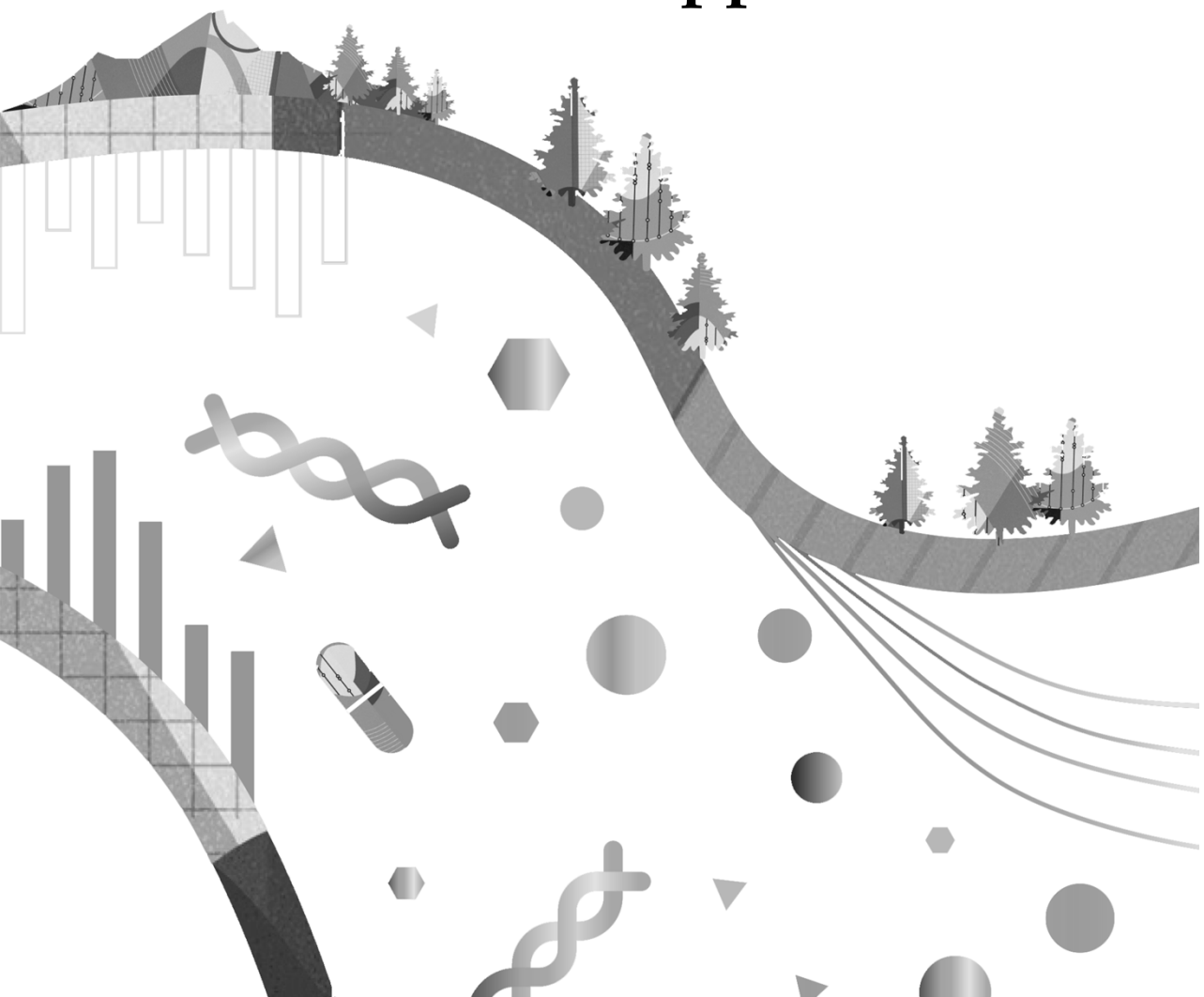
Insight into the pathophysiology of cardiometabolic diseases using multiple omics approaches

Wang, W.

