Insight into the pathophysiology of cardiometabolic diseases using multiple omics approaches

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Chapter 8

Appendices

Summary

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Acknowledgements

Summary

Despite extensive research, significant gaps remain in fully understanding and preventing the onset of cardiometabolic disease. In this thesis, we aimed to unravel part of the etiology and pathophysiology of cardiometabolic disease by investigating potential (causal) risk factors and the interplay between genetic and environmental factors in cardiometabolic diseases using cutting-edge statistical and epidemiological methods.

In **Chapter 1**, I provided an overview of the disease background, explained the relevant biological concepts, described the methodology employed and the study populations utilized in this thesis.

In **Chapter 2** of this thesis, we focused on insulin sensitivity of metabolomic measures. Although the association between insulin sensitivity and metabolomic measures has been extensively studied, the actual response of these measures to changes in insulin concentration in non-diabetic individuals has been understudied. We examined this response of metabolomic measures under different hyper-insulinemic levels while maintaining euglycemic conditions in middle-aged non-diabetic individuals. In this study, we compared the changes in the concentrations of 151 metabolomic measures after low- and high-dose insulin infusion compared to baseline levels by using linear mixed-effect models for repeated measures. The results showed that 90 out of the 151 metabolomic measures changed in concentration after low-dose insulin infusion compared with baseline, and 121 metabolomic measures changed in concentration after high-dose insulin infusion compared with baseline. Additionally, we compared the changes in concentrations of metabolomics measures after high-dose insulin infusion with those after low-dose insulin infusion. We found that some metabolomic measures seemed to reach their maximum response already at low-insulin infusion, while 99 out of 151 metabolomic measures showed an additional response at high-insulin dose infusion. In particular, the amino acids including leucine, isoleucine, valine, glutamine and tyrosine had a large decrease in concentration after high-dose insulin infusion when compare to baseline and low dose insulin infusion. In conclusion, metabolomic measures are differentially insulin sensitive and may thus be differentially affected by the development of insulin resistance. Those findings also suggested that the response to different insulin levels is likely organ-specific, which is reflected by changes in metabolomic measure after low-dose insulin infusion (primarily affecting metabolomic measures in the liver) and high-dose

insulin infusion (primarily affecting metabolomic measures in peripheral tissues). For patients, this could provide additional insight in the stage or the type of insulin resistance and thus potentially provide opportunities for personalized intervention.

In **Chapter 3** of this thesis, we investigated the relationship between blood mitochondrial function and type 2 diabetes (T2D). Mitochondria are essential organelles for the production of cellular energy and dysfunctional energy production may be cause and/or consequence of T2D. We associated mitochondrial DNA copy number (mtDNA-CN) as a measure for mitochondrial function and associated this measure with T2D incidence. We performed this analysis using multivariable-adjusted Cox proportional hazard models, and we found that a higher level of blood mtDNA-CN was associated with lower risk of developing T2D. To assess whether or not this association is causal, we performed bi-directional two-sample Mendelian Randomization (MR) analyses. The results showed that there was no evidence for a causal association between blood mtDNA-CN and type 2 diabetes in either direction. In order to investigate this relationship further, we analyzed the potential causal association between blood mtDNA-CN and BMI using bi-directional two-sample MR, but no indication for causal associations between these two features was found in either direction. Our hypothesis that blood mtDNA-CN drives the risk of T2D and obesity is based on the assumption that blood mtDNA-CN reflects overall mitochondrial function including mitochondrial function of tissues like liver and muscle that play critical roles in T2D. With some additional analyses, we found that higher blood mtDNA-CN was not associated with lean mass, nor with AST, ALT and ALP as measures of liver function. A slightly lower GGT was observed with an increase in blood mtDAN-CN. Altogether, these results suggest that the observed association between low blood mtDNA-CN and higher risk of type 2 diabetes is likely not causal. This could potentially be explained by our findings that blood mtDNA-CN is not associated with muscle mass and weakly with liver function and may thus not be a marker of muscle mitochondrial function and only a weak marker for liver mitochondrial function.

