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Metabolic hormones and ethnic aspects in obesity

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CHAPTER 8

GENERAL DISCUSSION AND FUTURE PERSPECTIVES

GENERAL DISCUSSION

Obesity is a chronic disease characterized by excessive fat deposits caused by a positive balance between energy intake and energy expenditure (1). The prevalence of obesity and its related diseases is rising, especially in certain ethnic populations (2, 3). In this thesis, we aimed to i) unravel the underlying causes of the disadvantageous metabolic profile of South Asians, and ii) comprehensively understand how different (non-) pharmacological interventions can modulate various circulating hormones and regulate overall energy metabolism in humans with different comorbidities. In this chapter, we discuss new insights based on experimental studies addressing these objectives, their implications for the general population and the South Asian population specifically in clinical practice, and future challenges.

Unraveling influential factors leading to increased risk of obesity and related diseases in the South Asian population

South Asians are prone to develop obesity and obesity-related diseases, including type 2 diabetes mellitus (T2DM), at a significantly younger age and lower body mass index (BMI) than other ethnic groups (4). As described in **Chapter 1**, their increased risk of developing T2DM is significantly influenced by their unfavorable metabolic phenotype, characterized by increased visceral fat mass, dyslipidemia, and reduced insulin sensitivity (5). Apart from this phenotype, several other factors likely contribute to their increased risk of developing T2DM, including lifestyle factors, inflammation, and hormonal dysregulation (5). In this thesis, inflammatory factors (**Chapter 2**) and various appetite-regulating hormones (**Chapter 3** and **Chapter 4**) were studied in South Asians compared to Europids.

Inflammation as a potential catalyzer of the South Asian metabolic phenotype

Inflammation plays a crucial role in the development of T2DM and its associated complications, often triggered by stress on the adipose tissue due to a positive energy balance. This stress leads to the attraction of different immune cell types, including monocytes, and the release of cytokines and chemokines that promote inflammation, exacerbating insulin resistance both locally in white adipose tissue (WAT) and systemically (6). While a more pro-inflammatory phenotype is already found in healthy South Asians, the precise differences in various immune cell types that coincide with the South Asian phenotype are still unknown (7, 8). This is important when developing specific treatment options for South Asians.

In **Chapter 2**, we demonstrated that the relative plasma levels of six inflammation-related proteins were higher in South Asians with T2DM compared to Europids, and

six proteins were lower among a large panel of inflammation-related proteins. Among them, fibroblast growth factor 21 (FGF21) was significantly lower in South Asians than Europids in both males and females (**Figure 1**). FGF21 is a pleiotropic hormone that affects glucose and lipid metabolism. It enhances insulin sensitivity in brown adipose tissue (BAT) and WAT and suppresses lipolysis in WAT (9). Furthermore, FGF21 has anti-inflammatory effects. These anti-inflammatory properties include targeting macrophages to shift from a pro-inflammatory to a pro-repair phenotype. This shift occurs through the paracrine effect of hepatocytes on Kupffer cells and in adipocytes via the secretion of adiponectin (10). The subsequent question arising from this study is whether the lower FGF21 levels in South Asians are a cause or a consequence of the already present metabolic complications in this cohort. To get more insight into this question, we also measured FGF21 levels in a small cohort of healthy South Asians since they had not developed metabolic complications as yet. Of note, in this cohort of males, FGF21 levels were comparable between South Asians and Europids, which may indicate that FGF21 regulation becomes disturbed later in life. However, since we only measured baseline FGF21 levels, we cannot exclude that stress-induced FGF21 release (e.g. following cold exposure) is disturbed in this population prior to the development of metabolic diseases. This may be of relevance since it implies that certain metabolic pathways may already be targeted in an early stage to prevent the development of metabolic diseases in this population (see below).

Next to measuring inflammation-related proteins, to better understand the increased pro-inflammatory phenotype observed in South Asians, it is crucial to focus on identifying specific immune cell types that may be involved. This can improve understanding of their vulnerable metabolic phenotype and give direction to possible intervention options. We previously showed that South Asians with T2DM had higher gene expression of B cell markers in blood than Europids (8). The role of B cells in developing insulin resistance has been investigated in mice, showing that a high-fed diet induces the accumulation of B cells in VAT after four weeks. Mice lacking mature B cells had body weight and VAT adipocyte size similar to those of mice with mature B cells, although with lower fasting glucose and insulin levels and improved glucose tolerance and insulin sensitivity (11). The accumulation of B cells in VAT may also contribute to the development of insulin resistance in humans with obesity, since B cells are known to stimulate the production of interferon-gamma (IFN-gamma) and tumor necrosis factor-alpha (TNF-alpha) from T-cells. These factors, in turn, are known to induce insulin resistance in other tissues than VAT, including skeletal muscle (12). Therefore, as increased B cells contribute to the development of insulin resistance, and South Asians with T2DM have higher expression of B cell markers in blood, this could have contributed to their insulin resistance (**Figure 1**). To further unravel this

hypothesis, future studies should focus on studying the presence of B cells and their activation status in WAT biopsies in South Asians. Moreover, determining whether the higher gene expression of B cell markers is a contributor to the development of T2DM in South Asians or merely a result of their T2DM requires research in a healthy population. Indeed, if increased B cell markers are already present in healthy South Asians, it would suggest that these markers are more likely a contributing factor rather than a consequence of T2DM.

