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1 General introduction and outline

1. ETIOLOGY OF CARDIOMETABOLIC DISEASE IN SOUTH ASIANS

The position of cardiometabolic disease in global health

Clustering of cardiometabolic risk factors, *e.g.* (visceral) adiposity, glucose intolerance, atherogenic dyslipidemia and hypertension, may culminate in clinical diseases including non-alcoholic fatty liver disease (NAFLD), type 2 diabetes (T2D) and cardiovascular disease (CVD). The occurrence of these cardiometabolic diseases has risen progressively during the past decades, thereby imposing a major and costly burden on the worldwide health-care system. Moreover, CVD is the leading global cause of death, with 18 million annual deaths by ischemic heart disease and stroke (1). Notably, people from South Asian descent, who form one quarter of the total world population, are particularly susceptible to develop T2D and CVD. The main aim of this thesis is to elucidate yet unknown factors that could contribute to the disadvantageous metabolic phenotype in South Asians, in search for new treatment strategies against cardiometabolic diseases.

Migration patterns of Dutch Hindostani in the last century

In this thesis we use the terms 'South Asians' and 'Hindostani' interchangeably, as all subjects who have participated in our studies were Dutch Hindostani. The word 'Hindostani' represents descendants of 'Hindostan', which is the Hindi term for British-India. British-India used to be an English colony on the Indian subcontinent until **1947**, whereafter it was divided into the countries currently known as India and Pakistan. Other countries of the Indian subcontinent include Bangladesh, Bhutan, Nepal and Sri Lanka. Subjects descending from India who participate in studies conducted in Western countries, such as the United Kingdom, United States and Canada, predominantly represent offspring from educated Indian migrants and are commonly referred to as 'Asian-Indians'. In contrary, the Dutch Hindostani mainly represent offspring from Indian contract workers in Surinam (2).

Surinam is located in South America and was colonized by the Dutch until **1975**. Surinam thrived on plantations, especially sugarcane, coffee and cocoa, but expected a shortage of fieldworkers after slavery was abolished in **1863**. Other Caribbean islands and mainlands that were colonized by the British and French at that time had therefore already started contracting British-Indians as fieldworkers. In **1872** the Dutch followed by signing the Dutch-British Treaty Pact (in Dutch '*Koelietraktaat*'); an agreement stating the rights and obligations of indentured laborers. A massive migration followed with over 34,000 British-Indians arriving in Surinam between **1873** and **1916** (3). The Dutch-British Treaty Pact was terminated approximately 50 years after its initiation, whereafter one third of the British-Indians contract workers returned home and two thirds remained in Surinam. Aside from this British-Indian majority, the Surinamese

population consisted of a minority of Javanese inhabitants (contract workers descending from the Indonesian Island of Java), Creol and Marron inhabitants (descendants of former African slaves) and to a lesser extent Chinese inhabitants (4). A large migration wave consisting of almost 40,000 Surinamese inhabitants to the Netherlands occurred when Suriname became independent in **1975**. A second migration wave consisting of over 15,000 people occurred around the **1980**s due to political reasons. Lastly, a smaller third migration wave took place during the early **1990**s, which is believed to be due to poor economic circumstances (4).

Estimating the number of Dutch Hindostani and their offspring living in the Netherlands is not clear cut, since Statistics Netherlands (in Dutch '*Centraal Bureau voor de Statistiek*') registers immigrants based on nationality and not ethnicity. Based on the available data it is suggested that approximately 340,000 Surinamese live in the Netherlands, who are mostly located in Amsterdam, followed by Rotterdam, the Hague, Almere and Utrecht (**Figure 1**). In an attempt to further identify these Surinamese immigrants into ethnic subgroups, Statistics Netherlands classified subjects based on their family names. These analyses point towards 150,000 subjects being Hindostani, which is 1% of the total Dutch population. This includes people of both first and second generations Hindostani, *i.e.* contract workers and their children, respectively. There are also an estimated 10,000 third generation Hindostani living in the Netherlands, *i.e.* grandchildren of contract workers, but as they are born in the Netherlands they have the Dutch nationality (5).

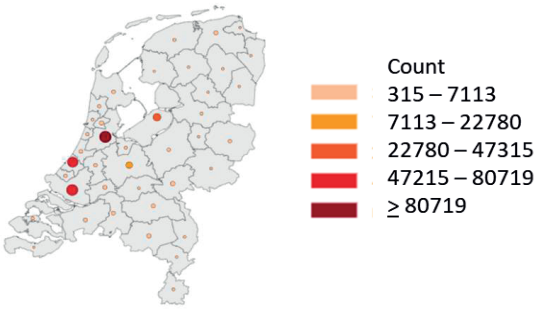

Figure 1. First and second generation Surinamese (Hindostani, Creoles, Javanese and Chinese) living in the Netherlands on January 1st 2019. Adapted from Statistics Netherlands (6).

