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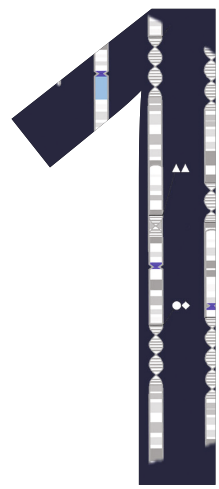
General introduction and outline of the thesis



GENERAL INTRODUCTION

The term "cardiometabolic disease" is firstly mentioned by Pescatello to describe a cluster of subclinical disorders that are shared by cardiovascular diseases and type 2 diabetes, including abdominal adiposity, hypertension, dyslipidaemia, hyperinsulinaemia and glucose intolerance (1). More recently, increasing evidence shed light on the link between cardiovascular diseases and type 2 diabetes, with substantial overlaps of risk factors leading to the diseases. Cardiometabolic diseases are multifactorial disorders involving both genetic and environmental factors, which substantially increases the complexity of understanding the physiological mechanisms of diseases and developing effective disease prognosis and diagnosis regimens. Recently, with the booming of high-throughput technology, multi-OMICS (i.e., integrating genetics, epigenetics, transcriptomes, metabolomics, proteomics with disease outcomes) approaches have been widely adopted in the clinical research to further investigate on the causes and prevention of cardiometabolic diseases.

In this thesis, the overall aims were to examine whether 1) postprandial metabolomics measures after a mixed meal are reliable and clinically informative, and to compare these measures to the most commonly used clinical fasting measures; 2) genetic analyses of postprandial (metabolomics) measures through genome-wide association studies (GWAS) provide insight in novel biological pathways; and 3) fasting glucose and cholesteryl ester transfer protein (CETP) concentrations are risks factors for a first event of venous thrombosis (VT) and to assess causality using Mendelian randomization analyses.

PART I The application of postprandial measures in epidemiological studies

Postprandial glucose and lipid metabolism

Cardiometabolic risk factors, including obesity, dyslipidaemia, insulin resistance, hypertension and low grade inflammation, are strongly associated with type 2 diabetes (T2D) and premature cardiovascular disease (2). Diabetes is a chronic disease that occurs when the pancreas does not produce enough insulin to regulate blood sugar levels. Continuous hyperglycaemia may damage nerves and blood vessels, ultimately leading to blindness, kidney failure, and cardiovascular diseases (CVDs) (3). Cardiovascular diseases are still ranking as the top cause of global mortality, accounting for more than one-third of the total deaths in 2016 (4). CVDs cover a broad range of disorders of the heart and blood vessels, ranging from coronary artery disease (CAD) to venous thrombosis (VT). Coronary artery disease manifests as a failure of blood vessels to supply the heart muscle with sufficient oxygen and nutrients. Venous thrombosis is caused by blood clots in veins, e.g., the deep veins of the legs. These clots can dislodge and travel through the heart into the arteries of the lungs leading

to pulmonary embolism (PE). In clinical practice, cardiometabolic risk factors, which can be determined in the blood, are commonly measured after an overnight fast for standardization purposes.

In modern society, it is common for most people to consume three meals during their waking hours, with a time interval of 4-5 hours between the meals, and to regularly eat smaller food items ('snack') between meals. Circulating lipids are elevated 5-8 hours in response to a fat-containing meal, meaning that our bodies reside in a postprandial state most of the time during a day (5). Since the postprandial response also increases exposure of our body to hyperlipidemia and hyperglycemia, the response measures have been the focus of a substantial interest (6). Associations between postprandial dyslipidaemia and hyperglycaemia and increased risk for cardiometabolic diseases have been established from both mechanistic experiments and population-based studies (7-13).