Part V

Discussion, Future perspective and Appendices



Chapter 7

Discussion and Future Perspective

The overarching aim of this thesis is to gain insight into the etiology and pathophysiology of cardiometabolic disease(s) by investigating potential (causal) risk factors and the interplay between genetic and environmental factors in cardiometabolic diseases. In this thesis, we employed cutting-edge statistical and epidemiological methodologies like clustered Mendelian Randomization (MR) and exploited multiple omics data (genomics and metabolomics) to unravel the mechanisms underlying the development of cardiometabolic diseases using established high-quality databases. In this chapter, I will summarize, interpret and discuss the findings from the different chapters of this thesis. In addition, limitations and future perspectives derived from those findings will be discussed to support progress for future prevention and treatment of cardiometabolic diseases.

MAIN FINDINGS

Insulin Sensitivity and Metabolomics

Insulin resistance plays a critical role in the development of type 2 diabetes (T2D) [1, 2], and is a risk factor for cardiovascular disease [1, 3]. Multiple studies have investigated metabolomic profiles in relation to insulin resistance [4, 5]. However, few studies have focused on the physiological effects of different doses of exogeneous administered insulin on metabolomic measures in individuals without diabetes. In **Chapter 2**, we examined the response of metabolomic measures (e.g., lipoprotein sub-particles and amino acids) to different hyper-insulinemic levels under euglycemic conditions in non-diabetic individuals. We found that the majority of metabolomic measures were sensitive to insulin, and a large fraction of them were differentially insulin-sensitive. This indicates that metabolites are likely to be differentially affected by the degree of hyperinsulinemia, due to insulin resistance. In particular, branched-chain amino acids (BCAAs), showing the highest reduction following high-dose insulin infusion in comparison to low-dose insulin infusion, were the most sensitive metabolomic measures to insulin in this study. Our results are in line with multiple studies showing elevated BCAAs in obese and insulin-resistant individuals [6-8]. In addition, multiple metabolomic measures observed as insulin-sensitive in this study have been described before to be associated with risk of development of cardiometabolic diseases. For instance, increased concentrations of BCAAs are associated with increased risk of coronary artery disease and T2D development [9, 10]. Especially, there is evidence from Mendelian randomization studies that the association between BCAAs and T2D is causal

[11]. As the majority of the studies focused on the pathophysiological effect of insulin resistance on metabolomic profiles, our study filled a gap by assessment of direct effects of hyperinsulinemia effect on metabolomic measures in individuals without diabetes.

Pathophysiology of Type 2 Diabetes Mellitus

Technological development of measuring techniques has made it possible to directly and indirectly quantify mitochondrial DNA more readily and affordably than ever before, which has led to wide availability of mitochondrial DNA related data [12]. As a result, an increasing number of epidemiological studies on the relationship between T2D and mitochondrial function have been published [13-15]. Most investigations have found an association between blood mitochondrial DNA copy number (mtDNA-CN) and T2D [16-19], but no study has investigated whether or not this association is potentially causal. In **Chapter 3** of this thesis, we investigated the potential causal association between blood mtDNA-CN and T2D and body mass index (BMI) using bi-directional two-sample MR analyses. Overall, the results did not provide evidence for a causal association between blood mtDNA-CN and T2D and BMI in either direction, despite being studied in multiple very large study samples (e.g., UK Biobank). Additional analyses indicated that blood mtDNA-CN may not reflect mtDNA-CN in T2D related organs like muscle and liver, which could explain the lack of a potential causal association. The mtDNA-CN in blood, serving as an indirect indicator of hematopoiesis and immunological processes, may not reflect the mitochondrial function in muscle or adipose tissues [12]. Consequently, exploring mtDNA-CN in muscle and adipose tissue, which do reflect mitochondrial function in T2D-related tissues, may offer a more robust way for investigating its potential role as a risk factor for T2D. To the best of our knowledge, this study is the first and largest investigation on the potential causal association and its direction between blood mtDNA-CN and T2D. Although blood mtDNA-CN may not be a biomarker for T2D, it might be valuable for studying the pathophysiology of other diseases. For instance, mtDNA-CN was found to be associated with cardiovascular diseases [20], aging [21] and Parkinson's disease [22], which might suggest its potential as a biomarker for elucidating the underlying pathological mechanisms of these diseases.

