta}_1^{\mathrm{OrLS}}\right) \\ &= g_i - \beta_1 \frac{E(g^2)}{E(\xi^2)+E((\beta_1\xi)^2)}\xi_i + \varepsilon_i - \beta_1\left(1+\frac{E(g^2)}{E(\xi^2)+E((\beta_1\xi)^2)}\right)\delta_i \end{aligned}$$

which shows that the association between the vertical OrLS residuals and the fasting levels ξ_i is much lower than for the OLS residuals. Importantly, only the vertical OrLS residuals provide an unbiased measure of genetic effects g_i and not the perpendicular ones that are minimized by orthogonal least squares regression. In fact, it can be shown that the values of e_i^{perp} are shrunk by a constant factor with respect to e_i^{OrLS}, which would therefore result in biased estimates of the genetic effect sizes γ_j

$$\begin{aligned} e_i^{\mathrm{perp}} &= \pm\sqrt{\left(x_i-\hat{\xi}_i\right)^2+\left(y_i-\hat{\beta}_1^{\mathrm{OrLS}}\hat{\xi}_i\right)^2} \\ &= \frac{1}{\sqrt{\left(\hat{\beta}_1^{\mathrm{OrLS}}\right)^2+1}}\left(y_i - x_i\cdot\hat{\beta}_1^{\mathrm{OrLS}}\right) \end{aligned}$$

where $\hat{\xi}_i$ are the estimated fasting levels, i.e. the observed fasting levels corrected for measurement noise

$$\hat{\xi}_i = \frac{1}{\left(\hat{\beta}_1^{\mathrm{OrLS}}\right)^2+1}x_i + \frac{\hat{\beta}_1^{\mathrm{OrLS}}}{\left(\hat{\beta}_1^{\mathrm{OrLS}}\right)^2+1}y_i$$

In order words, for the fitting of the regression line the summed squares of the orthogonal residuals are used, whereas for the association analyses the vertical (y-axis) residuals are used to estimate the effect of g_i.

As mentioned earlier, a more straightforward alternative for determining meal responses of metabolites is to calculate the difference (delta) between postprandial and fasting levels or to adjust postprandial levels for fasting levels as a covariate. From our discussion here it follows that although the delta approach is robust for cases in which only the intercept β_0 is affected by a meal but not the slope of the response (i.e. $\beta_1 \approx 1$), in cases in which the postprandial levels are proportional with a coefficient $\beta_1 \neq 1$ the delta measure is by definition associated with the fasting levels ξ_i. On the other hand, the covariate approach makes no assumptions about the size

of β_1, but since the estimate is biased due to measurement noise this adjustment will still induce an association with the fasting levels. After inspection of the fasting-postprandial scatterplots of all NMR metabolites, it was observed that both cases occur in the present dataset – i.e. metabolites with a coefficient β_1 different from 1 and metabolites that had substantial measurement error on fasting and postprandial values (Fig. S1A-C). Therefore, computing the vertical residuals of the orthogonal regression model is the best approach to cover all cases.

Importantly, it was observed that for a number of metabolites, amongst which glucose and several lipids and ketones, the postprandial levels were related in a strongly nonlinear fashion to the fasting levels (Fig. S1D-F). For these metabolites equation (1) has to be generalized to the nonlinear case. It was found that the most pronounced nonlinear responses showed saturation effects for high fasting levels, which could be adequately modelled by the formula

$$y_i = \beta_0 + \beta_1 \frac{\xi_i}{\xi_i \beta_2 + 1} + g_i + \varepsilon_i \quad (3)$$

$$x_i = \xi_i + \delta_i \quad (4)$$

Note that equation (3) has the linear relationship (1) as special case, namely, when $\beta_2 = 0$. Therefore, the nonlinear errors-in-variables model (3) and (4) was applied on all metabolite data in order to use a single statistical framework and also to model the response of metabolites with more subtle nonlinear effects. As mentioned earlier, only in cases when the correlation between postprandial and fasting levels was low ($R^2 < 0.2$) the linear model (1) and (2) was used to prevent overfitting.

A possible explanation for the nonlinear fasting-response relationship of certain metabolites is that health is an underlying factor that affects both the fasting levels and the height and time of the postprandial peak. For instance, blood glucose levels in normal glycemic subjects quickly respond to a meal and return relatively quickly to zero, whereas (pre)diabetic subjects have a much slower and more blunted glucose response. So where the postprandial time measurement of 150 min. lies relative to the peak in the total postprandial time curve will therefore determine in a nonlinear fashion what the value is of y_i.

3 References

1. de Mutsert R, den Heijer M, Rabelink TJ, Smit JW, Romijn JA, Jukema JW, de Roos A, Cobbaert CM, Kloppenburg M, le Cessie S, et al. The Netherlands Epidemiology of Obesity (NEO) study: study design and data collection. *Eur J Epidemiol.* 2013;28:513–523.

2. Anderson CA, Pettersson FH, Clarke GM, Cardon LR, Morris AP, Zondervan KT. Data quality control in genetic case-control association studies. *Nat Protoc*. 2010;5:1564–1573.

