Genetic determinants of cholesterol and energy metabolism : implications for cardiometabolic health

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Addendum

Summary

While most infectious diseases have been combatted in the past centuries, mankind is now facing epidemics of non-communicable diseases. This is strikingly reflected in the global rise of cardiometabolic disorders, which mainly comprise obesity, cardiovascular disease (CVD) and type 2 diabetes. To increase human life span, spent in good health, the development and improvement of preventive and curative strategies for cardiometabolic disease is eagerly warranted. With the studies describes in this thesis, we aimed to disentangle the interwoven physiological, environmental and genetic factors that determine cholesterol and energy metabolism to increase our understanding of their contribution to cardiometabolic disease risk, thereby facilitating the search for novel preventive and curative strategies. **Chapter 1** serves as a general introduction to cholesterol and energy metabolism, and their role in cardiometabolic health.

The first part of this thesis focussed on the cholesteryl ester transfer protein (CETP). CETP is able to transfer cholesteryl esters from high-density lipoprotein (HDL) towards apolipoprotein B containing triglyceride-rich lipoproteins, mainly very low-density lipoproteins (VLDL), and triglycerides from VLDL and chylomicrons towards triglyceride-poor particles, such as HDL. Since these lipid transfer properties of CETP induce a proatherogenic lipoprotein profile, CETP inhibitory molecules have been developed and tested in clinical trials for their capability to improve the lipoprotein profile and reduce CVD risk. To fully understand the role of CETP in CVD, its physiology and biological function should be fully unravelled. Strikingly, although CETP inhibitors have already been tested in clinical trials for over a decade, there was no consensus on the cellular source of circulating CETP, as both the liver and adipose tissue were regarded to be responsible for the production of CETP.

In **Chapter 2** we examined the contribution of body fat to circulating CETP concentration. To this end, we studied in 6,606 participants of the Netherlands Epidemiology of Obesity (NEO) study the associations of total body fat, body mass index, waist circumference, waist-to-hip ratio, abdominal subcutaneous and visceral adipose tissue assessed with magnetic resonance imaging (N=2,547), and total and trunk fat mass assessed with dual-energy X-ray absorptiometry (N=909) with serum CETP concentration. We concluded that, in this population-based study, there was no evidence for clinically relevant associations between these measures of body fat and serum CETP concentration, which implies that adipose tissue does not contribute to the CETP pool in serum. These data support

previous findings from our research group that circulating CETP concentration does not associate with waist circumference, and that circulating CETP concentration is mainly determined by the number of resident macrophages in the liver, which are referred to as Kupffer cells.

Based on the primary expression of *CETP* by these cells of the innate immune system and our previous finding that activation of Kupffer cells by the bacterial endotoxin lipopolysaccharide strongly decreases CETP expression, we subsequently studied if metabolic liver inflammation is also associated with a decrease in hepatic expression and plasma concentrations of CETP, the results of which are described in **Chapter 3**. To this end, we collected plasma and liver biopsy samples at various stages of non-alcoholic fatty liver disease from 93 obese individuals who underwent bariatric surgery. Liver lobular inflammation was histologically determined, and liver *CETP* expression, CETP positive cells, circulating CETP concentrations were quantified. We found no strong evidence for a negative association between metabolic liver inflammation and CETP-related outcomes, although we observed consistent trends towards lower measures of CETP in the presence of metabolic liver inflammation. From this, we concluded that metabolic and infection-induced liver inflammation have different effects on the expression and production of CETP by Kupffer cells. Acute and/or whole-body inflammatory responses to invading pathogens are thus probably required to induce a robust reduction in CETP production by Kupffer cells.

Next, to elucidate the genetic basis of serum CETP concentration, we performed the first genome-wide association study (GWAS) and exome-wide analysis on serum CETP concentration to identify common and rare genetic variants that determine circulating CETP. In **Chapter 4**, we describe the identification of three common independent variants mapped to the CETP gene (i.e. rs247616, rs12720922 and rs1968905) that together explain 16.4% of the total variation in CETP concentration, in a discovery cohort of the NEO study (N=4,248). These genetic variants were replicated in a separate subpopulation of the NEO study (N=1,458). Furthermore, in an exome-wide gene-based aggregation analysis (N=6,094), we identified rare variants (i.e. minor allele frequency <0.03) in the ABCA6 gene as a novel determinants of CETP concentration, as described in **Chapter 5**, from which we concluded that abundance or functionality of the ABCA6 protein regulates the circulating CETP concentration. Since ABCA6 is involved in cellular lipid and sterol transport and CETP is regulated by sterol derivatives via a liver X receptor α responsive element in its promoter region, we speculate that ABCA6 determines CETP production via intracellular regulation of lipid and sterol trafficking.