Of note, from a socioeconomical point of view, Hindostani living in the Netherlands are successfully integrated, with a generally high level of education and employment. The Dutch Hindostani do remain strongly connected to each other, which has led to the establishment of a large and deeply rooted Hindostani community with various active organizations in the Netherlands (2). Fascinatingly, South Asians both in India

and after migration to Western countries have a disadvantageous metabolic phenotype compared to that of white Caucasians. Albeit various research groups around the world have committed to exploring this topic, additional dedicated studies are necessary to fully grasp the underlying genetic and environmental factors leading to the high rate of cardiometabolic disease in South Asians.

Thin-fat phenotype and dysregulated energy balance in South Asians

The aforementioned susceptibility of South Asians to develop T2D and CVD is likely due, at least in part, to their relatively high amount and unfavorably distributed body fat, which is present already from a young age on. We will discuss factors contributing to this unfavorable metabolic phenotype in South Asians further below in this chapter.

When energy intake is equal to expenditure, the energy balance is considered neutral and body weight is kept constant. When energy intake however exceeds expenditure, the energy balance turns positive and results in weight gain. After a prolonged period of time this leads to excessive weight gain in terms of obesity. This excess energy is mainly stored in the form of triglycerides in white adipose tissue (WAT). WAT consists of depots just beneath the skin ('cutis'), *i.e.* subcutaneous WAT, and smaller compartments surrounding internal organs ('viscera'), *i.e.* visceral WAT. On the long-term, a positive energy balance results in both cellular hypertrophy (enlargement of white adipocytes) and hyperplasia (recruitment of new white adipocytes from precursor cells), thereby expanding WAT depots (7). Enlarged WAT depots, especially visceral, then become more and more saturated with lipids, followed by an influx of immune cells causing local insulin resistance. This leads to an overflow of lipids towards nonfatty tissues, *e.g.* the liver, skeletal muscles, heart, kidneys and pancreas, resulting in the lipotoxic formation of 'ectopic fat' (8).

Excessive storage of triglycerides in visceral WAT and the formation of ectopic fat in particular have detrimental consequences for metabolic health, as both are positively associated with insulin resistance and atherosclerosis, and their progression into T2D and CVD, respectively (9, 10). Interestingly, the predominant WAT storage pool for lipids varies between individuals. For example, there is a preference for visceral over subcutaneous lipid deposition in males *vs* females and in aged *vs* young people, which may partly explain the independent predictive value of sex and age on cardiometabolic disease (11). In addition, fat distribution is strongly affected by genetics, and the effect of genetic variants on fat distribution differs between sexes (12). Possibly, genetic variants also contribute to an unfavorable fat distribution pattern in South Asians, with perhaps detrimental consequences for cardiometabolic health.

Intriguingly, from a young age on, people from South Asian descent have a relatively high amount of fat mass, as reflected by a higher ratio of fat mass over lean mass, compared with white Caucasians. This phenomenon is already present at birth, with

South Asian neonates having a small gesture and low body weight compared with white Caucasian neonates, as characterized by a low muscle mass and high fat mass; the 'thin-fat' body phenotype. These relatively high amounts of body fat in South Asians are mainly located in their trunk rather than their extremities, as repeatedly shown by a higher subscapular skinfold thickness in South Asian neonates born in India compared with white Caucasian neonates (13, 14). This difference in body fat distribution remains preserved in following generations of South Asian neonates born in Surinam (15) and the United Kingdom (16), and to some extent in the Netherlands (17). Moreover, magnetic resonance imaging (MRI) analyses showed that the amounts of visceral and deep subcutaneous adipose tissue are larger in South Asian compared with white Caucasian neonates (18). This relatively higher amount of body fat and unfavorable fat distribution pattern is maintained throughout life and during progression to obesity, as demonstrated in South Asian children (19-21) and adults (22-27) in the United Kingdom, United States and Canada. Furthermore, the amount of ectopic fat is higher in South Asians compared with white Caucasians, as measured by lipid deposition in skeletal muscle (intramyocellular lipid; IMCL) (28-30), and in the liver resulting in NAFLD (31-34). The congenital high amount of body fat in South Asians, especially located in visceral and ectopic depots, is likely partly responsible for the high risk and progressive nature of cardiometabolic disease in this population throughout life. An important question however remains what the underlying cause of the unfavorable fat distribution pattern is in South Asians.

Various evolutionary hypotheses aim to answer this key question. The essence of these hypotheses is based on the gain of specific traits that increase chances of survival under difficult environmental conditions, for example by accumulation of adipose tissue as energy storage after having experienced famine (35, 36). Although beneficial in times of a negative energy balance, this may lead to the development of adiposity and cardiometabolic consequences under more prosperous and food-rich conditions, such as upon urbanization in India and after migration to Western countries (37). The contribution of genetics to the increased risk of cardiometabolic disease in South Asians is not yet known, since the still limited studies to date have not yielded pronounced and consistent differences in relevant genetic variants in South Asians compared with white Caucasians (35, 38).