Insulin is a hormone which plays an essential role in maintaining glucose homeostasis after a meal. Insulin is secreted by the beta cells of the pancreas, and reduces the postprandial glucose levels mainly via the promotion of glucose uptake by peripheral tissues and the reduction of glucose production by the liver (14). The insulin response to an intravenous glucose stimulus exhibits a biphasic pattern: a burst of insulin secretion in the first a couple of minutes is followed by a second wave of secretion based on the glucose load of the stimulus (15; 16). Early phase insulin secretion is important for postprandial glucose homeostasis and impaired early phase insulin secretion has been associated with the risk of type 2 diabetes (17). Clinically, the oral glucose tolerance test (OGTT) has been applied in the diagnosis of diabetes for decades, and the plasma glucose clearance rate after an OGTT is a reflection of beta cell function and insulin sensitivity. Another marker of glucose regulation is glycated haemoglobin or haemoglobin A1c (HbA1c), which is considered a representative marker for the average plasma glucose concentration over the preceding 8-12 weeks. In T2D prevention and management, targeting postprandial, has been shown to be more effective to achieve a specific HbA1c level as a goal to control hyperglycaemia rather than fasting glucose levels (12; 18).

Although it has long been recognized that both fasting and non-fasting lipid levels are associated with cardiovascular mortality, recently the risk associated with non-fasting lipid levels has been re-evaluated. This has led to the recommendation that non-fasting lipid measures are suitable for cardiovascular risk assessment and treatment decisions (5; 6; 11; 19; 20). The non-fasting state is a dynamic condition that is affected by many factors, including diet (e.g., composition, quantity), lifestyle (e.g., tobacco use, physical exercise), physiological factors (e.g., age, sex), pathological conditions (e.g., disease history), and genetics (21). Thus far, the genes and genetic loci that affect

postprandial glucose and lipid metabolism have not been fully understood. This is at least partly due to the complexity of performing and standardizing postprandial measurements in large scale epidemiological studies.

In the past decade there have been at least two population-based genome-wide studies on insulin response and 2-hour glucose excursion after an oral glucose tolerance test (22; 23). However, for these studies, it is important to note that a glucose bolus is different from any meal we eat, which is a combination of macro- and micro-nutrients rather than sugar alone. Postprandial glucose levels after a mixed meal are correlated with the glucose levels after the oral glucose tolerance test. However, the absolute levels and time response of glycaemia differ markedly between the two conditions (24). Regarding the genetic background of postprandial lipoprotein metabolism, no comprehensive genome-wide association study has been performed yet and most evidence to date came from candidate pathway/gene studies (25-29). We aim to fill in the void of depicting a genetic landscape of postprandial glucose and lipid metabolism.

Metabolomics in epidemiological studies

The suffix -OMICS indicates the comprehensive analyses of "all" molecules involved in a certain biological process, e.g., genomics for genes, proteomics for proteins, and metabolomics for metabolites. Metabolomics aims to measure "all" small molecules, i.e., metabolites, in the living system. To achieve this, both targeted and non-targeted metabolomics platforms have been developed. Targeted platforms identify a "known" set of metabolites, whereas non-targeted metabolomics platforms identify as many metabolites as possible, including "unknowns". Based on the nature of the metabolites, different analytical platforms can be considered, most importantly mass spectrometry (MS) coupled with chromatography (either gas or liquid) and nuclear magnetic resonance (NMR) spectroscopy based platforms (30). Both MS and NMR based platforms have their strengths and limitations. In this thesis, we will focus on two commercially available targeted platforms, the Biocrates AbsoluteIDQ™ p150 assay, a MS-based metabolomics platform and Nightingale, a NMR-based metabolomics platform. The p150 assay includes 163 metabolites from five substance classes: acylcarnitines (n = 41), sphingolipids (n = 15), glycerophosphocholines (n = 92), amino acids (n = 14) and hexoses (31). The Nightingale metabolomics platform provides 148 metabolites from eleven classes, mainly lipoprotein subclasses (n=98) (32).

Recently, researchers have started to implement multi-OMICS approaches, a transformation from a reductionist approach to a more holistic method, i.e., studying hundreds and thousands of biomarkers across different biological layers simultaneously (33). In contrast to conventional epidemiological studies addressing one hypothesis in a study, a large number of tests are performed at the same time in a multi-OMICS study,

with plentiful new hypotheses being generated. We aim to explore novel physiological pathways and risk factors of cardiometabolic diseases by applying the multi-OMICS approach in epidemiological studies.

