Combatting metabolic disease : ethnic aspects, mechanisms and novel treatment strategies

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CHAPTER 1

General introduction and outline

OBESITY AND RELATED DISORDERS

Obesity is one of the largest global health emergencies of the 21st century. According to the World Health Organisation, obesity is defined as a body mass index (BMI) of >30 kg/m^2. Since 1980, the prevalence of obesity has doubled worldwide to a number of 603.7 million adults (12.0% of the world population) in 2015 (1). Moreover, this number is expected to further increase in the coming years (2). Obesity deregulates metabolic processes including glucose and lipid handling and results in systemic inflammation. Therefore, obesity is a major risk factor for the development of type 2 diabetes mellitus (T2D), dyslipidemia and cardiovascular diseases (CVD). In line with this, the prevalence of diabetes is rapidly increasing as well, from 425 million adults with diabetes in 2017 to an estimated number of 629 million adults in 2045 (3), of whom 91% will have T2D. CVD are the most common causes of death among people with diabetes, currently accounting for over 2.5 million deaths worldwide (3).

WHITE AND BROWN ADIPOSE TISSUE

Obesity is the consequence of excessive storage of fat in the adipose tissue organ. Under 'lean' conditions, approximately 15% of body weight in men and about 25% of body weight in women is contributed by adipose tissue, while in obesity these percentages can increase up to >40% (4,5). The most abundant type of adipose tissue is white adipose tissue (WAT), which is located throughout the body and has a function in lipid storage and insulation. Two major WAT depots can be distinguished: subcutaneous WAT, located underneath the skin, and visceral adipose tissue, which surrounds the internal organs and is mainly located in the abdominal cavity (6). WAT stores excessive lipids and sugar in the form of triglycerides. Under conditions of high energy demands (*e.g.* exercise, fasting or cold exposure), WAT can release fatty acids derived from these triglycerides into the blood, via a process known as intracellular lipolysis. These fatty acids can subsequently be taken up by other tissues to fuel metabolism, *e.g.* skeletal muscle in case of exercise. Besides WAT, a different shade of adipose tissue exists, namely brown adipose tissue (BAT). For a long time, BAT was considered to be only present in neonates. However, since 2009, it has been established that human adults still have active BAT as well (7-9). BAT is predominantly located in the supraclavicular and para-aortic regions (8) and combusts fatty acids and to a lesser extent glucose into heat (*i.e.* thermogenesis), thereby maintaining body temperature under conditions below thermoneutrality (10). Although the total amount of WAT largely exceeds that of BAT (with an estimated average of 12-35 kg of WAT versus max. 200-300 g of BAT) (11), BAT has an extremely large capacity to convert energy stored in fatty acids and glucose into heat. It has been

estimated that fully activated BAT can produce up to 300 W/kg, whereas most other tissues produce only 1 W/kg (12).

AETIOLOGY OF INSULIN RESISTANCE AND RELATION WITH DYSLIPIDEMIA

While storage of large amounts of fat in WAT in obesity is not harmful *per se*, the accompanying dyslipidemia and insulin resistance can pose a health risk. Blood glucose levels are tightly regulated within a narrow range by balancing the endogenous glucose production and the uptake by peripheral tissues. Insulin is the most important hormone involved in the glucose homeostasis. Insulin is released by β-cells of the pancreas in response to high blood glucose levels. The main action of insulin is to lower blood glucose levels. This can be established by stimulating the uptake of glucose by peripheral tissues, including muscle and adipose tissue. During the development of obesity, WAT depots expand to increase the storage capacity. In this process adipocytes increase in size, a process called hypertrophy (13). Hypertrophic adipocytes release pro-inflammatory adipokines, including tumor necrosis factor α and interleukin 6 (14,15), that trigger the immune system resulting in infiltration of immune cells including macrophages (16). Pro-inflammatory cytokines can inhibit insulin signalling, which induces insulin resistance of the tissue (17). Eventually, when adipocytes become too large and blood supply is insufficient, hypoxia occurs which triggers cell death, thereby further attracting macrophages. Immune cell infiltration of WAT is one of the main characteristics of dysfunctional WAT in obesity. Moreover, dysfunctional adipocytes become insulin resistant leading to the release fatty acids (FA) into the circulation. High levels of circulating free FA inhibit glucose uptake and combustion (18,19), thereby elevating blood glucose and insulin levels in a process called insulin resistance, which may lead to T2D (18,20,21). In addition, an increased flux of free FA to the liver, combined with hepatic insulin resistance, stimulates hepatic triglyceride synthesis and VLDL secretion which further contributes to the development of dyslipidemia (22). Also, insulin resistance reduces the expression of lipoprotein lipase (LPL) in skeletal muscle and adipose tissue, which reduces VLDL catabolism, thereby further accelerating hypertriglyceridemia and dyslipidemia (23). When the maximum storage capacity of WAT is reached, triglycerides are stored in other organs (*e.g.* 'ectopic fat accumulation'), including liver, skeletal muscle and pancreas (24,25). In the pancreas local lipid accumulation causes pancreatic dysfunction, and eventually loss of insulin production, which even further exacerbates hyperglycemia and dyslipidemia (25,26). Of note, ectopic fat accumulation is considered one of the most important risk factors for the development of T2D (27,28).