Especially in populations that are predisposed to cardiometabolic disease, a healthy lifestyle is of utmost importance to maintain a neutral energy balance and thereby prevent obesity and its cardiometabolic complications. Various lifestyle aspects embedded in the South Asian culture may contribute to a positive energy balance. For example, traditional South Asian dishes typically include dense foods that are high in caloric content, refined carbohydrates, saturated fats and trans-fats (especially from cooking oils and deep-frying), and low in unsaturated fats and fibers (39). Moreover, consuming

fairly large portions of such foods during social engagements and religious gatherings is part of the traditional South Asian culture (40). In addition to excessive intake of unhealthy foods, South Asians in India generally show sedentary behavior (41), and studies conducted in Western countries also show lower physical activity levels in South Asians compared with white Caucasians (42-44). Since physical activity increases muscle mass, little physical activity may contribute to the low fat-free mass observed in adolescent and adult South Asians. Since fat-free mass is the most important component of body composition determining energy expenditure (45), low physical activity levels may thereby contribute to a low energy expenditure in South Asians. Indeed, healthy young South Asians have a lower resting energy expenditure compared with white Caucasians (46). Notably, in this study resting energy expenditure remained significantly lower in South Asians after correction for fat-free mass, also suggesting a role for additional unknown factors. One factor might be energy-combusting brown adipose tissue (BAT), which was lower in South Asians compared with white Caucasians in this study (46). We thus propose that less amounts of active BAT could contribute to a positive energy balance and thereby negatively affect cardiometabolic health in South Asians.

Tissue-specific insulin resistance and type 2 diabetes in South Asians

Both native and migrant South Asians are at particularly high risk to develop T2D (47, 48). Moreover, South Asians show a more rapid progression of prediabetes to overt diabetes (49-51), develop T2D already at a lower BMI (52, 53) and do so on average 5 to 10 years earlier compared with white Caucasians (49). Importantly, in South Asians, glycated hemoglobulin (HbA1c) levels also increase more rapidly and the onset of micro-vascular complications, *e.g.* retinopathy and nephropathy, is accelerated compared with white Caucasians (49). The American Diabetes Association has therefore advised to lower the BMI threshold for diabetes screening, from 25 kg/m^2 applied for whites, to 23 kg/m^2 for Asian Americans (54). The primary defect in T2D is generally resistance of metabolic organs, mainly liver, skeletal muscle and WAT, to the effects of insulin. Insulin resistance leads to compensatory insulin hypersecretion by the pancreas, which eventually deteriorates β-cell function and thereby impairs insulin secretion, which is further aggravated by lipid accumulation in the pancreas. Circulating insulin levels are indeed higher in South Asian children and adults compared with white Caucasians, and coincide with lower insulin sensitivity (35, 49). Notably, insulin levels are also already higher in umbilical cord blood of South Asian compared with white Caucasian neonates (13, 55, 56). These data suggest that the foundation for the high risk of T2D in South Asians is laid already upon birth, probably even in utero, and remains preserved throughout life.

A relatively high amount of fat mass *vs* fat-free mass is notorious to promote insulin resistance (10, 57, 58). Since such a body composition is characteristic for South Asians, as previously described in this chapter, this likely contributes for a large part to the high

rate of insulin resistance and T2D in this population. Insulin sensitivity however also remains lower in South Asians after correction for the amount of body fat (30, 59, 60). Other factors than the relative amount of body fat therefore also seem to play a role in the high degree of insulin resistance in South Asians, such as their fat distribution pattern. The formation of ectopic fat in particular negatively affects insulin sensitivity. More specifically, hepatic lipid deposition is detrimental for glucose homeostasis, and a clear negative effect of NAFLD on glucose metabolism and insulin sensitivity has been shown (10), also in South Asians (31, 32, 61). Ectopic lipid deposition in skeletal muscle may play only a limited role in the high degree of insulin resistance in South Asians, since no correlation between IMCL accumulation and insulin sensitivity in this ethnicity has been shown to date (30, 62). It may however also be the specific location of lipid deposition within the skeletal muscle rather than the total amount of IMCL that affects myocellular insulin sensitivity (10). To date, only one study demonstrated reduced gene expression and protein levels of components of the insulin signaling pathway in skeletal muscle in South Asians (60). To summarize, there is ample data suggesting that the relatively large amount of body fat and especially its ectopic deposition in the liver contributes to the high degree of insulin resistance in South Asians, whereas evidence for the involvement of skeletal muscle is scarce.