Another immune cell involved in the development of insulin resistance is the monocyte (**Figure 1**). Monocytes have been shown to play a role in the development of atherosclerosis and insulin resistance (13, 14). Monocytes contribute to the initiation, progression, and thrombus formation of atherosclerosis. Especially the $CD16^{+}$ monocytes are associated with the development of obesity and related diseases (15, 16). A recent paper showed that South Asians with intermediate risk for cardiovascular disease have more circulating classical monocytes ($CD14^{++}CD16^{-}$) than their Europid counterparts (17). This is of specific interest as previous research showed beneficial changes in different monocyte subsets in people living with obesity after 1.5 years of an intensive combined lifestyle intervention (CLI). This intervention included group therapy with nutritional advice, physical activity, and structural behavioral changes using a cognitive behavioral therapy-based approach (15). Therefore, an intensive CLI that decreases relative classical monocyte levels and reduces the expression of CD16 in intermediate and non-classical monocytes (15) could benefit South Asians. As the function of the monocytes was not assessed in the study, exposing the monocytes to a stimulus like lipopolysaccharide (LPS) could provide more information on possible CLI-induced changes in the function of the different monocyte subsets (15). In addition to the subtypes of monocytes, changes in the metabolism of these cells can also play a role in developing obesity-related diseases in South Asians. A recent study found that the monocytes of insulin-sensitive individuals are more dependent on non-oxidative glycolysis, compared to oxidative metabolism in insulin-resistant individuals (13). Research focusing on measuring the quantity and quality of monocytes, using flow cytometry and metabolic signatures to measure monocyte metabolism in different states of metabolic status, could provide more insight into the pro-inflammatory status of South Asians. While this proposed study design focuses more on specific cell types, an organ-on-a-chip system with microfluidics can model vascular disease e.g., thrombosis, atherosclerosis, and inflammation (18). A system designed to mimic the pathology of atherosclerosis development in South Asians, incorporating their monocytes and comparing the development of vascular disease with a model using monocytes from Europids, could provide more insight into the role of the monocytes in the development of atherosclerosis in South Asians.

A relevant question is whether treatments targeting inflammation, such as the B cells or monocytes, result in the prevention and/or improvement of obesity-related diseases in South Asians. Concerning potential treatments, a pharmacological intervention could be the anti-inflammatory drug salsalate. Salsalate is a salicylate known for treating inflammatory diseases like arthritis (19). It induces anti-inflammatory effects by inhibiting cyclooxygenase (COX) enzymes and the nuclear factor-κB (NF-κB) cascade, decreasing the release of pro-inflammatory cytokines (20, 21). Of specific interest is the fact that it decreases the chemokine monocyte chemoattractant protein-1 (MCP-1), which mediates the release of monocytes from the bone marrow and guides monocytes into VAT (22). In Europids with T2DM, treatment with salsalate decreases inflammatory mediators, lowers circulating leukocytes, neutrophils, and lymphocyte counts, and results in the improvement of glycemia (23). Since South Asians have a phenotype that includes decreased insulin sensitivity and more pro-inflammatory monocytes compared to Europids, salsalate could be particularly beneficial for South Asians. In addition to the improvement of glucose regulation, salsalate may also exert its beneficial effect via the activation of BAT. A previous study from our group showed that, in mice, salsalate prevents weight gain, and deterioration of glucose metabolism by improving fasting insulin levels and glucose tolerance during intravenous glucose tolerance tests, and activates BAT (24), making it of interest in South Asians as they have dyslipidemia and lower BAT volume. Altogether, this compound may be of specific interest to the South Asian population and this type of treatment may even be considered as a general first-line treatment in South Asians with prediabetes, with the aim to delay progression towards T2DM.

Finally, an important question to consider is why South Asians exhibit a more pro-inflammatory phenotype compared to Europids. One theory is that this pro-inflammatory phenotype could have evolved since it had a survival advantage in the past, possibly due to the high risk of exposure to infections in South Asia (25). A more pro-inflammatory profile could lead to faster and more effective eradication of various micro-organisms, thereby enhancing survival chances. On the other hand, South Asians living in the United Kingdom had a higher risk of mortality during the Coronavirus disease 2019 (COVID-19) pandemic compared to other ethnicities (26). However, many other factors could have influenced this higher mortality, such as socioeconomic position, racial discrimination in healthcare, and the presence of comorbidities (26).

Incretin hormones in the South Asian population

The incretin hormone glucagon-like peptide-1 (GLP-1) has gathered extensive interest over the years as it improves insulin sensitivity and stimulates satiety, and various GLP-1 receptor agonists have been shown to improve T2DM and result in weight loss

(27). However, the postprandial excursion of GLP-1 in South Asians has not been extensively studied. In **Chapter 3** we observed a biphasic peak of active GLP-1 and glucose-dependent insulinotropic polypeptide (GIP) during the MMTT in South Asian females, while a single peak was observed in Europids. In addition to the peak at 30 minutes, levels of active GLP-1 and active GIP were higher towards the end of the MMTT in South Asian compared to Europid females. A similar biphasic pattern, albeit less pronounced, was evident for total GLP-1 and GIP in South Asian females compared to Europids. Since glucose levels followed the same biphasic pattern in South Asians compared to Europids, we propose a mechanism that could be driven by biphasic emptying of the stomach in South Asians (28) (**Figure 1**). This is plausible since gastric emptying rate is known to influence glucose concentration and differences in gastric emptying rates have been described to differ between various ethnicities (28). The biphasic glucose excursion during the MMTT in South Asian females could have led to an observed second peak of especially active GLP-1 and GIP. Further research is needed to determine if the differences observed are indeed related to differences in the rates and patterns of gastric emptying between healthy South Asians and Europids and if they contribute to an increased risk of developing obesity and related diseases in this population. This could be explored by measuring the gastric emptying rate during an MMTT using a gastric emptying scintigraphy.

