Modulating energy metabolism: pathophysiological aspects and novel interventions

Straat, M.E.

CHAPTER 11

Appendices

SUMMARY

The global prevalence of obesity is increasing at alarming rate and has become a major public health problem. Obesity is a complex disease, characterized by excessive adiposity, and is linked to several cardiometabolic diseases, including type 2 diabetes (T2D) and cardiovascular diseases (CVD), that are among the leading causes of mortality worldwide. Obesity arises from a long-term positive energy balance and can be prevented or treated by maintaining or restoring energy homeostasis. Therefore, there is a need to further unravel the physiological aspects of energy metabolism, to investigate populations at risk for cardiometabolic diseases, and to explore novel treatment strategies aimed at beneficially modulating energy metabolism. In this thesis, we aimed to cover these topics by addressing two key objectives: 1) to gain more insight in various pathophysiological aspects of cardiometabolic diseases including in the disease-prone South Asian population, and 2) to study the physiological effects of cold exposure and identify a novel pharmacological approach to directly target brown adipose tissue (BAT).

Chapter 1 gives a general introduction into the physiology of energy metabolism, including a detailed description of lipid metabolism, and the consequences of a disturbed energy metabolism. We introduce the South Asian population, a population prone for cardiometabolic diseases, and we describe the importance of the biological clock for cardiometabolic health. Lastly, we present the application of cold exposure and direct activation of BAT as potential therapeutic interventions to combat cardiometabolic diseases.

In the following three chapters we focused on addressing key objective 1: to gain more insight in various pathophysiological aspects of cardiometabolic diseases. An independent risk factor for CVD is hypertriglyceridemia, which can result from various genetic mutations in the gene encoding lipoprotein lipase (*LPL*) or in genes that encode LPL regulators. In **chapter 2** we aimed to show detailed differences in the serum (apo)lipoprotein profile of patients with genetic hypertriglyceridemia *versus* normolipidemic controls. The concentration of a spectrum of apolipoproteins was assessed by multiplex liquid chromatography–mass spectrometry, and the concentration and size of lipoprotein subclasses and class-specific lipid composition was estimated using nuclear magnetic resonance spectroscopy. We found that patients with genetic hypertriglyceridemia compared to normolipidemic controls had higher levels of apolipoprotein (apoB)48 and the exchangeable apoC-I, apoC-II, apoC-III, and apoE, without altered apoB100. In addition, patients with genetic hypertriglyceridemia had higher concentrations of triglyceride (TG)-rich lipoproteins (*i.e.*, chylomicrons and

very-low-density lipoproteins [VLDL]), but lower low-density lipoprotein (LDL), of which medium and small-sized LDL particles appeared even absent. We concluded that dysfunctional LPL leads to accumulation of large TG-rich lipoproteins, whereas it prevents the formation of the small atherogenic lipoprotein remnant particles, probably contributing to the previously established lower CVD risk in patients with genetic hypertriglyceridemia.

In **chapter 3** we focused on the South Asian population, prone to develop cardiometabolic diseases. South Asians have a higher risk to develop T2D coinciding with earlier complications than Europids, partly explained by their higher insulin resistance and unfavorable body composition from a young age on. As inflammation plays a central role in the development and progression of T2D, we aimed to study whether circulating mRNA transcripts of immune genes are different between South Asian *versus* Europid patients with T2D. To this end, mRNA transcripts of 182 immune genes were measured in fasted blood samples from overweight-to-obese Dutch South Asian and Dutch Europid patients with T2D. We found that South Asians, compared to Europids, had higher mRNA levels of B cell and interferon (IFN) signaling genes. In South Asians, the IFN signaling pathway was the top canonical pathway and this was accompanied by higher plasma IFN-γ levels. Notably, the ethnic difference in gene expression was larger for females than males. We speculate that a more activated IFN signaling pathway may contribute to the more rapid progression of T2D in South Asians compared with Europids. Targeting the immune system, *e.g.* the IFN pathway, for the treatment of T2D using anti-inflammatory therapy may be especially beneficial in the South Asian population, which is an interesting topic for future research. Moreover, it remains to be elucidated whether the more pro-inflammatory state in South Asian *versus* Europid patients with T2D results from intrinsic ethnic differences or from a longer disease duration.

