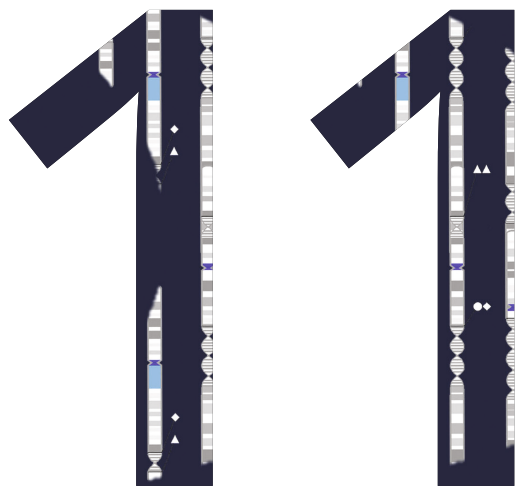
Discussion and future perspectives



DISCUSSION AND FUTURE PERSPECTIVES

Aims of this thesis

The overall aims of this thesis were to examine whether 1) postprandial metabolite measures after a mixed meal are reliable and clinically informative compared with the most commonly used clinical fasting measures; 2) genetic analyses of postprandial metabolomics measured through genome wide association studies (GWAS) provide insight in novel biological pathways associated with cardiometabolic disease; and 3) fasting glucose and CETP concentrations are risks factors for a first event of venous thrombosis (VT) and to determine their causality using Mendelian randomization analyses.

What have we learnt from postprandial measurements after a mixed meal?

In part I of this thesis, we focused on the added value of postprandial measures in disease prognosis and diagnosis, as well as understanding disease mechanisms. We provided solid evidence of utility for applying postprandial measurements after a mixed meal as biomarkers for disease diagnosis. In the past five years, epidemiological evidence has shown that, similar to fasting state measures, (random) non-fasting lipid levels are consistently associated with cardiovascular or all-cause mortality. This has led to the recommendation to also apply non-fasting lipid measures to cardiovascular risk assessment and treatment decisions (2-6). Phenotypically, we found postprandial metabolomic measurements after a mixed meal were as reliable and informative as fasting measures, in both reproducibility and risk stratification aspects. This highly similar performance between fasting and postprandial measurements can be well explained by the overlapping genetics, as we observed in this thesis. From a disease aetiological point of view, this overlap indicates that substantial novel insights from studies on postprandial versus fasting state metabolism may not be gained. Nonetheless, in clinical practice, a random postprandial measure is more feasible and flexible for patients than overnight fasting. Future research should extend from controlled postprandial to random postprandial states, and assess the genetic basis as well as prediction performance between fasting measures and random postprandial measures.

What have we learnt from response measurements after a mixed meal?

At the outset of the work in this thesis, we hypothesized that meal response measurements may open a new avenue of exploring disease biological pathways, in addition to fasting measures. Phenotypically, response measures appeared to be less informative and useable than either fasting or postprandial measures for disease risk stratification. However, by using response measures as outcomes, we found that

the genetic variant rs505922:C correlated with the early phase insulin response. This finding potentially explains why carriers of this allele have increased type 2 diabetes risk because it was associated with decreased early phase insulin secretion. At the same time, we identified several significant genetic associations to metabolite response measures. As one of the most interesting findings, rs10830963 in the melatonin receptor 1B (*MNTR1B)* gene showed a strong association with the postprandial glucose response. Taken together, these findings suggest that meal response measurements characterize some unique metabolic pathways in addition to fasting or postprandial measurements. Although these results are promising, we have to note that in the meal response metabolomics GWAS efforts described in this thesis, we adopted the commercial targeted NMR-based platform covering around 150 metabolites, accounting for less than 4% of the whole human metabolome (estimated with more than 4000 unique endogenous metabolites). To establish a complete genetic atlas of metabolite responses, we need to further increase the coverage of metabolites, and consider to include additional non-targeted metabolomics platforms in future studies.

What is still missing regarding the determinants of response measurements after a mixed meal?

Meal response measurements reflect an interplay between genetics and environment, and there is still a substantial amount of missing information on the determinants of response measurements. To determine the extent of variability in the meal response in the NEO study population, we performed an exploratory clustering analysis. Several subgroups were identified with distinct metabolite response trajectories after a mixed meal, which implies that intrinsic subtypes of meal response are present within a population. From our genetic investigations on metabolite response measurements, only a modest proportion of the variations in the meal response was explained by genetics (on average the SNP-wide genetically explained heritability of metabolite response = 12%). A recent study by whole genome sequencing reported that nearly all heritability estimated from pedigree studies on height and BMI was identified. This implies that the missing heritability of complex traits and diseases is likely due to rare variants, which are not captured by the current genetic variant detection and imputation methods (7). The identified heritability of the meal response may be higher when we continue to increase the study sample size with for example the data from large biobanks or use more advanced sequencing technology to explore the genetic variants with low-frequency or that are rare.