3. Blauw LL, Li-Gao R, Noordam R, de Mutsert R, Trompet S, Berbée JFP, Wang Y, van Klinken JB, Christen T, van Heemst D, et al. CETP (Cholesteryl Ester Transfer Protein) Concentration: A Genome-Wide Association Study Followed by Mendelian Randomization on Coronary Artery Disease. *Circ Genomic Precis Med*. 2018;11.

4. 1000 Genomes Project Consortium, Auton A, Brooks LD, Durbin RM, Garrison EP, Kang HM, Korbel JO, Marchini JL, McCarthy S, McVean GA, Abecasis GR. A global reference for human genetic variation. *Nature*. 2015;526:68–74.

5. Howie B, Fuchsberger C, Stephens M, Marchini J, Abecasis GR. Fast and accurate genotype imputation in genome-wide association studies through pre-phasing. *Nat Genet*. 2012;44:955–959.

6. Aulchenko YS, Struchalin M V, van Duijn CM. ProbABEL package for genome-wide association analysis of imputed data. *BMC Bioinformatics*. 2010;11:134.

7. Watanabe K, Taskesen E, van Bochoven A, Posthuma D. Functional mapping and annotation of genetic associations with FUMA. *Nat Commun.* 2017;8:1826.

8. Gómez-Rubio V. ggplot2 - Elegant Graphics for Data Analysis (2nd Edition). *J Stat Softw*. 2017;77:3–5.

9. Winkler TW, Kutalik Z, Gorski M, Lottaz C, Kronenberg F, Heid IM. EasyStrata: evaluation and visualization of stratified genome-wide association meta-analysis data. *Bioinformatics.* 2015;31:259–

261.

10. Soininen P, Kangas AJ, Würtz P, Suna T, Ala-Korpela M. Quantitative Serum Nuclear Magnetic Resonance Metabolomics in Cardiovascular Epidemiology and Genetics. *Circ Cardiovasc Genet*. 2015; 8:192–206.

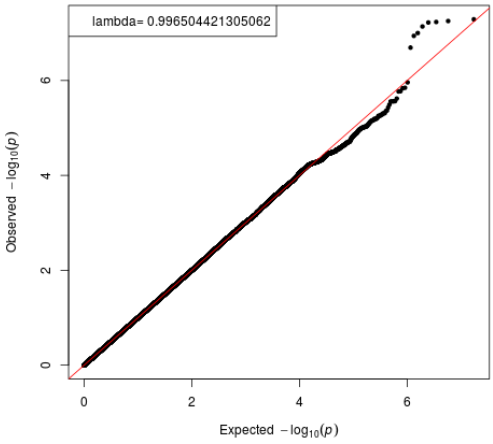
11. Kettunen J, Demirkan A, Würtz P, Draisma HH, Haller T, Rawal R, Vaarhorst A, Kangas AJ, Lyytikäinen LP, Pirinen M, et al. Genome-wide study for circulating metabolites identifies 62 loci and reveals novel systemic effects of LPA. Nat Commun. 2016;7:11122.

12. Blauw LL, Noordam R, Soidinsalo S, Blauw CA, Li-Gao R, de Mutsert R, Berbée JFP, Wang Y, van Heemst D, Rosendaal FR, et al. Mendelian randomization reveals unexpected effects of CETP on the lipoprotein profile. *Eur J Hum Genet*. 2019;27:422–431.

13. Li J, Ji L. Adjusting multiple testing in multilocus analyses using the eigenvalues of a correlation matrix. *Heredity (Edinb.)*. 2005; 95:221–227.

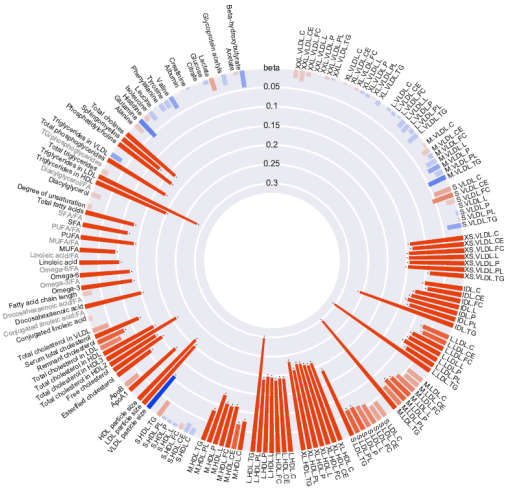
14. Fuller, W. A. (1987). Measurement Error Models. Wiley, New York.

Supplementary figure 1. Quantile-quantile plot TG response GWAS results in the NEO study

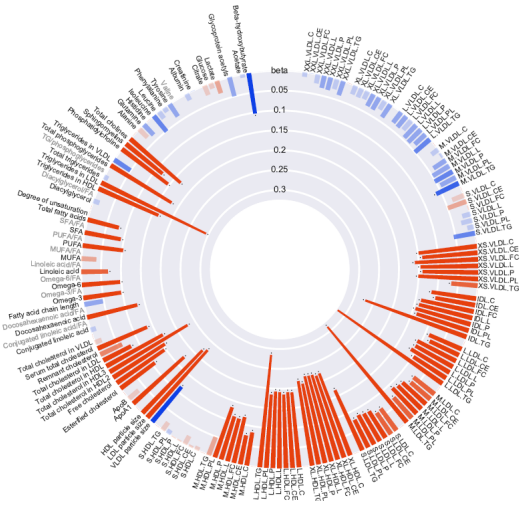


Supplementary figure 2A and 2B. The associations of rs7350789-A and fasting and postprandial NMR-based metabolic measures. Bar heights represent the magnitude of the beta coefficient from linear regression, which is expressed in standard deviation (SD) units. Red bars indicate positive betas and blue bars indicate negative betas. The transparency of the bars indicates the level of statistical significance. A p-value<0.00134 is regarded statistical significant, as represented by the black dots.