Despite higher levels of active GLP-1 and active GIP towards the end of the MMTT, the insulin response was not different in South Asian females compared to Europid females. This could indicate a lower insulin response to active GLP-1 in South Asian females, for instance, due to a lower pancreatic GLP-1 receptor sensitivity. Whether the sensitivity of these receptors changes during the development of T2DM in South Asians needs to be assessed with long-term follow-up studies assessing the receptor sensitivity before, during, and after the development of T2DM. This could be studied by infusing GLP-1 intravenously, followed by assessing the response of insulin and glucose levels. In addition, the role and function of GLP-1 in satiety among South Asians could be assessed by using questionnaires about feelings of hunger and satiety, or by providing *ad libitum* meals to measure the amount of food intake with and without prior intravenous GLP-1 infusion.

In contrast to the South Asian females, in **Chapter 3**, we found a lower area under the curve (AUC) of total GLP-1 levels already in young and lean South Asian males compared to Europids. Similar to the South Asian females, this did seem to affect the response of insulin, as insulin levels, if anything, were even higher in South Asian males compared to Europid males. This could fit with an enhanced GLP-1 receptor sensitivity in South Asian males. Alternatively, GIP could have compensated for the lower GLP-1 with respect to

the stimulation of insulin release. It is interesting to speculate on the underlying causes of the lower AUC of total GLP-1 in South Asian males. This could be due to a lower release of GLP-1 by the intestinal L-cells (**Figure 1**) or increased degradation of GLP-1 by the enzyme dipeptidyl-peptidase-4 (DPP-4) in the circulation. DPP-4 activity in South Asians can be assessed *in vitro*. If DPP-4 activity is higher in South Asian compared to Europid males, then treatment with DPP-4 inhibitors would be beneficial. This treatment could increase the GLP-1 concentration after a meal, thereby potentially improving the induction of satiety and stimulating insulin release postprandially (29). However, DPP-4 inhibitors increase active GLP-1 levels rather than total GLP-1 (29), and active GLP-1 levels were not significantly different during an MMTT in young and healthy South Asian males. In addition, active GLP-1 represents the endocrine function of GLP-1, while total GLP-1 represents the neural function as well (30), therefore increasing total GLP-1 would potentially be more beneficial as it targets satiety, shifting energy balance towards a negative one. Whether active GLP-1 levels differ further between South Asians and Europids living with obesity needs to be assessed in future studies, preferably combed with an MMTT as well.

The sex differences observed in postprandial GLP-1 regulation in South Asian males and females compared to Europids suggests the need for a more tailored made approach for using GLP-1 receptor agonists in this population, possibly even stratified by sex. For South Asian females, treatment with GLP-1 receptor (GLP-1R) agonists could be beneficial as GLP-1 R agonists have been shown to slow gastric emptying rate, thereby reducing postprandial glucose levels (31). Although the biphasic curve of glucose and active GLP-1 and GIP did not affect insulin excursions in our population of female South Asians, the AUC of glucose was already higher in healthy and lean South Asian compared to Europid females. This suggests that if South Asian females were to become more metabolically compromised, treatment with GLP-1R agonist might reduce glucose excursion by slowing the gastric emptying rate and thereby potentially improving insulin excursions postprandially. In **Chapter 3** we found a lower AUC of total GLP-1 in the South Asian males compared to the Europid males. People living with obesity typically have lower postprandial GLP-1 levels compared to individuals without obesity (32). The South Asian males in our cohort had a significantly higher BMI and higher cholesterol levels compared to Europid males, indicating a more metabolic comprised phenotype, more similar to people living with obesity. If the differences observed in the AUC of total GLP-1 between South Asian males and Europids are also observed or even more pronounced in South Asians living with obesity, this would support the use of GLP-1R agonists in the male South Asian population as well. Interestingly, the effects of the GLP-1R agonist liraglutide on weight loss and glucose regulation have been studied in South Asians compared to Europids with T2DM in the cohort described in **Chapters 2**

and **7**. South Asians and Europids with T2DM had a similar improvement in the glycemic index after treatment with liraglutide, indicating that both ethnicities with overweight and obesity and type 2 diabetes mellitus indeed benefited from this intervention (33, 34). Nonetheless, considering our finding of lower AUC of total GLP-1 already in young South Asian males likely resulting in lower activation of GLP-1 receptor pathways in peripheral tissues, at least South Asians males could potentially benefit from earlier treatment with GLP-1R agonists to prevent or delay the development of T2DM.

Leptin in the South Asian population

In addition to the differences observed in **Chapter 3**, which focused on incretin hormones and glucagon, we described in **Chapter 4** that leptin levels were significantly higher in both South Asian males and females compared to Europids. The average baseline leptin levels in South Asian and Europid males were 83 ± 54 *vs.* 22 ± 21 and females 410 ± 236 *vs.* 183 ± 128 ng/mL. Of note, these levels in lean South Asians, especially females, are in the range of leptin levels observed in Europid individuals living with obesity (35-38).

The increased fat percentage in South Asians in our cohort could be the reason for the significantly higher leptin levels, as we saw a positive correlation between leptin and fat percentage and fat mass in South Asians only (**Figure 1**). In addition, the location of the fat mass influences leptin concentrations. It has been described that subcutaneous adipose tissue (SAT) secretes more leptin than other fat depots like visceral adipose tissue (VAT) (39). However, this is contrary to the idea that South Asians have less SAT and store more fat ectopically. Whether South Asians have higher leptin expression in SAT compared to Europids, could be further examined by studying leptin expression in fat biopsies from subcutaneous depots. Furthermore, measuring leptin levels in cohorts with similar fat percentages and location of this fat could give a more definite answer if South Asians have indeed higher circulating leptin levels independently of fat percentage; however, matching South Asians with Europids for clinical studies remains a challenge, as described below.

