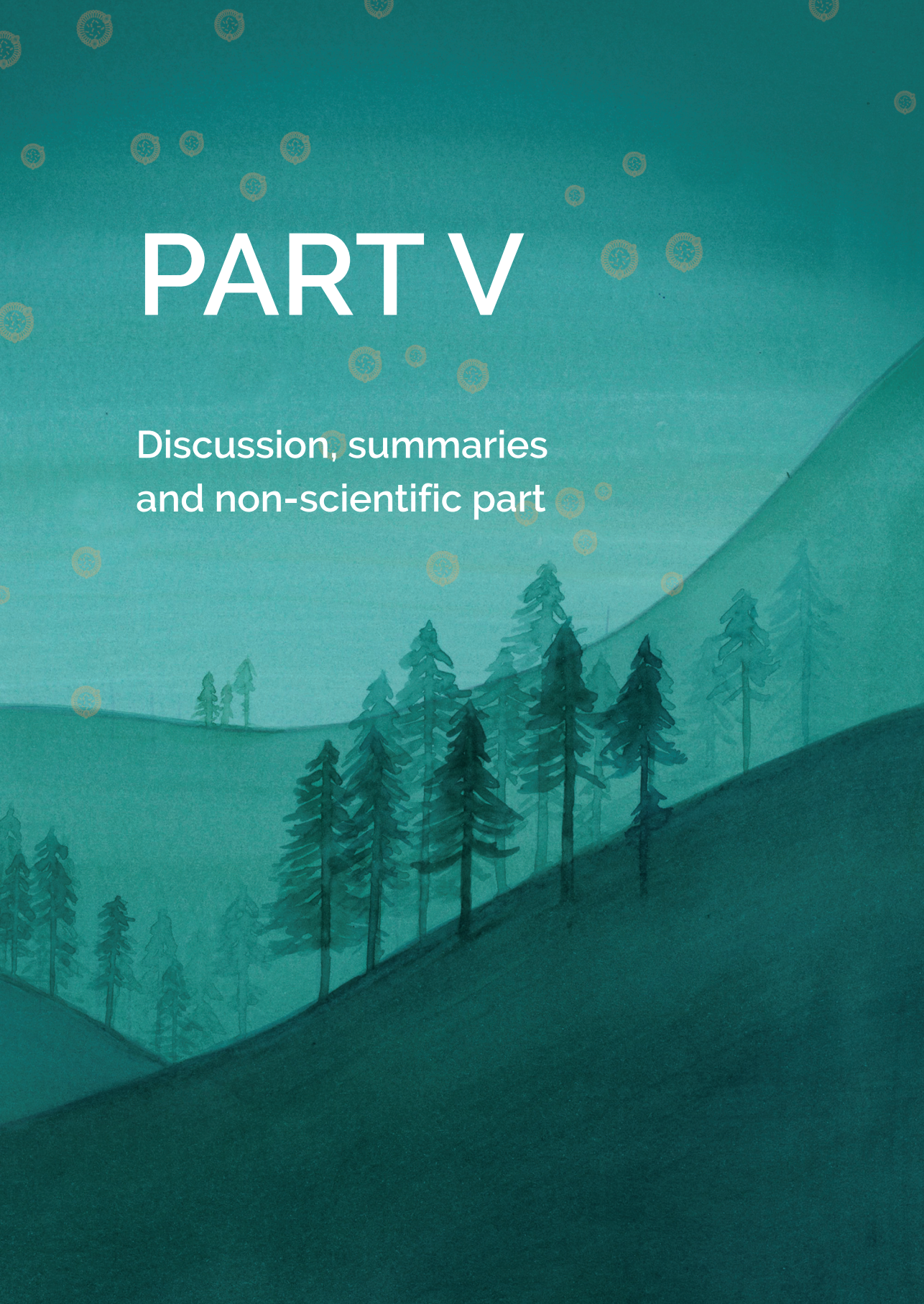
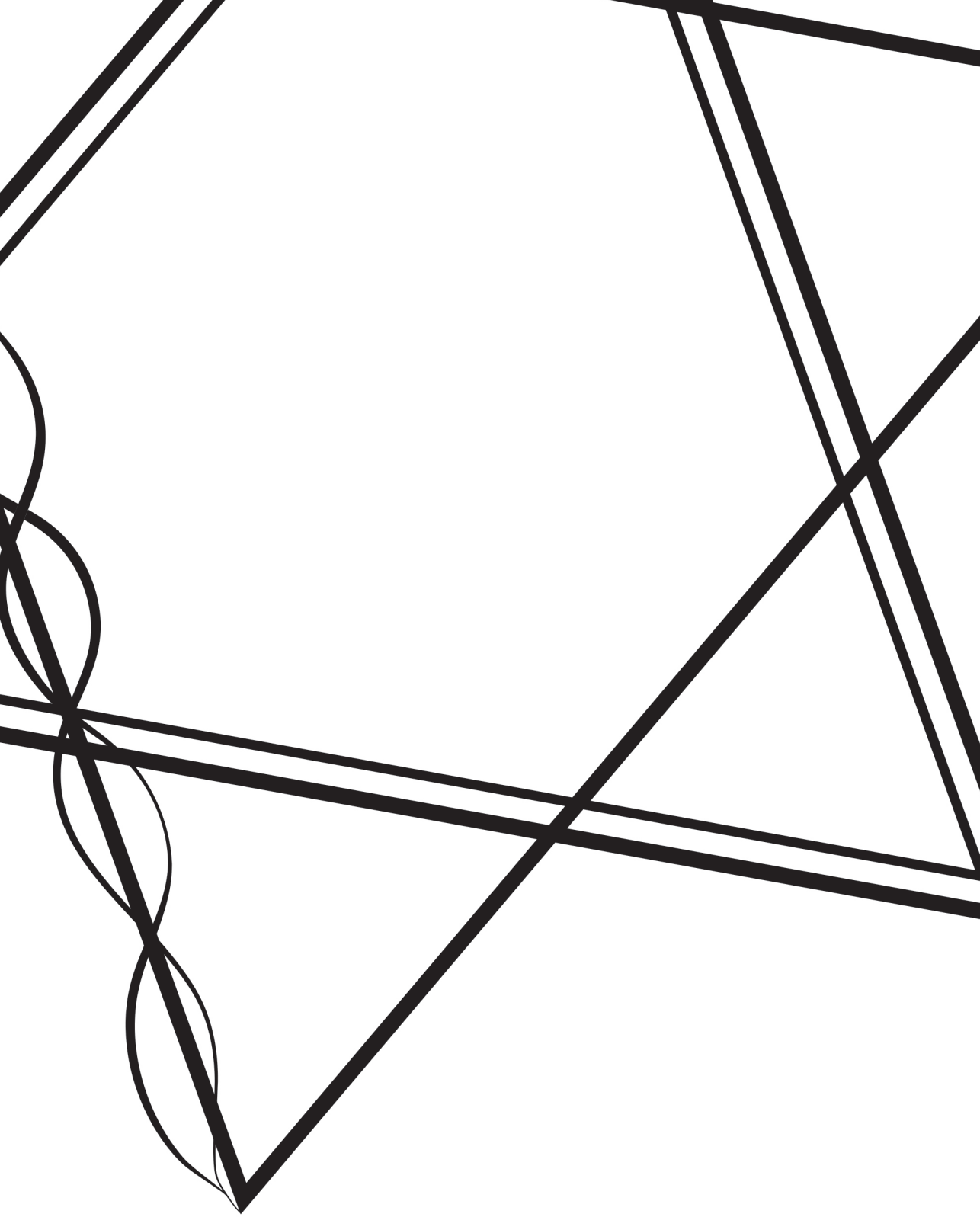
Genetic and environmental determinants of cardiometabolic health

