Genome-wide association study for circulating metabolite responses to a liquid meal: results from the NEO study

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ABSTRACT

Background

The genetics of fasting plasma metabolite concentrations has been extensively studied and yielded valuable insight in underlying pathways and associated pathophysiology. However, due to frequent food intake, humans spend the greater part of the day in a postprandial state. We set out to examine the genetic contribution to variation in postprandial plasma metabolite concentrations and responses to a liquid meal.

Methods

All participants in the Netherlands Epidemiology of Obesity study (N=5,705) consumed a liquid mixed meal after an overnight fast. Metabolomic measurements were performed in both fasting and postprandial (t=150 min) plasma samples using the Nightingale Proton NMR platform. Genome-wide association studies (GWAS) of fasting and postprandial metabolite concentrations were performed by linear regression. In addition, the genetics of the metabolite responses, as calculated by both baseline-adjusted nonlinear residuals and linear mixed models, were examined.

Results

GWAS of fasting metabolite concentrations replicated 36 out of 46 previously identified genetic associations. In comparison to fasting results, postprandial metabolite concentrations resulted in highly overlapping genetic signals. By using baseline-adjusted nonlinear residuals as metabolite responses, a strong association of rs10830963:G in the melatonin receptor 1B (*MTNR1B)* gene with the glucose response residual was identified (beta (SE): -0.23 (0.03), P-value: 2.2×10^{-19}), which was also observed using a linear mixed model based approach (beta (SE): 0.05 (0.01), P-value: 3.6×10^{-9}).

Conclusions

The genetics of fasting and postprandial metabolite concentrations overlap substantially. rs10830963 in the *MTNR1B* gene is a genetic determinant of the postprandial glucose response. Since the ligand of the MTNR1B receptor, melatonin, plays a role in the sleep wake cycle, this finding suggests a role for circadian rhythmicity in the plasma glucose response to a meal.

INTRODUCTION

Metabolites are considered intermediates between genes and clinical phenotypes (1). Recent developments in high-throughput metabolomic profiling based on mass spectrometry (MS) (1-4) and nuclear magnetic resonance (NMR) (2; 5-9) platforms have opened new avenues to explore gene-metabolite associations by genome-wide association studies (GWAS). In 2014, an atlas was generated involving 145 genetic loci associated with a broad spectrum of blood metabolites covering amino acids, carbohydrates, lipids, and peptides (10). A subsequent 2016 study, evaluating circulating fasting metabolites (mainly lipoprotein subclasses), observed over 60 genetic loci that are associated with at least one metabolite (11). These studies have provided considerable mechanistic insight into physiological pathways of diseases. However, the predominant focus of previous studies has been on the genetics of fasting metabolite concentrations. Here, we expand on these observations by including GWAS for postprandial abundances and metabolite response phenotypes.

Due to irregular meal intake, humans spend the majority of their waking hours in a non-fasting state. However, insight in the genes that affect plasma metabolites in response to food intake is limited. It seems more than likely that cumulative and prolonged exposure to specific plasma metabolites in response to food intake may have pathological consequences. This has been well documented for certain lipid metabolites (12-16), but may also be true for other metabolites. In clinical practice, an oral-glucose tolerance test (OGTT) is commonly used for the screening of suspected diabetes and is performed by determining glucose levels two hours after ingestion of a fixed dose of glucose. Previous GWAS investigations have identified genetic loci that are associated with glucose and insulin responses from the OGTT (17), which only partly overlap with the GWAS findings on fasting glucose and insulin measures. The measurements from the OGTT have expanded the understanding of genetics and pathophysiology of diabetes. However, our food intake is more than sugar alone, and similar to OGTT, meal responses reflected by metabolite profiles are likely to exhibit a large amount of variability, part of which will be determined by genetics. A recent candidate gene study showed that the genetics of fasting and postprandial metabolite concentrations are overlapping (18). Here we set out to extend those initial observations.

In the current study, we aimed to use GWAS to 1) identify novel and evaluate previously reported genetic contributions to variation in fasting metabolite concentrations, 2) discover genetic contributions to variation in postprandial metabolite concentrations, and 3) investigate the genetic basis of individual metabolite responses (the change from fasting state to postprandial state after a liquid mixed meal) in a large (N=5,705) population-based cohort study, the Netherlands Epidemiology of Obesity (NEO) study.

MATERIALS AND METHODS

Study design and study population

This study was performed in a population-based prospective cohort, the Netherlands Epidemiology of Obesity (NEO) study (19). All 6,671 participants gave written informed consent and the Medical Ethical Committee of the Leiden University Medical Center (LUMC) approved the study design. Initiated in 2008, the NEO study was designed to study pathways that lead to obesity-related diseases. Detailed information about the study design and data collection has been described elsewhere (19). Briefly, men and women aged between 45 and 65 years with a self-reported body mass index (BMI) of 27 kg/m^2 or higher living in the greater area of Leiden (in the west of the Netherlands) were eligible to participate in the NEO study. In addition, all inhabitants aged between 45 and 65 years from one municipality (Leiderdorp) were invited irrespective of their BMI. Participants were invited for a baseline visit at the NEO study center in the LUMC after an overnight fast. Prior to their visits, participants completed a questionnaire at home with demographic, lifestyle and clinical data. At the baseline visit, fasting blood samples were drawn. Within the next five minutes after the fasting blood draw, a liquid mixed meal (400mL with 600 kcal, with 16 percent of energy (En%) derived from protein, 50 En% carbohydrates, and 34 En% fat) was consumed and subsequent blood samples were drawn 30 and 150 minutes after the liquid mixed meal. Individuals were excluded from the analyses (Figure 1) when 1) taking any lipid-lowering medication, 2) violating overnight fasting, 3) violating liquid meal challenge protocol.

Genotyping and imputation

DNA was extracted from 6,671 venous blood samples obtained from the antecubital vein. Genotyping was performed in the Centre National de Génotypage (Evry Cedex, France), using Illumina HumanCoreExome-24 BeadChip (Illumina Inc., San Diego, California, United States of America). The detailed quality control process has been described previously (20). Genotypes were further imputed to the Haplotype Reference Consortium (HRC) release 1.1 (21). All genetic variants with an imputation quality below 0.4 or a minor allele frequency (MAF) below 0.01 were not considered for the analyses in the present study. As such a total of 5,705 individuals with genotype data for 7,701,731 variants were used in our association analysis.

NMR spectroscopy-based plasma metabolite quantification

Metabolomic measurements were performed in both fasting and postprandial (t=150 minutes after the liquid meal) plasma samples using the Nightingale high-throughput NMR metabolomics platform (22). No metabolomic measurements at the 30 minute sampling interval were measured. The metabolomics platform provides 148 metabolites

(Supplemental Table S1) from eleven substance classes: lipoprotein subclasses (n=98), lipoprotein particle sizes (n=3), apolipoproteins (n=2), fatty acids and saturation (n=11), cholesterol (n=9), glycerides and phospholipids (n=9), amino acids (n=8), ketone bodies (n=2), inflammation (n=1), glycolysis related metabolites (n=3), and fluid balance (n=2). The NMR-based metabolomics platform and the experimental procedure have been described in details previously (23).

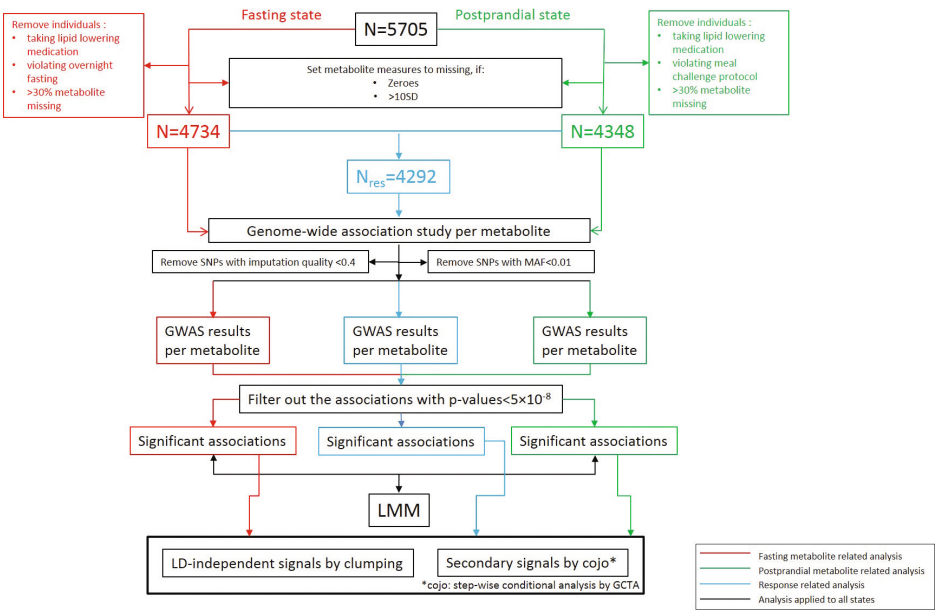

FIGURE 1. Analysis workflow.

Metabolite quality control and transformations

To remove samples with low quality and measurement errors, individuals were excluded when 1) metabolite concentrations deviated more than 10 standard deviation of the mean values derived from the entire NEO population and 2) more than 30% of missingness on all 148 metabolite concentrations under either fasting or postprandial states (Figure 1). Metabolite concentrations were inverse rank normal transformed (24) using an edited version of the rntransform() function from the GenABEL package (25), which randomly ranks tied values. When analysing metabolites in the fasting or postprandial states alone each state was transformed independently of each other. However, when analysing or deriving the response phenotype the data from the two states were merged prior to data transformation. As it was previously observed that sampling date (a compound variable composed of blood sampling year-month) has an appreciable effect on metabolite concentration in this data set, we included this variable as a covariate prior to / during linear regression (26).