THE SOUTH ASIAN POPULATION, A POPULATION AT INCREASED RISK FOR METABOLIC DISEASE

Obesity rates differ between ethnicities. Particularly in South Asians, originating from the Indian subcontinent and constituting 20% of the world population, obesity prevalence is estimated to reach 50% in urban areas (29), and obesity-associated complications including T2D are highly prevalent (30). Moreover, South Asians develop T2D at a younger age and lower BMI compared to white Caucasians (31,32). In addition, the South Asian ethnicity itself is considered an independent risk factor for CVD (33). The underlying mechanisms of this increased risk for metabolic disease are not completely understood, but cannot fully be explained by their higher prevalence of 'classical' risk factors including obesity and dyslipidemia (33-36). Therefore, it is likely that a combination of many additional risk factors explain the elevated risk on development of metabolic disease in this population (35,37). However, the nature of these additional risk factors have not been elucidated yet.

Obesity manifests differently in South Asians as compared to white Caucasians. With comparable BMI, South Asians have a higher body fat percentage than white Caucasians (38,39). In addition, body fat distribution is different in South Asians, with higher intra-abdominal and truncal subcutaneous adipose tissue dispositions and more ectopic fat disposition, possibly contributing to an increased risk on developing insulin resistance and eventually T2D (40-43). Not only the amount and distribution of body fat differs, it has also been proposed that adipocyte function is disturbed in South Asians (44,45).

Another 'classical' risk factor for metabolic disease, especially for CVD, is dyslipidemia. An unfavourable lipid profile, consisting of high levels of triglycerides and low-density-lipoprotein (LDL)-cholesterol (46) and low levels of high-density-lipoprotein (HDL)-cholesterol (46,47) is frequently present in South Asians. In addition, impaired endothelial function (*i.e.* reduced endothelium-dependent vasodilation and increased vessel stiffness) has been described in South Asians (48-50), which further predisposes to atherosclerosis development and thus CVD. Interestingly, already in cord blood of neonates elevated levels of triglycerides, non-HDL-cholesterol and E-selectin (marker of endothelial dysfunction) are observed (51), which underscores that (some) metabolic disturbances are already present early in life.

However, the above-mentioned 'classical' risk factors probably do not explain the full picture. 'Non-classical' risk factors such as differences in genetic make-up, diet, or differences in energy metabolism could therefore not be overlooked when exploring risk factors for the development of metabolic disease in the South Asian population. With respect to genetic make-up, so far no genetic polymorphisms have been identified that could account for the ethnic difference in predisposition for obesity or T2D (52). Neither do differences in dietary intake or exercise fully explain the increased risk (52). However,

there is increasing evidence that energy metabolism might be differently regulated in South Asians as compared to white Caucasians. We previously showed that healthy lean South Asians have 32% lower resting energy expenditure as compared to BMI-matched white Caucasians (53) and less energy-combusting BAT as assessed with [^{18}F]fluorodeoxyglucose positron emission tomography-computed tomography ([^{18}F]FDG PET/CT). These factors likely contribute to their lower energy metabolism and could thereby, at least in part, contribute to the development of metabolic disease.

Taken together, all of the above-mentioned 'classical' and 'non-classical' risk factors (summarized **Figure 1**) can potentially contribute to the increased susceptibility for metabolic disease in the South Asian population. However, the list is probably not complete. Additional studies are warranted to gain more insight in the factors that are involved in the increased risk on metabolic disease in this vulnerable population.