In **Chapter 4** of this thesis, we investigated the association between blood IGF-1 levels and T2D. Although it is biologically plausible that IGF-1 plays a role in T2D, previous studies have reported inconsistent findings on this association. We first performed Cox proportional hazard analyses on blood IGF-1 levels and T2D, and investigated a possible J-shape association between IGF-1 concentration and T2D, with low levels of IGF-1 being

associated with a higher risk of developing T2D. Subsequently, we performed two-sample MR analyses in the same study population, and observed that higher genetically-influenced IGF-1 was associated with higher risk of T2D. Considering the large heterogeneity in the estimated causal effects of the individual genetic instruments in the two-sample MR, we employed a novel method, clustered MR, to investigate the association between genetically-influenced IGF-1 level with T2D. We identified six clusters of genetically-influenced IGF-1, based on genetic instruments with similar estimated causal effects, to be associated with T2D, among which three clusters were related to a higher risk of T2D while the remaining three clusters were related to a lower risk of T2D. The main clusters in which a higher IGF-1 was associated with a lower risk of type 2 diabetes comprised instruments mapping to genes in the growth-hormone signaling pathway, whereas the main clusters in which a higher IGF-1 was associated with a higher risk of type 2 diabetes comprised instruments mapping to genes in pathways related to amino acid metabolism and genomic integrity. These results provide evidence that the effect of IGF-1 on T2D is dependent on the underlying metabolic/biomolecular pathways.

In **Chapter 5** of this thesis, we aimed to understand the effect of genetic factors and exposure to both long and short habitual sleep duration on dyslipidemia traits including high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c) and triglycerides (TG). In this study, 732,564 individuals from multiple population groups across 55 cohorts were involved. Through a 1-degree of freedom genome-wide variant-sleep interaction analyses on lipid levels, we identified 9 lead variants of which variants mapping to AMPD3 (LDL-c, LTST), ASPH (TG, LTST) and DLEU1 (TG, STST; HDL-c, STST) genes were most significant. Seven additional lead variants were identified by a 2-degree of freedom joint analyses, among which the lead variant mapping to SLC8A1 (TG, STST) which had been previously identified as a therapeutic target for reduction of ischemic damage following acute myocardial infarction. In conclusion, these results provided insight into the biological pathways underpinning the associations between disturbances in habitual sleep duration and lipid levels.

In **Chapter 6** of this thesis, in order to make best use of observational data and to understand how the drug concentration can associate with the outcome, we introduced a novel methodological framework, PHARMACOM-EPI, which can predict drug concentrations at occurrence of a clinical outcome. A warning was issued by the U.S. Food and Drug Administration in 2021 on the antiseizure drug lamotrigine, which claimed

that lamotrigine has the potential to increase risk of arrhythmias and related sudden cardiac death. Our hypothesis is that the increase risk of arrhythmias and related sudden cardiac death results from drug toxicity. By utilization of the PHARMACOM-EPI framework, lamotrigine concentrations for older patients with seizure were predicted at the time of death using a published population pharmacokinetic model which was selected based on demographic information and model accessibility. Patients were subsequently categorized into toxic and non-toxic groups based on the lamotrigine therapeutic range, after which the association between two groups and all-cause mortality were analyzed. This study found that a toxic plasma concentration of lamotrigine is associated with increased risk of all-cause death in the older lamotrigine users. Application of this novel framework PHARMACOM-EPI provided evidence to support our hypothesis that the increased risk of all-cause and cardiovascular death was associated with a toxic plasma concentration level of lamotrigine among older lamotrigine users. By leveraging this methodology, observational data could be harnessed more effectively.

In **Chapter 7**, I discussed the findings from Chapter 2 through Chapter 6 within the context of previous researches, their potential applications and future directions of research.