GLP-1 receptor agonism to improve cardiometabolic health

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Chapter 7

General discussion and future perspectives

GENERAL DISCUSSION

The prevalence of type 2 diabetes is rising steadily and is becoming one of the largest health problems in the world[1,2]. Type 2 diabetes is a chronic disease characterized by disturbed glucose regulation and is strongly associated with dyslipidaemia. Insulin resistance is a key player in the development of type 2 diabetes and obesity is a well-known risk factor for the development of insulin resistance. Mechanistically, accumulation of ectopic fat, which is the process of storage of triglycerides in non-adipose tissues, such as liver, muscles, kidneys and pancreas is crucial for the development of insulin resistance[3,4]. Type 2 diabetes can result in cardiovascular diseases as a consequence of micro- and macrovascular damage, and therefore it is of utmost importance to pursue optimal management of blood glucose levels, but also of blood lipid levels and blood pressure[5]. The cornerstone in achieving this is improvement of lifestyle, with focus on increased physical exercise and, in most patients, weight loss. Furthermore, a change in composition of the diet with high daily intake of dietary fibre can improve fasting blood glucose levels and reduce cardiovascular risk[6,7]. However, additional pharmacological treatment is often unavoidable. Unfortunately, even under tight pharmacological control, the risk to develop cardiovascular complications remains high[8]. Furthermore, most of the registered glucose-lowering agents including metformin and insulin do not induce weight loss and even increase body weight. This is then often accompanied by further increase in insulin resistance resulting in requirement of addition of other drugs or intensification of current (insulin) therapy. Glucagon-like peptide-1 (GLP-1) receptor agonists are a relatively novel treatment option for patients with type 2 diabetes[9-11]. Although it has been shown over the past decade that GLP-1 receptor agonists improve glycemic control and in fact reduce body weight[12,13], the effects of GLP-1 receptor agonists on different adipose tissue compartments, ectopic fat deposition, and cardiac function remained unclear.

In this thesis, we have investigated the effects of GLP-1 receptor agonists on cardiometabolic health. To this end we performed a clinical trial in which we studied effects of the GLP-1 receptor agonist liraglutide on ectopic fat accumulation (**Chapter 2**) and on cardiovascular function (**Chapter 3**) in patients with type 2 diabetes. We were the first to specifically describe these effects in South Asian subjects, a population with high risk to develop type 2 diabetes and pronounced ectopic fat deposition[14-16]. Furthermore, we aimed to clarify the mechanism(s) behind liraglutide-induced weight loss by investigating the effect of liraglutide on energy expenditure and brown adipose tissue (BAT), which might be activated by GLP-1 receptor agonism[17] (**Chapter 4**). In addition, we investigated the relationship between hepatic triglyceride content and circulating cholesteryl ester transfer protein (CETP), a protein associated with a pro-atherogenic profile (**Chapter**

5). Finally, we investigated effects of prolonged caloric restriction on cardiometabolic health and studied the relationship with endocannabinoids, which can contribute to accumulation of visceral adipose tissue (VAT) and induction of insulin resistance (**Chapter 6**). In this chapter I will discuss the results obtained from the studies described in the previous chapters of this thesis. Furthermore, the implications of our work are discussed and ideas are described for future research to improve cardiometabolic health in type 2 diabetes.