Bos, M.M.

PART V

Discussion, summaries and non-scientific part





CHAPTER 6

General discussion and future perspectives

Maxime M Bos

GENERAL DISCUSSION

The aim of this thesis was to study the role and interplay of genetic and environmental factors on cardiometabolic health. Hereby, we aimed to identify potential (causal) biological mechanisms that are of interest as future targets for primary or secondary preventive strategies for cardiometabolic diseases. The first part of this thesis focused on the use of untargeted metabolites in relation to early disturbances in glucose metabolism and insulin sensitivity in middle-aged individuals. The second part was aimed at studying, and potentially identifying, targets that are important in the relations between measures of habitual sleep with cardiometabolic health in the general population. In the third part, we focused on the *APOE* gene and the effect of oily fish intake, physical activity and polyunsaturated fatty acid intake on the onset of age-related diseases, especially coronary artery disease. In the last part of this thesis, we investigated the potential causal association of thyroid status with glucose homeostasis and type 2 diabetes mellitus. In this section of this thesis, I will discuss and further interpret the results from these different chapters. Moreover, the implications of the findings for future preventive and treatment strategies to decrease the burden of cardiometabolic diseases are discussed.

Main findings

The increase in overall life expectancy has resulted in a larger proportion of older individuals worldwide, which is projected to continue to increase[1]. Simultaneously, a rapid rise of individuals with multiple age-related cardiometabolic diseases such as type 2 diabetes mellitus is observed[2]. Therefore, there is an urgent need for the identification of risk factors in an early stage of this condition. In **Chapter 2**, we studied, through untargeted metabolomics, metabolites in relation to measures of insulin resistance in non-diabetic individuals and we replicated the identified metabolites in an independent cohort. We validated the metabolites by assessing their relationship with diabetes mellitus. The findings from **Chapter 2** of this thesis point towards biomarkers of early insulin resistance that may predict type 2 diabetes onset. In particular, several amino acids and saturated fatty acids may contribute to alterations in insulin sensitivity and increased insulin resistance[3]. These findings are in line with previous studies that show that several amino acids, sugar metabolites, and lipids have been associated with a higher risk for diabetes mellitus[4-7]. Especially, several (branched-chain) amino acids are consistently associated with diabetes mellitus[3,5]. Amino acids are essential for protein metabolism and muscle proteins in particular serve as an energy store. When the body is in the anabolic state, amino acids are added to the body's protein pool, while in the catabolic state energy is provided by the breakdown of endogenous proteins to provide the body with amino acids that are used for gluconeogenesis. This occurs when the body is energy deprived as well as when there is an excess of dietary protein. Both in

prediabetic and diabetic individuals, gluconeogenesis has mostly been observed to be higher as compared to controls[8]. Alanine and glutamine are the most important gluconeogenic precursors in the liver. Interestingly, genetic predisposition to type 2 diabetes is associated with increased levels of alanine and genetically-determined higher levels of alanine are associated with an increased risk for type 2 diabetes, thereby providing novel insights into promising causal paths to and from type 2 diabetes[9].

Habitual sleep is increasingly considered as an important factor contributing to cardiometabolic disease onset[10-13]. Previously, in epidemiological cohort studies, both a short and long habitual sleep duration have been associated with risk factors for an adverse cardiometabolic health, but likely via different biological mechanisms[10-13]. The findings as presented in **Chapter 3.1** and **3.2** indicate an observational relationship between habitual sleep duration and sleep quality with glucose metabolism and lipid metabolism. We additionally described that specifically a shorter sleep duration and a poorer sleep quality were associated with higher insulin resistance, higher fasting triglyceride levels and a higher hepatic triglyceride content. However, we demonstrated that it is not sleep duration or sleep quality per se, but rather BMI and associated sleep apnea that drive these associations, since these associations disappeared after adjustment for these factors. Moreover, an innovative research approach that is increasingly used to help disentangle questions of causality – Mendelian Randomization (MR) – was used to study these relations. We showed that using MR, no evidence for a causal association between total, short or long sleep duration, glycemic traits and type 2 diabetes was observed. Therefore, we conclude that previously observed associations of shorter sleep duration and poorer sleep quality with an adverse glucose metabolism and lipid profile, may be explained by BMI and sleep apnea, with no direct effect of sleep duration and quality on glucose metabolism. In a previous study, that assessed the causal association of total sleep duration with 22 prevalent diseases from the Electronic Medical Records in the Partners Biobank (n=16,033), associations were observed with congestive heart failure, obesity, hypertension, restless legs syndrome, and insomnia[14]. While after adjustment for obesity, associations with total sleep duration disappeared for hypertension and insomnia, these associations were maintained for congestive heart failure and restless legs syndrome. Taken together, findings suggest that sleep has no direct effect on energy metabolism and measures of glucose metabolism (and type 2 diabetes), however, sleep may have a direct effect on cardiovascular diseases. In line, another recent study showed that there is a causal effect of short sleep duration on myocardial infarction[15]. In **Chapter 3.3**, we aimed to elucidate the biology of sleep duration-associated cardiovascular risk through gene-lifestyle interactions. We performed short- and long-sleep-SNP interaction analyses in over 125,000 individuals in a large collaborative setting (notably within the Cohorts for