Insulin-like growth factor-1 (IGF-1) as a risk factor of T2D has been studied for years, but the epidemiological findings of their association are

inconsistent. We hypothesized that there might be distinct mechanisms underlying the association of high levels of IGF-1 with disease. Some of these mechanisms may contribute to an increased risk of developing T2D, while others might have no association with T2D or could potentially decrease the risk of T2D development. We hypothesized that these mechanisms might interact and can cancel out the overall impact of high IGF-1 levels on T2D. In **Chapter 4** of this thesis, we employed a novel method referred to as clustered MR, and identified six clusters of genetic variants representing IGF-1 levels, each of which showed a different association with T2D. Notably, three of those clusters suggested a positive causal effect of IGF-1 levels on T2D risk, while the remaining three clusters suggested a negative causal effect of IGF-1 levels on T2D risk. This result indicates that high level of IGF-1 likely affects the development of T2D in a context-dependent manner, depending on the biological pathways that increase IGF-1 levels in the blood. Before investigation of the potential causal association between IGF-1 and T2D, we also performed multivariable-adjusted regression analyses and observed a J-shape association between IGF-1 and T2D, with particularly low levels of IGF-1 being associated with an increased risk of developing T2D. Our observational findings are partly in line with several other studies showing a U-shape association between IGF-1 and T2D [23-25], but contrast with studies showing no evidence for this association [26, 27]. This difference in observational studies, to some extent, is in line with our finding in the clustered MR that the effect of high levels of IGF-1 on T2D is driven by different biological mechanism and that their association is context-dependent. Our study provides a possible reason for the inconsistent observational findings of the association between IGF-1 and type 2 diabetes. Given the use of diverse cohorts consisting of heterogenous participants in investigating the association between IGF-1 and T2D, the fluctuating participant numbers contributing to either increased or decreased risk of T2D development possibly result in inconsistent observational findings concerning the association between IGF-1 and T2D. Understanding the role of IGF-1 in T2D can help us to become more aware of the heterogeneity between individuals and direct the development of personalized medicine like targeted therapy or lifestyle modification.

Pathophysiology of Sleep-Associated Dyslipidemia

Habitual sleep is becoming increasingly recognized as a risk factor contributing to the development of cardiometabolic diseases and associated traits (i.e., dyslipidemia) [28]. Given the poorly understood biomolecular

mechanisms underpinning the association between habitual sleep duration and dyslipidemia, **Chapter 5** of this thesis examined the effect of genetic factors and habitual sleep duration on lipid traits including high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL) and triglycerides (TG). Through the analyses on approximately 732,564 (predominantly European) individuals from multiple population groups across 55 cohorts, our study revealed that the associations of several genetic variants with lipids were modified by either long or short habitual sleep duration. Among the identified lead variants, some were found to be located within the *ASPH* gene which is known to modulate cardiovascular risk by interacting with aspartic and succinic acid [29]. Notably, some additionally identified variants were mapped to the *SLC8A1* gene which has emerged as a therapeutic target for ischemic damage reduction following acute myocardial infarction [30, 31]. Despite the large sample size, especially in comparison with the previous effort [32], we only identified a relatively small number of genetic variants that were modified by differences in habitual sleep duration. Furthermore, most variants had low allele frequencies (i.e., below 1%). Therefore, sleep duration might not be the optimal phenotype to investigate the relationship between genetic variants and lipid levels as sleep is a highly multidimensional trait [33]. Nevertheless, this study offers novel insights into biological mechanism underlying the association between habitual sleep duration and lipid levels. Furthermore, the identified targets for treatment can provide novel insight into the prevention of atherosclerotic cardiovascular disease in individuals with disturbances in habitual sleep duration.

A novel framework - Integration of Pharmacometrics and Epidemiology

A novel methodological framework, PHARMACOM-EPI, was introduced in **Chapter 6** of this thesis. This framework integrated pharmacometrics and epidemiology, which enabled the prediction of drug concentration at the time of occurrence of clinical outcomes (i.e., mortality). In early 2021, a warning issued by U.S. Food and Drug Administration on the antiseizure drug lamotrigine, which claims 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. In **Chapter 6**, we applied the PHARMACOM-EPI framework to real-world data to investigate the association between plasma concentration of lamotrigine and the risk of death in older patients. The results showed that a toxic plasma concentration of lamotrigine was

associated with increased risk of all-cause and cardiovascular death in the older lamotrigine users, which supports our hypothesis. Our study is the first observational study using nationwide real-world data to predict plasma concentration to investigate the relationship between lamotrigine plasma concentrations and its related toxicity [34, 35]. This study introduced the PHARMACOM-EPI framework, which is capable of handling real-world data, thereby showcasing its potential applications in future research. By leveraging this methodology, observational data could be harnessed more effectively. Given ethical consideration and cost constrains, clinical trial data containing plasma concentration is not always available. This approach enables observational data to function as a valuable alternative under specific circumstances. When drug dosages are available in observational data, drug plasma concentrations can be predicted and applied in the pharmaco-epidemiological studies or as better-defined confounders that usually ignored in the epidemiological studies. Simultaneously, this study highlights the importance of personalized medicine within distinct population groups (older and pediatric populations) especially when dealing with drugs characterized by narrow therapeutic windows.