Interestingly, the pro-inflammatory phenotype of South Asians may influence leptin levels as well (40) (**Figure 1**). Chronic inflammation can impair leptin action by interfering with leptin receptor signaling, resulting in leptin resistance in the hypothalamus (40). Furthermore, as leptin receptors are present on different immune cells, such as monocytes, leptin binding to these receptors can result in low-grade inflammation. The hyperleptinemia observed in South Asians could, in turn, aggravate inflammation (40). The interplay between leptin and inflammation could lead to a higher tendency to develop obesity and obesity-related diseases in South Asians (**Figure 1**). Another

way to further study the interaction between leptin and the immune system is to expose monocytes to leptin *in vitro* to measure their sensitivity with respect to secretion of cytokines. A difference in sensitivity in leptin in other cell types like neurons and adipocytes could further explain the observed hyperleptinemia in South Asians. As a result of decreased leptin sensitivity in neurons and adipocytes, higher leptin levels are required to maintain a stable energy balance. *In vitro* studies can be done to assess the sensitivity of leptin receptors in South Asians of the neurons in the hypothalamus, adipocytes, and monocytes. Most studies determine leptin resistance based on the circulating leptin levels or focus on leptin signaling due to mutation of the leptin receptor gene, altered leptin transport across the blood-brain barrier, or decreased leptin receptor expression, which are all associated with the development of obesity (41, 42). Therefore, in addition to *in vitro* studies assessing leptin receptor sensitivity, measuring leptin receptor mRNA in neurons and protein levels or evaluating leptin receptors after prolonged fasting could provide more insight into the leptin receptors' sensitivity (41). However, these measurements in the South Asian population compared with Europids could not provide information on the location of leptin sensitivity. The leptin receptors are mainly located in hypothalamic neurons and are also expressed in smaller amounts in other tissues like adipocytes and skeletal muscle (39, 43-45). To pinpoint the location of leptin sensitivity, white fat biopsies, and muscle biopsies could be taken from South Asians, and leptin receptor sensitivity could be measured there.

In addition to the focus on the difference in the regulation of leptin in South Asians, leptin levels could potentially serve a more clinical function. Specifically in South Asians, our study showed that leptin represented the metabolic status of adiposity better than BMI. We found that the fat percentage was significantly higher in South Asians compared to Europids despite their normal BMI, and only the fat percentage positively correlated with leptin levels, not with BMI, in South Asian males and females. Therefore, leptin could serve as a metabolic biomarker for adiposity in this population. Standard measurements for predicting metabolic status, such as BMI and waist circumference, often misrepresent the actual metabolic status of South Asians. If leptin proves to be a more accurate marker, it could improve the assessment of their metabolic status. Indeed, the use of leptin as a predictive marker for metabolic syndrome has been described before (46). To evaluate leptin as a potential biomarker in South Asians, measurements of circulating leptin levels must be taken at baseline and followed longitudinally in large cohorts to monitor the development of obesity and obesity-related diseases.

Cortisol in the South Asian population

Besides GLP-1 and leptin, another hormone that may be of interest to South Asians is cortisol. As described in **Chapter 1,** the metabolic phenotype of South Asians, characterized by higher abdominal fat mass, increased insulin resistance, and lower muscle mass, is similar to symptoms seen in individuals with hypercortisolism, an extreme example of which is Cushing's syndrome (47). Our preliminary data described in **Chapters 3** and **4** showed that circulating plasma cortisol levels are lower in young and lean South Asian versus Europid females (4.2 ± 2.0 *vs.* 6.3 ± 2.1 µmol/L; P = 0.011). Previous research also found lower circulating cortisol levels in middle-aged (40-67 years) South Asian men compared to Europids (48).

A possible reason for the counterintuitively lower cortisol in South Asians compared to Europids is that they might have a higher sensitivity to cortisol (**Figure 1**). Cortisol binds to the nuclear glucocorticoid receptor (GR) and mineralocorticoid receptor (MR), resulting in the transactivation and transrepression of various genes. Of note, GR sensitivity differs between people; 4.5% and 38.0% of the Europid population are carriers of two distinct polymorphisms of the GR associated with increased glucocorticoid sensitivity (49, 50). This coincides with higher abdominal circumference and a disadvantageous glucose metabolism. GR sensitivity can be assessed using a bioassay *in vitro* in peripheral blood mononuclear cells, which would be highly interesting to perform in South Asians. Another option could be that the prevalence of the GR polymorphism associated with increased sensitivity of the GR is higher in South Asians compared to Europids. Two polymorphisms known to be associated with increased sensitivity to corticosteroids are a BclI polymorphism (rs41423247) and N363S (rs56149945) (51, 52). However, a previous study found a lower frequency of the N363S polymorphism in South Asians compared to Europids (53). If the sensitivity to cortisol is indeed increased in South Asians compared to Europids, treatment with a GR antagonist or a mineralocorticoid receptor antagonist could be an option to counteract the cortisol-induced metabolic dysfunctions observed in obesity (54).

In a physiological situation, the hypothalamic-pituitary-adrenal (HPA) axis consists of the secretion of hypothalamic corticotrophin-release hormone (CRH) to stimulate adrenocorticotrophic hormone (ACTH) from the pituitary and the secretion of glucocorticoids by the adrenal cortex (55). Different factors can disrupt the HPA-axis, like prolonged emotional stress (56). Our preliminary data suggest that South Asian females have higher perceived stress scores (PSS) based on the PSS-14 questionnaire. As prolonged emotional stress normally results in hypercortisolism, the results of higher PSS in South Asians are again in contrast with the observed lower circulating cortisol levels, again pointing towards possibly higher GR sensitivity (56, 57).

