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Summary

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SUMMARY

Cardiometabolic diseases such as type 2 diabetes (T2D) and cardiovascular diseases (CVD) put a major burden on the health care system, with CVD accounting for most annual deaths globally. People from South Asian descent are especially at risk to develop T2D and CVD. This may, at least in part, be due to their unfavorable body fat distribution with relatively large amounts of abdominal white adipose tissue (WAT) and ectopic fat deposition, contributing to insulin resistance and negatively affecting whole-body energy metabolism. Reducing the rates of obesity and its complications can be accomplished by shifting towards a negative energy balance. This can be achieved by lowering food intake, which is however challenging to maintain and on the long term most often results in weight regain. Parallel to lowering food intake, increasing energy expenditure can help to further induce and maintain weight loss. To this end, enhancing the thermogenic capacity of energy-combusting brown adipose tissue (BAT) is a promising novel treatment strategy. This could be especially useful in South Asians whom were previously shown to exhibit a lower BAT volume. In this thesis, we firstly investigated mechanisms underlying the unfavorable metabolic phenotype of South Asians. Secondly, we studied the potential of pharmacological agents to promote BAT activity and improve cardiometabolic health, in South Asian compared with white Caucasian men.

Chapter 1 serves as a general introduction into the South Asian heritage and their establishment in the Dutch society today. Here we describe the metabolic characteristics of South Asians and elaborate on pathophysiological aspects contributing to their high risk of T2D and CVD. We then continue to elaborate on the role of BAT in energy metabolism, and illustrate treatment strategies, either by implementing lifestyle changes or the use of pharmacological agents, that may improve cardiometabolic health by promoting BAT activity.

BAT generates heat by combusting fatty acids, which are stored within intracellular lipid droplets. To replenish these lipid pools, fatty acids are taken up from the circulation by lipoprotein lipase (LPL)-mediated hydrolysis of triglyceride-rich lipoproteins (TRLs). LPL activity can be inhibited by a family of angiopoietin-like proteins (ANGPTLs). More specifically, it has been shown that cold exposure modulates ANGPTL4 expression and thus LPL activity in a tissue-specific manner. ANGPTL4 acts in concert with its family members ANGPTL3 and ANGPTL8 to orchestrate lipid distribution between different metabolic tissues under alternating energy demands. In **chapter 2**, we investigated the effect of cold exposure on plasma levels of ANGPTL3 and ANGPTL8, in addition to ANGPTL4, in South Asian and white Caucasian men. We confirmed that cold exposure increases ANGPTL4 levels both in young healthy lean men and middle-aged men with overweight and prediabetes. We now also show that cold exposure increases ANGPTL3 and ANGPTL8 levels, but only in young healthy lean men. We previously proposed that

during cold exposure, ANGPTL4 may function to redirect TRLs away from WAT towards active BAT and skeletal muscle for uptake of TRL-derived fatty acids to facilitate thermogenesis. We here suggest that as a counter response, the increase in ANGPTL3 and ANGPTL8 may reduce the interaction of TRLs with thermogenic tissues to prevent excessive lipid combustion and/or accumulation in these tissues. This response could be absent in middle-aged men with overweight and prediabetes in an attempt to overcome reduced glucose uptake by BAT resulting from insulin resistance.

The Wnt signaling pathway is involved in embryonic development and oncogenesis. Interestingly, impaired Wnt signaling is also associated with adiposity and T2D in humans, and *in vitro* studies have indeed confirmed that Wnt signaling interacts with insulin signaling. Since South Asians are prone to develop obesity and insulin resistance, in **chapter 3** we next investigated whether Wnt signaling is impaired in subcutaneous WAT and skeletal muscle biopsies in overweight prediabetic South Asian men compared with white Caucasian men. We showed markedly higher plasma levels of the Wnt-inhibitor sclerostin in South Asians compared with white Caucasians. In addition, expression of various genes involved in Wnt and insulin signaling was lower in WAT in South Asians, and expression of genes involved in Wnt and insulin signaling were strongly positively correlated. In skeletal muscle, only *WNT10B* expression was lower in South Asians compared with white Caucasians, and the positive correlation between Wnt and insulin signaling gene expression was less pronounced. We conclude that Wnt signaling may be lower in WAT of South Asians compared with white Caucasians, thereby possibly contributing to impaired insulin signaling and development of T2D in South Asians.