Next, we switched our focus to another group of patients at risk for cardiometabolic disbalance, *i.e.* patients with the rare neurological sleep-wake disorder narcolepsy type 1, caused by the destruction of orexin-producing neurons that are located in the lateral hypothalamus. Notably, despite a normal to decreased food intake and comparable physical activity, a higher prevalence of obesity has been reported in patients with narcolepsy type 1 compared to healthy controls. In **chapter 4** we reviewed the current knowledge on the role of the orexin system in the control of energy balance, with specific focus on BAT metabolism in both preclinical and clinical studies. Although preclinical studies with orexin knock-out mice demonstrate a crucial role for the orexin system in the functionality of BAT and subsequently to an impaired energy homeostasis, convincing evidence from human studies about impaired BAT function in patients with

narcolepsy type 1 is lacking. Future studies are required to unravel the pathogenesis underlying the increased adiposity in patients with narcolepsy type 1, with specific focus on (brown/white) adipose tissue.

In the second part of this thesis, we addressed objective 2: to study the physiological effects of cold exposure and to identify a novel pharmacological approach to directly target BAT. In **chapter 5** we aimed to assess the effect of short-term cold exposure on transcript levels of a large panel of immune genes in blood from nonobese Dutch Europid and Dutch South Asian men. We found that cold exposure acutely upregulated mRNA levels of genes encoding cytotoxic proteins and pro-inflammatory chemokines. Of note, four markers of the nucleotide-binding oligomerization domain (NOD)-like receptor (NLR)-family, involved in inflammasomes, were lower in Dutch South Asians compared to Dutch Europids. In conclusion, short-term cold exposure acutely increases mRNA levels of genes involved in cytotoxicity of immune cells in blood. This could reflect a quick boost of the immune response towards a pro-inflammatory state, in preparation to fight an infection. It remains interesting for future research to investigate whether chronic cold exposure is able to modify immune cells towards an anti-inflammatory phenotype to ultimately serve as intervention for the prevention and treatment of cardiometabolic diseases that are characterized by low-grade inflammation.

Next, we shifted our focus from the effect of cold exposure on the immune system to the effect of cold exposure on lipid metabolism. Cold exposure mobilizes lipids as fuel for thermogenic processes in metabolic organs, including skeletal muscles and BAT. As such, the application of cold exposure has emerged as a potential intervention to enhance whole-body lipid catabolism. Although a beneficial effect of cold exposure on lipid metabolism has been shown in mice, the global effect of cold exposure on lipid metabolism in humans has been reported with mixed results depending on intensity and duration of cold. In **chapter 6**, we performed sequential lipidomic profiling during short-term cold exposure in serum of healthy, lean men. We found that cold exposure gradually increased circulating free fatty acids reaching a maximum at 60 min, and transiently decreased total TGs only at 30 min. A broad range of TG species was initially decreased, in particular unsaturated and polyunsaturated TG species with ≤5 double bonds, while after 120 min a significant increase was observed for polyunsaturated TG species with ≥6 double bonds. A mechanistic study in mice revealed that the cold-induced increase in polyunsaturated TGs was largely prevented by blocking adipose triglyceride lipase. We interpreted these findings as that cold exposure feeds thermogenic tissues with TG-derived fatty acids for combustion, resulting in a decrease of circulating TG species, followed by increased hepatic production of polyunsaturated TG species induced by liberation of free fatty acids stemming from adipose tissue. The

finding that cold exposure elicits modifications in lipid composition including an increase in PUFA-containing TAGs sheds new light on the beneficial effects of cold exposure and warrants future studies to determine the potential contribution of *e.g.* long-term repetitive cooling to cardiometabolic health.