Limitations and Future Perspective

Most of the results described in this thesis were limited to mainly European ancestry populations, and the results in this thesis should therefore be interpreted with caution for populations of non-European backgrounds. Future studies within non-European populations are warranted for those findings.

With the advent of new methods for estimation of causal effects, has two-sample Mendelian Randomization become less important?

Considering the limitations of conventional two-sample MR, more and more advanced methods have been developed to investigate the potential causal association between exposures and outcomes. Two-sample MR assumes that the exposure-outcome relation is linear. To address violations of this assumption, non-linear MR was put forward to investigate non-linear causal relationship between exposures and outcomes [36]. Two-step MR and multivariable MR was developed with the requirement to investigate the potential causal association between an exposure and an outcome with mediators [37, 38]. To address the pleiotropic effects limitation in two-sample MR, clustered MR was developed to explore different biological pathways between the exposure and outcome [39], especially in the case of large heterogeneity between the estimated causal effects of individual instruments on the study outcome. With the development of these advanced methods developed to estimate causal effects, does this mean that the conventional two-sample MR is no longer relevant? First of all, it is clear that all those advanced methods were developed based on the original principles of two-sample MR. This indicates conventional MR is the pillar of all the advanced methods. Besides, it is more logical to first perform two-sample MR before moving to some advanced methods. For example, in **Chapter 4**, we used the clustered MR to investigate the association between IGF-1 and type 2 diabetes. The rationale of using the advanced method, clustered MR, is that we observed heterogeneity of the estimated causal effects of instrumental variables in two-sample MR. Basic studies are always the foundation for more in-depth exploration. In summary, although two-sample MR analyses have their limitations, the role of this method in estimation of causal effect cannot be replaced.

Is habitual sleep duration as a lifestyle factor enough to capture the disturbance in lipid profiles?

In **Part III** of this thesis, **Chapter 5** focused on the effect of interaction between genetics and sleep duration on lipid traits in a large multiple-ethnic population. In this study, we solely examined the effect of sleep duration on lipids, and did not account for the complexity of sleep, which is a multidimensional trait that covers a wide spectrum of phenotypes including sleep duration, napping, insomnia, chronotype, etc. A significant amount of research has been focused on the effect of different sleep traits on cardiometabolic diseases. Some studies provided evidence that sleep duration [40, 41], napping [42-44], insomnia [45, 46] and chronotype [47, 48] were associated with risk of cardiometabolic diseases. MR analyses provided evidence that sleep duration, insomnia and daytime napping are causally associated with cardiometabolic diseases [42]. Although many studies have addressed the effect of sleep traits on cardiometabolic diseases, limited studies have taken into account the interaction between different sleep phenotypes [49-51]. These studies suggested that sleep duration is not the only sleep trait contributing to the risk of cardiometabolic diseases. In future studies, we may need to focus more on other sleep traits and on the combination of different sleep phenotypes affecting cardiometabolic diseases. In addition to sleep, it is also important to examine the effect on cardiometabolic diseases from other related lifestyle factors such as exercise, diet, and explore their potential interactions.

Is medication use overlooked in epidemiological observational studies? Can we benefit from multi-disciplinary approaches to handle this confounder?

One of the major challenges in epidemiological observational studies of cardiometabolic disease is medication use by the study participants. According to the paper published by *Jungyeon, et.al.*, many epidemiological studies have not considered or given sufficient attention to medication use, which may have impacted the results leading to bias or wrong interpretation [52]. In this paper, etiological studies from high-ranked journals published between 2015 and 2019 focusing on blood pressure, glucose or lipid measurements were assessed. The results indicated that a large proportion of studies (47%) did not report information on medication use or only provided medication use for part of the variables affected (14%) [52]. One of the main reasons for not considering medication use is absence or insufficiency of data

for medication use in the respective databases. To address this issue, we need to raise awareness about the importance of recording medication use during data collection. **Chapter 6** in **Part IV** of this thesis introduced a novel framework integrating pharmacometrics and epidemiological disciplines to predict plasma concentrations based on dosages and then associated predicted drug concentration with clinical outcomes of interest. Given that the dosage information is accessible, **Chapter 6** may provide the opportunity to address the issue of the lack of medication concentration data. Further efforts are required to determine how to deal with insufficient medication data and how to handle medication use in observational research.