The metabolic characteristics of adipocytes also affect their lipid storage capacity and insulin sensitivity. The lipolysis rate is increased in hypertrophic subcutaneous adipocytes, resulting in an overspill of fatty acids towards visceral WAT compartments and nonfatty tissues, especially in the context of inflammation caused by infiltrating immune cells. Additionally, a large size of abdominal adipocytes is associated with insulin resistance and T2D (63). Subcutaneous abdominal adipocytes are indeed larger in South Asians compared with white Caucasians (32, 64, 65), and negatively associate with the glucose disposal rate in South Asians as demonstrated previously by the euglycemic-hyperinsulinemic clamp technique (64). Furthermore, a high amount of small and less differentiated adipocytes is considered an unhealthy form of WAT expansion and is associated with an impaired intracellular lipid storage capacity and insulin resistance (66, 67). There is indeed evidence pointing towards less matured subcutaneous adipocytes in South Asians compared with white Caucasians (65, 68). These studies suggest that unfavorable adipocyte characteristics and a potentially infiltrated immune cell pattern also contribute to the high degree of insulin resistance in South Asians. Further research investigating intracellular signaling pathways in metabolic tissues that may be altered in South Asians are needed. One interesting pathway that is involved in adipogenesis and insulin resistance is Wnt signaling, which is mainly known for its osteoanabolic effects by promoting differentiation of precursor cells into osteoblasts rather than adipocytes (69). Since impairing mutations in the Wnt signaling pathway are linked to both adiposity (70) and diabetes in humans (71), it would be interesting to investigate whether Wnt

signaling is reduced in South Asians and could thereby contribute to fat accumulation and insulin resistance in this population.

In addition to the key role of WAT, the previously mentioned lifestyle factors may be implicated in the high rate of insulin resistance and its progression to T2D in South Asians. Importantly, physical activity and cardiorespiratory fitness levels, *i.e.* the supply of oxygen to skeletal muscle during physical activity, are inversely associated with insulin resistance, and are indeed lower in South Asians compared with white Caucasians (24, 60). Promoting physical activity could therefore be a successful treatment strategy to improve cardiometabolic health in South Asians, and regular exercise was indeed shown to reduce diabetes risk (72), also in South Asians (73). Underlying mechanisms include stimulating non-insulin-mediated glucose uptake by skeletal muscle and improving cardiorespiratory fitness, as well as improving whole-body insulin sensitivity, also independent from weight loss (72, 74). In addition to stimulating physical activity and exercise, promoting healthy dietary habits has been shown to reduce diabetes risk, also in South Asians (73). This includes limiting caloric intake, increasing the intake of healthy dietary components such as fibers and unsaturated fatty acids, and minimizing the intake of saturated fats, added sugars and processed foods (75, 76), which could be particularly relevant for those consuming typical South Asian foods.

Atherosclerotic cardiovascular disease in South Asians

In addition to their exceptionally high risk of developing T2D, South Asians are at increased risk to develop atherosclerotic CVD compared with other ethnic groups, including other Asians and whites (38, 47). Similar to T2D, CVD occurs earlier in South Asians compared with white Caucasians, with a first myocardial infarction presenting on average 10 years earlier in South Asians (77). Moreover, once CVD has established, South Asians have a higher hospitalization rate and mortality risk compared with whites (38). T2D is a well-known risk factor for CVD (78), and the high occurrence of T2D in South Asians is therefore likely an important mediator of their increased CVD risk (77, 79, 80). Furthermore, the aforementioned adiposity in South Asians likely plays a role, as this does not only negatively affect glucose regulation but also promotes other classical CVD risk factors including hypertension and dyslipidemia (81, 82). This holds true especially for the visceral WAT depot, as the waist-to-hip ratio is strongly independently positively associated with the risk of myocardial infarction in South Asians (77, 83). Furthermore, adiposity promotes CVD by directly affecting the cardiovascular system via secreting pro-inflammatory cytokines. Interestingly, there is a fat depot directly surrounding the heart, *i.e.* epicardial fat, which is located between the myocardium and the visceral layer of the pericardium, that secretes a variety of adipokines, cytokines and other factors that promote atherosclerosis and thereby negatively impact cardiovascular function (82). Notably, the volume of this fat depot is higher in South Asians compared with

white Caucasians (84). Epicardial adipose tissue might therefore be involved in the pronounced atherosclerosis in South Asians, and therefore it would be highly interesting to investigate whether this fat depot indeed possesses more pro-inflammatory and pro-atherogenic characteristics in South Asians compared with white Caucasians. Collecting biopsies from this region is however difficult for ethical reasons.