TABLE 1. LD-independent significant signals by nonlinear residual metabolite responses to a liquid meal.

Metabolite	SNP	Chr	POS	Gene	EA/ NEA	EAF	Response			Fasting			Postprandial		
							beta	se	p-value	beta	se	p-value	beta	se	p-value
Lead signals															
Glc*	rs10830963	11	92708710	*MTNR1B*, intronic	G/C	0.26	0.19	0.02	2.15E-19	0.19	0.02	1.85E-15	-0.02	0.03	3.78E-01
Glc	rs7936247	11	92690032	intergenic	T/G	0.32	0.14	0.02	2.35E-13	0.14	0.02	2.21E-10	-0.01	0.02	6.21E-01
XXLVLDLC*	rs458741	5	55807837	AC022431.2, intronic	C/T	0.77	-0.02	0.03	5.76E-10	-0.02	0.03	4.76E-01	0.04	0.03	8.64E-02
XXLVLDLCE*	rs458741	5	55807837	AC022431.2, intronic	C/T	0.77	-0.03	0.03	9.74E-10	-0.03	0.03	1.95E-01	0.04	0.03	1.35E-01
Suggestive signals															
XXLVLDLL*	rs467022	5	55805639	CTC-236F12.4, upstream gene variant	T/C	0.77	-0.01	0.03	1.73E-09	-0.01	0.03	6.86E-01	0.05	0.03	5.99E-02
XXLVLDLP*	rs467022	5	55805639		T/C	0.77	-0.01	0.03	2.11E-09	-0.01	0.03	6.90E-01	0.05	0.03	5.88E-02
XXLVLDLTG*	rs467022	5	55805639		T/C	0.77	-0.01	0.03	2.58E-09	-0.01	0.03	7.38E-01	0.05	0.03	5.65E-02
XXLVLDLFC*	rs173964	5	55809465	AC022431.2, intronic	G/A	0.78	-0.01	0.03	4.26E-09	-0.01	0.03	7.47E-01	0.05	0.03	7.58E-02
XXLVLDLPL*	rs173964	5	55809465		G/A	0.78	-0.01	0.03	5.32E-09	-0.01	0.03	7.33E-01	0.05	0.03	8.00E-02
DHA*	rs143754716	11	92131010	*FAT3*, intronic	G/C	0.02	-0.05	0.09	7.00E-09	-0.05	0.09	5.59E-01	0.17	0.09	7.07E-02
XLVLDLCE*	rs467022	5	55805639	CTC-236F12.4, upstream gene variant	T/C	0.77	0.01	0.03	7.49E-09	0.01	0.03	5.68E-01	0.04	0.03	1.29E-01
His*	rs7982187	13	27470380	intergenic	G/T	0.24	-0.08	0.02	9.80E-09	-0.08	0.02	1.16E-03	0.05	0.03	3.75E-02
XLVLDLC*	rs173964	5	55809465	AC022431.2, intronic	G/A	0.77	0.01	0.03	1.11E-08	0.01	0.03	6.19E-01	0.03	0.03	1.83E-01
SHDLFC*	rs114652642	5	105906380	intergenic	C/A	0.03	0.24	0.07	1.23E-08	0.24	0.07	7.21E-04	0.09	0.07	2.38E-01
XLVLDLFC*	rs173964	5	55809465	AC022431.2, intronic	G/A	0.77	0.02	0.03	2.00E-08	0.02	0.03	5.22E-01	0.04	0.03	1.46E-01
FreeC*	rs143642501	16	82387230	intergenic	T/C	0.02	-0.20	0.11	2.07E-08	-0.20	0.11	6.59E-02	0.13	0.12	2.63E-01
SLDLCE*	rs28855728	4	31252214	intergenic	A/G	0.14	0.01	0.03	2.66E-08	0.01	0.03	8.65E-01	-0.03	0.03	2.76E-01
VLDLC	rs138698912	11	121783017	intergenic	A/G	0.01	-0.28	0.13	3.21E-08	-0.28	0.13	3.25E-02	0.01	0.14	9.19E-01
LDLTG*	rs2037053	12	101646796	intergenic	G/C	0.04	0.06	0.06	3.74E-08	0.06	0.06	3.19E-01	-0.07	0.06	2.59E-01
SLDLC*	rs28855728	4	31252214	intergenic	A/G	0.14	0.01	0.03	4.18E-08	0.01	0.03	8.15E-01	-0.03	0.03	3.33E-01
MLDLTG*	rs2037053	12	101646796	intergenic	G/C	0.04	0.06	0.06	4.20E-08	0.06	0.06	2.63E-01	-0.07	0.06	2.12E-01

TABLE 1. Continued.

Metabolite	SNP	Chr	POS	Gene	EA/ NEA	EAF	Response			Fasting			Postprandial		
							beta	se	p-value	beta	se	p-value	beta	se	p-value
SHDLFC*	rs150441681	13	100184791	*TM9SF2*, intronic	G/A	0.04	-0.03	0.06	4.30E-08	-0.03	0.06	5.40E-01	0.10	0.06	9.79E-02
XLHDLFC*	rs116717021	5	168758491	intergenic	T/C	0.03	0.05	0.07	4.44E-08	0.05	0.07	4.17E-01	-0.04	0.07	5.71E-01
MVLDLCE	rs138698912	11	121783017	intergenic	A/G	0.01	-0.29	0.13	4.52E-08	-0.29	0.13	2.52E-02	0.03	0.14	8.40E-01
IDLTG*	rs118039629	12	101803806	*ARL1*, upstream gene variant	A/G	0.02	0.03	0.08	4.78E-08	0.03	0.08	7.00E-01	-0.13	0.08	1.06E-01
IDLTG*	rs71473282	10	88666064	*BMPR1A*, intronic	A/G	0.02	0.08	0.07	4.86E-08	0.08	0.07	2.84E-01	-0.04	0.07	5.50E-01

SNP: single nucleotide polymorphism; Chr: chromosome; EA/NEA: effect allele (coding allele)/non-effect allele (non-coding) allele; EAF: effect allele frequency/coding allele frequency; beta: effect size per coding allele; SE: standard error.

* All secondary signals that were identified by step-wise conditional analysis in GCTA were highlighted with a star in the first column called Metabolite.

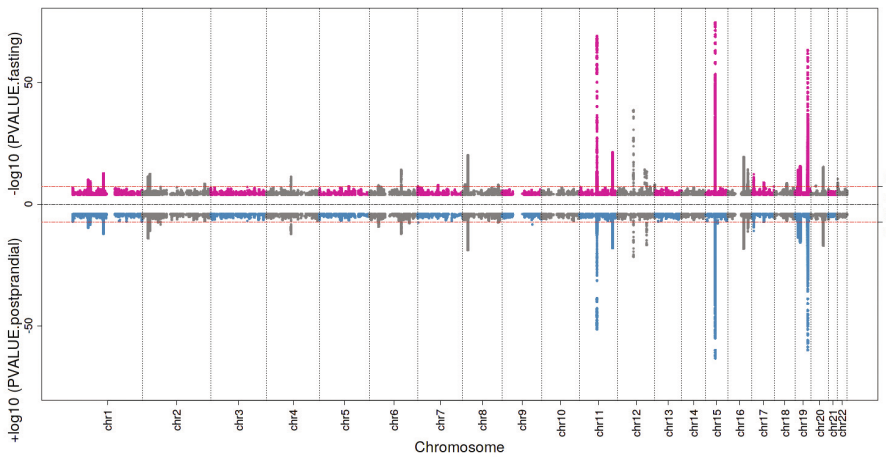

FIGURE 2. Miami plot of 148 fasting and postprandial metabolites.

Defining metabolite response to a liquid mixed meal

Metabolite response, or the change in metabolite concentration between a fasting and postprandial state was analysed in two manners. First and foremost, for each metabolite, we derived a response phenotype defined as the residuals of an orthogonal nonlinear least squares (OrNLS, Supplemental Materials) regression where the postprandial state was set as the dependent variable and the fasting state is the independent variable in a univariate analysis. Response could have been defined as a simple estimation of change or a delta between postprandial and fasting states. However, we observed that for 30 of the 148 metabolites analysed the data were best explained by a non-linear curve as opposed to a linear one, possibly because a physiological plateauing effect for some metabolites (Supplemental Figure 1). As such, a simple delta estimates of change or residuals derived from simple linear regressions between the two states would not accurately capture the variation of response, or metabolite change.

Alternatively, and only for genotype-phenotype associations discovered in the fasting or postprandial state, we also evaluate response via linear mixed models (LMM) that included an interaction term between dietary state and genotype alongside individual random effects. We reasoned that genotype-phenotype associations identified in either the fasting or postprandial state would be good candidates for response or variability in genotypic effects across the two states. Under this framework we simultaneous measure the effect of dietary state, genotype, and the interaction of state and genotype on metabolite concentration. Here, the interaction term provides a measurement of genotype on response, or specifically a differential effect across the two states. Given the computational expense of the LMM, we choose not to implement

this second procedure genome-wide but rather only on those associations with any previous evidence of effect on metabolite concentration, thereby also reducing our testing burden.