From genome-wide association studies to causal inference

Genome-wide association studies (GWAS) have been, and still are, extensively applied to investigate the genetics of a wide variety of traits. GWAS exploit single nucleotide polymorphisms (SNPs), variations of a single nucleotide at a specific position in the genome, in a population. The principle of GWAS is based on linkage disequilibrium (LD) in the genome. LD is the non-random association of (SNP) alleles at separated chromosomal loci in a population. Thus, GWAS test the hypothesis that a particular SNP either is the causative mutation itself or in the close vicinity (in LD) of the causative mutation that is associated with the trait. The large majority of currently genotyped SNPs are located in the intronic and intergenic regions of the genome, which are generally assumed to be non-coding regions. To increase the chance of finding causal genetic variants that are in protein coding regions, exome chips were developed that are enriched with genetic variants located in the exonic regions of the genome (34).

As of September 2018, the NHGRI-EBI GWAS Catalogue archived 5687 GWAS related to 71,673 variant-trait associations (35). This number is expected to increase with several orders of magnitude in the next a couple of years, with the contributions from GWAS on additional high-throughput OMICS measures, thereby broadening the phenotypic spectrum. However, a critical and challenging task remains, i.e., to disentangle the functions of identified variants and their translational potential for disease diagnosis, prognosis, and prevention as well as drug development. As over 80% of the tag SNPs genotyped on the arrays are thought to influence gene expression instead of being in a protein coding region, the identification of causal variants by fine mapping and other functional analysis methods has become especially important in the post-GWAS era (36).

GWAS have provided novel insight in the molecular mechanisms underlying many traits (37). In addition, GWAS have given us tools to study causal inference by means of Mendelian randomization (MR) studies (38). MR studies utilize genetic variants to mimic a randomized controlled trial (RCT) with randomly selected treated and untreated arms. The genetic variants are presumably randomly allocated from parents to offspring at conception, and are exploited as instrumental variables to determine whether an exposure is causally associated with an outcome. One of the well-known applications of MR studies in causal inference is analyses of the causal role of HDL-cholesterol in CVD. Cholesteryl ester transfer protein (CETP) decreases HDL-cholesterol and was therefore adopted by many pharmaceutical companies as a new candidate drug target. CETP

inhibitors were developed and tested at great effort and expense, but nearly all the clinical trials on CETP inhibitors did not show substantial benefits on reducing the risk of cardiovascular diseases. After the fact, these negative findings were corroborated by evidence from MR studies between HDL-cholesterol levels and the risk of cardiovascular diseases, i.e., HDL-cholesterol is not likely to be causal and thus not a drug target for cardiovascular diseases (39-42). We aim to identify novel genetic variants through GWAS on underexplored measurements (e.g., non-fasting measures) and apply these variants to infer the causal relationships to diseases risks by MR approach.

PART II Cardiometabolic risk factors for venous thrombosis

Venous thrombosis is characterized by the formation of blood clots, mostly in the deep veins of the leg. These clots can travel in the circulation to the lungs, leading to pulmonary embolism, which causes substantial morbidity and mortality. Venous thrombosis and atherosclerotic cardiovascular disease are traditionally regarded as two separate diseases, with their own pathophysiology, epidemiology, and treatment (43). However, in recent years, several studies reported that patients with VT were at increased risk of subsequent arterial disease (44; 45). Results of these studies indicated that VT and CVD might share common risk factors, including some known cardiovascular risk factors such as hypertension, obesity, diabetes mellitus hallmarked by hyperglycaemia, and dyslipidaemia (43; 46). However, it is still unclear whether hyperglycaemia and dyslipidaemia are risk factors for venous thrombosis since epidemiological studies reported inconsistent findings. Diverse study designs and limited adjustment for confounding could explain the conflicting data in the literature. Therefore, further large population-based studies are warranted, in order to estimate the presence and the strength of the association, and in addition to adjust for all potential confounders. A recently performed MR study demonstrating the causal association between obesity and the risk of VT (47) showed that using a strong genetic instrument and a well-powered sample size may be a useful approach to conclusively answer the question whether hyperglycemia and dyslipidemia are causally associated with VT. We aim to further understand the role of hyperglycaemia and dyslipidaemia in the risk of VT by both observational and MR studies.