(A)



(B)



Supplementary tables

Supplementary table 1. Genome-wide significant SNPs associated with fasting TG and postprandial TG at 150 min after a mixed-meal intake

SNP	CHR	GENE	EA	EAF	BETA (SE)	PVALUE
Fasting TG						
rs780094	2	*GCKR*	C	0.64	-0.13(0.019)	6.7E-11
rs117910839	8	*LPL*	A	0.034	-0.32(0.057)	3.1E-08
rs3916027	8	*LPL*	A	0.28	-0.21(0.020)	1.3E-23
rs7005359	8	*LPL*	G	0.18	-0.17(0.028)	8.0E-10
rs6999158	8	*LPL*	A	0.31	-0.12(0.020)	4.3E-09
rs964184	11	*ZPR1,APOA1,APOA4,APOA5,APOC3*	C	0.86	-0.28(0.026)	1.6E-26
rs739846	19	*SUGP1, MAU2, (CILP2)*	A	0.072	-0.23(0.036)	1.5E-10
rs438811	19	*APOE*	T	0.25	0.15(0.021)	6.9E-13
Postprandial TG						
rs780094	2	*GCKR*	C	0.64	-0.13(0.019)	1.2E-11
rs71556736	7	*MLXIPL*	T	0.14	-0.17(0.027)	1.2E-10
rs75218485	8	*LPL*	T	0.23	-0.42(0.070)	3.0E-09
rs6999158	8	*LPL*	A	0.31	-0.13(0.020)	6.6E-11
rs117910839	8	*LPL*	A	0.034	-0.33(0.057)	5.4E-09
rs3208305	8	*LPL*	T	0.30	-0.22(0.020)	3.4E-28
rs964184	11	*ZPR1, APOA1,APOA4,APOA5,APOC3*	C	0.86	-0.29(0.026)	2.1E-28
rs8107974	19	*SUGP1, MAU2, (CILP2)*	T	0.073	-0.21(0.036)	8.1E-09
rs5117	19	*APOE*	C	0.24	0.15(0.022)	3.8E-11

SNP, single nucleotide polymorphism; CHR, chromosome; POS, position; EA, effect allele; EAF, effect allele frequency; BETA, beta coefficient; SE, standard error

Supplementary table 2. Summary statistics of the suggestive lead SNPs associated triglyceride response in response to a mixed-meal in NEO

Lead SNP	CHR	POSITION	MAPPED GENE	EA	EAF	BETA (SE)	PVALUE
rs80315981	1	50913388	*FAF1*	A	0.03	-0.30 (0.06)	2.8E-06
rs79134551	3	30588306	*TGFBR2*	A	0.07	0.22 (0.05)	1.7E-06
rs189635654	7	145998641	*CNTNAP2*	T	0.01	0.50 (0.10)	2.4E-06
rs7179279	15	26981846	*GABRB3*	G	0.81	0.11 (0.02)	2.7E-06
rs16964414	15	37258615	*MEIS2*	A	0.07	-0.19 (0.04)	1.7E-06
rs7350789	15	58679668	*LIPC*	A	0.36	-0.11 (0.02)	5.1E-08
rs73530333	19	29787769	*CTC-525D6.1*	T	0.12	0.14 (0.03)	3.2E-06

Threshold for the suggestive signals is $1x10^{-6}$.

CHR, chromosome; SNP, single nucleotide polymorphism; EA, effect allele; EAF, effect allele frequency; BETA, beta coefficient; SE, standard error

* Beta coefficient expressed in standard deviation (SD) units

Supplementary table 3. Association of the lead SNPs identified in fasting and postprandial TG GWAS analyses with TG response to a mixed-meal in NEO

Lead SNP	CHR	GENE	EA	EAF	BETA (SE)	PVALUE
rs780094	2	*GCKR*	C	0.64	0.00013 (0.02)	0.99
rs117910839	8	*LPL*	A	0.034	-0.0061 (0.059)	0.92
rs964184	11	*ZPR1,APOA1,APOA4,APOA5,APOC3*	C	0.86	0.024 (0.027)	0.37
rs739846	19	*SUGP1, MAU2, (CILP2)*	A	0.072	0.077 (0.037)	0.036
rs438811	19	*APOE*	T	0.25	-0.042 (0.022)	0.06

Threshold for genome-wide significance is 5x10-8. Threshold for the suggestive signals is 1x10-6.