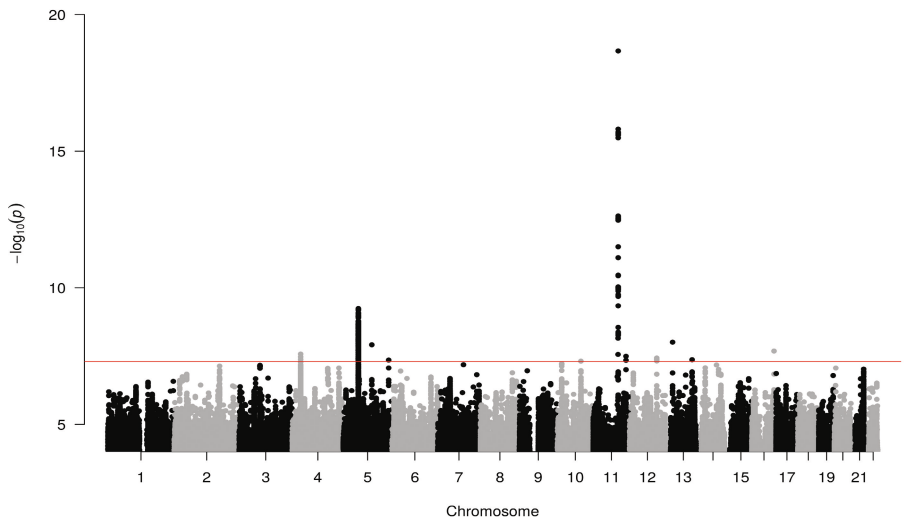

FIGURE 3. Manhattan plot of 148 metabolite response measured by OrNLSr nonlinear residuals.

Genome-wide association analyses of metabolites under different prandial states

Three unique GWAS, fasting, postprandial, and response were performed on 148 metabolites across 4734, 4348, and 4292 individuals, respectively (Figure 1), and 7,701,731 genetic variants across the 22 autosomal chromosomes. Linear regression analyses, assuming an additive genetic model, were performed with SNPTESTv2 (27) on the residuals of inverse rank normal transformed metabolite concentrations after adjusting for age, sex, the first ten principal components and the batch effect variable sampling date (a compound variable composed of blood sampling year-month).

SNP-metabolite associations that reached genome-wide significance (i.e., P-value $<5\times10^{-8}$) in either the fasting or postprandial state GWAS were further evaluated in a LMM framework to test for response, or an interaction between genotype and dietary state. The linear mixed model was applied using the function lmer in the R package "lme4" (28), fitted using restricted maximum likelihood (REML), and P-values of LMM significance were derived by Satterthwaite approximations, to control for type 1 error rates (29). A significant association from LMMs was determined by controlling the false discovery rate at 5%, i.e. controlling the expected proportion of false discoveries among the rejected hypotheses less than 5%, by R function "p.adjust(method="fdr")".

To identify independent loci in each GWAS and LMM tested signals, we first identified linkage disequilibrium (LD)-independent blocks by clumping all variants with a standard GWAS significance level of P-value<5×10^{-8} in PLINK (30). All genetic variants with a P-value below the threshold ('-clump-p1') were set as "index" SNPs, and all the other SNPs were clustered into different clumps or LD blocks based on their linkage disequilibrium (LD) and physical proximity to the "index" SNPs controlled by 'clump-r2' and 'clump-kb' separately in the command (4). In the current analyses, the following parameters were adopted: '--clump-p1' 5.0×10^{-8}, '--clump-r2' 0.5, '--clump-kb' 1000. Linkage disequilibrium patterns were based on the 1000 Genome v3 20101123 reference set of Utah Residents (CEPH) with Northern and Western European Ancestry (CEU) population (31).

Secondary signals were subsequently identified through step-wise conditional analyses using the genome-wide complex trait analysis (GCTA) tool version 1.24.4 (32), with a parameter of minor allele frequency (MAF) >0.01. A conditional P-value <5×10^{-8} was considered to be genome-wide significant. The NMR-based platform used here focuses heavily on lipoprotein subclasses that exhibit strong intercorrelations (Supplemental Figure 2). To account for these intercorrelations in FDR based multiple test corrections, we applied the variance decomposition method proposed by Li et al (33). This resulted in 39/38/44 independent components underlying 148 metabolites in the fasting state, the postprandial state, and the response as determined by OrNLS respectively. Accordingly, metabolome-wide significance was set to 1.28×10^{-9}, 1.32×10^{-9}, 1.14×10^{-9} for fasting, postprandial and nonlinear residual metabolite response GWAS, respectively, where the standard GWAS significance level (5×10^{-8}) was divided by the number of independent components underlying 148 metabolites in each state determined by variance decomposition (33). The analysis workflow is shown in Figure 1. The associations with P-value <5×10^{-8} were defined as suggestive signals and considered for identifying LD-independent blocks and secondary signals. For the associations reached metabolome-wide significance, they were called the lead signals and reported in the Results.

Estimates of heritability

The narrow-sense SNP-based heritability for each metabolite under fasting, postprandial and OrNLS derived response states was estimated by restricted maximum likelihood (GREML) under the framework of genome-wide complex trait analysis (GCTA). Genetic variants (both genotyped and imputed genotype data) with a minor allele frequency (MAF) >1% were retained for the analysis (32).

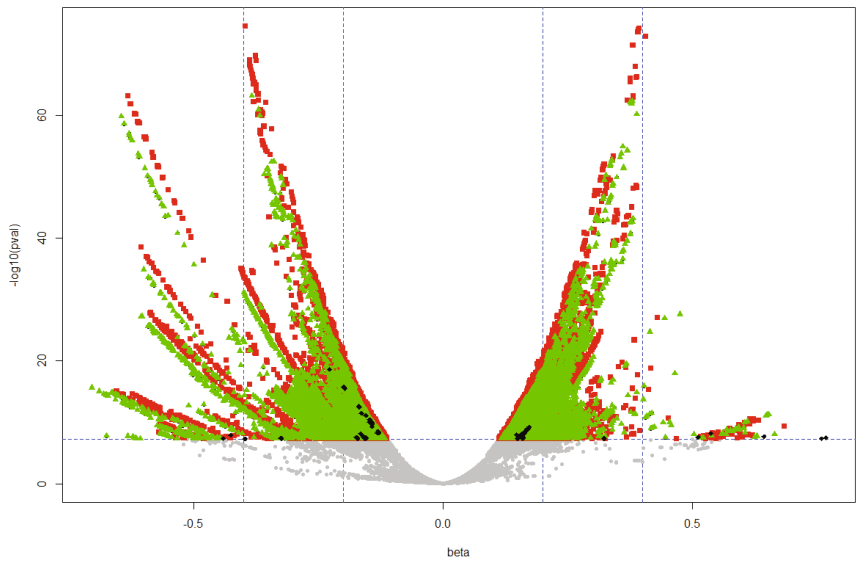

FIGURE 4. Volcano plot of all signals reached genome-wide association threshold (P-value<5×10^{-8}) on different fasting status. The horizontal dash line corresponds to p-value = 5×10^{-8}.

RESULTS

Description of metabolomics data under different prandial states

Postprandial metabolite concentrations were correlated with their fasting levels (median absolute Pearson's r = 0.29, interquartile range [0.12, 0.59]). Moreover, for 30 metabolites, these associations were non-linear (Supplemental Figure 1). When determining a meal response parameter for a metabolite conditional on the fasting level, the fasting and postprandial associations should be taken into account. For this, we adopted orthogonal nonlinear least squares (OrNLS) regressions to estimate the metabolite responses to a liquid meal. Interestingly, the metabolite response as determined by OrNLS showed low correlations to either fasting (median absolute Pearson's r = 0.088, interquartile range [0.043, 0.14]) or postprandial (median absolute Pearson's r = 0.11, interquartile range [0.047, 0.19]) state measures.

Fasting GWAS

In total 32,212 SNP-fasting metabolite concentration associations were discovered at a P-value less than 5×10^{-8} across 144 of the 148 tested metabolites (Supplementary Table 3), which were further identified LD-independent blocks by clumping. These variants map to 2249 unique loci, where variants were clustered into 348 linkage disequilibrium

blocks given a linkage disequilibrium r2 greater than 0.5 (Supplementary Table 4). In addition, 759 secondary signals were identified using joint conditional analysis (Supplementary Table 5). Among the 62 associations previously reported by Kettunen et al. (11) between SNPs and fasting metabolites, 46 could be tested in the current fasting metabolite GWAS (the other 16 associations could not be evaluated due to our quality control: SNP MAF<0.01 or imputation quality <0.4). Among these 46 identified associations, 36 were successfully replicated (FDR corrected P-value < 0.05) in the NEO study (Supplemental Table 2).

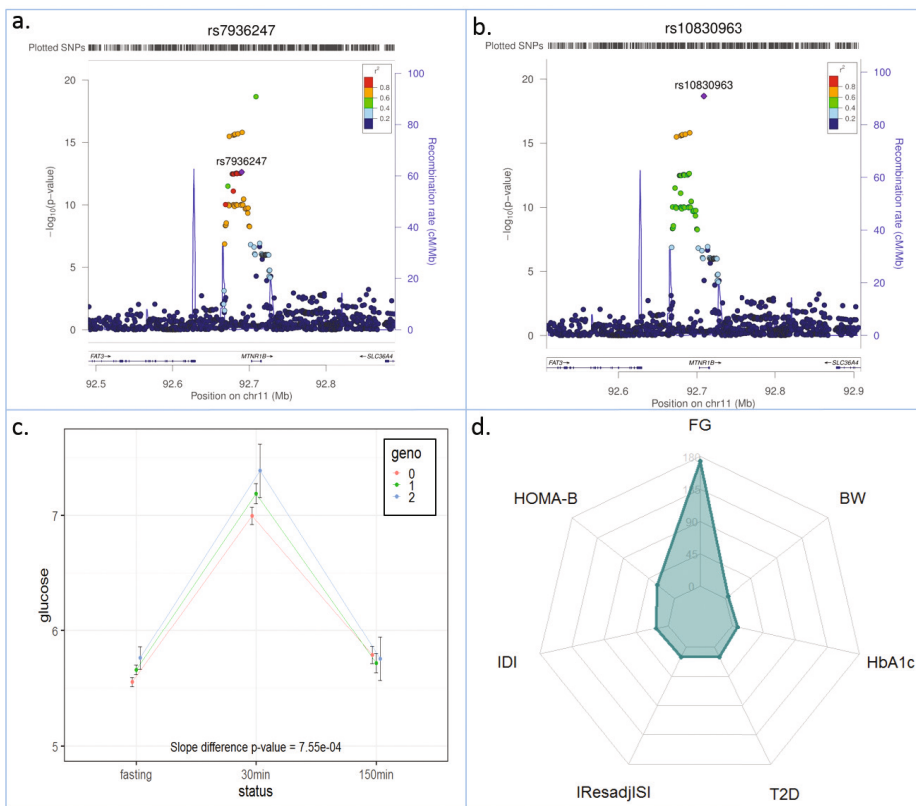

FIGURE 5. Combinatorial plot of glucose response signals identified from GWAS. (a). Regional plot for the lead signal rs7936247; (b). Regional plot for the lead signal rs10830963; (c). Glucose levels at fasting, 30 minutes and 150 minutes after a liquid mixed meal in the NEO cohort, stratified by rs10830963 genotype; (d). The -log10(p-values) of associations between rs10830963 and several glycaemic traits from literature; FG: fasting glucose; BW: birth weight; HbA1c: Hemoglobin A1C (HbA1c) test; T2D: type 2 diabetes; IResadjISI: corrected insulin response adjusted for insulin sensitivity index; IDI: Insulin disposition index; HOMA-B: the Homeostasis Model Assessment (HOMA) estimates steady state beta cell function (%B).