Heart and Ageing Research in Genomics Epidemiology[16,17]) to obtain novel insights in the biological background of sleep duration-associated adverse lipid profiles. A total of 59 novel loci were identified in relation to lipid traits. These loci were previously described in relation to adiposity, inflammation and neuropsychiatric traits. Importantly, the novel lipid loci that we identified with short sleep duration were not identified with long sleep duration, and *vice versa*. Our findings suggest that the biological mechanisms that underlie the relation between short sleep and an adverse lipid profile are distinct from those of long sleep and an adverse lipid profile.

Large initiatives have been performed to investigate the genetic contribution to CVD pathogenesis [18]. Genetic variation in the *APOE* gene has been widely recognized to increase the risk of CVD, which has also been confirmed by genome-wide association studies[18-20]. Genetic variation in *APOE* is a well-established CVD risk factor; however, in some individuals from a number of non-European ancestries (e.g., sub-Saharan), this relationship is not observed[21,22], possibly due to cultural, environmental and lifestyle differences. In **Chapter 4.1**, a hypothesis is proposed regarding potential effect modification by oily fish intake and physical activity on the risk of cardiometabolic and brain diseases associated with genetic variation in *APOE*. We hypothesized a higher level of physical activity and a higher intake of oily fish to decrease the adverse health effects associated with genetic variation in *APOE*, especially in carriers of the ApoE ε4 isoform. This hypothesis was further tested in the largest study to date, embedded in the population-based UK Biobank[23], in **Chapter 4.2**. With a higher level of physical activity and a higher intake of oily fish, we observed a lower incidence of coronary artery disease (CAD) in all ApoE isoform groups. Our results indicate that not only carriers of the ApoE ε4 isoform may benefit from a higher intake of oily fish and a higher physical activity, but that all individuals may benefit from a higher level of physical activity and a higher oily fish intake in regard to CAD incidence. However, this study did not find support for the previous hypothesis that lifestyle interactions are specifically present in individuals carrying the ApoE ε4 isoform. In line, another study that assessed potential interaction with lifestyle factors and *APOE* in relation to cognition failed to prove the existence of a gene-environment interaction[24].

Several studies have observed associations between thyroid status and diabetes mellitus, however causality remained unknown[25]. In **Chapter 5.1**, we focussed on genetically-determined TSH and fT4 in relation to measures of glucose metabolism and type 2 diabetes using MR. We showed that there is no causal association between genetically-determined higher TSH and fT4 with measures of glucose metabolism and type 2 diabetes. However, we found some evidence for an association between genetic variation in the *DIO1* gene and measures of glucose metabolism. In **Chapter 5.2**,

we examined whether the findings of **Chapter 5.1** could be replicated using stronger instruments in a large cohort study of the UK Biobank. Since diabetes mellitus is a heterogeneous disease, which is often not taken into consideration, we took into account different subtypes of diabetes mellitus. Moreover, we assessed the associations in relation to genetically-determined BMI, since BMI is the main risk factor for diabetes mellitus onset. We observed that genetically-determined higher fT4 was associated with diabetes mellitus only in those individuals who had a younger age at diagnosis and did not use insulin or insulin analogues within the first year after diagnosis. When we stratified based on genetically-determined BMI, a higher genetically-determined TSH was associated with diabetes mellitus, however, only in the subgroup with a low genetically-determined BMI. Taken together, these findings may indicate that a higher fT4 level may protect from diabetes mellitus only at a younger age and that a higher TSH level may protect from diabetes mellitus onset, however, only if individuals have a lower BMI.

Conclusions and Future perspectives

In this thesis, we identified markers of early disturbances of glucose metabolism which may reflect diabetes mellitus onset. The findings of **Chapter 2** point to the possibility of using biomarkers that are indicative of risk of future disease. Early identification of individuals at high risk for diabetes is of importance for prevention, mainly because routine screening misses many cases of prediabetes and early type 2 diabetes[26]. These developments point towards the usage of metabolomics in clinical practice. Since high-throughput metabolomics is an easily assessable and relatively cheap method, it is relevant to study the potential usage of this method in clinical practice since an increase in usage of this method is expected. For example, several metabolites may be used in strategies involved in risk stratification of subjects at increased risk of type 2 diabetes. Moreover, the usage of metabolomics for insulin sensitivity and diabetes onset may be implemented in risk stratification of patients with difficult glucose control. Whether such markers provide accurate prognostic information for individuals without diabetes mellitus is subject to further study.