Another explanation for the finding of lower circulating cortisol in South Asians compared to Europids in our and other studies is a disrupted cortisol rhythm in South Asians. Circulating cortisol levels differ throughout the day, with the highest peak occurring in the morning, rising before waking (58). Physical activity, diet, and sleep all influence the body's circadian rhythm. South Asians exercise less and have more sleep disturbances due to for example sleep apnea, described in **Chapter 1**, which could lead to a blunted rhythm in circadian cortisol with lower morning cortisol levels (59). Another option is that different ethnicities have their own (genetically determined) cortisol rhythm. A meta-analysis comparing the circadian rhythm of Blacks, Hispanics, and Whites found differences in the cortisol rhythm between each ethnicity. Blacks and Hispanics had a more blunted cortisol rhythm that increased towards bedtime, with especially a lower peak cortisol in the morning (60). A flatter circadian cortisol rhythm is associated with decreased insulin sensitivity and T2DM (61). Therefore, South Asian males and females could potentially have a flatter cortisol rhythm than Europids, contributing to their higher risk of developing T2DM. The cortisol rhythm in South Asians has not been extensively researched thus far, making this an interesting topic for future studies.

Sex hormones in the South Asian population

In **Chapter 3** and **4**, we found noticeable differences between males and females of South Asian descent**.** For instance, active GLP-1 and GIP increased towards the end of the MMTT only in South Asian females, while plasma cortisol and perceived stress were lower only in females. A potential influence could be differences in sex hormones. Such differences in South Asians have been described previously and could potentially contribute to their increased risk of developing obesity and obesity-related diseases (62, 63).

During menopause, estrogen levels decrease, leading to an increase in abdominal fat and contributing to the development of obesity (64). In South Asians, it has been described that the mean age of menopause is about 3 years lower than in Europids and that South Asian females can experience menopause-related symptoms differently (65, 66). During menopause, the risk of obesity-related diseases like CVD and T2DM increases due to the changes in estrogen, and earlier menopause is associated with increased development of obesity-related diseases (67). Together with the already present increased risk of developing metabolic diseases in the South Asian population, (see **Chapters 1** and **8**)**,** screening every South Asian female peri/post-menopausal for obesity-related diseases could potentially prevent such diseases or allow earlier treatment of these diseases, resulting in long-term health improvement. In addition, hormone replacement treatment (HRT) has been shown to significantly reduce the

risk of CVD and overall mortality in females when started before 60 years of age (68). HRT may also be an interesting option for South Asians that should be further studied.

Certain diseases affecting sex hormones are potentially more prevalent in South Asians, although the prevalence of these diseases varies across studies (69-71) (**Figure 1**). Examples include endometriosis and polycystic ovarium syndrome (PCOS) (72, 73). South Asian women with endometriosis often report less pain and a better quality of life but tend to have more advanced stages of the disease at diagnosis (72). Even though endometriosis was not associated with the development of T2DM in some studies, there are indications that there is a relation with insulin sensitivity, as women with endometriosis have an increased risk of gestational diabetes mellitus (74, 75). Additionally, there are indications that endometriosis is linked with abnormal metabolic measurements, like higher insulin levels (76). However, before we can speculate about the impact of endometriosis on insulin sensitivity in the South Asian population, the mechanisms underlying the pathology of endometriosis need to be further investigated. PCOS is associated with insulin resistance and hyperinsulinemia and was found to be more prevalent in South Asians (77). South Asian women with PCOS often present with anovulation at a younger age, more severe hirsutism, and a higher prevalence of acanthosis nigricans than Europids (71). Metformin, the first-line treatment for T2DM is also registered for people with PCOS (78). As South Asians already have an increased risk of developing T2DM with signs of insulin resistance when they are young and lean, starting metformin in South Asian females with PCOS could potentially decrease the risk of developing T2DM later in life (79, 80).