Postprandial GWAS

At a P-value less than 5×10^{-8}, the postprandial metabolite GWAS identified 30,747 genotype-metabolite concentration associations (Supplementary Table 6), mapping to 286 LD-independent autosomal regions (Figure 2, Supplementary Table 7). In addition 689 unique secondary signals were identified (Supplementary Table 8). Among the 30,747 associations, 26,419 of them were also identified in the fasting state, largely mirroring the observations of fasting level metabolites (Figure 4 and Supplemental Figure 3). Another 4328 associations are unique in the postprandial state. In addition, effect estimates are stronger in the postprandial state (Wilcoxon rank sum test of abs(beta) estimates P-value<2.2×10^{-16}) relative to the fasting state (Figure 4).

Response GWAS by orthogonal nonlinear least squares regression (OrNLSr)

In this genome-wide analysis, we identified 234 genetic variants that contributed to variation in metabolite response to a meal (P-value <5×10^{-8}, Supplementary Table 9), mapping to 16 LD-independent genomic regions across 23 metabolites (Table 2). In addition, 23 secondary associations were also identified (Supplementary Table 10). Only two of the LD-independent associations (rs10830963:fasting glucose levels; rs7936247:fasting glucose levels) were also observed in either fasting or postprandial states (Supplemental Figure 3 and Figure 4).

Two regions, one on chromosome 5 and a second on chromosome 11 harboured the strongest associations for metabolite response (Figure 3). Association signals are attributed to four LD-independent loci. Two signals, rs10830963 (MAF: 0.26, beta (SE): 0.19 (0.02), P-value: 2.2×10^{-19}) and rs7936247 (MAF: 0.32, beta (SE): 0.14 (0.02), P-value: 2.4×10^{-13}), on chromosome 11 are located at the *MTNR1B* locus (rs10830963 and rs7936247; LD r2 = 0.49; Figure 5a-b and Table 2) and are associated with glucose response. Another signal rs458741 located on chromosome 5 was associated with extremely large VLDL total cholesterol levels (XXLVLDLC) (MAF: 0.23, beta (SE): -0.02 (0.03), P-value: 2.76×10^{-10}) and extremely large VLDL cholesterol esters levels (XXLVLDLCE) (MAF: 0.23, beta (SE): -0.03 (0.03), P-value: 9.74×10^{-10}), respectively.

Given the observed association between rs10830963 and glucose response, glucose as measured by a clinical chemistry laboratory at fasting state, 30 minutes and 150 minutes after a liquid mixed meal were examined in the NEO cohort by stratifying on rs10830963 genotype. The postprandial glucose excursions, defined as the change in glucose concentrations from before to after a meal, showed a significant difference (fasting status and genotype interaction p-value: 7.6×10^{-4}) (Figure 5c). rs10830963 has been linked to several glycaemic traits in previous large-scale GWAS meta-analysis, with the strongest signals on fasting glucose (Figure 5d), followed by HOMA-β.

TABLE 2. LD-independent signals by linear mixed model after FDR correction.

Metabolite	SNP	Chr	POS	Gene	EA/ NEA	EAF	LMM			Fasting			Postprandial			Response		
							beta	se	p-value	beta	se	p-value	beta	se	p-value	beta	se	p-value
Glc	rs10830963	11	92708710	*MTNR1B*, intronic	G/C	0.26	0.05	0.01	3.64E-09	0.19	0.02	1.85E-15	-0.02	0.03	3.78E-01	-0.23	0.03	2.15E-19
Glc	rs2121650	11	92679138	intergenic	A/G	0.35	-0.06	0.01	2.11E-06	0.13	0.02	1.52E-09	-0.02	0.02	4.41E-01	-0.15	0.02	7.94E-12
HDL3C	rs55696635	15	58709750	*LIPC*, intronic	A/G	0.19	0.05	0.01	1.82E-04	0.22	0.03	1.05E-16	0.26	0.03	1.61E-21	0.08	0.03	2.90E-03
HDLTG	rs10468017	15	58678512	*LIPC*, intronic	T/C	0.30	-0.06	0.01	3.59E-07	0.22	0.02	1.09E-23	0.18	0.02	1.80E-14	-0.09	0.02	6.41E-05
HDLTG	rs443401	15	58692095	*LIPC*, intronic	G/C	0.48	0.05	0.01	5.07E-06	-0.19	0.02	5.57E-19	-0.16	0.02	1.35E-12	0.08	0.02	2.90E-04
HDLTG	rs35128881	15	58692148	*LIPC*, intronic	T/G	0.20	0.60	0.13	7.56E-06	0.22	0.03	2.81E-17	0.18	0.03	1.42E-11	-0.10	0.03	1.83E-04
HDLTG	rs792902	15	58716044	*LIPC*, intronic	G/A	0.52	0.06	0.01	4.06E-05	-0.18	0.02	1.94E-13	-0.14	0.03	1.64E-08	0.08	0.03	1.79E-03
LVLDLC	rs73004967	19	19717056	*PBX4*, intronic	G/A	0.07	0.06	0.01	8.05E-05	-0.27	0.04	5.90E-11	-0.21	0.04	9.13E-07	0.13	0.04	2.03E-03
LVLDLCE	rs17217098	19	19702384	*PBX4*, intronic	A/G	0.07	-0.15	0.04	2.47E-05	-0.27	0.04	7.37E-11	-0.22	0.04	4.40E-07	0.12	0.04	4.52E-03
LVLDLFC	rs73004967	19	19717056	*PBX4*, intronic	G/A	0.07	0.06	0.01	2.89E-05	-0.28	0.04	2.49E-11	-0.20	0.04	1.67E-06	0.14	0.04	1.53E-03
LVLDLFC	rs146782026	6	140383624	RP3-332B22.1	C/T	0.01	0.04	0.01	9.12E-05	-0.44	0.11	8.62E-05	-0.63	0.11	1.75E-08	-0.30	0.11	7.10E-03
LVLDLL	rs17217098	19	19702384	*PBX4*, intronic	A/G	0.07	-0.06	0.02	5.19E-05	-0.27	0.04	1.17E-10	-0.20	0.04	2.12E-06	0.13	0.04	3.66E-03
MVLDLC	rs17216693	19	19666574	intergenic	C/T	0.14	-0.06	0.02	1.58E-04	-0.18	0.03	1.85E-09	-0.15	0.03	4.41E-06	0.08	0.03	8.34E-03
MVLDLC	rs12274192	11	116652351	*ZNF259*, intronic	G/A	0.07	-0.14	0.04	2.06E-04	0.27	0.04	5.06E-11	0.24	0.04	2.95E-08	-0.10	0.04	2.43E-02
MVLDLCE	rs61905086	11	116614863	*BUD13*, downstream gene variant	C/T	0.07	-0.11	0.03	1.35E-04	0.25	0.04	1.08E-10	0.23	0.04	4.66E-08	-0.11	0.04	6.54E-03

TABLE 2. Continued.

Metabolite	SNP	Chr	POS	Gene	EA/ NEA	EAF	LMM			Fasting			Postprandial			Response		
							beta	se	p-value	beta	se	p-value	beta	se	p-value	beta	se	p-value
MVLDLFC	rs146782026	6	140383624	RP3-332B22.1	C/T	0.01	-0.15	0.04	9.86E-05	-0.42	0.11	9.80E-05	-0.61	0.11	4.57E-08	-0.31	0.11	5.02E-03
Phe	rs7295308	12	103295482	*PAH*, intronic	G/A	0.15	0.08	0.02	8.60E-05	-0.10	0.03	9.43E-04	-0.19	0.03	4.72E-10	-0.03	0.03	3.55E-01
SerumTG	rs146782026	6	140383624	RP3-332B22.1	C/T	0.01	0.10	0.02	6.75E-05	-0.42	0.11	1.11E-04	-0.62	0.11	2.10E-08	-0.33	0.11	2.44E-03
TotPG	rs11604424	11	116651115	*ZNF259*, intronic	T/C	0.79	-0.08	0.02	6.68E-05	-0.15	0.03	9.25E-09	-0.07	0.03	5.72E-03	0.10	0.03	9.84E-05
XLHDLTG	rs964184	11	116648917	*ZNF259*, 3'prime UTR variant	C/G	0.87	-0.07	0.02	7.98E-06	-0.18	0.03	4.66E-09	-0.14	0.03	1.65E-05	0.00	0.03	9.14E-01
XXLVLDLC	rs12280753	11	116613660	intergenic	T/C	0.08	-0.08	0.02	1.99E-05	0.25	0.04	1.63E-09	0.23	0.04	1.51E-08	-0.11	0.04	1.50E-02
XXLVLDLL	rs12280753	11	116613660	intergenic	T/C	0.08	-0.07	0.02	2.59E-04	0.25	0.04	1.93E-09	0.23	0.04	2.94E-08	-0.09	0.04	3.72E-02
XXLVLDLP	rs12280753	11	116613660	intergenic	T/C	0.08	0.05	0.01	3.39E-05	0.25	0.04	1.91E-09	0.23	0.04	3.11E-08	-0.10	0.04	2.25E-02
XXLVLDLTG	rs12280753	11	116613660	intergenic	T/C	0.08	-0.06	0.01	2.52E-04	0.25	0.04	1.90E-09	0.23	0.04	3.49E-08	-0.10	0.04	2.60E-02

SNP: single nucleotide polymorphism; Chr: chromosome; POS: position; EA/NEA: effect allele (coding allele)/non-effect allele (non-coding) allele; EAF: effect allele frequency/coding allele frequency; beta: effect size per coding allele; SE: standard error.