CHR, chromosome; SNP, single nucleotide polymorphism; EA, effect allele; EAF, effect allele frequency; BETA, beta coefficient; SE, standard error

* Beta coefficient expressed in standard deviation (SD) units

Supplementary table 4. Sensitivity analyses. Association of rs7350789-A with TG response : crude, adjusted for BMI, adjusted for lipid-lowering medication and stratified analyses in women and men

Crude	Adjusted for BMI	Lipid-lowering medication	Stratified analysis	
			Men	Women
-0.11 (0.020), p=5.24E-08	-0.11 (0.019), p=3.2E-08	-0.11 (0.020), p=5.9E-08	-0.11 (0.030), p=1.10E-04	-0.10 (0.026), p=8.2E-05

Supplementary table 5. Associations between rs7350789-A and postprandial response of 149 metabolic measures in the NEO study (n=5744)

METABOLIC MEASURE	BETA	SE	PVALUE
Very-low-density lipoproteins (VLDL)			
XXL.VLDL.C	-0.089	0.022	4.91E-05
XXL.VLDL.CE	-0.084	0.022	1.20E-04
XXL.VLDL.FC	-0.085	0.022	9.85E-05
XXL.VLDL.L	-0.065	0.022	3.08E-03
XXL.VLDL.P	-0.067	0.022	2.16E-03
XXL.VLDL.PL	-0.080	0.022	2.55E-04
XXL.VLDL.TG	-0.059	0.022	7.15E-03
XL.VLDL.C	-0.081	0.021	1.28E-04
XL.VLDL.CE	-0.079	0.021	1.82E-04
XL.VLDL.FC	-0.084	0.021	8.38E-05
XL.VLDL.L	-0.072	0.021	7.02E-04
XL.VLDL.P	-0.070	0.021	1.05E-03
XL.VLDL.PL	-0.084	0.021	8.09E-05
XL.VLDL.TG	-0.064	0.021	2.58E-03
L.VLDL.C	-0.075	0.020	1.76E-04
L.VLDL.CE	-0.074	0.020	2.21E-04
L.VLDL.FC	-0.077	0.020	1.26E-04
L.VLDL.L	-0.062	0.020	1.88E-03
L.VLDL.P	-0.061	0.020	2.56E-03
L.VLDL.PL	-0.072	0.020	3.12E-04
L.VLDL.TG	-0.054	0.020	7.47E-03
M.VLDL.C	-0.087	0.020	1.13E-05
M.VLDL.CE	-0.086	0.020	1.29E-05
M.VLDL.FC	-0.073	0.020	2.08E-04
M.VLDL.L	-0.043	0.020	2.56E-02
M.VLDL.P	-0.058	0.020	3.16E-03
M.VLDL.PL	-0.071	0.020	3.47E-04
M.VLDL.TG	-0.043	0.020	2.91E-02
S.VLDL.C	-0.046	0.020	2.01E-02
S.VLDL.CE	-0.014	0.020	4.71E-01
S.VLDL.FC	-0.081	0.020	3.80E-05
S.VLDL.L	-0.080	0.020	5.05E-05
S.VLDL.P	-0.077	0.020	8.58E-05
S.VLDL.PL	-0.079	0.020	6.02E-05
S.VLDL.TG	-0.053	0.020	7.51E-03
XS.VLDL.C	0.013	0.020	5.18E-01
XS.VLDL.CE	0.008	0.020	6.98E-01
XS.VLDL.FC	0.030	0.020	1.24E-01
XS.VLDL.L	-0.000	0.020	9.83E-01
XS.VLDL.P	-0.010	0.020	6.23E-01

XS.VLDL.PL	0.006	0.020	7.77E-01
XS.VLDL.TG	-0.082	0.020	3.18E-05
Intermediate-density lipoproteins (IDL)			
IDL.C	0.018	0.020	3.58E-01
IDL.CE	0.012	0.020	5.50E-01
IDL.FC	0.028	0.020	1.58E-01
IDL.L	0.005	0.020	7.80E-01
IDL.P	0.017	0.020	3.91E-01
IDL.PL	0.018	0.020	3.61E-01
IDL.TG	0.013	0.020	5.01E-01
Low-density lipoproteins (LDL)			
L.LDL.C	0.003	0.019	8.76E-01
L.LDL.CE	0.009	0.019	6.55E-01
L.LDL.FC	0.036	0.020	6.75E-02
L.LDL.L	0.001	0.019	9.45E-01
L.LDL.P	0.025	0.020	2.04E-01
L.LDL.PL	0.023	0.020	2.47E-01
L.LDL.TG	0.035	0.020	6.92E-02
M.LDL.C	0.041	0.020	3.77E-02
M.LDL.CE	0.041	0.020	3.65E-02
M.LDL.FC	0.040	0.020	4.22E-02
M.LDL.L	0.013	0.020	5.00E-01
M.LDL.P	0.034	0.020	8.27E-02
M.LDL.PL	0.009	0.020	6.55E-01
M.LDL.TG	0.034	0.020	8.15E-02
S.LDL.C	0.043	0.020	2.81E-02
S.LDL.CE	0.043	0.020	2.77E-02
S.LDL.FC	0.034	0.020	8.33E-02
S.LDL.L	0.034	0.020	8.50E-02
S.LDL.P	0.032	0.020	1.02E-01
S.LDL.PL	0.008	0.020	6.75E-01
S.LDL.TG	-0.056	0.020	4.25E-03
High-density lipoproteins (HDL)			
XL.HDL.C	0.012	0.020	5.50E-01
XL.HDL.CE	0.012	0.020	5.42E-01
XL.HDL.FC	0.016	0.021	4.30E-01
XL.HDL.L	-0.002	0.020	9.40E-01
XL.HDL.P	-0.003	0.020	8.73E-01
XL.HDL.PL	-0.017	0.020	4.13E-01
XL.HDL.TG	-0.089	0.020	1.37E-05
L.HDL.C	-0.030	0.021	1.46E-01
L.HDL.CE	-0.029	0.021	1.56E-01
L.HDL.FC	-0.031	0.021	1.28E-01
L.HDL.L	0.029	0.019	1.18E-01
L.HDL.P	-0.038	0.021	6.18E-02
L.HDL.PL	-0.038	0.021	6.49E-02
L.HDL.TG	-0.034	0.020	9.30E-02