Study population

To address the main research questions of this thesis, two population-based epidemiological studies were used for the analyses: the Netherlands Epidemiology of Obesity (NEO) study and the Multiple Environmental and Genetic Assessment of risk factors for VT (MEGA) study.

The NEO study

The NEO study was used in **Chapters 2, 3, 4, 5, 6, and 8.** The NEO study is a population-based prospective cohort study. Initiated in 2008, the NEO study was designed to study pathways that lead to obesity-related diseases (48). 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 Leiden University Medical Center (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. DNA was abstracted from venous blood samples obtained from the antecubital vein. Genotyping was performed using Illumina HumanCoreExome-24 BeadChip (Illumina Inc., San Diego, California, United States of America).

The MEGA study

The MEGA study was used in **Chapter 7 and 9**. The MEGA study is a population-based case-control study with the aim of studying the aetiology of VT. From March 1999 to September 2004, 4956 consecutive patients aged between 18 and 70 years with an objectively confirmed first event of VT (deep venous thrombosis of the leg or pulmonary embolism) were recruited from six anticoagulation clinics in the Netherlands (49). The control subjects were recruited from two sources, i.e., partners of VT patients when between 18 and 70 years of age and without a history of VT (n=3297); and from the general population, by random-digit dialling (RDD), further frequency matched for age and sex with the VT cases (n=3000). For logistic reasons, a blood sample was provided only by patients and controls recruited before June 2002. Of the participants included after June 2002 and those who were not available for a blood draw, buccal swabs were collected for DNA analysis.

OUTLINE OF THE THESIS

The thesis is structured in two parts based on the aims of the studies. In the first part, there are five chapters analysing the additional value of postprandial measures after a liquid mixed meal. In **Chapter 2,** the predictive power of metabolite profiles for

glucose dysregulation is compared between the fasting and postprandial state. Since we observe that the predictors selected in the fasting and postprandial metabolite profiles are entirely overlapping, we hypothesize that the genetics of fasting and postprandial state metabolite measures are also overlapping. In **Chapter 3** we perform a candidate SNP analysis on all the published fasting metabolite-SNP associations and replicate these associations with postprandial metabolite measures. **Chapter 4, 5, 6** are hypothesis-free studies to explore the reliability and genetic basis of postprandial glucose and lipid metabolism in the NEO study. As a quality assessment, **Chapter 4** analyses the reproducibility of repeated postprandial state metabolite measures from the NMR platform after both short- and long-term intervals and compare these with fasting state measures. **Chapter 5** describes GWAS analyses on 148 circulating metabolites from the NMR metabolomics platform. We analyse the genetic associations to the fasting and postprandial state metabolite concentrations and the response between fasting and postprandial state measures. The aim of the study is to obtain a snapshot of genetic landscape of postprandial metabolites. **Chapter 6** focuses on the genetic basis of postprandial glucose haemostasis, and we conduct a series of GWAS on early-phase insulin response measures.

In the second part of the thesis, three studies are reported that revisited several, but currently still controversial, hypotheses in venous thrombosis research, i.e. whether hyperglycaemia or dyslipidaemia are risk factors for venous thrombosis. To address the association between hyperglycaemia as well as type 2 diabetes and the risk of VT, **Chapter 7** is conducted in the MEGA study. As CETP is an important target in the lipoprotein metabolism to increase HDL-C levels and reduce the risk of cardiovascular diseases, we perform the first GWAS on serum CETP concentrations, which is described in **Chapter 8**. In addition to standard lipid profiles, circulating CETP concentrations as well as CETP genotypes have also been associated with higher risks of VT. In **Chapter 9**, we use the genetic variants identified in **Chapter 8** to perform a MR study in the MEGA study to understand the role of CETP in VT. **Chapter 10** provides a summary of all the findings from this thesis and **Chapter 11** discusses the clinical implications as well as future directions of research.