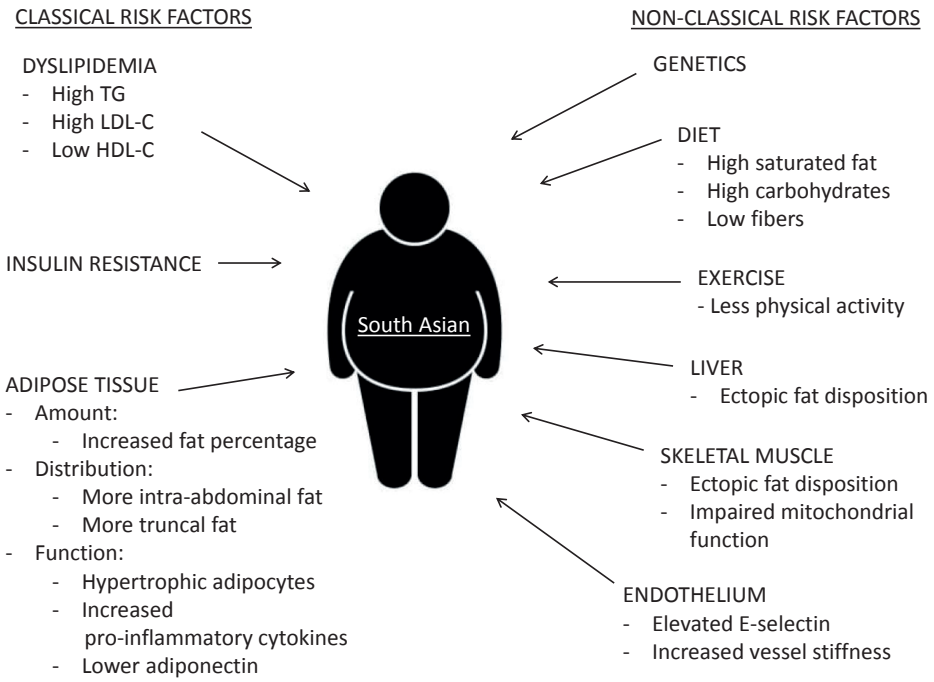

Figure 1. Known 'classical' and 'non-classical' risk factors for metabolic disease in the South Asian population. HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides.

When additional risk factors for the development of metabolic disease will have been identified, specific treatment strategies can be developed to target these diseases. Current treatment strategies for obesity are often focussed on reducing food intake via dieting or on increasing energy expenditure via increased physical activity. However,

these interventions are generally not effective on the long-term. In fact, the only effective anti-obesity intervention thus far is invasive bariatric surgery (54). Therefore, novel treatment strategies are warranted. Since BAT is able to combust lipids and glucose, thereby increasing energy expenditure, modulation of this tissue is considered to be an interesting target to combat obesity and T2D.

PHYSIOLOGICAL ACTIVATION OF BAT

In line with its function to produce heat, the main physiological activator of BAT is cold exposure (10). BAT is highly innervated by sympathetic neurons. Upon cold exposure, the sympathetic outflow from the hypothalamus towards BAT is increased. Sympathetic neurons release norepinephrine that binds to β3-adrenergic receptors on the brown adipocyte membrane, at least in mice (55-57). In humans it is still under debate what receptor(s) is (are) involved in BAT activation. As a result, the uncoupling protein-1 (UCP-1) in the inner membrane of the mitochondria becomes activated. UCP-1 uncouples mitochondrial respiration from adenosine triphosphate production resulting in the dissipation of chemical energy as heat (58,59). At the same time, BAT releases endocannabinoids, which are believed to act on endocannabinoid (CB) receptors at the presynaptic terminal of sympathetic nerve endings to inhibit noradrenalin signalling (60,61). This sequence of events likely serves as a feedback mechanism to prevent excessive activation of BAT by cold (depicted in **Figure 2**). Interestingly, circulating endocannabinoid levels are elevated in obesity (62-64). It remains to be determined whether circulating endocannabinoid levels also differs between South Asians and white Caucasians and if endocannabinoids could contribute to increased risk on metabolic disease in the South Asian population.