In addition to adiposity, one of the most important classical risk factors for CVD is dyslipidemia (85-87). South Asians are particularly susceptible to develop dyslipidemia, in particular high triglyceride levels and low HDL-cholesterol levels (88-90), and often show a trend towards increased LDL-cholesterol levels (88). Small dense LDL-particles are enriched in triglycerides and are strong predictors of atherosclerotic CVD (91, 92). Therefore, assessing the quality and quantity of LDL particles rather than measuring total LDL-cholesterol levels has gained much interest in terms of predicting CVD. Specifically, levels of apoB, a protein present on atherogenic lipoproteins, are strongly positively associated with the risk of and mortality from atherosclerotic CVD (91). Such atherogenic dyslipidemia is also generally present in South Asians, as multiple studies have shown that South Asians have more small dense LDL particles (93), higher apoB levels (94) and a higher apoB/apoA-I ratio (94-96) compared with white Caucasians. Furthermore, lipoprotein(a) (Lp(a)), a lipoprotein structurally similar to LDL but containing an additional apo(a) (97), is an independent risk factor for CVD (98). Also in South Asians, Lp(a) levels are often higher compared with whites (85, 99-102) and are strongly positively associated with CVD risk (103, 104). Since the Lp(a) concentration is strongly genetically determined (105), genetic variants probably explain the increased Lp(a) levels in South Asians. Altogether, atherogenic dyslipidemia may contribute for an important part to the susceptibility of South Asians to develop CVD. Interestingly, the susceptibility of LDL to aggregate has recently been described as a novel risk factor for CVD (106), but differences in LDL aggregation between ethnicities are currently unknown.

The lifestyle factors described previously in this chapter also likely contribute to the high CVD risk in South Asians. A sedentary lifestyle and unhealthy diet stimulate development and progression of CVD via promoting classical risk factors such as adiposity, diabetes and dyslipidemia. Targeting these lifestyle factors by increasing physical activity levels and implementing a healthy diet reduces CVD risk and mortality, also independently from their beneficial effects on glucose regulation, albeit future studies specifically including South Asians are warranted (47, 78). Not unimportantly, tobacco smoking accelerates atherosclerosis via various mechanisms, and active smoking even doubles 10-year CVD mortality and reduces life expectancy with an average of 10 years (47). Also in South Asians smoking is the most important risk factor for myocardial infarction (38), and quitting smoking is the most cost-effective evidence-based lifestyle change to attenuate CVD risk (47). Since tobacco products are frequently used in the

South Asian culture (38), quitting smoking is particularly important to lower the risk and burden of CVD in South Asians.

The combined risk factors for T2D and CVD in South Asians described in this paragraph are summarized in **Figure 2**.

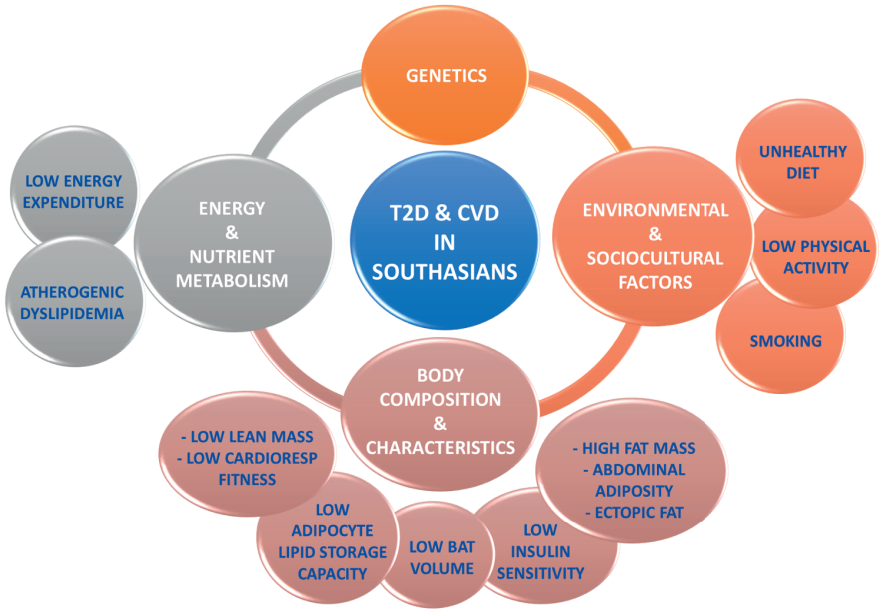

Figure 2. Risk factors for type 2 diabetes (T2D) and cardiovascular disease (CVD) in South Asians

2. TARGETING CARDIOMETABOLIC DISEASE IN SOUTH ASIANS: BROWN ADIPOSE TISSUE ACTIVATION

Brown adipose tissue physiology

Lowering body weight by inducing a negative energy balance is an effective strategy to reduce obesity and its cardiometabolic complications. A negative energy balance can be achieved by lowering food intake, which is however notoriously difficult to adhere to, especially in the current society with 24/7 access to excessive amounts of unhealthy foods, unfortunately often resulting in weight regain on the long term. Increasing energy expenditure, for example by performing exercise, can therefore aid in successfully maintaining a healthy body weight. In addition, stimulating energy-combusting BAT activity is a promising treatment strategy to further enhance energy expenditure. BAT and WAT form the two main distinct types of adipose tissue in humans. As described previously in this chapter, WAT is abundantly represented in humans and stores excess energy contained in fatty acids and glucose in the form of triglycerides. In contrary to

WAT, BAT is present in much lower amounts in humans, with an estimated average of 50-150 mL in adults (107). BAT is located mainly in the neck area and around the aorta in adult humans (108), likely in order to efficiently distribute its generated heat throughout the body. Whereas white adipocytes have one unilocular lipid droplet and sparse mitochondria, brown adipocytes are characterized by multilocular (multiple small) lipid droplets and many mitochondria that express the unique protein uncoupling protein-1 (UCP-1). In addition, scattered within WAT, so-called 'beige' or 'brite' adipocytes exist which have an intermediate and flexible phenotype. Under basal conditions these adipocytes morphologically resemble white adipocytes, however under (cold) stimulated conditions they gain a brown-like phenotype with multilocular lipid droplets and increased numbers of mitochondria high in UCP-1 expression (109).