REFERENCES

1. Pescatello LS, VanHeest JL: Physical activity mediates a healthier body weight in the presence of obesity. Br J Sports Med 2000;34:86-93
2 Vasudevan AR, Ballantyne CM: Cardiometabolic risk assessment: an approach to the prevention of cardiovascular disease and diabetes mellitus. Clin Cornerstone 2005;7:7-16
3. Forbes JM, Cooper ME: Mechanisms of diabetic complications. Physiol Rev 2013;93:137-188
4. Cardiovascular diseases (CVDs) [article online], 2017. Available from https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds).
5. Lairon D, Lopez-Miranda J, Williams C: Methodology for studying postprandial lipid metabolism. Eur J Clin Nutr 2007;61:1145-1161
6. Pappas C, Kandaraki EA, Tsirona S, Kountouras D, Kassi G, Diamanti-Kandarakis E: Postprandial dysmetabolism: Too early or too late? Hormones (Athens) 2016;15:321-344
7. Zilversmit DB: Atherogenesis: a postprandial phenomenon. Circulation 1979;60:473-485
8. Patsch JR, Miesenbock G, Hopferwieser T, Muhlberger V, Knapp E, Dunn JK, Gotto AM, Jr., Patsch W: Relation of triglyceride metabolism and coronary artery disease. Studies in the postprandial state. Arterioscler Thromb 1992;12:1336-1345
9. Ryu JE, Howard G, Craven TE, Bond MG, Hagaman AP, Crouse JR, 3rd: Postprandial triglyceridemia and carotid atherosclerosis in middle-aged subjects. Stroke 1992;23:823-828
10. 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
11. Karpe F: Postprandial lipoprotein metabolism and atherosclerosis. J Intern Med 1999;246:341-355
12. Monnier L, Lapinski H, Colette C: Contributions of fasting and postprandial plasma glucose increments to the overall diurnal hyperglycemia of type 2 diabetic patients - Variations with increasing levels of HbA(1c). Diabetes care 2003;26:881-885
13. Shiraiwa T, Kaneto H, Miyatsuka T, Kato K, Yamamoto K, Kawashima A, Kanda T, Suzuki M, Imano E, Matsuhisa M, Hori M, Yamasaki Y: Post-prandial hyperglycemia is an important predictor of the incidence of diabetic microangiopathy in Japanese type 2 diabetic patients. Biochemical and biophysical research communications 2005;336:339-345
14. Gavin JR, 3rd: Pathophysiologic mechanisms of postprandial hyperglycemia. Am J Cardiol 2001;88:4H-8H
15. Gerich JE: Is reduced first-phase : Insulin release the earliest detectable abnormality in individuals destined to develop type 2 diabetes? Diabetes 2002;51:S117-S121
16. Caumo A, Luzi L: First-phase insulin secretion: does it exist in real life? Considerations on shape and function. Am J Physiol-Endoc M 2004;287:E371-E385
17. Del Prato S, Tiengo A: The importance of first-phase insulin secretion: implications for the therapy of type 2 diabetes mellitus. Diabetes Metab Res 2001;17:164-174
18. Ceriello A: Point: postprandial glucose levels are a clinically important treatment target. Diabetes care 2010;33:1905-1907
19. Langsted A, Nordestgaard BG: Nonfasting Lipid Profiles: The Way of the Future. Clin Chem 2015;61:1123-1125
20. Langsted A, Nordestgaard BG: Nonfasting versus fasting lipid profile for cardiovascular risk prediction. Pathology 2018;
21. Lopez-Miranda J, Williams C, Lairon D: Dietary, physiological, genetic and pathological influences on postprandial lipid metabolism. Br J Nutr 2007;98:458-473