During thermogenesis, BAT combusts FA derived from intracellular lipid stores, thereby lowering intracellular lipid levels. As a consequence, the intracellular lipid stores become depleted and need to be replenished. To this end, BAT takes up free FA and triglyceride (TG)-derived FA from TG-rich lipoproteins in the blood (65). The TG-derived FA uptake by BAT is dependent on LPL, which is present on the vessel wall (66,67). LPL is regulated by many factors including angiopoietin-like 4 (ANGPTL4) (68,69). ANGPTL4 inhibits LPL thereby preventing the uptake of TG-derived FA by metabolic tissues (68) and at the same time stimulates intracellular lipolysis in WAT to further increase circulating free FA levels (70). ANGPTL4 is suggested to be differently regulated in BAT and WAT. Preclinical studies have shown that upon cooling, ANGPTL4 levels increase in WAT while they decrease in BAT in order to facilitate shuttling of FA from WAT to BAT (71). However, the effects of cold exposure on ANGPTL4 in humans is still unknown. In addition, cold exposure increases sympathetic outflow towards WAT which adds to enhanced lipolysis

and more release of FA into the bloodstream (72). These FA can be directly taken up by BAT or travel to the liver, where there are used for the synthesis into triglycerides. Triglycerides can subsequently be secreted into the blood in the form of VLDL. Notably, pre-clinical studies indicate the presence of a feed-forward mechanism in which FA, released by WAT, in the brain, further increases sympathetic outflow to BAT to stimulate the combustion of FA for thermogenesis (73).

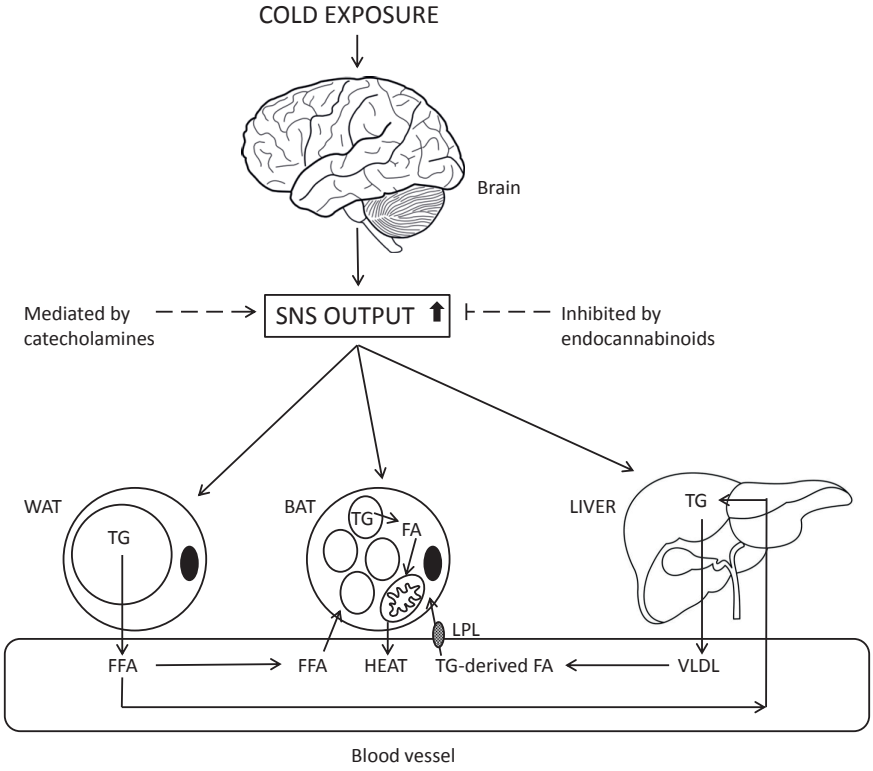

Figure 2. Link between the sympathetic nervous system and the endocannabinoid system during cold exposure. Cold exposure increases output of the sympathetic nervous system to peripheral organs. Sympathetic simulation of white adipose tissue (WAT) stimulates lipolysis which results in release of free fatty acids (FFA) into the bloodstream. In addition, sympathetic stimulation of the liver increases the production and release of very-low-density lipoproteins (VLDL) into the bloodstream. Sympathetic stimulation of brown adipose tissue (BAT) induces thermogenesis thereby combusting intracellular lipid stores. The intracellular lipid stores can be replenished through uptake of WAT and liver-derived (triglyceride-derived)-fatty acids from the blood. BAT, brown adipose tissue; FFA, free fatty acids; SNS, sympathetic nervous system; TG, triglyceride; VLDL, very low density lipoproteins; WAT, white adipose tissue.

