Investigating insulin resistance in human obesity with transcriptomics

Summary

The prevalence of overweight and obesity is increasing worldwide. Obesity is officially recognized as a disease with a great impact on health and well-being. Obesity is associated with insulin resistance (IR), which is a major risk factor for the development of type 2 diabetes and cardiovascular disease. A plethora of evidence suggests that adipose tissue mass and function and the distribution of body fat are the most important contributing factors, determining an individual's risk to develop obesity-associated IR, type 2 diabetes and cardiometabolic disease. Importantly, IR may develop separately in multiple organs and the severity may vary between organs. Sub-typing obese individuals based on their IR phenotype, may lead to a better understanding of the relationship between insulin resistance and type 2 diabetes and cardiometabolic risk. This may give leads to precision-based prevention and treatment strategies.

The primary goal of this thesis was to investigate IR phenotypes in human obesity with transcriptomic data from the adipose tissue and whole blood. For the purposes of this thesis, we applied data-driven computational approaches (e.g. differential gene expression analysis, biological pathway and network analysis).

In **Chapter 2**, we investigated abdominal subcutaneous adipose tissue (SAT) gene expression and clinical profiles in individuals with more pronounced IR in skeletal muscle or liver. We demonstrated distinct adipose tissue transcriptome profiles in tissue-specific IR. We showed that an altered ECM gene expression profile in SAT was present in overweight and obese individuals with pronounced hepatic IR. Furthermore, an upregulation of inflammatory gene expression was particularly present in individuals with pronounced muscle IR. We subsequently hypothesized that an increased SAT inflammatory gene expression, as observed in the muscle IR group, may lead to the secretion of pro-inflammatory adipokines in the circulation and a systemic pro-inflammatory profile inducing subsequently peripheral IR. In line with this hypothesis, in two cohorts with similar characteristics, the CODAM and Maastricht Study, we showed that low-grade inflammation scores of plasma inflammatory markers, were inversely associated with muscle insulin sensitivity (MISI), after adjustment for sex and body composition, while we did not observe an association between the systemic low-grade inflammation score and hepatic IR (HIRI).

In **Chapter 3**, we investigated SAT gene expression, clinical and metabolic profiles of individuals who were discordant for IR in their adipose tissue and skeletal muscle. We found that 40% of the study participants were discordant for IR in adipose tissue and muscle. Furthermore, we found that adipose tissue IR was characterized by an upregulation of inflammatory and ECM genes, and a worse metabolic and inflammatory profile as compared to adipose insulin sensitive individuals, regardless of concurrent presence of muscle IR. On the other hand, within individuals with a relative insulin sensitive adipose

tissue and no major alterations in adipose tissue gene expression and systemic metabolic profile, a considerable group of individuals did develop muscle IR.

The data presented in **Chapter 2** and **3**, open new exciting avenues showing distinct IR subgroups in overweight and obese individuals in the development of type 2 diabetes and cardiovascular disease. Our findings could help identify functional subgroups of obese individuals with different risk profiles, which may represent a starting point for future research aimed at identifying novel, more effective precision-based prevention and treatment strategies of obesity and its complications.

In **Chapter 4**, using a publicly available collection of seven adipose tissue transcriptomics datasets and the bioinformatics tool TissueDecoder we presented an approach to assess the contribution of macrophages to the overall subcutaneous adipose tissue gene expression. We observed lower frequencies of adipocytes and higher frequencies of adipose stem cell in individuals characterised by high macrophage frequencies. Adipose tissue of individuals with high macrophage frequencies had a higher expression of genes involved in complement activation, chemotaxis, focal adhesion and oxidative stress. Similarly, we observed a lower expression of genes involved in lipid metabolism, fatty acid synthesis and oxidation and mitochondrial respiration. We hypothesized that increased macrophage and adipose stem cell percentage and the decreased percentage of adipocytes reflects adipose tissue inflammation and impaired pre-adipocyte differentiation, possibly reflective of a limited capacity for hyperplasia and adipose tissue dysfunction that contributes to an unfavourable metabolic profile.

In **Chapter 5**, we presented an approach to investigate the whole blood transcriptome of insulin resistant and insulin sensitive individuals, independent of white blood cell (WBC) profile. We showed the relative amount of monocytes to be significantly greater in the insulin resistant compared to the insulin sensitive participants. We demonstrated a distinct blood transcriptome profile in insulin resistance, independent of WBC profile. We observed that, the expression of interferon-stimulated genes (ISGs) in the whole blood was higher in insulin resistant as compared to insulin sensitive individuals. We hypothesised that this interferon related signature might indicate increased systemic inflammation possibly due to an innate immune response and whole-body insulin resistance, which can be a cause or a consequence of IR. We additionally observed a lower expression of genes involved in cellular differentiation in the whole blood of insulin resistant individuals. Hence, we think that the systemic inflammation in combination with the downregulation of cellular differentiation and remodelling of actin cytoskeleton we observed in the whole blood of the insulin resistant individuals may reflect obesity and/or insulin resistance related organ dysfunction, such as adipose tissue or gut.

Collectively in **Chapters 4** and **5**, we have shown the additive value of integrating publicly available datasets in combination with the useful application of cell-type composition in SAT gene expression (Chapter 4) and whole blood transcriptome analysis of IR (Chapter 5). In addition to identifying functional subgroups of obese individuals with different risk profiles, the contribution of macrophage frequencies and other cell types to organ/tissue dysfunction and plasticity, can offer targets and biomarkers for novel precision-based prevention and treatment strategies of obesity and its complications.

In conclusion, the present thesis investigated IR phenotypes in human obesity with transcriptomics data from the adipose tissue and the whole blood, and have offered insights which may represent a starting point for future research aimed at identifying novel, more effective precision-based prevention and treatment strategies of obesity and its complications. Future research should investigate the usefulness of stratification on IR phenotypes, single-cell approaches to further elucidate IR phenotypes and finally enable a data reuse culture. Precision-based strategies, either data-driven, knowledge-driven, or a combination of both, have the potential to change the conventional standards of care in human obesity. Nevertheless, parallel efforts from governments, policy makers and food industry are needed to make environments less obesity-promoting and more supportive of healthy eating and physical activity. Simply, making the healthy choice the easy choice.