In addition to the genetic component, environmental factors are also important in determining the meal response. In essence, a meal response reflects the flexibility of sugar and lipid metabolism after nutrient intake, and it is well established that, amongst

others, physical activity, previous diet and the gut microbiome affect whole-body insulin sensitivity and lipid metabolism (8-11). Extensive efforts are currently underway to take these environmental factors into account and the results from these efforts may explain additional meal response variation on top of the genetic determinants.

In the past decade, increasing evidence has suggested that the gut microbiome plays an important role in influencing body mass index (BMI) and blood lipid levels (12). Individuals with unfavourable lipid profiles (high triglyceride and low HDL level) exhibited low microbial diversity, a high abundance of some taxa from the phylum Actinobacteria, and a lower abundance of many taxa from the phyla Proteobacteria and Bacteroidetes (13). Another recent study showed a causal association between increased levels of the bacterially derived short chain fatty acid butyrate in faeces and improved insulin response (14). These findings indicate that gut microbiome may be an important player in determining an individuals' meal response. Further investigations are needed to characterize the microbiome composition (i.e., diversity and abundance) and activity of the subtypes of meal response as we observed in the NEO study population, which will further shed light on the role of gut microbiota in the meal response.

Are these findings generalizable to the other ethnicities?

All the findings covered in this thesis were based on Western study populations, and it is desirable to know whether these findings also hold for other ethnicity groups, for instance through comparative studies. The prevalence of T2D differs significantly among ethnicities, which is likely caused by both different environmental and genetic factors. In the past 5 years, numerous studies have focused on multi-ancestry GWAS for T2D. We performed a candidate SNP study in a Saudi Arabian cohort of 1,578 individuals (659 T2D cases and 919 controls), aiming to replicate 122 previously reported T2D-related variants from 84 loci discovered in the Caucasian population. With a stringent Bonferroni threshold of $P = 4.1 \times 10^{-4}$ (= 0.05/122), none of the previously identified T2D variants reached the significance level in the study of the Saudi Arabian population. This indicates that large-scale multi-ethnicity studies are urgently needed to identify additional ethnicity-specific disease-associated variants (11).

Are we moving towards precision medicine?

The fundamental question of precision medicine seeks to address "whom", "when", and "how" to medically intervene. In this thesis, we used GWAS to identify genetic variants that are associated with response measures. The results presented here may contribute to move precision medicine forward to identify "whom". We are all different, as a result of our inherently unique genetic background and living environment. Our data show that some individuals have a genetic predisposition towards an excess

glucose response after a meal, which may translate to the development of impaired insulin response when these individuals live a sedentary lifestyle or consume large amounts of sugar-sweetened soft drinks. In these individuals, a personalized advice to increase exercise or decrease consumption of sugar rich foods and beverages may be particularly effective. However, whether knowledge of genetic predisposition to disease on the long term is having sufficient motivation for these individuals to adhere to a changed lifestyle remains to be determined. However, it is likely that mobile health (m-health) devices and applications for smart phones and wearable devices will greatly facilitate personalised lifestyle interventions in daily life.

Based on multi-omics profiling (i.e., integrating genetics, epigenetics, metagenomics, proteomics and metabolomics), disease diagnosis can be more precise and timely. Implementation of these results in personalized advice to patients addresses the question of "when" to intervene. In this thesis, we showed that it is feasible to distinguish pre-diabetic individuals from healthy controls using plasma metabolite profiles, also providing the possibility of early detection followed by early intervention. It can be expected that improving the precision of the prediction is key for implementation of precision medicine. We are only at the beginning of dissecting the complex interactions between the different layers of -omics measurements. Since these omics measurements are likely to increasingly replace single clinical measurements, this insight will develop.

The last question is "how" to perform interventions. Emerging pharmacogenomic evidence showed that dose requirements vary widely among individuals because of our genetic variation. Genotype-guided therapy has proved to be more effective than traditional "one pill for everyone solution" for some medications, such as warfarin and targeted cancer therapies based on genetic profiling (15; 16). With regard to cardiometabolic disease, genotype-guided therapy seems more distant, but genotype information will be increasingly available in patients. This will inevitably lead to additional insight and hopefully application of genotype-guided therapy for cardiometabolic disease.

The future of precision medicine might look like the proof-of-concept study, already published in 2012, "*The Integrative Personal Omics Profile study*". In this study, in one individual, transcriptome, proteome, and metabolome were traced over 14 months continuously. In this individual, prediabetes was readily diagnosed. The further development of disease could be averted by prompting the individual to adjust his lifestyle (17). We are still on the journey of embedding multi-omics approaches into precision medicine, determining our individual risk of developing disease and assessing the most effective interventions to help improve our health.

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