1. GLP-1 receptor agonists in the treatment of type 2 diabetes

GLP-1 is a peptide hormone that is formed by posttranslational processing of proglucagon, and is mainly produced and released by enteroendocrine L-cells after food ingestion. GLP-1 delays gastric emptying, induces satiety, and stimulates glucose-dependent insulin secretion and inhibits glucagon release by the pancreas[18]. Together, these actions of GLP-1 prevent excessive hyperglycemia upon a meal. The physiological role of GLP-1 in energy metabolism is steadily being unraveled and recent studies have shined a new light on its function. Recently, it has been suggested that, since GLP-1 is quickly degraded by dipeptidyl peptidase 4 (DPP-4) expressed by endothelial cells in the gut, the main function of endogenous GLP-1 is to activate sensory afferent fibers of the vagal nerve. Via this way, nutritional abundance is signaled to the brain, with subsequent reduction of appetite and inhibition of parasympathetic outflow resulting in inhibition of gastric emptying[19]. Furthermore, the incretin effect, i.e. the augmented insulin secretion upon oral glucose intake caused by incretin hormones, which was historically thought to be the main "function" of GLP-1, has recently been studied more extensively and seems to play a less significant role than previously assumed. Interestingly, intravenous peripheral administration of a specific antagonist for the GLP-1 receptor, exendin(9-39)NH_2, resulted in only a small decrease in insulin secretion upon oral glucose intake, suggesting a minor contribution of GLP-1 to the incretin effect. In contrast, antagonism of glucose dependent insulinotropic peptide (GIP), another well-known incretin hormone produced by intestinal K cells, using GIP(3-30)NH_2, markedly reduced insulin secretion and even induced glucose intolerance[20,21]. Of course, it is important to note that GLP-1 receptor agonists that are used in the pharmacological treatment of type 2 diabetes and obesity result in supraphysiological GLP-1 receptor agonism, because these agonists are designed to be more resistant to degradation by DPP-4 compared to endogenous GLP-1. Therefore, physiological effects of endogenous GLP-1 are not necessarily equal to pharmacological effects. Indeed, in addition to the effects of endogenous GLP-1 on vagal nerve activation and subsequent induction of satiety and inhibition of gastric emptying, pharmacological treatment with GLP-1 receptor agonists has substantial direct effects on the pancreas to inhibit glucagon release and enhance glucose-induced insulin secretion resulting in more pronounced reduction of blood glucose levels[19,22]. The current

view of the proposed effects of GLP-1 on various organs is schematically depicted in **Figure 1**.

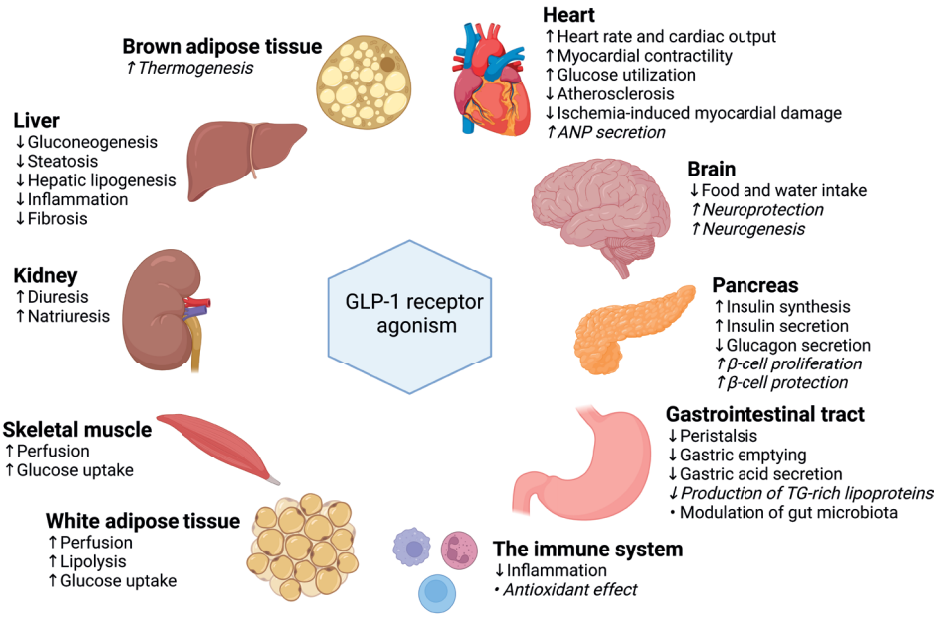

Figure 1. Schematic depiction of the proposed effects of GLP-1 on various organs. See section 1 for explanation. Effects not reported (as yet) in humans but only in pre-clinical models are italicized. ANP, atrial natriuretic peptide; TG, triglyceride. Created with BioRender.com.

In 2002, the first results of continuous subcutaneous infusion of native GLP-1 for a prolonged period of 6 weeks in patients with type 2 diabetes were published, showing inhibition of gastric emptying and reduction of body weight. Furthermore, infusion of GLP-1 improved insulin sensitivity and β-cell function and, consequently, improved glycemic control[23]. Since then, GLP-1 receptor agonists have been developed and have now taken an important position in pharmacological treatment of type 2 diabetes and, more recently, also in the treatment of obesity. Clinical trials with GLP-1 receptor agonists have shown that GLP-1 receptor agonism effectively induces weight loss and improves glycemic control[24,25] and in the studies we performed these effects were also present. We investigated this specifically in a South Asian population, in which the risk to develop type 2 diabetes as well as cardiovascular diseases is exceptionally high. We were the first to report results of a randomized placebo-controlled clinical trial in this group. Although studies on effects of GLP-1 receptor agonists in South Asians have not been extensively performed, a recent review of several observational studies with Indian subjects reported a larger reduction in HbA1c and body weight compared to the