Thus, sympathetic activation of BAT following cold exposure results in combustion of TG-derived FA towards heat. Evidence for the importance of active BAT in energy metabolism comes from pre-clinical and clinical trials. In rodent studies, cold exposure

activates BAT, increases energy expenditure, decreases fat mass and increases glucose tolerance and insulin sensitivity (10,66,74). Human trials have also shown that 10 days (75) or 4 weeks (76) of intermittent cold acclimatisation enhances BAT activity and increases energy expenditure in both lean (75,76) and obese individuals (77). Moreover, intermittent cold exposure increases BAT activity and alleviates peripheral insulin resistance in T2D patients (78), suggesting a pivotal role for BAT in whole-body metabolism. These data underscore that activation of BAT by cold exposure could be a valuable tool to for the treatment of obesity and related diseases such as T2D and CVD.

PHARMACOLOGICAL ACTIVATION OF BAT

Albeit that the metabolic benefits of BAT activation by means of cold exposure have been well established, prolonged cold exposure is not a very convenient treatment option for humans. Therefore, more suitable therapeutic modalities such as pharmaceutical activation of BAT are currently being investigated. Promising therapeutic approaches are either direct modulation of activating receptors on the brown adipocyte itself or indirect modulation of BAT activity by manipulating the sympathetic outflow towards BAT (**Figure 3**). An example of a drug that directly stimulates the β3-adrenergic receptor on brown adipocytes is mirabegron. Mirabegron is already on the market for the treatment of overactive bladder disease and clinical studies indicate that one dose of mirabegron (200 mg) activates BAT as effectively as acute cold exposure in young healthy lean men (79). It remains to be determined if long-term administration of this compound indeed improves glucose and lipid profiles and prevents/ reverses obesity and diabetes development. In addition, it would be interesting to investigate if mirabegron is also effective in different risk populations, including South Asians.

A second class of drugs that has the potential to activate BAT are drugs that modulate incretin hormone signalling, such as dipeptidyl peptidase-4 (DPP4) inhibitors and glucagon-like peptide-1 (GLP-1) receptor agonists. DPP4 inhibitors enhance the bioavailability of incretin hormones including GLP-1, while GLP-1 receptor agonists directly stimulate the GLP-1 receptors. GLP-1 receptor stimulation in the hypothalamus increases sympathetic outflow to peripheral metabolic tissues in rodents (80) and is thereby able to modulate BAT activity. Pre-clinical studies indeed show increased BAT activity, increased energy expenditure, an improved glucose and lipid metabolism upon oral administration with the DPP4 inhibitor sitagliptin (81) or central administration of the GLP-1 receptor agonist exenatide (82,83). If this is also the case in humans, and whether different effects would be observed between ethnicities, e.g. between South Asians versus white Caucasians, still needs to be elucidated.

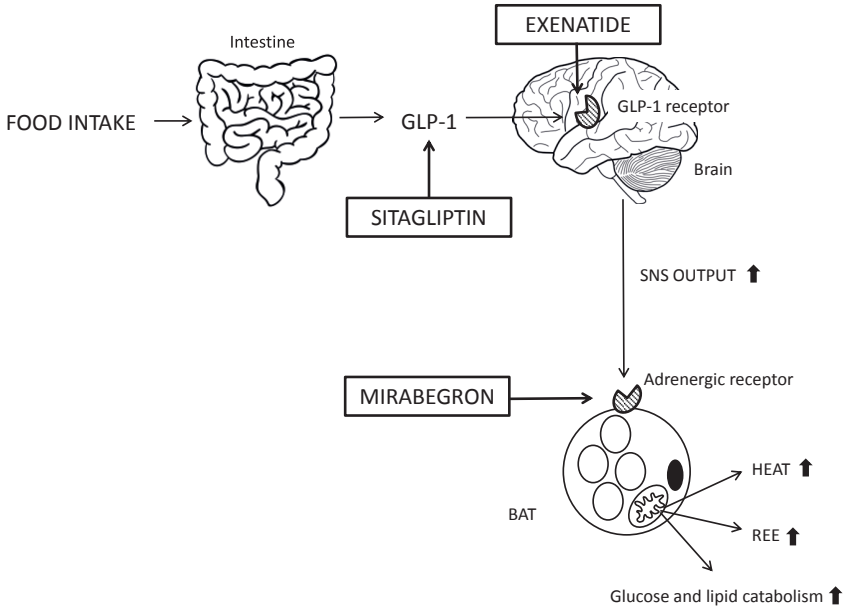

Figure 3. Novel pharmacological approaches to directly and indirectly activate brown adipose tissue. Sitagliptin and exenatide indirectly activate brown adipose tissue through increasing endogenous glucagon-like-peptide-1 and activating central GLP-1 receptors, respectively. Mirabegron directly activates brown adipose tissue through modulation of adrenergic receptors on brown adipocytes. BAT, Brown adipose tissue GLP-1, glucagon-like-peptide-1; REE, resting energy expenditure; SNS, sympathetic nervous system.