Testing for response effects by linear mixed model

To further determine whether genetic variants associated with fasting or postprandial metabolite concentrations affect response, we performed a targeted linear mixed model analysis for response on these genotype-to-metabolites associations. In total 36541 genotype-metabolite associations were tested, 204 of which exhibited a response effect (genotype-by-dietary state interaction) at an FDR of 5% (Supplemental Table 11). These associations map to 17 genomic regions across 19 metabolites (Table 2). The strongest effect was observed for glucose levels and maps to rs10830963 in the intron of *MTNR1B* gene (genotype-by-dietary state interaction: beta (SE): 0.05(0.01), P-value: 3.64×10^{-9}), which is consistent with the finding by OrNLSr.

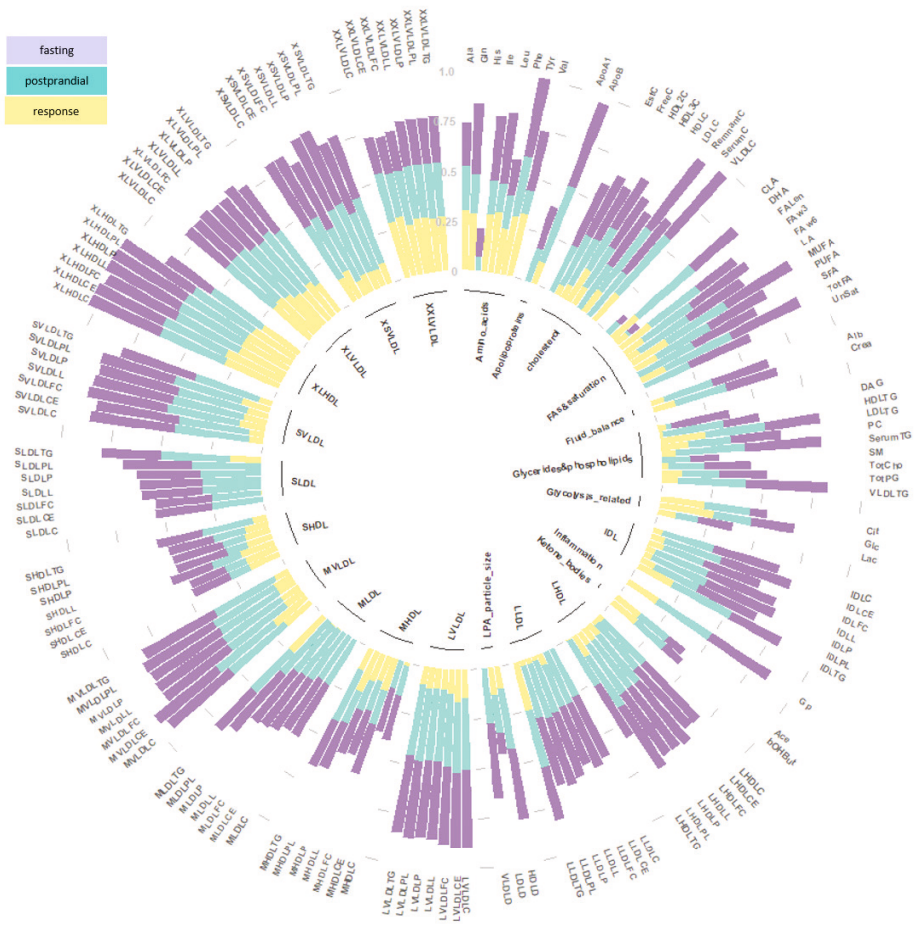

FIGURE 6. Heritability of 148 metabolites on different fasting status

Heritability

To understand the proportion of postprandial metabolite concentrations as well as metabolite responses attributable to genetics, narrow-sense heritability was estimated from genome-wide SNPs in the NEO population. On average, the SNP heritability was 31% for fasting metabolites, which was higher than the average SNP heritability for postprandial metabolites concentrations and response measures (27% and 12%, respectively). Heritability for fasting state metabolites was higher than the counterpart postprandial measures (paired one-sided Wilcoxon-Rank test, p-value=2.2×10^{-11}). Overall, the heritability of metabolite responses was much smaller than either fasting or postprandial state measures. However, the response measures of the amino acids (except for histidine), XXLVLDL, XLVLDL and XLHDL have a heritability estimate of around 25% (Figure 6).

DISCUSSION

To increase our understanding of the genetics of postprandial metabolite concentrations as well as metabolite responses to a liquid meal, we performed GWAS on metabolites measured before and after a liquid mixed meal and on the response itself. We replicated the majority of the fasting metabolite-SNP associations reported previously. Subsequently, we observed highly overlapping genetic association signals between 148 fasting and postprandial metabolite measures. By using baseline-adjusted nonlinear residuals to determine the meal response, rs10830963 located in the intron of *MTNR1B* gene was associated with glucose response, which was also found using a linear mixed model based approach. Since the ligand of the MTNR1B receptor, melatonin, plays a role in the sleep wake cycle, this association suggests a role of circadian rhythmicity in the glucose response after a meal.

When the genetics of metabolite responses after a meal are assessed, it is important to realize that these responses are likely to be affected by their fasting baseline concentrations. This could be addressed by adjustment for baseline measures. However, this may result in collider bias (34) and lead to spurious associations from inflated type 1 error. Baseline adjustment has been extensively addressed in the literature with both empirical simulation studies and theoretical analyses using directed acyclic graphs (DAGs) (34; 35). Linear mixed models, which control for a modelled baseline, were proposed to reduce potential bias introduced by baseline adjustment (36). Here, we exploited two approaches to investigate the genetics of metabolite responses. We firstly adopted an orthogonal nonlinear least squares regression (*OrNLSr)* model to derive measures of postprandial responses that are statistically independent from baseline levels. In addition, we ran linear mixed models for the candidate significant associations

that were identified in either fasting or postprandial metabolite GWAS. With both of the response modelling approaches, rs10830963-G located in the intron of *MTNR1B* gene exhibited the strongest signal associated with the glucose response. Therefore, this finding is not likely due to bias from baseline adjustment.

Melatonin receptors (MTNR) belong to the G protein-coupled receptor (GPCR) family, with two common subtypes being found in humans (37), i.e., G-protein coupled receptors MT1 (MTNR1A) and MT2 (MTNR1B). MTNR1A is primarily associated with sleep promotion, whereas MTNR1B is associated with the regulation of the internal circadian clock to accommodate diurnal rhythms (38). rs10830963-G, located in the middle of the single intron of the *MTNR1B* gene, was found to be associated with fasting glucose levels and the type 2 diabetes risk in GWAS studies nearly a decade ago (39; 40). Concurrently, this variant was shown to be associated with decreased early-phase insulin secretion (41; 42), which is normally considered as the earliest detectable abnormality in individuals that are prone to type 2 diabetes (43). In line with this, G-allele carriers were observed to have a 20% increased risk to develop pre-diabetes (hazard ratio [HR] (95% confidence interval [95%CI]: 1.20 [1.15, 1.27]), whereas no additional risk was observed for the progression to type 2 diabetes from an impaired fasting glucose state (HR [95%CI]: 0.98 [0.89, 1.07]). In the current study, the glucose response was induced by a liquid meal, which more closely resembles regular meal intake. We observed a strong effect of rs10830963-G risk allele on plasma glucose levels at 30 minutes after a meal. This translates to a life-long accumulative effect from this genotype on glucose exposure after meals and likely plays an important role in the development of glucose intolerance and insulin resistance.

The plasma melatonin concentration fluctuates across the day following a diurnal pattern, with high levels during the night and being nearly undetectable in the early morning (44). All the blood samples in the NEO study were collected between 8 am and 12 pm, which is at the trough of plasma melatonin levels. This would imply that melatonin is thus unlikely to mediate the circadian effect of rs10830963-G on glucose response in our study. A previous small intervention study addressed the effect of exogenous melatonin administration on glucose intolerance. This was determined by the oral glucose tolerant test (OGTT; 75g) and individuals were stratified on the rs10830963-G genotype and measured both in the morning and evening (45). In contrast to expectation, the results suggested an interaction effect between the rs10830963-G genotype and exogenous melatonin administration on glucose intolerance, which was only observed in the morning, but not in the evening, when endogenous melatonin levels are elevated. This implies variability in glucose response after a meal in carriers of the rs10830963-G genotype, which remains to be determined.

Several methodological aspects should be considered. The main strength of this study is the liquid meal that was provided to all the NEO participants, which more closely resembles normal meal consumption during the day than a glucose tolerance test, to assess glucose metabolism after a meal. We generated novel insight in the genetic basis for fasting and postprandial metabolite concentrations in a general population. Moreover, to assess metabolite responses, we used two different methods to account for the potential bias introduced by baseline adjustment. Nonetheless, the sample size of the genome-wide association study was relatively small to identify genetic variants with low-frequency and rare variants. In addition, it is still unclear whether the association between rs10830963-G genotype and glucose response is generalizable at different time periods during a day.