Our findings from **Chapter 3.1** and **3.2** suggest that a shorter sleep duration and a poorer sleep quality are not associated with glucose metabolism and the lipid profile once BMI and sleep apnea are taken into consideration and no causal association between sleep duration, type 2 diabetes and glycemic traits is observed. In **Chapter 3.3**, we demonstrated that we were able to identify additional lipid loci once we take into account interaction with total sleep duration. The new identified loci mainly associated with adiposity and psychological measures. These findings suggest that shifting the focus to anthropometric and psychiatric factors that are related to adverse habitual

sleep may improve risk management and treatment strategies in cardiometabolic disease prevention. That being said, an adverse sleep profile may still be used as a risk factor in risk stratification for cardiometabolic disease. Collectively, the findings of our study provide insights in sleep-associated lipid biology that are of very high interest in follow-up studies. In order to increase insights into sleep, further studies should also consider the complexity of sleep. Habitual sleep is a reflection not only of sleep duration, but also of factors such as chronotype and insomnia. In order to gain better insights in the biology of sleep in relation to cardiometabolic health, studies should incorporate this multidimensional nature of sleep in their researches. Moreover, metabolomics studies are of interest in relation to sleep, since these methods are reflective of the metabolic state of an individual. Additionally, in the studies as described in this thesis, we only used self-reported measures of sleep. These measures may be vulnerable to biases and therefore studies using more objective measures are warranted. One could think of studies that use accelerometer-derived variables. As a final suggestion, future studies could focus on several lifestyle factors in a jointly manner. Sleep is affected by many factors, including food intake and physical activity, which should all be considered when assessing sleep in relation to cardiometabolic health.

Despite the introduction of cholesterol lowering medication, cardiovascular disease (CVD) is still one of the most common causes of morbidity and mortality in the general population[27]. Much of the current research has been focused on disentangling the biology of CVD pathogenesis and the identification of novel targets for disease prevention. The findings from **Chapter 4.1** and **4.2** of this thesis point toward a possible direct link of physical activity, oily fish intake and polyunsaturated fatty acid intake with incident CVD and CAD. While a higher physical activity, fish intake and PUFA intake both decreased the risk of CAD, no evidence for a statistical environment-*APOE* interaction was observed. Therefore, it seems unlikely that interventions intended to reduce cardiovascular risk show different effects depending on *APOE* genotype. Importantly, we demonstrated that individuals that are at a high genetic risk for CVD and CAD can still benefit from a healthier lifestyle. Thus, the addition of lifestyle advice to existing treatment options for cardiovascular disease and to prevention strategies should be further investigated.

In the last part of this thesis, we focussed on the association of thyroid status with diabetes mellitus and insulin resistance. In **Chapter 5.1**, we demonstrated that there is no evidence for a causal association between circulating TSH and fT4 with type 2 diabetes mellitus and glycemic traits. In **Chapter 5.2**, we provide evidence that in a larger population a higher genetically-determined TSH is causally associated with diabetes mellitus, however, only in participants with a genetically-determined low BMI. This may imply that there still may be a causal association between thyroid status and diabetes,

however, BMI may be a stronger risk factor for diabetes mellitus, thereby overruling the potential protective effect of a higher TSH. Future studies should investigate this hypothesis. Moreover, in the current study, we only assessed the effects of circulatory TSH and fT4 on DM onset. Variations in circulating TSH and fT4 are thought to predominantly reflect the sensitivity of the thyroid gland to feedforward stimulation and the sensitivity of the pituitary gland to feedback inhibition. In contrast, deiodinases have a critical role in the activation of thyroid hormones in target tissues[28]. In **Chapter 5.1**, we demonstrated that genetic variation in *DIO1* may affect glucose metabolism[29]. This result may indicate a role of reduced bioavailability of thyroid hormone in target tissues (such as liver) in glucose metabolism. We therefore propose that future studies should focus on the role of deiodinases and availability of thyroid hormones in target tissues on the risk of DM.