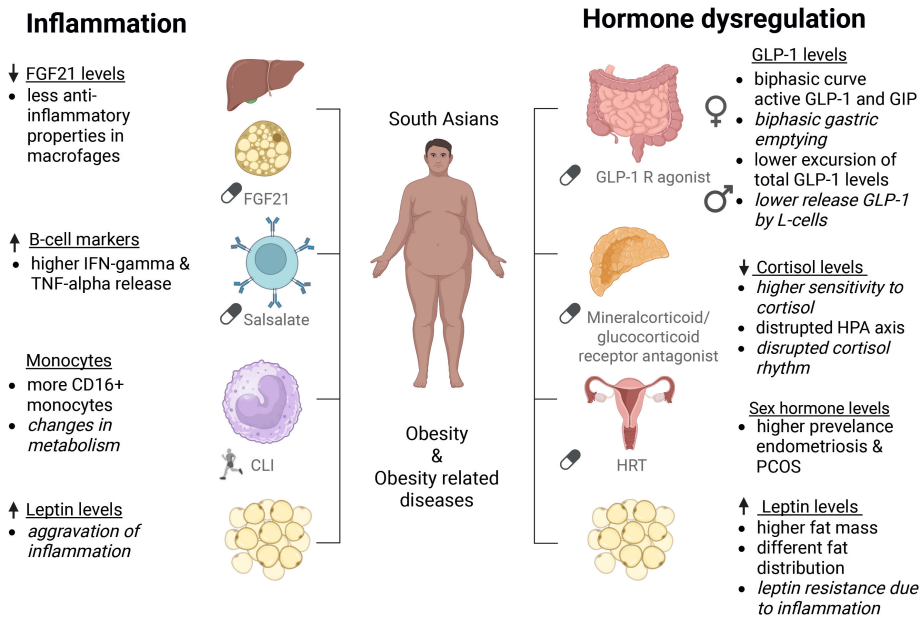

Figure 1. Inflammatory and hormonal factors that may underlie the high risk of developing obesity and associated diseases in the South Asian population. The pro-inflammatory phenotype in South Asians includes lower circulating FGF21 levels, higher B cell markers, more CD16+ monocytes, and increased circulating leptin levels. In addition, hormonal dysregulation in the South Asian population includes biphasic active GLP-1 and GIP excursions to a MMTT in females, lower circulating total GLP-1 levels in males, higher circulating leptin levels, lower cortisol levels, and a higher prevalence of diseases that influence sex hormones. Changes in the metabolism of monocytes causing a shift from non-oxidative glycolysis to oxidative metabolism, and the aggravation of inflammation by increased circulating leptin levels, are proposed hypotheses contributing to the inflammatory factors of the increased risk of South Asians to develop obesity and related diseases. We hypothesize that a lower release of GLP-1 by the intestinal L-cells, a higher sensitivity to cortisol with a disrupted cortisol rhythm, and leptin resistance due to inflammation contribute further to the increased risk in this population. Proposed treatment options for the underlying mechanisms contributing to the development of obesity and obesity-related diseases in the South Asian population are shown in grey. CLI, combined lifestyle intervention; FGF21, fibroblast growth factor 21; GLP-1, glucagon-like peptide 1; GLP-1R agonist, glucagon-like peptide-1 receptor agonist; HPA axis, hypothalamic-pituitary-adrenal; HRT, hormone replacement therapy; IFN-gamma, interferon-gamma; PCOS, polycystic ovarium syndrome; TNF-alpha; tumor necrosis factor-alpha.

Challenges in researching the South Asian population

In the study described in **Chapters 3** and **4,** we compared lean and young South Asians and Europids who were mainly matched on BMI and age. However, in those cohorts, it was evident that South Asians had a higher fat percentage than Europids with the same BMI range. These differences in body composition may have influenced the results. For example, the higher fat percentage of South Asians could explain the difference in leptin levels observed in **Chapter 4**. In addition, in the cohort of individuals with T2DM, in **Chapters 2** and **7**, participants were included based on age (18-74 years), BMI ≥ 25 kg/m^2, and HbA_{1c} ≥ 6.5% and ≤ 11.0%). Despite the South Asians having a lower BMI and smaller waist circumference, their fat percentage was not significantly different. Furthermore, South Asians had a significantly longer duration of T2DM, which implies longer exposure to the disease and its associated treatments. Therefore, matching South Asians with Europids by BMI likely is not optimal. We could overcome this by having stricter BMI cut-offs. For example, the upper BMI limit for the lean cohorts could be decreased from 25 to 23 kg/m^2. However, since it is generally challenging to find a sufficient number of participants of South Asian descent for studies, it should be realized that lowering BMI criteria could further hamper inclusion. In addition, the average BMI in our cohort described in **Chapters 3** and **4** was 23 kg/m^2. Despite matching based on BMI, we already observed significant metabolic differences. Of note, these metabolic differences are part of the South Asian phenotype and are part of the driving factors behind our research. Another option would be matching based on waist circumference. An increased waist circumference is an indicator of more central obesity and is associated with type 2 diabetes, hypertension, and metabolic syndrome (81). However, waist circumference was not significantly different between South Asians and Europids in our cohort, despite the metabolic difference. However, a potential difference might have been masked by the height difference between both populations with the Europids being consistently taller compared to the South Asians. Therefore, matching on waist circumference is also not a viable option.

Other options for matching are based on fat mass, fat percentage, or a metabolic biomarker in blood. However, not every research facility has the possibility to measure fat mass or fat percentage. In addition, individuals willing to participate are often unaware of their fat mass, leading to significantly more screening and potentially more exclusion of participants from the study. Similarly, the circulating levels of a biomarker are also not known before screening. An intervention with a venous puncture to draw blood is necessary to acquire it, leading to an extra burden for individuals willing to participate in a study. Using fat mass and leptin measurements in the assessment of the metabolic status of individuals could be more useful clinically when assessing the potential risk of developing obesity and obesity-related diseases.

In addition to the challenge of matching South Asians based on their metabolic status, other factors could contribute to differences between cohorts in studies, including South Asians. For example, South Asians have a different lifestyle compared to Europids. South Asians are known to exercise less than Europids and smoke more (82). Both exercise and smoking influence many metabolic processes, such as lipid metabolism and systemic inflammation (83, 84). Therefore, individuals who engage in vigorous exercise and smokers are often excluded from research.

We experienced that the inclusion of South Asian males was especially challenging as they responded less to advertisements. Ideally, collaborating with researchers who are well-acquainted with the culture of South Asians, are integrated into their community, and potentially of South Asian heritage themselves could spread the word about new research and their potential benefit for the South Asian community and help recruit new study participants. Building databases with the contact information of willing participants of South Asian descent could improve accessibility for recruitment, although privacy issues remain a current challenge.

Novel therapeutic targets in the treatment of obesity

Many intervention options currently available to combat obesity are based on lifestyle interventions (85). These interventions are often difficult to adhere to and almost always result in weight regain after a prolonged period (86). Additionally, most treatment options are not specifically tailored to the underlying cause of the individual who is living with obesity. Therefore, effective preventative or treatment options with long-term health benefits should be developed considering the person's underlying mechanism(s) contributing to obesity.

Activating brown adipose tissue

In **Chapter 1**, we described a potential beneficial effect of activating BAT in decreasing ectopic fat mass and slightly increasing resting energy expenditure. This could be especially beneficial for individuals with low BAT activity, such as South Asians (87).