We next used the GWAS-identified common CETP variants as genetic instruments in Mendelian randomization analyses to investigate the causal effects of an increase in CETP concentration on the lipoprotein metabolite profile (NEO study) and coronary artery disease risk (CARDIoGRAMplusC4D consortium), which is relevant to better understand the outcomes of the CETP inhibitor trials that aim to reduce CVD. We showed in **Chapter 6** that an increase in circulating CETP causally decreases the concentration of mainly large HDL particles, and increases the classes of small VLDL particles without increasing the classes and composition of low-density lipoprotein (LDL) particles. Additionally, increased CETP causally associated with a higher risk of coronary artery disease with an odds of 1.08 (95% CI 0.94, 1.23) (**Chapter 4**). Interestingly, it was previously reported that small and very small VLDL particle concentrations (reflecting triglyceride-rich lipoprotein remnants) and cholesterol in VLDL (reflecting remnant cholesterol) are among the lipoprotein components that most strongly associate with an increased CVD risk. Thus, contrary to the current dogma that CETP increases CVD risk via increasing LDL-cholesterol, our results imply that the increased CVD risk associated with higher CETP concentrations is explained by an increase of small VLDL (remnant) particles.

The focus of the second part of this thesis was on the role of energy metabolism in cardiometabolic health. Specifically, we aimed to study the association of environmental and genetic factors, which were previously described to influence brown adipose tissue (BAT) activity, with energy expenditure and disease outcomes. Activation of BAT increases energy expenditure, as this tissue is capable to produce heat in response to cold by combusting energy that is stored in glucose and triglycerides. Based on a recent landmark paper, which showed that a 10 day cold acclimatisation period activates BAT and improves insulin sensitivity in patients with type 2 diabetes, we assessed in **Chapter 7** the association between mean annual temperature and diabetes incidence during 1996-2009 in the United States of America. On a global scale, we also assessed the association between mean annual temperature and the prevalence of glucose intolerance using data from 2014. We demonstrated that on average per 1°C increase in temperature age-adjusted diabetes incidence increased with 0.314 (95% CI 0.194, 0.434) per 1000. Similarly, the worldwide prevalence of glucose intolerance increased by 0.170% (95% CI 0.107, 0.234) per 1°C rise in temperature. These findings emphasize the importance of future research into the effects of environmental temperature on glucose metabolism and the onset of diabetes, especially in view of the global rise in temperatures.

Since it was shown in rats that nicotine increases energy combustion in BAT, we studied in **Chapter 8** the association between smoking and resting energy expenditure in 1,189 study participants from the NEO study. We showed that resting energy expenditure expressed per fat free mass was higher in current smokers compared with never smokers. There was no difference in resting energy expenditure between never and former smokers or between never and occasional smokers and no association with pack years, from which we concluded that smoking has primarily acute effects on resting energy expenditure.

Finally, we studied in **Chapter 9** the association between rs1421085-risk allele carriage and whole-body fat oxidation in 1,246 participants of the NEO study. We initiated this study based on a landmark paper from 2015 that described a potential genetic basis for BAT activity, by showing that T-to-C substitution in the rs1421085 variant of the fat mass and obesity-associated gene (*FTO*) gene reduced browning of pre-adipocytes *in vitro*. We hypothesized that humans who carry the C-allele of rs1421085 may have reduced browning of their white fat depots, which possibly leads to a lower fat oxidation, since brown fat cells selectively enhance the oxidation of fat over glucose. However, we observed no evidence for associations of rs1421085 in *FTO* with resting whole-body fat oxidation, despite higher measure of obesity in risk allele carriers. This implies that the contribution of browning of white adipocytes to total energy metabolism is apparently too small to affect whole-body fat oxidation. Thus, likely, the rs1421085 C-allele in *FTO* induces obesity via other pathways than via reduced fat oxidation.

To conclude this thesis, the study findings, their implications for future research, and the perspectives for preventive and therapeutic strategies of cardiometabolic disease are discussed in **Chapter 10**. All in all, the results of the studies described in this thesis have increased our understanding of the physiological, environmental and genetic factors that determine cholesterol and energy metabolism, and provided insight in their contribution to cardiometabolic health.