This thesis provides novel insights in several associations that are potentially of importance in cardiometabolic health. However, we should keep in mind that cardiometabolic diseases are very complex and multiple pathways act together in order to maintain cardiometabolic health. For example, another study demonstrated that sleep duration tended to be positively associated with free thyroxine levels and negatively associated with HbA1c and CRP[30]. Moreover, these findings showed that short-sleeping UK adults are more likely to have obesity, a disease with many comorbidities. This extraordinary level of complexity calls for more sophisticated approaches in order to deepen our understanding of the mechanisms that underlie cardiometabolic health and disease. For example, national mega-biobanks such as the UK Biobank, the Million Veteran Program and the China Kadoorie Biobank offer a variety of possibilities to perform research with genomic data in large well-phenotyped populations. In combination with other omics approaches (e.g. metabolomics, proteomics), these studies may provide valuable insights in cardiometabolic health. Identification of genetic determinants of cardiometabolic health, assisted by reliable and cost-effective biomarkers, can help in the further understanding of the individual risk differences in developing cardiometabolic disease. Moreover, the tremendous amount of data that will be generated by these studies asks for more complex modelling of data. Therefore, more advanced machine-learning methods such as methods to perform advanced clustering and classification may be of special interest in this era of big data. Because of the exponential increase in data (complexity), the application of the more traditional bioinformatic tools may become soon outdated. By using more advanced data modelling techniques, several types of data (e.g. clinical and omics) may be analysed simultaneously. When these methods are then used to predict individual risks for disease onset, this may be of value in personalized medicine. In the future, these efforts may lead to a healthcare system in which the risk for an individual (or group of individuals) for certain diseases can be estimated more precisely. Then, future and preventive strategies may be implemented

specifically tailored to an individual's genetic profile to more effectively prevent and treat several cardiometabolic diseases. Eventually, by considering the multidimensionality of cardiometabolic health and the adaptation of future preventive and curative strategies based upon this complexity may add to a more effective prevention and treatment of cardiometabolic disease.

REFERENCES

1. Kontis V, Bennett JE, Mathers CD, Li G, Foreman K, Ezzati M. Future life expectancy in 35 industrialised countries: projections with a Bayesian model ensemble. *Lancet.* 2017;389(10076):1323-1335.
2. Gorelick PB, Furie KL, Iadecola C, et al. Defining Optimal Brain Health in Adults: A Presidential Advisory From the American Heart Association/American Stroke Association. *Stroke.* 2017;48(10):e284-e303.
3. Würtz P, Soininen P, Kangas AJ, et al. Branched-Chain and Aromatic Amino Acids Are Predictors of Insulin Resistance in Young Adults. 2013;36(3):648-655.
4. Del Coco L, Vergara D, De Matteis S, et al. NMR-Based Metabolomic Approach Tracks Potential Serum Biomarkers of Disease Progression in Patients with Type 2 Diabetes Mellitus. *J Clin Med.* 2019;8(5).
5. Gar C, Rottenkolber M, Prehn C, Adamski J, Seissler J, Lechner A. Serum and plasma amino acids as markers of prediabetes, insulin resistance, and incident diabetes. *Crit Rev Clin Lab Sci.* 2018;55(1):21-32.
6. Mook-Kanamori DO, de Mutsert R, Rensen PC, et al. Type 2 diabetes is associated with postprandial amino acid measures. *Arch Biochem Biophys.* 2016;589:138-144.
7. t Hart LM, Vogelzangs N, Mook-Kanamori DO, et al. Blood Metabolomic Measures Associate With Present and Future Glycemic Control in Type 2 Diabetes. *J Clin Endocrinol Metab.* 2018;103(12):4569-4579.
8. Chung ST, Hsia DS, Chacko SK, Rodriguez LM, Haymond MW. Increased gluconeogenesis in youth with newly diagnosed type 2 diabetes. *Diabetologia.* 2015;58(3):596-603.
9. Liu J, van Klinken JB, Semiz S, et al. A Mendelian Randomization Study of Metabolite Profiles, Fasting Glucose, and Type 2 Diabetes. *Diabetes.* 2017;66(11):2915-2926.
10. Wong PM, Manuck SB, DiNardo MM, Korytkowski M, Muldoon MF. Shorter sleep duration is associated with decreased insulin sensitivity in healthy white men. *Sleep.* 2015;38(2):223-231.
11. Reutrakul S, Van Cauter E. Interactions between sleep, circadian function, and glucose metabolism: implications for risk and severity of diabetes. *Annals of the New York Academy of Sciences.* 2014;1311:151-173.
12. Cappuccio FP, Taggart FM, Kandala NB, et al. Meta-analysis of short sleep duration and obesity in children and adults. *Sleep.* 2008;31(5):619-626.
13. Cappuccio FP, D'Elia L, Strazzullo P, Miller MA. Quantity and quality of sleep and incidence of type 2 diabetes: a systematic review and meta-analysis. *Diabetes Care.* 2010;33(2):414-420.
14. Dashti HS, Redline S, Saxena R. Polygenic risk score identifies associations between sleep duration and diseases determined from an electronic medical record biobank. *Sleep.* 2018;42(3).
15. Daghlas I, Dashti HS, Lane J, et al. Sleep Duration and Myocardial Infarction. *J Am Coll Cardiol.* 2019;74(10):1304-1314.
16. Rao DC, Sung YJ, Winkler TW, et al. Multiancestry Study of Gene-Lifestyle Interactions for Cardiovascular Traits in 610 475 Individuals From 124 Cohorts: Design and Rationale. *Circ Cardiovasc Genet.* 2017;10(3):e001649.
17. Psaty BM, O'Donnell CJ, Gudnason V, et al. Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium: Design of prospective meta-analyses of genome-wide association studies from 5 cohorts. *Circ Cardiovasc Genet.* 2009;2(1):73-80.

