Mechanisms of cold-induced improvements in glucose homeostasis

Summary

The high prevalence of obesity has led to a global escalation in the number of people diagnosed with type 2 diabetes mellitus (T2DM). Non-medical treatment options to improve glucose homeostasis in these patients mainly consist of lifestyle interventions, such as exercise- and dietary regimes. Nevertheless, these forms of interventions are not widely enthused by most humans, as evidenced by the low long-term adherence rates. Therefore, there is the need for new therapies to improve glucose homeostasis in patients with T2DM.

In search for alternative strategies to improve the disease state of T2DM patients, our research group has previously shown that repeated exposure to mild cold (i.e. mild cold acclimation) improves insulin-stimulated glucose disposal by ~43% in T2DM patients. Further elaboration on the mechanisms underlying these improvements revealed that these effects were primarily attributed to an increased uptake of glucose by skeletal muscle, although the exact intracellular pathways involved remained unknown. Nevertheless, given the pronounced effects of cold exposure on glucose homeostasis, identification of the underlying mechanisms mediating skeletal muscle glucose uptake upon cold exposure could reveal new therapeutic avenues for the treatment of T2DM. In this thesis, it was therefore investigated whether activation of β_2-adrenergic receptors (β_2-ARs) or the occurrence of shivering could underlie these observed improvements in skeletal muscle glucose uptake and glucose homeostasis following cold acclimation.

This thesis primarily focusses on potential mechanisms by which cold enhances skeletal muscle glucose uptake. However it is also possible that other metabolic tissues contribute to cold-induced improvements in glucose homeostasis. To gain more insights into the contribution of different tissues to cold-induced effects on glucose homeostasis, a comprehensive literature study was performed in **Chapter 2**. In this chapter, emphasis was put on the effects of cold exposure on the pancreas, liver, (white) adipose tissue, and skeletal muscle – the primary organs regulating glucose homeostasis – whereas only limited attention was paid to brown adipose tissue (BAT), as the abundance of this tissue is relatively low in overweight/obese adult humans. Based on the studies reviewed in this chapter, it was concluded that adaptations within skeletal muscle, resulting in an increased glucose uptake, are key for the effects of cold exposure on glucose homeostasis, although a potential role for the liver and white adipose tissue should not be overlooked.

The first mechanism by which cold exposure could enhance skeletal muscle glucose uptake is via activation of the sympathetic nervous system and subsequent activation of the highly expressed β_2-AR located on skeletal muscle cells. Activation of β_2-ARs has previously been associated with an increased glucose uptake *in vitro*. However, whether activation of β_2-ARs also stimulates skeletal muscle glucose uptake *in vivo* was entirely unknown. In **Chapter 3**, diet-induced obese, insulin resistant mice were treated with the selective β_2-agonist clenbuterol. It was found that clenbuterol could stimulate glucose uptake by skeletal muscle *in vivo*, thereby highlighting a physiological relevance of this receptor in mediating skeletal muscle glucose uptake. Furthermore, treatment with clenbuterol markedly improved both glucose and insulin tolerance in these mice, indicating a potential therapeutical relevance as well.

Although the findings in **Chapter 3** are highly promising, it could be hypothesized that these effects are partly mediated via activation of BAT, as clenbuterol has a low affinity towards the primary receptors involved in BAT activation (i.e. β_1- and β_3-AR), and rodents are characterised by large volumes of this metabolically active tissue. In terms of a potential therapeutical relevance for T2DM patients, it is highly important to investigate the contribution of BAT in these β_2-agonist-mediated improvements in glucose homeostasis, as overweight/obese individuals generally have small BAT depots. In **Chapter 4**, the effects of clenbuterol treatment on whole-body glucose homeostasis were therefore investigated in diet-induced obese, insulin resistance mice ablated of UCP1$^{-/-}$, the key protein mediating BAT thermogenesis. Remarkably, clenbuterol treatment significantly improved glucose homeostasis even in the absence of thermogenically functional BAT, indicating that BAT is not indispensable for β_2-agonist-mediated improvements in glucose homeostasis. These findings suggest that other tissues than BAT are responsible for β_2-adrenergic stimulated improvements in glucose homeostasis, and therefore suggests that such an approach may also be effective in humans who are characterized by low amounts of BAT.

In **Chapter 5**, the findings of these rodent studies were translated into a clinical setting to investigate whether treatment with a β_2-agonist could boost skeletal muscle glucose uptake in humans. Thus, healthy, young, male subjects were treated with clenbuterol or a placebo for 2-weeks in a randomised, placebo-controlled, double-blinded cross-over study, after which insulin-stimulated skeletal muscle glucose uptake was determined with the golden standard hyperinsulinemic-euglycemic clamp technique. Interestingly, even in these healthy subjects that do not have impairments in their glucose homeostasis, clenbuterol treatment could

significantly enhance insulin-stimulated skeletal muscle glucose uptake. Unfortunately, the molecular mechanisms involved in these effects could not be identified. The findings in **Chapter 5** are nevertheless highly important, as they potentially indicate that similar beneficial effects could occur in individuals with an impaired glucose homeostasis, such as T2DM patients.

Besides activation of β_2-ARs, shivering during cold exposure could also explain the previously observed improvements in skeletal muscle glucose uptake and glucose homeostasis upon cold acclimation. Thus, in our previous mild cold acclimation study, subjects self-reported the occurrence of tense muscles (i.e. low-grade shivering), and a follow-up study with measures to prevent shivering was unable to replicate the beneficial effects of mild cold acclimation in T2DM patients, hinting towards an important role for shivering. However, studies investigating the effects of repeated cold-induced shivering on glucose homeostasis are largely lacking. In **Chapter 6**, overweight/obese subjects were therefore exposed to cold-induced shivering for 10 days (with at least 1 hour of shivering per day) and effects on glucose homeostasis were investigated before, the day after a single cold exposure, and after cold acclimation with shivering by means of oral glucose tolerance tests (OGTT). It was found that cold acclimation with shivering improved glucose clearance during an OGTT in these overweight/obese subjects, suggesting an improvement in glucose homeostasis. In addition, profound reductions were observed in fasting glucose, free-fatty acids, and triglyceride concentrations, whereas also robust improvements in blood pressure were reported. Combined, these results indicate that repeated cold-induced shivering could potentially be an alternative strategy to improve not only glucose homeostasis in T2DM patients, but also components of the metabolic syndrome associated with overweight/obesity.

When combining the results described in **Chapters 3-6**, it can be concluded that both activation of β_2-ARs and the occurrence of shivering could explain our previously observed improvements in skeletal muscle glucose uptake and whole-body glucose homeostasis following mild cold acclimation in T2DM patients. In addition, the results of these studies strongly suggest that both these mechanisms could potentially be exploited as an alternative treatment strategy to improve the disease state of individuals with prediabetes or T2DM patients. However, further studies are needed to investigate the clinical relevance of these therapeutic avenues.