M.HDL.C	0.012	0.020	5.45E-01
M.HDL.CE	0.012	0.020	5.57E-01
M.HDL.FC	0.010	0.020	5.95E-01
M.HDL.L	-0.000	0.020	9.81E-01
M.HDL.P	-0.003	0.020	8.76E-01
M.HDL.PL	-0.000	0.020	9.83E-01
M.HDL.TG	-0.075	0.020	1.31E-04
S.HDL.C	0.047	0.020	1.62E-02
S.HDL.CE	0.055	0.020	4.96E-03
S.HDL.FC	-0.025	0.020	2.03E-01
S.HDL.L	0.002	0.020	9.27E-01
S.HDL.P	-0.003	0.020	8.71E-01
S.HDL.PL	-0.051	0.020	9.26E-03
S.HDL.TG	-0.079	0.020	6.01E-05
Lipoprotein particle size			
VLDL particle size	-0.025	0.020	1.99E-01
LDL particle size	0.033	0.020	9.27E-02
HDL particle size	0.069	0.019	2.83E-04
Apolipoproteins			
ApoA1	0.003	0.020	8.85E-01
ApoB	-0.046	0.020	1.86E-02
Cholesterols			
Esterified cholesterol	0.028	0.020	1.64E-01
Free cholesterol	0.015	0.020	4.63E-01
Total cholesterol in HDL2	0.011	0.020	5.75E-01
Total cholesterol in HDL3	0.039	0.020	4.96E-02
Total cholesterol in HDL	0.011	0.020	5.79E-01
Total cholesterol in LDL	0.037	0.020	6.19E-02
Remnant cholesterol	-0.048	0.020	1.51E-02
Serum total cholesterol	0.023	0.020	2.36E-01
Total cholesterol in VLDL	-0.093	0.020	2.65E-06
Fatty acids			
Conjugated linoleic acid	-0.029	0.025	2.43E-01
Docosahexaenoic acid	-0.018	0.020	3.64E-01
Fatty acid chain length	-0.065	0.023	4.52E-03
Omega-3	-0.044	0.020	2.63E-02
Omega-6	-0.035	0.020	7.65E-02
Linoleic acid	-0.048	0.020	1.54E-02
MUFA	-0.124	0.020	4.71E-10
PUFA	-0.038	0.020	5.48E-02
SFA	-0.038	0.020	6.16E-02
Total fatty acids	-0.073	0.020	2.63E-04
Degree of unsaturation	-0.001	0.020	9.78E-01

Glycerides			
Diacylglycerol	-0.076	0.028	7.25E-03
Triglycerides in HDL	-0.099	0.020	4.46E-07
Triglycerides in LDL	0.021	0.020	2.84E-01
Total triglycerides	-0.099	0.020	4.46E-07
Total phosphoglycerides	0.019	0.020	3.35E-01
Triglycerides in VLDL	-0.062	0.020	1.61E-03
Phospholipids			
Phosphatidylcholine	-0.014	0.021	5.05E-01
Sphingomyelins	0.012	0.020	5.59E-01
Total cholines	0.012	0.020	5.39E-01
Kidney function			
Albumin	-0.025	0.020	2.07E-01
Creatinine	-0.013	0.020	4.98E-01
Amino acids			
Alanine	0.002	0.019	9.08E-01
Glutamine	0.018	0.019	3.54E-01
Histidine	-0.024	0.020	2.29E-01
Isoleucine	-0.024	0.018	1.94E-01
Leucine	-0.024	0.018	1.86E-01
Phenylalanine	0.002	0.019	9.04E-01
Tyrosine	0.005	0.018	7.97E-01
Valine	-0.003	0.019	8.90E-01
Glycolysis			
Citrate	0.022	0.020	2.72E-01
Glucose	0.012	0.019	5.32E-01
Lactate	-0.012	0.020	5.29E-01
Inflammation			
Glycoprotein acetyls	-0.055	0.020	4.76E-03
Ketone bodies			
Acetate	-0.024	0.020	2.33E-01
Beta-hydroxybutyrate	-0.102	0.019	4.05E-08