18. Nikpay M, Goel A, Won HH, et al. A comprehensive 1,000 Genomes-based genome-wide association meta-analysis of coronary artery disease. *Nature genetics.* 2015;47(10):1121-1130.
19. CARDIoGRAMplusC4D Consortium, Deloukas P, Kanoni S, et al. Large-scale association analysis identifies new risk loci for coronary artery disease. *Nature genetics.* 2013;45(1):25-33.
20. Song Y, Stampfer MJ, Liu S. Meta-analysis: apolipoprotein E genotypes and risk for coronary heart disease. *Annals of internal medicine.* 2004;141(2):137-147.
21. Chen CH, Mizuno T, Elston R, et al. A comparative study to screen dementia and APOE genotypes in an ageing East African population. *Neurobiology of aging.* 2010;31(5):732-740.
22. Gureje O, Ogunniyi A, Baiyewu O, et al. APOE epsilon4 is not associated with Alzheimer's disease in elderly Nigerians. *Annals of neurology.* 2006;59(1):182-185.
23. Sudlow C, Gallacher J, Allen N, et al. UK Biobank: An Open Access Resource for Identifying the Causes of a Wide Range of Complex Diseases of Middle and Old Age. *PLOS Medicine.* 2015;12(3):e1001779.
24. Lyall DM, Celis-Morales C, Lyall LM, et al. Assessing for interaction between APOE ε4, sex, and lifestyle on cognitive abilities. *Neurology.* 2019;92(23):e2691-e2698.
25. Chaker L, Ligthart S, Korevaar TIM, et al. Thyroid function and risk of type 2 diabetes: a population-based prospective cohort study. *BMC Med.* 2016;14(1):150-150.
26. Wilson PWF, Meigs JB, Sullivan L, Fox CS, Nathan DM, D'Agostino RB, Sr. Prediction of Incident Diabetes Mellitus in Middle-aged Adults: The Framingham Offspring Study. *JAMA Internal Medicine.* 2007;167(10):1068-1074.
27. Andersson C, Vasan RS. Epidemiology of cardiovascular disease in young individuals. *Nature reviews Cardiology.* 2018;15(4):230-240.
28. Gereben B, Zavacki AM, Ribich S, et al. Cellular and molecular basis of deiodinase-regulated thyroid hormone signaling. *Endocr Rev.* 2008;29(7):898-938.
29. Bos MM, Smit RAJ, Trompet S, van Heemst D, Noordam R. Thyroid Signaling, Insulin Resistance, and 2 Diabetes Mellitus: A Mendelian Randomization Study. *J Clin Endocrinol Metab.* 2017;102(6):1960-1970.
30. Potter GDM, Cade JE, Hardie LJ. Longer sleep is associated with lower BMI and favorable metabolic profiles in UK adults: Findings from the National Diet and Nutrition Survey. *PloS one.* 2017;12(7):e0182195-e0182195.