CONCLUSION

The genetics of fasting and postprandial metabolite concentrations after a liquid meal are highly overlapping. rs10830963 in the *MTNR1B* gene is a genetic determinant of the postprandial glucose response after a liquid meal, which implies a role for circadian rhythmicity in the plasma glucose response to a meal.

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SUPPLEMENTARY INFORMATION

SUPPLEMENTAL TABLE 1. List of measured metabolites on the platform, clustered into eleven subclasses.

Short name	Full name	Remark
Lipoprotein subclasses		
Chylomicrons and extremely large VLDL particles: with an average particle diameter over 75 nm		
XXLVLDLP	Particle concentration (mmol/l)	
XXLVLDLL	Total lipids (mmol/l)	
XXLVLDLPL	Phospholipids (mmol/l)	
XXLVLDLC	Total cholesterol (mmol/l)	
XXLVLDLCE	Cholesterol esters (mmol/l)	
XXLVLDLFC	Free cholesterol (mmol/l)	
XXLVLDLTG	Triglycerides (mmol/l)	
Very large VLDL particles: with an average particle diameter of 64 nm		
XLVLDLP	Particle concentration (mmol/l)	
XLVLDLL	Total lipids (mmol/l)	
XLVLDLPL	Phospholipids (mmol/l)	
XLVLDLC	Total cholesterol (mmol/l)	
XLVLDLCE	Cholesterol esters (mmol/l)	
XLVLDLFC	Free cholesterol (mmol/l)	
XLVLDLTG	Triglycerides (mmol/l)	
Large VLDL particles: with an average particle diameter of 53.6 nm		
LVLDLP	Particle concentration (mmol/l)	
LVLDLL	Total lipids (mmol/l)	
LVLDLPL	Phospholipids (mmol/l)	
LVLDLC	Total cholesterol (mmol/l)	
LVLDLCE	Cholesterol esters (mmol/l)	
LVLDLFC	Free cholesterol (mmol/l)	
LVLDLTG	Triglycerides (mmol/l)	
Medium VLDL particles: with an average particle diameter of 44.5 nm		
MVLDLP	Particle concentration (mmol/l)	
MVLDLL	Total lipids (mmol/l)	
MVLDLPL	Phospholipids (mmol/l)	
MVLDLC	Total cholesterol (mmol/l)	
MVLDLCE	Cholesterol esters (mmol/l)	
MVLDLFC	Free cholesterol (mmol/l)	

SUPPLEMENTAL TABLE 1. Continued.

Short name	Full name	Remark
MVLDLTG	Triglycerides (mmol/l)	
Small VLDL particles: with an average particle diameter of 36.8 nm		
SVLDLP	Particle concentration (mmol/l)	
SVLDLL	Total lipids (mmol/l)	
SVLDLPL	Phospholipids (mmol/l)	
SVLDLC	Total cholesterol (mmol/l)	
SVLDLCE	Cholesterol esters (mmol/l)	
SVLDLFC	Free cholesterol (mmol/l)	
SVLDLTG	Triglycerides (mmol/l)	
Very small VLDL particles: with an average particle diameter of 31.3 nm		
XSVLDLP	Particle concentration (mmol/l)	
XSVLDLL	Total lipids (mmol/l)	
XSVLDLPL	Phospholipids (mmol/l)	
XSVLDLC	Total cholesterol (mmol/l)	
XSVLDLCE	Cholesterol esters (mmol/l)	
XSVLDLFC	Free cholesterol (mmol/l)	
XSVLDLTG	Triglycerides (mmol/l)	
IDL particles: intermediate-density lipoprotein particles with an average particle diameter of 28.6 nm		
IDLP	Particle concentration (mmol/l)	
IDLL	Total lipids (mmol/l)	
IDLPL	Phospholipids (mmol/l)	
IDLC	Total cholesterol (mmol/l)	
IDLCE	Cholesterol esters (mmol/l)	
IDLFC	Free cholesterol (mmol/l)	
IDLTG	Triglycerides (mmol/l)	
Large LDL particles: low-density lipoprotein particles with an average particle diameter of 25.5 nm		
LLDLP	Particle concentration (mmol/l)	
LLDLL	Total lipids (mmol/l)	
LLDLPL	Phospholipids (mmol/l)	
LLDLC	Total cholesterol (mmol/l)	
LLDLCE	Cholesterol esters (mmol/l)	
LLDLFC	Free cholesterol (mmol/l)	
LLDLTG	Triglycerides (mmol/l)	
Medium LDL particles: low-density lipoprotein particles with an average particle diameter of 23.0 nm		

SUPPLEMENTAL TABLE 1. Continued.

Short name	Full name	Remark
MLDLP	Particle concentration (mmol/l)	
MLDLL	Total lipids (mmol/l)	
MLDLPL	Phospholipids (mmol/l)	
MLDLC	Total cholesterol (mmol/l)	
MLDLCE	Cholesterol esters (mmol/l)	
MLDLFC	Free cholesterol (mmol/l)	
MLDLTG	Triglycerides (mmol/l)	
Small LDL particles: low-density lipoprotein particles with an average particle diameter of 18.7 nm		
SLDLP	Particle concentration (mmol/l)	
SLDLL	Total lipids (mmol/l)	
SLDLPL	Phospholipids (mmol/l)	
SLDLC	Total cholesterol (mmol/l)	
SLDLCE	Cholesterol esters (mmol/l)	
SLDLFC	Free cholesterol (mmol/l)	
SLDLTG	Triglycerides (mmol/l)	
Very large HDL particles: high-density lipoprotein particles with an average particle diameter of 14.3 nm		
XLHDLP	Particle concentration (mmol/l)	
XLHDLL	Total lipids (mmol/l)	
XLHDLPL	Phospholipids (mmol/l)	
XLHDLC	Total cholesterol (mmol/l)	
XLHDLCE	Cholesterol esters (mmol/l)	
XLHDLFC	Free cholesterol (mmol/l)	
XLHDLTG	Triglycerides (mmol/l)	
Large HDL particles: high-density lipoprotein particles with an average particle diameter of 12.1 nm		
LHDLP	Particle concentration (mmol/l)	
LHDLL	Total lipids (mmol/l)	
LHDLPL	Phospholipids (mmol/l)	
LHDLC	Total cholesterol (mmol/l)	
LHDLCE	Cholesterol esters (mmol/l)	
LHDLFC	Free cholesterol (mmol/l)	
LHDLTG	Triglycerides (mmol/l)	
Medium HDL particles: high-density lipoprotein particles with an average particle diameter of 10.9 nm		
MHDLP	Particle concentration (mmol/l)	

SUPPLEMENTAL TABLE 1. Continued.

Short name	Full name	Remark
MHDLL	Total lipids (mmol/l)	
MHDLPL	Phospholipids (mmol/l)	
MHDLC	Total cholesterol (mmol/l)	
MHDLCE	Cholesterol esters (mmol/l)	
MHDLFC	Free cholesterol (mmol/l)	
MHDLTG	Triglycerides (mmol/l)	
Small HDL particles		
SHDLP	Particle concentration (mmol/l)	
SHDLL	Total lipids (mmol/l)	
SHDLPL	Phospholipids (mmol/l)	
SHDLC	Total cholesterol (mmol/l)	
SHDLCE	Cholesterol esters (mmol/l)	
SHDLFC	Free cholesterol (mmol/l)	
SHDLTG	Triglycerides (mmol/l)	
Lipoprotein particle sizes		
VLDLD	Mean diameter of VLDL particles (nm)	calculated as the particle concentration weighted average of the XXL-, XL-, L-, M-, S, and XS-VLDL subclass diameters
LDLD	Mean diameter of LDL particles (nm)	calculated as the particle concentration weighted average of all the LDL and the IDL subclass diameters
HDLD	Mean diameter of HDL particles (nm)	calculated as the particle concentration weighted average of all the HDL subclass diameters
Cholesterol		
SerumC	Serum total cholesterol (mmol/l)	
VLDLC	Total cholesterol in VLDL(mmol/l)	
RemnantC	Remnant cholesterol (non-HDL, non-LDL-cholesterol) (mmol/l)	
LDLC	Total cholesterol in LDL (mmol/l)	
HDLC	Total cholesterol in HDL (mmol/l)	
HDL2C	Total cholesterol in HDL2 (mmol/l)	HDL particles within the density range of 1.063-1.125 g/mL

SUPPLEMENTAL TABLE 1. Continued.

Short name	Full name	Remark
HDL3C	Total cholesterol in HDL3 (mmol/l)	HDL particles within the density range of 1.125-1.210 g/mL
EstC	Esterified cholesterol (mmol/l)	
FreeC	Free cholesterol (mmol/l)	
Glycerides & phospholipids		
SerumTG	Serum total triglycerides (mmol/l)	
VLDLTG	Triglycerides in VLDL (mmol/l)	
LDLTG	Triglycerides in LDL (mmol/l)	
HDLTG	Triglycerides in HDL(mmol/l)	
DAG	Diacylglycerol (mmol/l)	
TotPG	Total phosphoglycerides (mmol/l)	
PC	Phosphatidylcholine and other cholines (mmol/l)	
SM	Sphingomyelins (mmol/l)	
TotCho	Total cholines (mmol/l)	
Apolipoproteins		
ApoA1	Apolipoprotein A-I (g/l)	
ApoB	Apolipoprotein B (g/l)	
Fatty acids (FA) & saturation		
TotFA	Total fatty acids (mmol/l)	
FALen	Estimated description of fatty acid chain length, not actual carbon number	
UnSat	Estimated degree of unsaturation	
DHA	22:6, docosahexaenoic acid (mmol/l)	
LA	18:2, linoleic acid (mmol/l)	
CLA	Conjugated linoleic acid - mmol/l	
FAw3	Omega-3 fatty acids (mmol/l)	
FAw6	Omega-6 fatty acids (mmol/l)	
PUFA	Polyunsaturated fatty acids (mmol/l)	
MUFA	Monounsaturated fatty acids; 16:1, 18:1 (mmol/l)	
SFA	Saturated fatty acids (mmol/l)	
Glycolysis related metabolites		
Glc	Glucose (mmol/l)	
Lac	Lactate (mmol/l)	
Cit	Citrate (mmol/l)	
Amino acids		
Ala	Alanine (mmol/l)	

SUPPLEMENTAL TABLE 1. Continued.