The excessive risk of CVD in South Asians cannot be fully explained by classical risk factors such as increased LDL-cholesterol levels. Interestingly, it was recently shown that the susceptibility of circulating LDL particles to aggregate *in vitro* is related to their lipid composition, and that the presence of such aggregation-prone LDL particles is associated with mortality from cardiovascular events in patients with CVD. In **chapter 4** we therefore assessed the aggregation susceptibility and lipid composition of LDL particles isolated from plasma of healthy South Asians compared with white Caucasians. LDL particles from South Asians were indeed considerably more prone to aggregate. In addition, body fat percentage, which was higher in South Asians, correlated positively with LDL aggregation. Furthermore, when investigating the LDL lipidome, we observed that body fat percentage positively correlated with LDL-sphingomyelins, especially sphingomyelin 24:0 which was also higher in South Asians. We conclude that LDL aggregation susceptibility is higher in South Asians compared with white Caucasians, which may be explained in part by their higher body fat percentage leading to enrichment of LDL particles with sphingomyelins. Presence of such aggregation-prone circulating LDL particles could contribute to the higher CVD risk in South Asians later in life.

We next switched our focus to studying the effect of two different pharmacological compounds on BAT metabolism in both South Asian and white Caucasian subjects. Firstly, in **chapter 5** we performed a randomized placebo-controlled trial in which we compared the effects of cold exposure and one dose of 200 mg of the beta-3-adrenergic receptor (β_3-AR) agonist mirabegron on BAT and markers of energy metabolism in healthy lean South Asian and white Caucasian men. In both ethnicities, cold exposure increased the skin temperature in the supraclavicular area, as a proxy for BAT thermogenesis, lowered the fat fraction of the supraclavicular adipose tissue depot, increased free fatty acid levels, and increased resting energy expenditure due to enhanced lipid oxidation. Mirabegron also increased the supraclavicular skin temperature in both ethnicities, and lowered the fat fraction of the supraclavicular adipose tissue depot after pooling data from all subjects. Furthermore, mirabegron increased free fatty acid levels in both ethnicities, and resting energy expenditure when pooling data from all subjects, due to an increase in lipid oxidation which was only significant in white Caucasians. Recently, it has been shown that the beneficial metabolic effects of mirabegron on BAT are mediated via β_2-AR signaling rather than β_3-AR signaling. Investigating the potential of a selective β_2-AR agonist to enhance BAT activity would therefore be very interesting, also in the South Asian population.

In addition to directly stimulating β-ARs on brown adipocytes, mimicking incretin hormones is a promising treatment strategy to indirectly stimulate BAT thermogenesis. More specifically, central agonism of the glucagon-like peptide-1 (GLP-1) receptor agonist activates BAT in mice, and GLP-1 receptor agonists lower body weight and improve glucose and lipid levels in patients with T2D. To investigate whether GLP-1 receptor agonism also activates BAT in humans, in **chapter 6** we determined the effect of 12 weeks administration of the GLP-1 receptor agonist exenatide on BAT and markers for energy metabolism in nondiabetic men, and compared this between South Asians and white Caucasians. Exenatide lowered body weight, without affecting energy expenditure or substrate oxidation rates, and lowered serum triglycerides and total cholesterol, as well as plasma glucose. Notably, exenatide increased the metabolic volume and standardized uptake value of BAT measured with [^{18}F]FDG-PET/CT scan, whereas the fat fraction of the supraclavicular adipose tissue depot measured with MRI remained unaltered. The overall effect of exenatide on these metabolic parameters was comparable between ethnicities. These results show that also in humans GLP-1 receptor agonism has multiple beneficial metabolic effects in addition to improving glucose levels, and that this may at least be partly mediated by enhanced glucose disposal by BAT.

Lastly, in **chapter 7** we placed the results of the studies performed in this thesis into the perspective of the available evidence described in the scientific literature. We described novel pathophysiological aspects contributing to T2D and CVD, with a focus on the South Asian population. We then shifted our focus towards BAT and firstly

described mechanisms involved in its lipid uptake. Thereafter, we expanded on the potential of pharmacological compounds that could improve cardiometabolic health in part by stimulating BAT thermogenesis. Finally, we briefly highlighted methodological challenges in the quantification of BAT functionality, and discussed methods that are currently being developed in an attempt to overcome these issues in future BAT research. We concluded that continuing to unravel underlying mechanisms contributing to the high risk of T2D and CVD in South Asians remains important in the search for novel treatment options to tackle these cardiometabolic diseases. Whether successfully stimulating BAT thermogenesis can be of significant aid in the treatment of cardiometabolic disease on the long term, remains to be elucidated in the forthcoming years.

In summary, the studies described in this thesis provide novel insight into the effect of cold exposure on the lipid-trafficking regulating ANGPTLs, underlying mechanisms contributing to cardiometabolic disease in South Asians in terms of Wnt signaling in metabolic tissues and the aggregation susceptibility as well as the lipidome composition of LDL, and the effect of the two promising pharmacological treatment strategies of β-AR agonism and GLP-1 receptor agonism on BAT activity and whole-body energy metabolism. As such, our studies contribute to further unraveling risk factors for cardiometabolic diseases and the development of novel therapeutic handles in strategies to combat these diseases, especially in the vulnerable South Asian population.