Optimizing the timing of BAT activation could lead to higher efficacy in improving metabolic health. In the cohort described in **Chapter 5**, we observed more effective cold-induced thermogenesis in the morning in males only. Still, we found that cold only increased FGF21 levels in the evening (88). This indicates that different sexes or people from different ethnicities could benefit more from personalized timing of exposure to cold. For example, males might benefit more from cold exposure in the morning to increase energy expenditure. In contrast, given their lower energy expenditure and lower FGF21 levels, South Asians could benefit from exposure both in the morning (to

increase energy expenditure) and evening (to increase FGF21 levels). To assess if cold exposure in the morning and evening can indeed increase energy expenditure and FGF21 levels, studies should be conducted on individuals exposed to cold for extended periods at different times of the day.

Furthermore, BAT contributes to diet-induced thermogenesis (DIT), which is influenced by the diurnal rhythm of BAT (89). DIT, especially fat oxidation, increases after breakfast, moderately increases after lunch, and does not increase after dinner. DIT increases more after breakfast and lunch in people with high BAT activity than in individuals with low BAT (89). Combining cold exposure and higher relative food intake in the morning compared to other times of the day could increase thermogenesis and fat oxidation even more, especially in males. A study with cold exposure in unfasted conditions throughout the day while measuring energy expenditure could provide more information on the effectiveness of these combined interventions.

In addition to cold exposure, pharmacological methods to active BAT have also been studied. As described in **Chapter 6**, we found that a single intravenous dose of the ADRB2 agonist salbutamol activates BAT, as indicated by increased glucose uptake assessed via an [^{18}F]FDG PET/CT scan, which was accompanied by an increase in resting energy expenditure. To assess the benefits of pharmacological activation of BAT, long-term studies need to be conducted to determine if chronic ADRB2 agonism can continuously activate BAT and to evaluate the duration of the side effects, as we noticed an increase in heart rate and blood pressure after a single dose of salbutamol. Ideally, targeting the ADRB2 on brown adipocytes by nanotechnology could prevent such cardiovascular side effects. Different mechanisms using nanotechnology to improve the efficacy of salbutamol have been studied to treat asthma, such as the use of a nanocarrier attached to salbutamol to increase the interaction with the pleura of the longs or liposomes to increase the retention of salbutamol in the longs (90, 91). The use of these nanoparticles with incorporated salbutamol could possibly be constructed to target specifically the ADRB2 on brown adipocytes.

In addition to targeting BAT with salbutamol, incorporating sex differences could benefit the pharmacological activation of BAT. Our study was performed only in young and lean males; these results should also be assessed in females. Differences in the effectiveness of salbutamol between males and females have been demonstrated as salbutamol inhalation decreased fat mass and increased lean mass only in healthy female athletes using salbutamol to prevent exercise-induced bronchoconstriction, and not in males (92). In addition, protein turnover was greater in response to the ADRB2 agonist formoterol in females than in males, which was attributed to the difference in sex

steroids (92, 93). Therefore, females could potentially experience a greater activation of BAT by an ADRB2 agonist than males. Furthermore, our cohort was young and lean, however, obesity is associated with lower expression of the ADRB2 gene in peripheral blood samples (94). Therefore, people living with obesity could have a reduced benefit of salbutamol to activate BAT. Future research should focus on the effectiveness of salbutamol to activate BAT in males and females living with obesity.

We noticed that half of the 10 males included in our study responded better to salbutamol with respect to glucose uptake by BAT compared to the other half. In previous studies using larger cohorts of individuals receiving salbutamol for asthma similar effects were noticed, with less effective treatment in about half of the population (95). Those studies identified two polymorphisms that results in amino acid changes (rs1042713, c.G46A, p.Gly16Arg; rs1042714, c.G79C, p.Gln27Glu) of the ADRB2 receptor that could explain this observation (96). These changes cause less effective ADRB2 receptors than other variants and have a prevalence of about 28% for the Gly16Arg polymorphism and 27% for the Gln27Glu polymorphism in the Europid population (96-98). Ideally, we would measure both ADRB2 polymorphisms in the saliva of all participants in our previous cohort described in **Chapter 6** and study whether the participants with lower effectiveness were carriers of these polymorphisms. This could lead to a more effective application of activating BAT as we would beforehand identify those individuals who would benefit from cold exposure or an ADRB2 agonist if they are not carriers of the polymorphisms. Furthermore, activating BAT via cold or pharmacological intervention could complement other treatment options that work through different pathways for treating obesity and obesity-related diseases.

Incretin hormone-based drugs as a treatment for obesity

As described in **Chapter 1**, GLP-1R agonists are pharmacological treatment options for obesity and T2DM that significantly affect weight loss and improve the glycemic index. Combining GLP-1R agonists with long-acting analogs of other hormones such as glucose-dependent insulinotropic polypeptide (GIP) and glucagon is even more beneficial, and new potential treatment options are emerging using these mechanisms (99, 100).

In 2023, the dual GLP-1R and GIP receptor (GIPR) agonist tirzepatide received Food and Drug Administration (FDA) approval for type 2 diabetes, and subsequently for obesity (101). Combining GLP-1R and GIPR agonism enhances appetite suppression compared to GLP-1R agonism alone (99). The mean change in body weight at 72 weeks of treatment in people living with obesity with tirzepatide was -21% (99). The most common side effect of GLP-1R agonists is nausea (102). GIP-based therapy seems

to reduce this side effect when used in combination with GLP-1R agonists. Although the mechanism behind the anti-emetic effects of GIPR agonists remains unclear, it is hypothesized that the location of GIPRs in the brain may play a role (103). A systematic review comparing the effectiveness of tirzepatide in Asians (consisting of Chinese, South Koreans, and Indians) and non-Asians with T2DM showed that tirzepatide was more effective in reducing body weight in Asians. However, they experienced more gastrointestinal side effects (104). In non-Asians, tirzepatide was more effective in lowering fasting blood glucose levels and HbA_{1c} (104). A clinical trial comparing the effect of tirzepatide in South Asians with Europids could unravel the differential effect of tirzepatide between these ethnicities. The finding that tirzepatide improved glycemic parameters more effectively in Europids than in Asians could be due to possible longer exposure to T2DM in Asians. This would be similar to what we observed in our South Asian cohort described in **Chapter 7,** indicating a more metabolically compromised state. Comparing the effectiveness of a GLP-1R agonist to tirzepatide, especially when administered earlier in the disease progression of T2DM, could determine the most effective treatment of the two for South Asians with T2DM. This comparison should also assess whether combining GLP-1R and GIPR agonism within Tirzepatide results in fewer side effects compared to GLP-1R agonism and if adding GIPR agonism can lower ectopic fat in South Asians.