In **chapter 7**, we specifically pointed our attention to the thermogenic organ BAT. Here, we described that rodent and probably also human BAT activity is subjected to circannual and diurnal rhythms, with a peak in its activity in winter and around the onset of the active period. We discussed the genetic, neuronal and endocrine generation of these rhythms in BAT. We addressed how disruption of circadian rhythms, for instance through genetic clock deficiency in mice, prolonged light exposure, and (mimicking) shift work, are associated with cardiometabolic problems, often accompanied by declined BAT function. Although the exact contribution of BAT dysfunction to these phenotypes is not known, unravelling the affected mechanistic pathways may lead to the discovery of novel therapeutic targets to combat cardiometabolic diseases. Moreover, these rhythms should probably be considered when measuring BAT function and when therapeutically targeting BAT.

Since cold exposure is the physiological activator of BAT, in **chapter 8** we aimed to investigate whether cold-induced thermogenesis is subjected to a diurnal variation in humans. To this end, we performed a randomized crossover study in twenty-four young and lean males and females. Participants underwent short-term cold exposure in the morning and evening, with one day in between. We found that only in males, cold-induced thermogenesis, as assessed by the increase in energy expenditure and supraclavicular skin temperature upon cold, was higher in the morning than in the evening. Nonetheless, females showed a better cold tolerance in the morning (*e.g.*, lower temperature at the start of shivering) and a higher increase in circulating triglycerides, total cholesterol, and high-density lipoprotein (HDL) cholesterol levels after cold exposure in the morning, than in the evening. Although differently regulated between males and females, these findings suggest that the cold responses of thermogenic tissues, such as BAT, are subjected to a diurnal variation which may be of importance when applying cold exposure to improve whole-body metabolism.

Although the application of cold exposure using for example cooling vests is feasible at home, it may not be a suitable strategy for everyone. Therefore, directly targeting the thermogenic BAT by mimicking the adrenergic effect of cold exposure may provide a solution. BAT is activated by the beta-3-adrenergic receptor (ADRB3) in rodents, while the ADRB2 is dominantly present in human BAT biopsies and responsible for noradrenergic activation of human brown adipocytes. In **chapter 9**, we assessed

whether ADRB2 agonism activates human BAT by performing a randomized double-blinded crossover trial in young and lean males to compare a single intravenous bolus of salbutamol without and with the ADRB1/2 antagonist propranolol. Salbutamol, compared to salbutamol with propranolol, increased glucose uptake by BAT as assessed by a dynamic 2-[^{18}F]fluoro-2-deoxy-D-glucose ([^{18}F]FDG) positron emission tomography-computed tomography (PET-CT) scan, heart rate and whole-body energy expenditure, with the salbutamol-induced glucose uptake by BAT positively associated with the increase in energy expenditure. Notably, participants with high salbutamol-induced glucose uptake by BAT had a lower body fat mass, waist-hip ratio and serum LDL-cholesterol concentration. Our data show that the ADRB2 activates human BAT and warrant investigation of ADRB2 activation in long-term studies.

Finally, in **chapter 10** we evaluated the results described in this thesis in the light of current scientific literature, we discussed their implications in current clinical practice, and highlighted remaining challenges for the future. Specifically, the results described in this thesis provide new insights in the cardiometabolic disease profile of South Asians and highlight inflammation as a previously underexplored factor and potential treatment target. Moreover, we propose that cold exposure as strategy to enhance whole-body energy metabolism can be best applied in the morning and we provide mechanistic insight in how cold exposure accelerates lipoprotein metabolism. Lastly, we provide evidence that the ADRB2 is the adrenergic receptor that activates human BAT *in vivo*, which may result in new therapeutic strategies that target human BAT to combat cardiometabolic diseases. In the upcoming years it will become clear whether novel prevention and treatment strategies are effective enough to halt the disturbingly increasing rate of obesity and cardiometabolic diseases.