Does multi-omics pave the road toward achieving precision medicine?

The findings presented in **Chapter 4** of this thesis provide evidence for considerable individual heterogeneity, and **Chapter 6** stressed the importance of personalized medicine especially for medication with a narrow therapeutic window. These observations provide arguments to further develop the discipline of precision medicine. According to US Food and Drug Administration (FDA), precision medicine is an approach to provide a precise strategy for prevention, diagnosis and treatment of disease taking into account people's differences in genes, lifestyles and environments [53]. In the current thesis, genomics and metabolomics were used to identify genetic variants that were associated with cardiometabolic disease, and to unravel underlying biological pathways of cardiometabolic disease to increase our awareness of personalized medicine. However, neither genomics alone nor other single omics cannot capture the complex holistic biological processes underlying most diseases [54]. With the advancement of different omics techniques, high-throughput data in the disciplines of genomics, epigenomics, transcriptomics, proteomics, and metabolomics can now be relatively easily and cheaply obtained [55]. Integration of multiple omics data (which is called multi-omics) needs to be applied in future studies to provide a more comprehensive view of the etiology and pathophysiology of cardiometabolic diseases [56]. With the increasing availability of multi-omics data and awareness of the importance of biological interconnections, network-based approaches are rapidly emerging as a promising method which represent biological systems as interconnected networks of bio-molecules (including genes, proteins, metabolites, etc.) [57]. Employing network-based approaches for analyzing multi-omics data enables the integration of biochemical process which includes gene regulation of protein production, protein interaction with each other to carry out cellular functions

and the interconnectivity of metabolites across different metabolic pathways [58]. By comprehensive elucidation of disease pathophysiology, this approach has the potential to significantly advance both personalized prevention and treatment of diseases [59]. The realization of personalized or stratified medicine can be achieved through patient stratification or disease subtyping based on identified mechanisms and identification of risk factors for distinct populations.

Reference

[1]Ginsberg HN (2000) Insulin resistance and cardiovascular disease. J Clin Invest 106(4): 453-458. 10.1172/jci10762
[2]Goldstein BJ (2002) Insulin resistance as the core defect in type 2 diabetes mellitus. The American Journal of Cardiology 90(5, Supplement 1): 3-10. https://doi.org/10.1016/S0002-9149(02)02553-5
[3]Kosmas CE, Bousvarou MD, Kostara CE, Papakonstantinou EJ, Salamou E, Guzman E (2023) Insulin resistance and cardiovascular disease. J Int Med Res 51(3): 3000605231164548. 10.1177/03000605231164548
[4]Gu X, Al Dubayee M, Alshahrani A, et al. (2020) Distinctive Metabolomics Patterns Associated With Insulin Resistance and Type 2 Diabetes Mellitus. Front Mol Biosci 7: 609806. 10.3389/fmolb.2020.609806
[5]Palmer ND, Stevens RD, Antinozzi PA, et al. (2015) Metabolomic Profile Associated With Insulin Resistance and Conversion to Diabetes in the Insulin Resistance Atherosclerosis Study. The Journal of Clinical Endocrinology & Metabolism 100(3): E463-E468. 10.1210/jc.2014-2357
[6]Tai ES, Tan ML, Stevens RD, et al. (2010) Insulin resistance is associated with a metabolic profile of altered protein metabolism in Chinese and Asian-Indian men. Diabetologia 53(4): 757-767. 10.1007/s00125-009-1637-8
[7]Newgard CB, An J, Bain JR, et al. (2009) A branched-chain amino acid-related metabolic signature that differentiates obese and lean humans and contributes to insulin resistance. Cell Metab 9(4): 311-326. 10.1016/j.cmet.2009.02.002
[8]Kim JY, Park JY, Kim OY, et al. (2010) Metabolic profiling of plasma in overweight/obese and lean men using ultra performance liquid chromatography and Q-TOF mass spectrometry (UPLC-Q-TOF MS). J Proteome Res 9(9): 4368-4375. 10.1021/pr100101p
[9]Chen X, Yang W (2015) Branched-chain amino acids and the association with type 2 diabetes. J Diabetes Investig 6(4): 369-370. 10.1111/jdi.12345
[10]Flores-Guerrero JL, Osté MCJ, Kieneker LM, et al. (2018) Plasma Branched-Chain Amino Acids and Risk of Incident Type 2 Diabetes: Results from the PREVEND Prospective Cohort Study. Journal of Clinical Medicine 7(12): 513
[11]Lotta LA, Scott RA, Sharp SJ, et al. (2016) Genetic Predisposition to an Impaired Metabolism of the Branched-Chain Amino Acids and Risk of Type 2 Diabetes: A Mendelian Randomisation Analysis. PLoS Med 13(11): e1002179. 10.1371/journal.pmed.1002179
[12]Picard M (2021) Blood mitochondrial DNA copy number: What are we counting? Mitochondrion 60: 1-11. https://doi.org/10.1016/j.mito.2021.06.010
[13]Poulton J, Luan J, Macaulay V, Hennings S, Mitchell J, Wareham NJ (2002) Type 2 diabetes is associated with a common mitochondrial variant: evidence from a population-based case-control study. Hum Mol Genet 11(13): 1581-1583. 10.1093/hmg/11.13.1581
[14]Park KS, Chan JC, Chuang LM, et al. (2008) A mitochondrial DNA variant at position 16189 is associated with type 2 diabetes mellitus in Asians. Diabetologia 51(4): 602-608. 10.1007/s00125-008-0933-z
[15]Jiang W, Li R, Zhang Y, et al. (2017) Mitochondrial DNA Mutations Associated with Type 2 Diabetes Mellitus in Chinese Uyghur Population. Scientific Reports 7(1): 16989. 10.1038/s41598-017-17086-7
[16]Memon AA, Sundquist J, Hedelius A, Palmér K, Wang X, Sundquist K (2021) Association of mitochondrial DNA copy number with prevalent and incident type 2 diabetes in women: A population-based follow-up study. Sci Rep 11(1): 4608. 10.1038/s41598-021-84132-w