BAT burns fatty acids and glucose into heat in order to maintain core body temperature under cold conditions (110). Firstly, when cold is sensed by transient receptor potential channels in nerve endings in the skin, a signal is forwarded to the hypothalamic temperature regulating center. This promotes sympathetic outflow, which stimulates the release of noradrenalin by nerve endings that densely innervate brown adipocytes. Noradrenalin subsequently binds to β-adrenergic receptors (β-ARs) on these brown adipocytes, inducing an intracellular signaling cascade that promotes hydrolysis of triglycerides stored within the intracellular lipid droplets. The fatty acids that are then released enter the mitochondria to stimulate UCP-1-mediated uncoupled respiration, thereby preventing ATP generation and resulting in dissipation of energy as heat (110). Upon prolonged stimulation, the intracellular lipid stores of brown adipocytes become depleted and need to be replenished. Replenishment occurs via the uptake of circulating fatty acids and glucose by BAT, the latter probably mainly for de novo lipogenesis (107). BAT takes up fatty acids mainly via lipoprotein lipase (LPL)-mediated hydrolysis of triglyceride-rich lipoproteins (TRLs) (111). The uptake of TRL-derived fatty acids by metabolic tissues, such as BAT and WAT, is under strict regulation of several factors, including the family of angiopoietin-like proteins (ANGPTLs) of which the LPL inhibitor ANGPTL4 is the most studied. Mouse studies established that cold exposure regulates *Angptl4* expression in a tissue-specific manner, *i.e.* being increased in WAT and decreased in BAT. As a result, during cold exposure, TRLs are shuttled away from WAT towards heat-producing BAT for LPL-mediated hydrolysis and subsequent uptake of fatty acids (112). Furthermore, the novel family members ANGPTL3 and ANGPTL8 are linked to T2D and CVD (113). How these ANGPTLs exactly affect not only lipid distribution but also glucose regulation however still warrants further investigation. For example, the effect of cold exposure on ANGPTL3 and ANGPTL8 levels in humans is yet unknown, which would be especially interesting to investigate in insulin-resistant subjects such as South Asians. Stimulating BAT thermogenesis is a potentially promising treatment strategy to target obesity, hyperlipidemia and hyperglycemia, via increasing energy expenditure and

stimulating the uptake of circulating lipids and glucose, respectively (114). We propose that this holds true especially for populations with a high prevalence of cardiometabolic disease, such as South Asians.

Brown adipose tissue activation to improve cardiometabolic health

Only a decade ago it was discovered that adult humans have metabolically active BAT that takes up glucose during cold exposure, as measured by ^{18}F-fluorodeoxyglucose ([^{18}F]FDG) positron emission tomography computed tomography (PET/CT) scan (115-117). Since then, much research has focused on determining BAT substrate uptake preferences (107) and optimizing BAT visualization methods (118). For example, MRI is a promising imaging method which can differentiate BAT from WAT based on its lower amount of intracellularly stored triglycerides and higher amount of water, *i.e.* the 'fat fraction'. The fat fraction lowers when active BAT utilizes its triglycerides for thermogenic purposes (119). Since humans possess large amounts of WAT and only little BAT, in contrast to rodents, it is of specific interest to study the possibility of stimulating beige adipocytes to gain a brown-like phenotype to enhance their thermogenic capacity, *i.e.* 'browning'. The ultimate goal is to discover strategies with the potential to activate BAT that could thereby improve cardiometabolic health (114).

From the beginning of this BAT era, clinical trials have focused on cold exposure as a potent method to activate and recruit BAT, mostly in healthy young subjects, and to a lesser extent in overweight or obese older subjects with (120) or without T2D (121). Since cold acclimation is however not a particularly attractive treatment option for humans, studies have also focused on other lifestyle interventions, such as adjusting dietary composition (114). Of all the investigated dietary components, most evidence pointing towards browning has been derived from the use of polyphenols. Polyphenols consist of a group of natural occurring compounds that are present in amongst others fruits and vegetables, whole grains and chocolate (122). Interestingly, various polyphenols enhance browning and improve metabolism in mice. Moreover, in humans, the polyphenol subgroups of capsinoids, *i.e.* analogues of capsaicine that are naturally present in red chili peppers, and green tea catechins also increase energy expenditure, promote lipid oxidation, and enhance BAT activity as mostly demonstrated by [^{18}F]FDG-PET/CT scan (114). These results were however not always consistent (123), and it remains to be studied whether dietary compounds in feasible amounts for human consumption also improve cardiometabolic health on the long term. In addition to cold exposure and dietary compounds, promoting physical activity has been a treatment strategy of interest aiming to stimulate BAT activity and browning of WAT. Numerous studies in rodents indicate that exercise stimulates browning of WAT. The effect of exercise on BAT in rodents is however controversial, which also holds true for the effect of exercise on thermogenic tissues in humans (114).