OUTLINE OF THIS THESIS

As is evident from this **chapter**, obesity and related metabolic diseases including T2D and CVD are a growing health care concern. Especially South Asian individuals are at increased risk for the development of metabolic diseases, but the underlying mechanisms are still not fully elucidated. Furthermore, current intervention strategies for the treatment of obesity are not effective on the long-term, apart from bariatric surgery. Although increasing energy expenditure by modulation of BAT seems a promising novel approach, modulation of BAT activation by cold exposure in humans is far from optimal. Therefore, the studies described in this thesis were aimed at 1) unravelling the underlying mechanisms that could explain the increased predisposition for metabolic disease in the South Asian population and 2) identifying novel treatment strategies that activate BAT and increase energy expenditure in risk population, including South Asians and individuals with overweight and prediabetes, with the ultimate goal to combat obesity, T2D and CVD.

In the first part of this thesis, we focused on identification of factors that could, at least in part, explain the enhanced susceptibility for the development of metabolic disease in the South Asian population. First, we focused on the endocannabinoid system. The endocannabinoid system is known to play an important role in energy metabolism by regulating appetite, intracellular lipolysis and energy expenditure and is found to be over-activated in subjects with obesity. In addition, endocannabinoids are thought to act in a negative feedback loop to prevent excessive BAT activity. Theoretically, differences in endocannabinoid signalling between South Asians and white Caucasians can thus contribute to the difference in predisposition for metabolic disease. Therefore, in **Chapter 2**, we first investigated whether endocannabinoid tone is higher in subjects from South Asian descent by studying circulating endocannabinoid levels in young health lean white Caucasian and South Asian men. Since endocannabinoid tone is a reflection of local endocannabinoid regulation in metabolically active organs, we next focussed on differences in local endocannabinoid signalling in WAT and skeletal muscle. To this end, we studied, in **Chapter 3**, gene expression of cannabinoid receptors and enzymes involved in endocannabinoid synthesis and degradation in middle-aged, overweight, prediabetic white Caucasian and South Asian individuals. Next, we shifted our focus to other factors that could possibly explain the difference in predisposition for metabolic disease between South Asians and white Caucasians. BAT is a metabolic organ which takes up lipids from the circulation to fuel thermogenesis. Angiopoietin-like (ANGPTL4) inhibits LPL-dependent uptake of TG-derived fatty acids by metabolic tissues. Since differences in substrate uptake by BAT could explain a difference in BAT function, we investigated, in **Chapter 4**, the effect of BAT activation (by means of short-term cold exposure) on circulating ANGPTL4 levels in white Caucasians and South Asians. As combustion of lipids by BAT results in the generation of lipid-associated metabolites, we next explored the effect of short-term cooling on lipid-associated metabolites in blood. In **Chapter 5** we investigated the changes in metabolites upon mild cooling and whether these responses differed between the two ethnicities.

In the second part of this thesis, we focussed on two promising pharmacological strategies to activate BAT. In **Chapter 6**, the effect of the β3-adrenergic receptor agonist mirabegron was investigated on BAT activity and energy expenditure. To this end, young healthy lean white Caucasian and South Asian men participated in a three day randomized cross-over study in which they were exposed to either short-term cooling, administration of mirabegron or placebo. The acute effects on lipid and glucose metabolism, energy expenditure and BAT activity were studied and potential differences in responses between the two ethnicities were assessed. In **Chapter 7**, we aimed at investigating the effect of chronic administration of the DPP4 inhibitor sitagliptin on lipid and glucose metabolism, energy expenditure and metabolism of BAT, WAT and skeletal muscle in another population at high risk for metabolic disease (*e.g.* overweight, prediabetic sub-

jects). A double-blinded randomized placebo-controlled trial was performed in which 30 overweight, prediabetic white Caucasian men received either sitagliptin or placebo for a duration of 12 weeks. Pre- and post-treatment blood samples were collected, indirect calorimetry was performed to measure energy expenditure, and an oral bolus of glucose was given to determine glucose tolerance. In addition, a skeletal muscle biopsy was taken to assess effects of sitagliptin on expression of genes involved in glucose and lipid metabolism and mitochondrial function. Furthermore, glucose uptake by BAT was assessed using [^{18}F]FDG PET/CT.

Finally, the results from these studies and their implications are discussed in **Chapter 8**.

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