The most recent development in incretin-based pharmacological intervention for obesity is the triple GLP-1/GIP/glucagon receptor agonist, retatrutide (100). Adding a glucagon receptor- activating modality further decreases satiety and increases energy expenditure (105). A clinical study in individuals living with obesity showed that 48 weeks of retatrutide treatment can decrease body weight by up to 24% from baseline in the high dose group of 12 mg subcutaneously once weekly (100). These results are almost comparable to bariatric surgery, where individuals typically lose up to 30 % of their body weight within one year (106). Combining GLP-1R agonism with glucagon receptor agonism could especially benefit people with lower energy expenditure, such as the South Asian population.

As we showed in **Chapter 7**, the GLP-1R agonist liraglutide does not increase circulating levels of the satiety hormone growth differentiation factor 15 (GDF15), suggesting GLP-1 and GDF15 may have independent effects on energy metabolism. Therefore, combining GDF15-based therapy with liraglutide or another GLP-1R agonist could have additive effects on reducing satiety. In mice and rodents, this combination has indeed shown promising results thus far in reducing food intake and body weight, fasting glucose, insulin, and triglycerides (107). Similarly, combining GLP-1R agonists with FGF21 has benefited rodents, especially in treating metabolic dysfunction-associated

steatohepatitis (MASH), a disorder characterized by steatosis, hepatocyte ballooning, inflammation, and fibrosis (108). Many of these combinations of incretin hormones could especially benefit the South Asian population. As we described in **Chapters 1-3**, young and healthy South Asian males have lower GLP-1 levels after an MMT, lower energy expenditure, and lower levels of FGF21. However, whether this population benefits more from these treatment options compared to Europids remains to be investigated.

Concluding remarks and future perspectives

Obesity is a complex chronic disease with many physical and mental consequences. Although treatment options are improving, effective options with long-term efficacy are still sparse. To further combat obesity, understanding its underlying mechanisms, identifying and comprehending at-risk individuals and populations, and finding novel approaches for its treatment are therefore of utmost importance. In this thesis, we have addressed further underlying mechanisms contributing to the increased risk of South Asians developing obesity and obesity-related diseases and have studied potential novel approaches to target obesity.

Based on the results of this thesis, we conclude that a pro-inflammatory phenotype is part of the underlying mechanism of the unfavorable metabolic phenotype of South Asians. We anticipate that identifying if B cell markers are increased in healthy South Asians and measuring the quantity and quality of monocytes could provide insight into the increased risk of developing obesity and related diseases in South Asians. Another part of the underlying mechanism is their hormone dysregulation, with a biphasic excursion of active GLP-1 and GIP in South Asian females to an MMTT, with a lower AUC GLP-1 levels in males, and higher leptin levels in both sexes. Furthermore, there are indications that South Asians have an altered stress response, resulting in lower cortisol levels. In addition to the differences in circulating hormone levels, the sensitivity to these hormones could have be altered in South Asians. CLI for the modulation of immune cell subsets or anti-inflammatory medications like salsalate and GLP-1R agonist treatment could be especially beneficial in reversing the pro-inflammatory phenotype and hormone disruption in South Asians. More research is needed to test the effectiveness of these mediations in this population with the challenges considered of matching South Asians with Europids during the selection of participants. We propose using alternative methods of matching, like fat mass or fat percentage, or a metabolic biomarker like leptin instead of using BMI.

To combat obesity, effective and sustainable (non)-pharmacological treatment options are needed. When using cold exposure to activate metabolically favorable BAT, its diurnal rhythm must be considered. Our results that FGF21 only increased after

cold exposure in the evening indicate that the moment of BAT activation could be adapted to the personable phenotype of the individual. If exposure to cold is not feasible, using the ADRB2 agonist salbutamol to activate BAT might be indicated. Cold exposure and pharmacological options for the activation of BAT must be explored by chronic treatment of individuals with different metabolic statuses and ethnicities. Especially for the pharmacological treatments, finding options to specifically target the ADRB2 receptor on brown adipocytes could improve dyslipidemia and reduce ectopic fat deposition without major (cardiovascular) side effects. Furthermore, activation of BAT could be combined with current treatments for obesity, like GLP-1R agonists. Other combinations with GLP-1R agonists, such as long-acting analogs of FGF21 and GDF15, could further benefit people living with obesity. The latter is supported by our observation that GDF15 acts via a different pathway than the GLP-1R agonist liraglutide. Recent developments in treatment options that combine GLP-1 with GIP and glucagon-based therapies could, therefore, lead to novel combined therapies. These therapies may provide options to prevent obesity-related diseases, especially in South Asians. Improving access to diagnostics and treatment for all people living with obesity could improve the success rate of sustainable weight loss and improve metabolic parameters in people with obesity. Furthermore, providing a personalized approach with specific interventions for the phenotype of an individual living with obesity could lead to the first steps toward ending the obesity epidemic.

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