BETA, beta coefficient; SE, standard error

Supplementary table 6. Associations between rs7350789-A and fasting and postprandial TG levels of 149 metabolic measures in the NEO study (n=5744)

	Fasting			Postprandial		
METABOLIC MEASURE	**BETA**	**SE**	**PVALUE**	**BETA**	**SE**	**PVALUE**
Very-low-density lipoproteins (VLDL)						
XXL.VLDL.C	0.022	0.018	2.35E-01	-0.011	0.019	5.57E-01
XXL.VLDL.CE	0.029	0.018	1.16E-01	-0.001	0.019	9.62E-01
XXL.VLDL.FC	0.013	0.018	4.92E-01	-0.023	0.019	2.29E-01
XXL.VLDL.L	0.002	0.018	9.33E-01	-0.031	0.019	9.93E-02
XXL.VLDL.P	0.000	0.018	9.94E-01	-0.032	0.019	8.58E-02
XXL.VLDL.PL	0.009	0.018	6.41E-01	-0.026	0.019	1.71E-01
XXL.VLDL.TG	-0.005	0.018	7.84E-01	-0.037	0.019	5.07E-02
XL.VLDL.C	0.004	0.018	8.14E-01	-0.023	0.019	2.24E-01
XL.VLDL.CE	0.003	0.018	8.90E-01	-0.024	0.019	2.02E-01
XL.VLDL.FC	0.006	0.018	7.25E-01	-0.022	0.019	2.51E-01
XL.VLDL.L	-0.008	0.018	6.81E-01	-0.033	0.019	7.66E-02
XL.VLDL.P	-0.009	0.018	6.24E-01	-0.035	0.019	6.67E-02
XL.VLDL.PL	0.002	0.018	8.92E-01	-0.025	0.019	1.83E-01
XL.VLDL.TG	-0.013	0.018	4.69E-01	-0.038	0.019	4.21E-02
L.VLDL.C	-0.007	0.019	7.09E-01	-0.033	0.019	8.24E-02
L.VLDL.CE	-0.010	0.019	5.93E-01	-0.035	0.019	6.53E-02
L.VLDL.FC	-0.005	0.019	8.05E-01	-0.031	0.019	1.08E-01
L.VLDL.L	-0.022	0.019	2.34E-01	-0.043	0.019	2.18E-02
L.VLDL.P	-0.024	0.019	1.93E-01	-0.045	0.019	1.74E-02
L.VLDL.PL	-0.013	0.019	5.04E-01	-0.036	0.019	5.58E-02
L.VLDL.TG	-0.031	0.019	1.01E-01	-0.050	0.019	8.71E-03
M.VLDL.C	0.006	0.019	7.38E-01	-0.020	0.019	2.86E-01
M.VLDL.CE	0.022	0.019	2.56E-01	-0.006	0.019	7.73E-01
M.VLDL.FC	-0.014	0.019	4.67E-01	-0.037	0.019	5.44E-02

M.VLDL.L	-0.029	0.019	1.18E-01	-0.048	0.019	1.05E-02
M.VLDL.P	-0.034	0.019	7.44E-02	-0.051	0.019	6.57E-03
M.VLDL.PL	-0.021	0.019	2.58E-01	-0.043	0.019	2.35E-02
M.VLDL.TG	-0.049	0.019	9.02E-03	-0.063	0.019	8.98E-04
S.VLDL.C	0.046	0.019	1.71E-02	0.032	0.019	1.03E-01
S.VLDL.CE	0.056	0.019	3.82E-03	0.050	0.020	1.15E-02
S.VLDL.FC	0.023	0.019	2.26E-01	0.002	0.019	9.04E-01
S.VLDL.L	-0.001	0.019	9.60E-01	-0.021	0.019	2.67E-01
S.VLDL.P	-0.008	0.019	6.79E-01	-0.028	0.019	1.40E-01
S.VLDL.PL	0.006	0.019	7.64E-01	-0.016	0.019	3.99E-01
S.VLDL.TG	-0.037	0.019	4.82E-02	-0.054	0.019	4.04E-03
XS.VLDL.C	0.139	0.019	8.28E-13	0.148	0.020	5.11E-14
XS.VLDL.CE	0.134	0.019	6.12E-12	0.140	0.020	1.23E-12
XS.VLDL.FC	0.144	0.019	1.19E-13	0.160	0.020	3.69E-16
XS.VLDL.L	0.145	0.019	9.90E-14	0.146	0.020	1.47E-13
XS.VLDL.P	0.143	0.019	2.03E-13	0.141	0.020	9.69E-13
XS.VLDL.PL	0.146	0.019	3.93E-14	0.151	0.019	1.02E-14
XS.VLDL.TG	0.089	0.019	3.38E-06	0.067	0.019	5.57E-04
Intermediate-density lipoproteins (IDL)						
IDL.C	0.099	0.019	2.73E-07	0.114	0.019	4.54E-09
IDL.CE	0.090	0.019	3.52E-06	0.102	0.019	1.78E-07
IDL.FC	0.120	0.019	3.96E-10	0.136	0.019	1.02E-12
IDL.L	0.127	0.019	5.33E-11	0.139	0.019	8.04E-13
IDL.P	0.134	0.019	4.50E-12	0.145	0.019	9.10E-14
IDL.PL	0.125	0.019	8.02E-11	0.138	0.019	6.80E-13
IDL.TG	0.258	0.019	1.36E-40	0.271	0.019	6.17E-44
Low-density lipoproteins (LDL)						