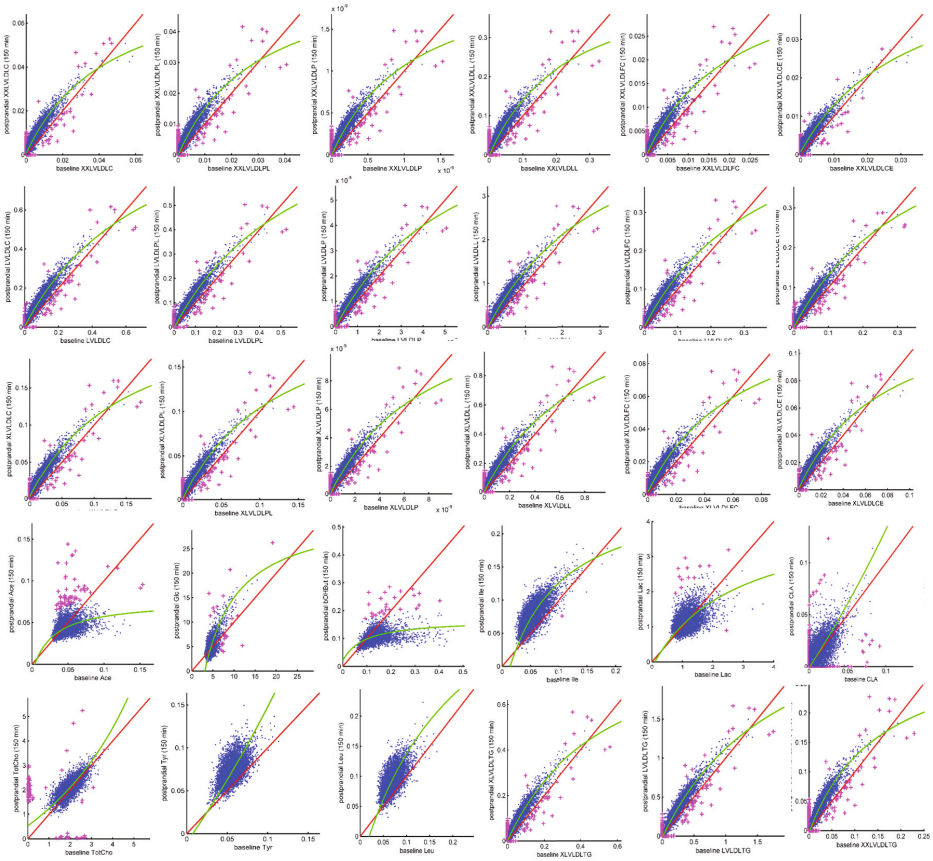
Short name	Full name	Remark
Gln	Glutamine (mmol/l)	
His	Histidine (mmol/l)	
Ile	Isoleucine (mmol/l)	
Leu	Leucine (mmol/l)	
Val	Valine (mmol/l)	
Phe	Phenylalanine (mmol/l)	
Tyr	Tyrosine (mmol/l)	
Ketone bodies		
Ace	Acetate (mmol/l)	
bOHBut	3-hydroxybutyrate (mmol/l)	
Fluid balance		
Crea	Creatinine (mmol/l)	
Alb	Albumin	
Inflammation		
Gp	Glycoprotein acetyls, mainly a1-acid glycoprotein (mmol/l)	

SUPPLEMENTAL TABLE 2. Replication of associations between fasting metabolites and SNPs that were reported previously.

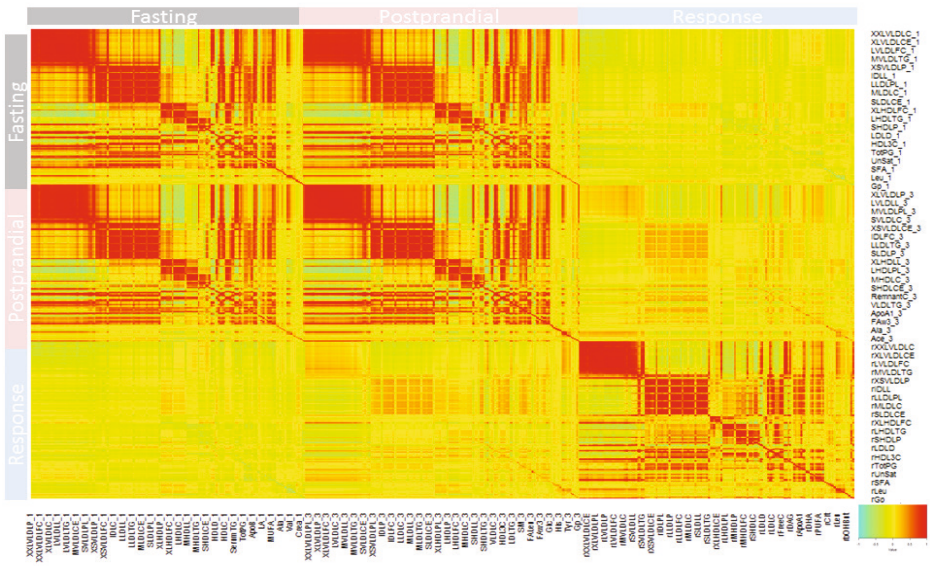
Kettunen's results											NEO fasting				NEO postprandial		
No	**Metabolite**	**Gene**	**Chr**	**Position**	**EA/ NEA**	**EAF**	**beta**	**SE**	**p-value**	**Gene**	**EAF**	**beta**	**SE**	**p-value**	**beta**	**SE**	**p-value**
1	XLHDLPL	rs4503368	1	161190250	C/T	0.53	-0.07	0.01	3.2 E -11	*USF1*	0.54	-0.01	0.02	8.05E-1	0.01	0.02	6.38E-1
2	MHDLFC	rs590820	1	230309619	G/A	0.44	-0.06	0.01	1.6 E -9	*GALNT2*	0.42	-0.07	0.02	**2.10E-3**	-0.05	0.02	1.53E-2
3	IDLFC	rs952275	2	21221399	G/T	0.43	0.11	0.01	1.5 E -27	*APOB*	0.50	0.09	0.02	**9.31E-6**	0.11	0.02	2.74E-7
4	LDLC	rs6756629	2	44065090	A/G	0.08	-0.14	0.02	1.2 E -14	*ABCG5*	0.06	-0.17	0.04	**6.41E-5**	-0.12	0.04	4.44E-3
5	VLDLD	rs10455872	6	161010118	G/A	0.04	-0.20	0.03	1.3 E 12	*LPA*	0.07	-0.03	0.04	3.74E-1	-0.04	0.04	2.84E-1
6	LLDLPL	rs73066442	7	21592973	G/A	0.25	0.08	0.01	9.7 E 11	*DNAH11*	0.23	0.04	0.02	1.01E-1	0.05	0.03	5.79E-2
7	VLDLD	rs17145750	7	73026378	T/C	0.15	-0.13	0.01	4.3 E -20	*MLX1PL*	0.16	-0.11	0.03	**3.25E-5**	-0.10	0.03	4.04E-4
8	SVLDLTG	rs115849089	8	19912370	A/G	0.11	-0.18	0.02	1.6 E -25	*LPL*	0.13	-0.28	0.03	**1.11E-18**	-0.29	0.03	6.39E-19
9	XSVLDLTG	rs2954029	8	126490972	T/A	0.48	-0.09	0.01	2.8 E -16	*TRIB1*	0.49	-0.08	0.02	**7.83E-5**	-0.09	0.02	7.93E-5
10	XLHDLFC	rs686030	9	15304782	A/C	0.87	0.09	0.01	2.0 E -9	*TTC39B*	0.85	0.01	0.03	7.01E-1	0.03	0.03	3.26E-1
11	XLHDLC	rs2575876	9	107665739	A/G	0.19	-0.10	0.01	2.7 E -15	*ABCA1*	0.25	-0.04	0.02	9.38E-2	-0.04	0.03	1.12E-1
12	SVLDLTG	rs964184	11	116648917	C/G	0.86	-0.24	0.01	7.6 E -66	*ZNF259*	0.87	-0.27	0.03	**1.42E-18**	-0.27	0.03	1.91E-17
13	HDLD	rs67053123	12	125353810	A/T	0.15	0.11	0.01	9.8 E -13	*SCARB1*	0.14	0.02	0.03	4.51E-1	0.05	0.03	1.60E-1
14	XLHDLTG	rs1532085	15	58683366	G/A	0.6	-0.26	0.01	9.2 E -155	*LIPC*	0.62	-0.25	0.02	**5.40E-31**	-0.22	0.02	6.16E-22
15	HDLC	rs247617	16	56990716	A/C	0.3	0.23	0.01	5.8 E -97	*CETP*	0.33	0.18	0.02	**8.07E-16**	0.19	0.02	1.34E-16
16	HDLD	rs6507939	18	47176261	C/A	0.84	0.11	0.01	8.3 E -16	*LIPG*	0.86	0.07	0.03	**2.21E-2**	0.08	0.03	1.29E-2
17	VLDLD	rs116843064	19	8429323	A/G	0.03	-0.22	0.03	2.8 E -10	*ANGPTL4*	0.02	-0.21	0.07	**3.55E-3**	-0.14	0.08	6.47E-2
18	LLDLFC	rs142130958	19	11190652	A/G	0.11	-0.24	0.02	6.5 E -47	*LDLR*	0.13	-0.23	0.03	**4.70E-14**	-0.24	0.03	4.42E-14
19	MVLDLC	rs72999033	19	19366632	T/C	0.06	-0.18	0.02	1.0 E -16	*CILP*	0.07	-0.30	0.04	**3.73E-13**	-0.25	0.04	1.18E-8
20	LLDLFC	rs7412	19	45412079	T/C	0.06	-0.59	0.03	2.0 E -120	*APOE*	0.08	-0.63	0.04	**5.78E-64**	-0.64	0.04	1.36E-60
21	XSVLDLPL	rs1883711	20	39179822	C/G	0.06	0.17	0.02	2.3 E -11	*MAFB*	0.03	0.07	0.08	4.30E-1	0.08	0.09	3.40E-1
22	SHDLP	rs6073958	20	44551855	C/T	0.2	0.20	0.01	2.6 E -55	*PLTP*	0.21	0.17	0.02	**2.63E-12**	0.22	0.03	1.08E-17
23	SerumTG	rs1168041	1	62960250	C/T	0.72	0.10	0.01	3.4 E -17	*ANGPTL3*	0.64	0.08	0.02	**2.49E-4**	0.06	0.02	1.03E-2
24	Ala	rs1260326	2	27730940	C/T	0.64	-0.10	0.01	7.4 E -26	*GCKR*	0.63	-0.15	0.02	**1.02E-12**	-0.10	0.02	9.34E-6
25	Val	rs10211524	2	65208074	A/G	0.41	0.09	0.01	5.2 E -20	*SLC1A4*	0.42	0.11	0.02	**2.76E-7**	0.12	0.02	3.36E-8
26	Glc	rs560887	2	169763148	C/T	0.7	0.12	0.01	3.4 E -32	*G6PC2*	0.68	0.15	0.02	**3.01E-11**	0.05	0.02	3.82E-2
27	Val	rs9637599	4	89206230	C/A	0.47	0.11	0.01	1.7 E -35	*PPM1K*	0.49	0.13	0.02	**2.49E-10**	0.14	0.02	1.02E-10