Parallel to studies investigating the effect of lifestyle interventions on BAT activity and browning of WAT, the urge for more effective treatments has stimulated the search for pharmacological agents with such properties. A key paper from Cypess *et al.* (124) showed that a single dose of the β_3-AR agonist mirabegron increases energy expenditure and [^{18}F]FDG uptake by BAT in healthy young subjects. Mirabegron is prescribed in clinical practice to patients with overactive bladder disease due to its relaxation effect on smooth muscle tissue. The dosage prescribed in clinical practice is however 4 times lower (50 mg) than was needed to show effects on energy expenditure and BAT activity (200 mg) in this proof-of-principle study (124). This may explain, at least in part, why other β_3-AR agonists in previous clinical trials did not clearly improve the metabolic phenotype (125-127). Promising, a recent study from this same group showed that treatment of healthy young women for a prolonged period (4 weeks) with mirabegron in a dose of 100 mg per day increased metabolically active BAT, as measured by [^{18}F]FDG-PET/CT, and resting energy expenditure, and even improved markers for whole-body insulin sensitivity, pancreatic β-cell insulin secretion and glucose disposal (128). These data further support that β-AR agonism has the potential to improve energy metabolism, possibly in part via increasing nutrient uptake by BAT. It remains to be elucidated how β-AR agonism exactly affects the various pathways in lipid metabolism and whether it truly increases lipid uptake and thermogenesis by BAT in humans. Furthermore, it would be relevant to determine whether the effect of β-AR agonism is different in cardiometabolically compromised patients, for example in South Asians. Another promising treatment strategy that improves cardiometabolic health possibly in part via BAT activation, is agonism for the glucagon-like peptide-1 (GLP-1) receptor. GLP-1 is an incretin hormone that is produced and released into the blood by intestinal L-cells after food intake, and subsequently stimulates pancreatic insulin secretion to lower postprandial blood glucose levels. Once entering the blood, GLP-1 is degraded within minutes by the enzyme dipeptidyl peptidase-4 (DPP-IV) (129), which has led to the development of DPP-IV inhibitors that increase endogenous GLP-1 levels. In fact, both GLP-1 receptor agonists and DPP-IV inhibitors are successfully used in clinical practice to lower blood glucose levels in patients with T2D. Interestingly, these compounds exert many beneficial metabolic effects in addition to lowering blood glucose levels, including a reduction in body weight, lowering of lipid levels, increase in lipid utilization and possibly also an increase in energy expenditure (129-132). Notably, we (133) and others (130) have shown that central agonism of the GLP-1 receptor in mice stimulates sympathetic outflow towards BAT and increases glucose and lipid uptake by BAT, which is accompanied by a reduction in body weight and blood glucose and lipid levels. BAT activation therefore likely mediates part of these beneficial effects on cardiometabolic parameters. Whether GLP-1 receptor agonism also stimulates BAT activity in humans is however still unknown, which could be especially relevant for South Asians. The various

lifestyle factors and pharmacological compounds described in this paragraph that aim to activate BAT and thereby improve cardiometabolic health are summarized in **Figure 3**.

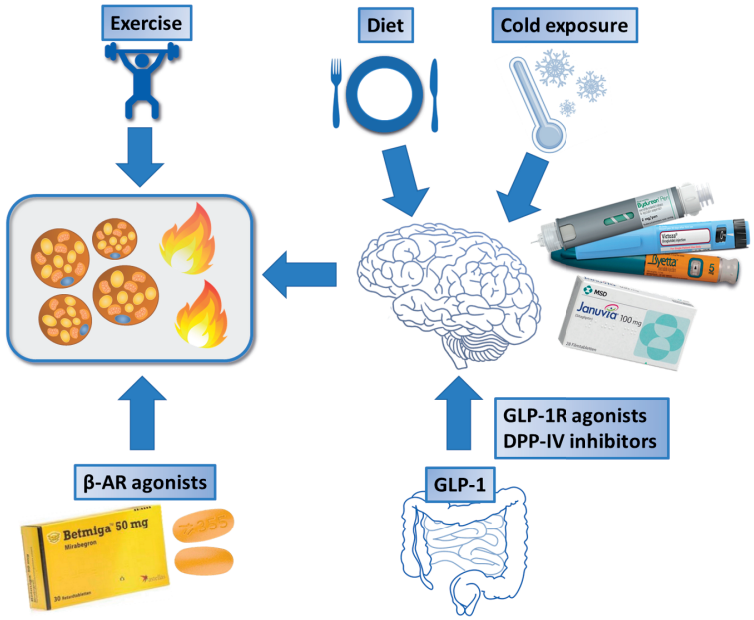

Figure 3. Lifestyle factors and the promising pharmacological agents of beta-adrenergic receptor (β-AR) agonists, glucagon-like peptide-1 receptor (GLP-1R) agonists and dipeptidyl peptidase-4 (DPP-IV) inhibitors as treatment strategies to activate BAT and brown WAT.