L.LDL.C	0.073	0.019	1.77E-04	0.090	0.019	4.16E-06
L.LDL.CE	0.066	0.019	6.12E-04	0.082	0.020	2.82E-05
L.LDL.FC	0.089	0.019	3.64E-06	0.110	0.019	1.13E-08
L.LDL.L	0.092	0.019	1.88E-06	0.108	0.019	2.61E-08
L.LDL.P	0.098	0.019	3.57E-07	0.114	0.019	4.97E-09
L.LDL.PL	0.084	0.019	1.39E-05	0.098	0.019	4.08E-07
L.LDL.TG	0.302	0.019	3.39E-57	0.322	0.019	1.04E-64
M.LDL.C	0.047	0.019	1.47E-02	0.068	0.020	5.45E-04
M.LDL.CE	0.045	0.019	2.17E-02	0.065	0.020	9.01E-04
M.LDL.FC	0.059	0.019	2.38E-03	0.080	0.020	3.92E-05
M.LDL.L	0.066	0.019	7.22E-04	0.083	0.020	2.11E-05
M.LDL.P	0.072	0.019	2.19E-04	0.088	0.020	5.91E-06
M.LDL.PL	0.061	0.019	1.68E-03	0.069	0.020	4.56E-04
M.LDL.TG	0.296	0.019	1.81E-55	0.312	0.018	1.09E-62
S.LDL.C	0.045	0.019	1.99E-02	0.068	0.020	5.18E-04
S.LDL.CE	0.043	0.019	2.86E-02	0.065	0.020	9.27E-04
S.LDL.FC	0.057	0.019	3.49E-03	0.079	0.019	5.50E-05
S.LDL.L	0.061	0.019	1.67E-03	0.080	0.019	3.85E-05
S.LDL.P	0.065	0.019	7.30E-04	0.084	0.019	1.71E-05
S.LDL.PL	0.065	0.019	8.47E-04	0.078	0.020	6.72E-05
S.LDL.TG	0.184	0.019	1.70E-21	0.166	0.019	1.72E-17
High-density lipoproteins (HDL)						
XL.HDL.C	0.214	0.017	1.15E-34	0.220	0.017	1.49E-37
XL.HDL.CE	0.211	0.018	5.03E-33	0.218	0.017	5.41E-36
XL.HDL.FC	0.215	0.017	7.85E-37	0.223	0.017	9.68E-40
XL.HDL.L	0.222	0.017	8.51E-39	0.223	0.017	5.08E-40
XL.HDL.P	0.223	0.017	1.92E-39	0.224	0.017	1.86E-40
XL.HDL.PL	0.209	0.017	2.11E-35	0.207	0.017	7.77E-35
XL.HDL.TG	0.233	0.019	1.64E-34	0.216	0.019	5.75E-30
L.HDL.C	0.191	0.017	7.31E-30	0.190	0.017	1.34E-29
L.HDL.CE	0.193	0.017	2.94E-30	0.191	0.017	5.18E-30
L.HDL.FC	0.187	0.017	1.42E-28	0.185	0.017	3.21E-28

L.HDL.L	0.196	0.017	1.84E-31	0.192	0.017	2.30E-30
L.HDL.P	0.198	0.017	4.52E-32	0.193	0.017	8.01E-31
L.HDL.PL	0.190	0.017	1.21E-29	0.185	0.017	2.73E-28
L.HDL.TG	0.297	0.017	1.35E-67	0.284	0.017	9.27E-65
M.HDL.C	0.075	0.018	2.40E-05	0.084	0.018	2.59E-06
M.HDL.CE	0.065	0.018	2.60E-04	0.074	0.018	3.69E-05
M.HDL.FC	0.109	0.018	5.62E-10	0.119	0.018	2.39E-11
M.HDL.L	0.107	0.018	1.72E-09	0.111	0.018	4.32E-10
M.HDL.P	0.110	0.018	5.73E-10	0.113	0.018	1.99E-10
M.HDL.PL	0.130	0.018	1.83E-13	0.133	0.018	4.46E-14
M.HDL.TG	0.082	0.020	2.56E-05	0.049	0.020	1.35E-02
S.HDL.C	-0.019	0.019	3.27E-01	0.009	0.019	6.26E-01
S.HDL.CE	-0.026	0.019	1.79E-01	0.005	0.019	7.87E-01
S.HDL.FC	0.017	0.019	3.74E-01	0.023	0.019	2.32E-01
S.HDL.L	-0.008	0.019	6.59E-01	0.003	0.019	8.78E-01
S.HDL.P	-0.008	0.019	6.84E-01	0.002	0.019	9.17E-01
S.HDL.PL	-0.008	0.019	6.68E-01	-0.011	0.019	5.55E-01
S.HDL.TG	0.046	0.019	1.60E-02	0.024	0.019	2.16E-01
Lipoprotein particle size						
VLDL particle size	-0.107	0.019	9.49E-09	-0.115	0.019	7.56E-10
LDL particle size	0.200	0.019	1.59E-26	0.228	0.019	7.08E-34
HDL particle size	0.214	0.017	3.21E-36	0.219	0.017	7.55E-38
Apolipoproteins						
ApoA1	0.161	0.017	3.55E-20	0.174	0.017	1.65E-23
ApoB	0.043	0.019	2.64E-02	0.032	0.020	1.01E-01
Cholesterols						
Esterified cholesterol	0.108	0.019	1.52E-08	0.108	0.019	1.93E-08
Free cholesterol	0.153	0.019	1.05E-15	0.146	0.019	2.67E-14
Total cholesterol in HDL2	0.140	0.017	4.07E-16	0.155	0.017	2.71E-19
Total cholesterol in HDL3	0.210	0.018	8.93E-32	0.229	0.018	3.97E-37
Total cholesterol in HDL	0.148	0.017	1.14E-17	0.163	0.017	5.29E-21