Kettunen's results											NEO fasting				NEO postprandial		
No	**Metabolite**	**Gene**	**Chr**	**Position**	**EA/ NEA**	**EAF**	**beta**	**SE**	**p-value**	**Gene**	**EAF**	**beta**	**SE**	**p-value**	**beta**	**SE**	**p-value**
28	His	rs3733402	4	187158034	A/G	0.57	0.08	0.01	9.1 E -15	*KLKB1*	0.52	0.01	0.02	5.86E-1	-0.04	0.02	1.08E-1
29	Cit	rs2921604	5	14867948	C/T	0.44	0.10	0.01	3.7 E -25	*ANKH*	0.46	0.09	0.02	**1.83E-5**	0.09	0.02	2.84E-5
30	Phe	rs2731672	5	176842474	C/T	0.74	0.09	0.01	3.9 E -16	*F12*	0.75	-0.02	0.02	3.17E-1	-0.01	0.02	7.50E-1
31	Tyr	rs14399	6	111543944	A/C	0.39	-0.10	0.01	1.4 E -24	*SLC16A10*	0.44	-0.09	0.02	**6.31E-6**	-0.12	0.02	4.51E-8
32	Glc	rs878521	7	44255643	A/G	0.22	0.09	0.01	8.0 E -14	*GCK*	0.26	0.14	0.02	**2.11E-8**	0.08	0.03	2.53E-3
33	Crea	rs10265221	7	151414329	C/T	0.24	0.07	0.01	7.5 E -11	*PRKAG2*	0.28	0.07	0.02	**3.79E-3**	0.04	0.02	7.57E-2
34	Gln	rs7078003	10	99359412	T/C	0.2	0.07	0.01	3.0 E -10	*HOGA1*	0.17	0.14	0.03	**4.57E-7**	0.05	0.03	8.17E-2
35	Glc	rs10466351	11	92697981	T/C	0.38	0.07	0.01	3.3 E -13	*MTNR1B*	0.37	0.13	0.02	**1.00E-8**	-0.02	0.02	3.45E-1
36	Gln	rs2657879	12	56865338	G/A	0.18	-0.22	0.01	3.3 E -70	*GLS2*	0.19	-0.34	0.03	**2.83E-39**	-0.26	0.03	2.93E-22
37	Phe	rs1718309	12	103242396	G/A	0.6	-0.08	0.01	2.5 E -15	*PAH*	0.61	-0.16	0.02	**1.23E-13**	-0.18	0.02	7.78E-17
38	Gp	rs28929474	14	94844947	T/C	0.02	-0.29	0.04	2.7 E -13	*SERPINA1*	0.02	-0.30	0.08	**1.63E-4**	-0.28	0.08	6.92E-4
39	Crea	rs61524473	15	45646283	C/T	0.26	0.09	0.01	1.2 E -15	*GATM*	0.27	0.10	0.02	**3.40E-5**	0.08	0.02	1.47E-3
40	Gp	rs77303550	16	72079657	T/C	0.19	0.15	0.01	4.0 E -30	*HP*	0.22	0.11	0.03	**8.27E-6**	0.10	0.03	1.73E-4
41	Cit	rs172642	17	6595398	C/A	0.48	0.08	0.01	4.8 E -18	*SLC13A5*	0.52	0.13	0.02	**2.28E-10**	0.13	0.02	1.97E-9
42	Crea	rs2079742	17	59465697	C/T	0.18	0.10	0.01	8.6 E -14	*BCAS3*	0.15	0.07	0.03	**1.92E-2**	0.08	0.03	2.22E-2
43	Cit	rs2040771	22	19161935	T/C	0.48	-0.09	0.01	1.3 E -22	*SLC25A1*	0.47	-0.12	0.02	**2.10E-9**	-0.10	0.02	2.51E-6
44	Ala	rs4554975	12	47201814	G/A	0.64	−0.07	0.01	6.1 E −13	*SLC38A4*	0.57	-0.04	0.02	6.31E-2	-0.05	0.02	3.74E-2
45	His	rs7954638	12	96314795	A/C	0.48	−0.08	0.01	7.3 E −15	*HAL*	0.47	-0.15	0.02	**2.08E-13**	-0.10	0.02	7.88E-6
46	His	rs1998848	14	21492229	A/G	0.05	0.15	0.02	4.9 E −10	*NDRG2*	0.01	0.45	0.12	**1.73E-4**	0.08	0.12	5.21E-1

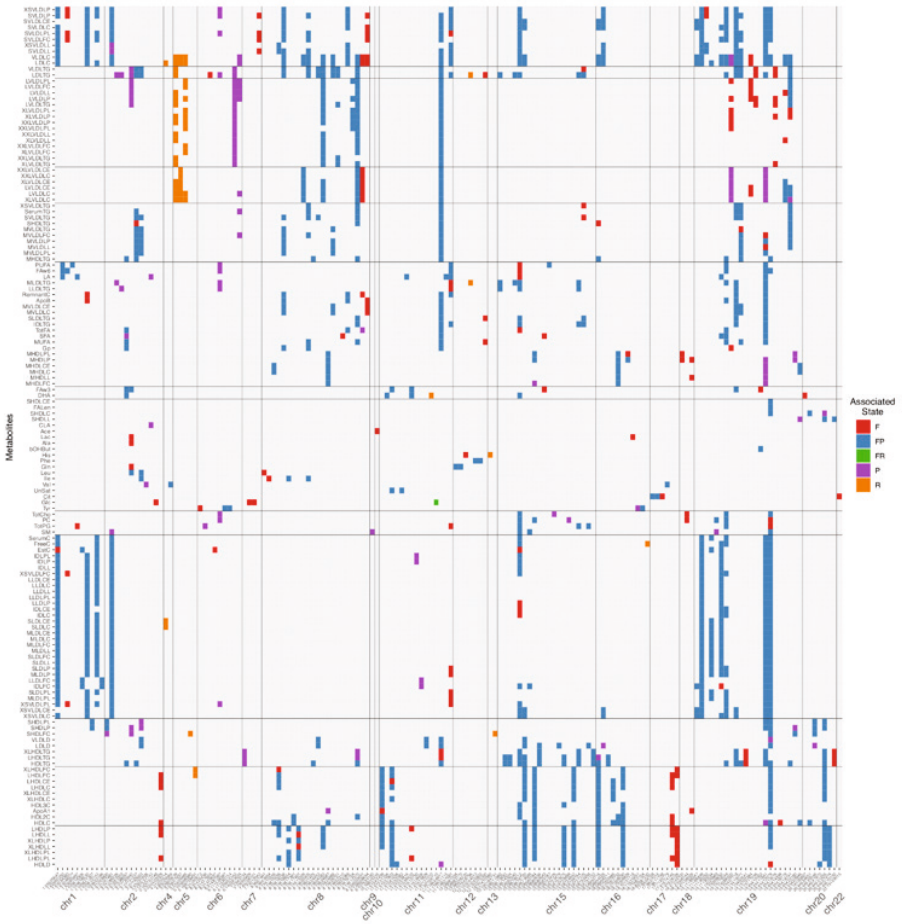
SNP: single nucleotide polymorphism; Chr: chromosome; EA/NEA: effect allele (coding allele)/non-effect allele (non-coding) allele; EAF: effect allele frequency/coding allele frequency; info: imputation quality; beta: effect size per coding allele; SE: standard error.



SUPPLEMENTAL FIGURE 1. Metabolite concentrations (N=30) between fasting and postprandial states with non-linear associations.



SUPPLEMENTAL FIGURE 2. Intra- and inter-state Pearson correlations between 444 metabolites (148×3) under fasting, postprandial and response.



SUPPLEMENTAL FIGURE 3. Overall of all the identified signals from GWAS on different fasting status.