OUTLINE OF THIS THESIS

In **this chapter** we described the etiology of the cardiometabolic epidemic that the world is currently facing. Notably, people from South Asian descent are particularly susceptible to develop T2D and CVD compared with other ethnicities including whites, and suffer to a disproportionally large extent from morbidity and mortality due to these diseases. Aside from having many classical cardiometabolic risk factors, South Asians also possess additional risk factors that may explain at least part of their high cardiometabolic risk. Treatment of T2D and CVD is of utmost importance due to their burden on global health care, and therapies should primarily be aimed at preventing their onset by targeting cardiometabolic risk factors. One currently widely investigated and potentially promising treatment strategy to improve energy metabolism is stimulating BAT thermogenesis. In the studies described in this thesis, we therefore firstly aimed to further unravel underlying mechanisms contributing to T2D and CVD particularly

in South Asians. We then aimed to explore the potential of different pharmacological agents to activate BAT in South Asians compared with white Caucasians.

Firstly, in **chapter 2** we investigated the effect of cold exposure on a subset of ANGPTLs. ANGPTLs are proteins involved in lipid trafficking between metabolic tissues. In this chapter we aimed to explore cold-induced changes in plasma levels of ANGPTLs and their relation with lipid and glucose levels and BAT activity. To investigate how age and unfavorable metabolic characteristics, *e.g.* adiposity and insulin resistance, affect the cold-induced response in ANGPTLs, we also compared these observations between healthy young lean and middle-aged overweight prediabetic subjects. Moreover, since we previously observed a lower cold-induced increase in serum free fatty acids in South Asians compared with white Caucasians, possibly indicating less sympathetic outflow in this ethnicity, we also compared the effect of cold on ANGPTLs between South Asians and white Caucasians. We then focused on Wnt signaling, a cell signaling transduction route which is mainly known for its role in oncogenesis and embryonic development. Since mutations in Wnt genes and blood levels of Wnt family proteins have also been linked to glucose regulation and diabetes, with evidence suggesting interaction between Wnt and insulin signaling in white adipocytes, in **chapter 3** we studied whether Wnt signaling is impaired in South Asians compared with white Caucasians. To this end we assessed expression of Wnt signaling genes in skeletal muscle and subcutaneous abdominal WAT biopsies and plasma levels of the Wnt inhibitor sclerostin, as well as the relation of these parameters with measures for insulin signaling in overweight prediabetic South Asian and white Caucasian men. **In chapter 4**, we continued aiming to unravel underlying mechanisms that could contribute to the excess CVD risk in South Asians. It was recently shown that LDL particle aggregation predicts future cardiovascular events in patients with CVD. We therefore assessed LDL aggregation susceptibility in healthy young South Asians and compared this with white Caucasians, as a possible predictor for future CVD.

We then shifted our focus in **chapters 5** and **6** towards investigating the ability of pharmacological agents to improve cardiometabolic health by stimulating BAT activity in South Asians and white Caucasians. More specifically, in **chapter 5**, we performed a placebo-controlled randomized trial evaluating the effect of the β_3-AR agonist mirabegron on BAT activity and energy expenditure compared with cold exposure in South Asian and white Caucasian men. We measured the effect of mirabegron on BAT activity by means of its fat fraction with MRI. We additionally investigated the effect of mirabegron and cold exposure on serum lipidomics, aiming to gain mechanistic insight into the changes in different lipid species during BAT activation. Next, in **chapter 6** we studied the effect of GLP-1 receptor agonism on BAT activity. We hypothesized that GLP-1 receptor agonism stimulates BAT activity in humans, since it was shown in mice that central agonism for the GLP-1 receptor stimulates thermogenesis and increases lipid and glucose uptake by BAT. We therefore evaluated the effect of the GLP-1 receptor ago-

nist exenatide on BAT activity and energy expenditure in healthy young South Asian and white Caucasian men, in addition to its effects on body composition and blood glucose and lipid levels. In this study we visualized BAT by measuring its glucose uptake via [^{18}F] FDG-PET/CT scan as well as by assessing its fat fraction with MRI. Lastly, in **chapter 7** the results of our studies are put in the context of the available literature to date and future perspectives for our field of research are discussed.

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