[17]Liu X, Longchamps RJ, Wiggins KL, et al. (2021) Association of mitochondrial DNA copy number with cardiometabolic diseases. Cell Genomics 1(1): 100006. https://doi.org/10.1016/j.xgen.2021.100006
[18]Reiling E, Ling C, Uitterlinden AG, et al. (2010) The association of mitochondrial content with prevalent and incident type 2 diabetes. J Clin Endocrinol Metab 95(4): 1909-1915. 10.1210/jc.2009-1775
[19]Fazzini F, Lamina C, Raftopoulou A, et al. Association of mitochondrial DNA copy number with metabolic syndrome and type 2 diabetes in 14 176 individuals. Journal of Internal Medicine n/a(n/a). https://doi.org/10.1111/joim.13242
[20]Luo J, Noordam R, Jukema JW, et al. (2023) Low leukocyte mitochondrial DNA abundance drives atherosclerotic cardiovascular diseases: a cohort and Mendelian randomization study. Cardiovasc Res 119(4): 998-1007. 10.1093/cvr/cvac182
[21]Mengel-From J, Thinggaard M, Dalgård C, Kyvik KO, Christensen K, Christiansen L (2014) Mitochondrial DNA copy number in peripheral blood cells declines with age and is associated with general health among elderly. Human Genetics 133(9): 1149-1159. 10.1007/s00439-014-1458-9
[22]Pyle A, Anugrha H, Kurzawa-Akanbi M, Yarnall A, Burn D, Hudson G (2016) Reduced mitochondrial DNA copy number is a biomarker of Parkinson's disease. Neurobiology of Aging 38: 216.e217-216.e210. https://doi.org/10.1016/j.neurobiolaging.2015.10.033
[23]Schneider HJ, Friedrich N, Klotsche J, et al. (2011) Prediction of incident diabetes mellitus by baseline IGF1 levels. Eur J Endocrinol 164(2): 223-229. 10.1530/eje-10-0963
[24]Sandhu MS, Heald AH, Gibson JM, Cruickshank JK, Dunger DB, Wareham NJ (2002) Circulating concentrations of insulin-like growth factor-I and development of glucose intolerance: a prospective observational study. Lancet 359(9319): 1740-1745. 10.1016/s0140-6736(02)08655-5
[25]Friedrich N, Thuesen B, Jørgensen T, et al. (2012) The association between IGF-I and insulin resistance: a general population study in Danish adults. Diabetes Care 35(4): 768-773. 10.2337/dc11-1833
[26]Drogan D, Schulze MB, Boeing H, Pischon T (2016) Insulin-Like Growth Factor 1 and Insulin-Like Growth Factor–Binding Protein 3 in Relation to the Risk of Type 2 Diabetes Mellitus: Results From the EPIC–Potsdam Study. American Journal of Epidemiology 183(6): 553-560. 10.1093/aje/kwv188
[27]Similä ME, Kontto JP, Virtamo J, et al. (2019) Insulin-like growth factor I, binding proteins -1 and -3, risk of type 2 diabetes and macronutrient intakes in men. British Journal of Nutrition 121(8): 938-944. 10.1017/S0007114519000321
[28]St-Onge MP, Grandner MA, Brown D, et al. (2016) Sleep Duration and Quality: Impact on Lifestyle Behaviors and Cardiometabolic Health: A Scientific Statement From the American Heart Association. Circulation 134(18): e367-e386. 10.1161/cir.0000000000000444
[29]Pell VR, Chouchani ET, Frezza C, Murphy MP, Krieg T (2016) Succinate metabolism: a new therapeutic target for myocardial reperfusion injury. Cardiovascular Research 111(2): 134-141. 10.1093/cvr/cvw100
[30]Jang IK, Weissman NJ, Picard MH, et al. (2008) A randomized, double-blind, placebo-controlled study of the safety and efficacy of intravenous MCC-135 as an adjunct to primary percutaneous coronary intervention in patients with acute myocardial infarction: Evaluation of MCC-135 for left ventricular salvage in acute myocardial infarction (EVOLVE). Am Heart J 155(1): 113.e111-118. 10.1016/j.ahj.2007.08.020
[31]Kawasumi H, Satoh N, Kitada Y (2007) Caldaret, an intracellular Ca2+ handling modulator, limits infarct size of reperfused canine heart. J Pharmacol Sci 103(2): 222-233. 10.1254/jphs.fp0060765