22. Saxena R, Hivert MF, Langenberg C, Tanaka T, Pankow JS, Vollenweider P, Lyssenko V, Bouatia-Naji N, Dupuis J, Jackson AU, Kao WH, Li M, Glazer NL, Manning AK, Luan J, Stringham HM, Prokopenko I, Johnson T, Grarup N, Boesgaard TW, Lecoeur C, Shrader P, O'Connell J, Ingelsson E, Couper DJ, Rice K, Song K, Andreasen CH, Dina C, Kottgen A, Le Bacquer O, Pattou F, Taneera J, Steinthorsdottir V, Rybin D, Ardlie K, Sampson M, Qi L, van Hoek M, Weedon MN, Aulchenko YS, Voight BF, Grallert H, Balkau B, Bergman RN, Bielinski SJ, Bonnefond A, Bonnycastle LL, Borch-Johnsen K, Bottcher Y, Brunner E, Buchanan TA, Bumpstead SJ, Cavalcanti-Proenca C, Charpentier G, Chen YD, Chines PS, Collins FS, Cornelis M, G JC, Delplanque J, Doney A, Egan JM, Erdos MR, Firmann M, Forouhi NG, Fox CS, Goodarzi MO, Graessler J, Hingorani A, Isomaa B, Jorgensen T, Kivimaki M, Kovacs P, Krohn K, Kumari M, Lauritzen T, Levy-Marchal C, Mayor V, McAteer JB, Meyre D, Mitchell BD, Mohlke KL, Morken MA, Narisu N, Palmer CN, Pakyz R, Pascoe L, Payne F, Pearson D, Rathmann W, Sandbaek A, Sayer AA, Scott LJ, Sharp SJ, Sijbrands E, Singleton A, Siscovick DS, Smith NL, Sparso T, Swift AJ, Syddall H, Thorleifsson G, Tonjes A, Tuomi T, Tuomilehto J, Valle TT, Waeber G, Walley A, Waterworth DM, Zeggini E, Zhao JH, consortium G, investigators M, Illig T, Wichmann HE, Wilson JF, van Duijn C, Hu FB, Morris AD, Frayling TM, Hattersley AT, Thorsteinsdottir U, Stefansson K, Nilsson P, Syvanen AC, Shuldiner AR, Walker M, Bornstein SR, Schwarz P, Williams GH, Nathan DM, Kuusisto J, Laakso M, Cooper C, Marmot M, Ferrucci L, Mooser V, Stumvoll M, Loos RJ, Altshuler D, Psaty BM, Rotter JI, Boerwinkle E, Hansen T, Pedersen O, Florez JC, McCarthy MI, Boehnke M, Barroso I, Sladek R, Froguel P, Meigs JB, Groop L, Wareham NJ, Watanabe RM: Genetic variation in GIPR influences the glucose and insulin responses to an oral glucose challenge. Nature genetics 2010;42:142-148
23. Prokopenko I, Poon W, Magi R, Prasad BR, Salehi SA, Almgren P, Osmark P, Bouatia-Naji N, Wierup N, Fall T, Stancakova A, Barker A, Lagou V, Osmond C, Xie W, Lahti J, Jackson AU, Cheng YC, Liu J, O'Connell JR, Blomstedt PA, Fadista J, Alkayyali S, Dayeh T, Ahlqvist E, Taneera J, Lecoeur C, Kumar A, Hansson O, Hansson K, Voight BF, Kang HM, Levy-Marchal C, Vatin V, Palotie A, Syvanen AC, Mari A, Weedon MN, Loos RJ, Ong KK, Nilsson P, Isomaa B, Tuomi T, Wareham NJ, Stumvoll M, Widen E, Lakka TA, Langenberg C, Tonjes A, Rauramaa R, Kuusisto J, Frayling TM, Froguel P, Walker M, Eriksson JG, Ling C, Kovacs P, Ingelsson E, McCarthy MI, Shuldiner AR, Silver KD, Laakso M, Groop L, Lyssenko V: A central role for GRB10 in regulation of islet function in man. PLoS genetics 2014;10:e1004235
24. Meier JJ, Baller B, Menge BA, Gallwitz B, Schmidt WE, Nauck MA: Excess glycaemic excursions after an oral glucose tolerance test compared with a mixed meal challenge and self-measured home glucose profiles: is the OGTT a valid predictor of postprandial hyperglycaemia and vice versa? Diabetes Obes Metab 2009;11:213-222
25. Dworatzek PDN, Hegele RA, Wolever TMS: Postprandial lipemia in subjects with the threonine 54 variant of the fatty acid-binding protein 2 gene is dependent on the type of fat ingested. Am J Clin Nutr 2004;79:1110-1117
26. Gomez P, Miranda JL, Marin C, Bellido C, Moreno JA, Moreno R, Perez-Martinez P, Perez-Jimenez F: Influence of the-514C/T polymorphism in the promoter of the hepatic lipase gene on postprandial lipoprotein metabolism. Atherosclerosis 2004;174:73-79
27. Jang Y, Kim JY, Kim OY, Lee JE, Cho H, Ordovas JM, Lee JH: The -> 1131T -> C polymorphism in the apolipoprotein A5 gene is associated with postprandial hypertriacylglycerolemia; elevated small, dense LDL concentrations; and oxidative stress in nonobese Korean men. Am J Clin Nutr 2004;80:832-840
28. Lopez-Miranda J, Cruz G, Gomez P, Marin C, Paz E, Perez-Martinez P, Fuentes FJ, Ordovas JM, Perez-Jimenez F: The influence of lipoprotein lipase gene variation on postprandial lipoprotein metabolism. J Clin Endocr Metab 2004;89:4721-4728
29. Cardona F, Morcillo S, Gonzalo-Marin M, Tinahones FJ: The apolipoprotein E genotype predicts postprandial hypertriglyceridemia in patients with the metabolic syndrome. J Clin Endocr Metab 2005;90:2972-2975
30. Liggi S, Griffin JL: Metabolomics applied to diabetes - lessons from human population studies. Int J Biochem Cell B 2017;93:136-147