Total cholesterol in LDL	0.059	0.019	2.26E-03	0.079	0.019	5.38E-05
Remnant cholesterol	0.075	0.019	1.06E-04	0.061	0.020	2.08E-03
Serum total cholesterol	0.123	0.019	1.20E-10	0.136	0.019	1.11E-12
Total cholesterol in VLDL	0.043	0.019	2.44E-02	0.018	0.019	3.63E-01
Fatty acids						
Conjugated linoleic acid	0.010	0.019	5.97E-01	-0.014	0.019	4.77E-01
Docosahexaenoic acid	0.151	0.019	3.14E-15	0.130	0.019	1.26E-11
Fatty acid chain length	0.026	0.019	1.73E-01	-0.048	0.019	1.21E-02
Omega-3	0.133	0.019	2.44E-12	0.096	0.019	5.35E-07
Omega-6	0.130	0.019	9.85E-12	0.097	0.019	5.45E-07
Linoleic acid	0.104	0.019	5.47E-08	0.068	0.019	4.92E-04
MUFA	0.093	0.019	1.50E-06	0.037	0.019	5.42E-02
PUFA	0.139	0.019	3.77E-13	0.103	0.019	1.08E-07
SFA	0.119	0.019	8.70E-10	0.093	0.020	2.36E-06
Total fatty acids	0.130	0.019	1.90E-11	0.081	0.020	3.23E-05
Degree of unsaturation	0.012	0.019	5.25E-01	0.001	0.019	9.73E-01
Glycerides						
Diacylglycerol	0.027	0.019	1.57E-01	-0.016	0.019	3.98E-01
Triglycerides in HDL	0.199	0.019	7.41E-25	0.165	0.020	3.05E-17
Triglycerides in LDL	0.283	0.019	3.09E-50	0.294	0.019	1.46E-53
Total triglycerides	0.019	0.019	3.22E-01	-0.011	0.019	5.62E-01
Total phosphoglycerides	0.175	0.019	1.04E-20	0.180	0.019	9.88E-22
Triglycerides in VLDL	-0.031	0.019	9.42E-02	-0.049	0.019	9.98E-03
Phospholipids						
Phosphatidylcholine	0.171	0.019	9.00E-20	0.146	0.018	3.33E-15
Sphingomyelins	0.123	0.018	1.50E-11	0.119	0.018	5.69E-11
Total cholines	0.166	0.019	7.02E-19	0.169	0.018	8.20E-20
Kidney function						
Albumin	0.002	0.019	9.07E-01	-0.017	0.020	3.77E-01
Creatinine	-0.005	0.016	7.71E-01	-0.001	0.016	9.55E-01
Amino acids						

Alanine	0.028	0.019	1.45E-01	0.029	0.019	1.33E-01
Glutamine	-0.057	0.019	2.61E-03	-0.035	0.020	7.31E-02
Histidine	0.016	0.019	4.13E-01	0.001	0.020	9.57E-01
Isoleucine	-0.027	0.017	1.18E-01	-0.051	0.019	7.22E-03
Leucine	-0.002	0.017	9.10E-01	-0.021	0.019	2.72E-01
Phenylalanine	-0.022	0.019	2.57E-01	-0.012	0.019	5.25E-01
Tyrosine	-0.023	0.019	2.23E-01	-0.037	0.019	5.07E-02
Valine	-0.034	0.017	4.66E-02			
Glycolysis						
Citrate	0.000	0.019	9.86E-01	0.026	0.019	1.88E-01
Glucose	-0.012	0.018	5.05E-01	0.014	0.019	4.54E-01
Lactate	0.034	0.019	7.11E-02	0.032	0.019	9.73E-02
Inflammation						
Glycoprotein acetyls	-0.029	0.019	1.42E-01	-0.049	0.020	1.13E-02
Ketone bodies						
Acetate	0.009	0.019	6.48E-01	-0.009	0.020	6.60E-01
Beta-hydroxybutyrate	-0.044	0.019	2.13E-02	-0.103	0.018	1.36E-08

BETA, beta coefficient; SE, standard error