[32]Noordam R, Bos MM, Wang H, et al. (2019) Multi-ancestry sleep-by-SNP interaction analysis in 126,926 individuals reveals lipid loci stratified by sleep duration. Nature Communications 10(1): 5121. 10.1038/s41467-019-12958-0
[33]Chung J, Goodman M, Huang T, Bertisch S, Redline S (2021) Multidimensional sleep health in a diverse, aging adult cohort: Concepts, advances, and implications for research and intervention. Sleep Health 7(6): 699-707. 10.1016/j.sleh.2021.08.005
[34]Christensen J, Trabjerg BB, Dreier JW (2022) Cardiac morbidity and mortality associated with the use of lamotrigine. Epilepsia 63(9): 2371-2380. 10.1111/epi.17339
[35]Diederich S, Hemmeter U, Paulmann M, Mockenhaupt M (2023) Effects of dosage in new users of lamotrigine inducing epidermal necrolysis: Results of the German Registry of Severe Skin Reactions. Epilepsia 64(5): 1259-1265. https://doi.org/10.1111/epi.17563
[36]Staley JR, Burgess S (2017) Semiparametric methods for estimation of a nonlinear exposure-outcome relationship using instrumental variables with application to Mendelian randomization. Genetic epidemiology 41(4): 341-352. 10.1002/gepi.22041
[37]Burgess S, Thompson SG (2015) Multivariable Mendelian randomization: the use of pleiotropic genetic variants to estimate causal effects. Am J Epidemiol 181(4): 251-260. 10.1093/aje/kwu283
[38]Carter AR, Sanderson E, Hammerton G, et al. (2021) Mendelian randomisation for mediation analysis: current methods and challenges for implementation. European Journal of Epidemiology 36(5): 465-478. 10.1007/s10654-021-00757-1
[39]Foley CN, Mason AM, Kirk PDW, Burgess S (2020) MR-Clust: Clustering of genetic variants in Mendelian randomization with similar causal estimates. Bioinformatics. 10.1093/bioinformatics/btaa778
[40]Yan B, Yang J, Zhao B, Fan Y, Wang W, Ma X (2021) Objective Sleep Efficiency Predicts Cardiovascular Disease in a Community Population: The Sleep Heart Health Study. J Am Heart Assoc 10(7): e016201. 10.1161/jaha.120.016201
[41]Yiallourou SR, Maguire GP, Carrington MJ (2021) Sleep quantity and quality and cardiometabolic risk factors in Indigenous Australians. J Sleep Res 30(2): e13067. 10.1111/jsr.13067
[42]Jia Y, Guo D, Sun L, et al. (2022) Self-reported daytime napping, daytime sleepiness, and other sleep phenotypes in the development of cardiometabolic diseases: a Mendelian randomization study. Eur J Prev Cardiol 29(15): 1982-1991. 10.1093/eurjpc/zwac123
[43]Lin D, Sun K, Li F, et al. (2014) Association between habitual daytime napping and metabolic syndrome: a population-based study. Metabolism 63(12): 1520-1527. 10.1016/j.metabol.2014.08.005
[44]Leng Y, Cappuccio FP, Surtees PG, Luben R, Brayne C, Khaw KT (2016) Daytime napping, sleep duration and increased 8-year risk of type 2 diabetes in a British population. Nutr Metab Cardiovasc Dis 26(11): 996-1003. 10.1016/j.numecd.2016.06.006
[45]Laugsand LE, Strand LB, Platou C, Vatten LJ, Janszky I (2014) Insomnia and the risk of incident heart failure: a population study. Eur Heart J 35(21): 1382-1393. 10.1093/eurheartj/eht019
[46]Laugsand LE, Vatten LJ, Platou C, Janszky I (2011) Insomnia and the risk of acute myocardial infarction: a population study. Circulation 124(19): 2073-2081. 10.1161/circulationaha.111.025858
[47]Li T, Xie Y, Tao S, et al. (2023) Prospective study of the association between chronotype and cardiometabolic risk among Chinese young adults. BMC Public Health 23(1): 1966. 10.1186/s12889-023-16902-2
[48]Baldanzi G, Hammar U, Fall T, et al. (2022) Evening chronotype is associated with elevated biomarkers of cardiometabolic risk in the EpiHealth cohort: a cross-sectional study. Sleep 45(2). 10.1093/sleep/zsab226