31. Romisch-Margl W, Prehn C, Bogumil R, Rohring C, Suhre K, Adamski J: Procedure for tissue sample preparation and metabolite extraction for high-throughput targeted metabolomics. Metabolomics 2012;8:133-142
32. Soininen P, Kangas AJ, Wurtz P, Tukiainen T, Tynkkynen T, Laatikainen R, Jarvelin MR, Kahonen M, Lehtimaki T, Viikari J, Raitakari OT, Savolainen MJ, Ala-Korpela M: High-throughput serum NMR metabonomics for cost-effective holistic studies on systemic metabolism. Analyst 2009;134:1781-1785
33. Chen R, Mias GI, Li-Pook-Than J, Jiang LH, Lam HYK, Chen R, Miriami E, Karczewski KJ, Hariharan M, Dewey FE, Cheng Y, Clark MJ, Im H, Habegger L, Balasubramanian S, O'Huallachain M, Dudley JT, Hillenmeyer S, Haraksingh R, Sharon D, Euskirchen G, Lacroute P, Bettinger K, Boyle AP, Kasowski M, Grubert F, Seki S, Garcia M, Whirl-Carrillo M, Gallardo M, Blasco MA, Greenberg PL, Snyder P, Klein TE, Altman RB, Butte AJ, Ashley EA, Gerstein M, Nadeau KC, Tang H, Snyder M: Personal Omics Profiling Reveals Dynamic Molecular and Medical Phenotypes. Cell 2012;148:1293-1307
34. Page CM, Baranzini SE, Mevik BH, Bos SD, Harbo HF, Andreassen BK: Assessing the Power of Exome Chips. PloS one 2015;10:e0139642
35. Buniello A, MacArthur JAL, Cerezo M, Harris LW, Hayhurst J, Malangone C, McMahon A, Morales J, Mountjoy E, Sollis E, Suveges D, Vrousgou O, Whetzel PL, Amode R, Guillen JA, Riat HS, Trevanion SJ, Hall P, Junkins H, Flicek P, Burdett T, Hindorff LA, Cunningham F, Parkinson H: The NHGRI-EBI GWAS Catalog of published genome-wide association studies, targeted arrays and summary statistics 2019. Nucleic Acids Res 2018;
36. Gallagher MD, Chen-Plotkin AS: The Post-GWAS Era: From Association to Function. American journal of human genetics 2018;102:717-730
37. Visscher PM, Wray NR, Zhang Q, Sklar P, McCarthy MI, Brown MA, Yang J: 10 Years of GWAS Discovery: Biology, Function, and Translation. American journal of human genetics 2017;101:5-22
38. Davey Smith G, Hemani G: Mendelian randomization: genetic anchors for causal inference in epidemiological studies. Hum Mol Genet 2014;23:R89-98
39. Wu ZJ, Lou YQ, Qiu XC, Liu Y, Lu L, Chen QJ, Jin W: Association of cholesteryl ester transfer protein (CETP) gene polymorphism, high density lipoprotein cholesterol and risk of coronary artery disease: a meta-analysis using a Mendelian randomization approach. BMC medical genetics 2014;15
40. Pikula A, Beiser AS, Wang J, Himali JJ, Kelly-Hayes M, Kase CS, Yang Q, Seshadri S, Wolf PA: Lipid and lipoprotein measurements and the risk of ischemic vascular events Framingham Study. Neurology 2015;84:472-479
41. Ference BA, Kastelein JJP, Ginsberg HN, Chapman MJ, Nicholls SJ, Ray KK, Packard CJ, Laufs U, Brook RD, Oliver-Williams C, Butterworth AS, Danesh J, Smith GD, Catapano AL, Sabatine MS: Association of Genetic Variants Related to CETP Inhibitors and Statins With Lipoprotein Levels and Cardiovascular Risk. Jama 2017;318:947-956
42. Blauw LL, Li-Gao R, Noordam R, de Mutsert R, Trompet S, Berbee JFP, Wang Y, van Klinken JB, Christen T, van Heemst D, Mook-Kanamori DO, Rosendaal FR, Jukema JW, Rensen PCN, Willems van Dijk K: CETP (Cholesteryl Ester Transfer Protein) Concentration: A Genome-Wide Association Study Followed by Mendelian Randomization on Coronary Artery Disease. Circ Genom Precis Med 2018;11:e002034
43. Lijfering WM, Flinterman LE, Vandenbroucke JP, Rosendaal FR, Cannegieter SC: Relationship between Venous and Arterial Thrombosis: A Review of the Literature from a Causal Perspective. Semin Thromb Hemost 2011;37:884-895
44. Becattini C, Vedovati MC, Ageno W, Dentali F, Agnelli G: Incidence of arterial cardiovascular events after venous thromboembolism: a systematic review and a meta-analysis. J Thromb Haemost 2010;8:891-897
45. Roach REJ, Lijfering WM, Flinterman LE, Rosendaal FR, Cannegieter SC: Increased risk of CVD after VT is determined by common etiologic factors. Blood 2013;121:4948-4954