[49]Goodman MO, Dashti HS, Lane JM, et al. (2023) Causal Association Between Subtypes of Excessive Daytime Sleepiness and Risk of Cardiovascular Diseases. J Am Heart Assoc 12(24): e030568. 10.1161/jaha.122.030568
[50]Li J, Yin J, Luo Y, et al. (2022) Association of healthy sleep pattern with the risk of cardiovascular disease and all-cause mortality among people with diabetes: A prospective cohort study. Diabetes Res Clin Pract 186: 109822. 10.1016/j.diabres.2022.109822
[51]He L, Ma T, Cheng X, Bai Y (2023) The association between sleep characteristics and the risk of all-cause mortality among individuals with cardiometabolic multimorbidity: a prospective study of UK Biobank. J Clin Sleep Med 19(4): 651-658. 10.5664/jcsm.10404
[52]Choi J, Dekkers OM, le Cessie S (2022) How measurements affected by medication use are reported and handled in observational research: A literature review. Pharmacoepidemiol Drug Saf 31(7): 739-748. 10.1002/pds.5437
[53]FDA US (2018) Precision Medicine. Available from https://www.fda.gov/medical-devices/in-vitro-diagnostics/precision-medicine
[54]Karczewski KJ, Snyder MP (2018) Integrative omics for health and disease. Nat Rev Genet 19(5): 299-310. 10.1038/nrg.2018.4
[55]Dai X, Shen L (2022) Advances and Trends in Omics Technology Development. Front Med (Lausanne) 9: 911861. 10.3389/fmed.2022.911861
[56]Kreitmaier P, Katsoula G, Zeggini E (2023) Insights from multi-omics integration in complex disease primary tissues. Trends Genet 39(1): 46-58. 10.1016/j.tig.2022.08.005
[57]Zhou G, Li S, Xia J (2020) Network-Based Approaches for Multi-omics Integration. Methods Mol Biol 2104: 469-487. 10.1007/978-1-0716-0239-3_23
[58]Yan J, Risacher SL, Shen L, Saykin AJ (2018) Network approaches to systems biology analysis of complex disease: integrative methods for multi-omics data. Briefings in Bioinformatics 19(6): 1370-1381. 10.1093/bib/bbx066
[59]Han J-DJ (2008) Understanding biological functions through molecular networks. Cell Research 18(2): 224-237. 10.1038/cr.2008.16