46. Ageno W, Becattini C, Brighton T, Selby R, Kamphuisen PW: Cardiovascular risk factors and venous thromboembolism - A meta-analysis. Circulation 2008;117:93-102
47. Lindstrom S, Germain M, Crous-Bou M, Smith EN, Morange PE, Vlieg AV, de Haan HG, Chasman D, Ridker P, Brody J, de Andrade M, Heit JA, Tang WH, DeVivo I, Grodstein F, Smith NL, Tregouet D, Kabrhel C, Consortium I: Assessing the causal relationship between obesity and venous thromboembolism through a Mendelian Randomization study. Hum Genet 2017;136:897-902
48. de Mutsert R, den Heijer M, Rabelink TJ, Smit JW, Romijn JA, Jukema JW, de Roos A, Cobbaert CM, Kloppenburg M, le Cessie S, Middeldorp S, Rosendaal FR: The Netherlands Epidemiology of Obesity (NEO) study: study design and data collection. European journal of epidemiology 2013;28:513-523
49. Roach RE, Lijfering WM, van Hylckama Vlieg A, Helmerhorst FM, Rosendaal FR, Cannegieter SC: The risk of venous thrombosis in individuals with a history of superficial vein thrombosis and acquired venous thrombotic risk factors. Blood 2013;122:4264-4269