'Liraglutide Effect and Action in Diabetes' (LEAD) program, a phase 3 controlled clinical trial program conducted in a western population[26]. However, the observational studies did not include a control arm, which may have affected the results. The degree of improvement of glycemic control and reduction of body weight in the trial we performed in a South Asian population was similar to those observed in other populations[27,28]. Also, when compared to the Western European subjects of our MAGNA VICTORIA trial no difference in liraglutide efficacy on glycemic endpoints was observed[29]. Importantly, however, the average BMI of our South Asian population was relatively low (29 kg/m^2), indicating that not only obese, but also overweight patients with type 2 diabetes from South Asian descent can similarly benefit from treatment with liraglutide or other GLP-1 receptor agonists.

Additional beneficial effects of GLP-1 receptor agonism on different aspects of cardiometabolic health have recently been revealed. For example, liraglutide was shown to reduce hepatic steatosis in patients with nonalcoholic steatohepatitis (NASH)[30]. In addition, treatment of obese patients with type 2 diabetes with exenatide or liraglutide for 2 weeks decreased postprandial dyslipidemia, which is an important aspect of diabetic dyslipidemia[31,32]. Moreover, treatment of subjects with type 2 diabetes with oral semaglutide for 12 weeks reduced postprandial triglycerides, apolipoprotein (Apo) B-48 and very-low-density lipoprotein (VLDL)[33]. Also, cardiovascular outcome trials have provided evidence for decreased cardiovascular and non-cardiovascular mortality, as well as renoprotective effects[34]. Our present findings resulting from studying the effects of GLP-1 receptor agonist liraglutide, provide further insight in potential mechanisms of GLP-1 receptor agonism in improving cardiometabolic health, as will be discussed in the next sections.

2. The mechanism behind weight loss induced by GLP-1 receptor agonism

In the MAGNA VICTORIA trials that we have performed, liraglutide (1.8 mg/day) reduced body weight in both South Asian and Western European patients with type 2 diabetes by 3.9 and 4.3 kg, respectively, which is in line with previous studies investigating effects of liraglutide (1.8 mg/day) on body weight, showing a similar weight reduction (i.e. 2.8-5.2 kg) after similar duration (i.e. 24-26 weeks) of treatment[27,35-37]. In general, weight loss is the consequence of a negative energy balance, caused by an imbalance between energy intake and expenditure. Effects of GLP-1 receptor agonists on energy intake have been studied extensively, showing decreased food intake by induction of satiety [38-41]. Studies in rats have shown that GLP-1 reduces appetite by activating specific regions in the hypothalamus and the amygdala[42]. Furthermore, delayed gastric emptying leads to gastric distension with activation of gastric mechanoreceptors, which induces satiation signals

and results in reduction of food intake[43-45]. Although it is thus well known that GLP-1 reduces food (energy) intake, it was still unclear how GLP-1 receptor agonism affects energy expenditure, the other side of the energy balance. Indeed, another mechanism by which GLP-1 was presumed to contribute to weight loss, is by increase of energy expenditure. In rodents, intracerebroventricular injection of GLP-1 receptor agonists induces weight loss mostly independent of its anorexigenic effects by increasing BAT thermogenesis[17,46,47]. In humans, one study showed increased energy expenditure after long-term (1 year) treatment with GLP-1 receptor agonists exenatide and liraglutide in addition to metformin[17], while most studies in humans showed no effect on energy expenditure during infusion with native GLP-1[48,49] or after prolonged (up to 12 weeks) treatment with GLP-1 receptor agonists[39,50,51]. In this thesis we describe effects of treatment with liraglutide on energy expenditure after both short and prolonged treatment, providing insight in effects over time, and are the first to relate this to effects on BAT in humans as measured by MRI (**Chapter 4**). In a population of Western European subjects with type 2 diabetes, we showed that liraglutide does not increase energy expenditure and even decreases energy expenditure after 4 and 12 weeks of treatment compared to energy expenditure at baseline. After 26 weeks, the resting energy expenditure was still decreased, although not significantly (p=0.056). A possible explanation for the decreased energy expenditure that we reported can be a reduction of diet-induced thermogenesis upon decreased food intake (and body weight) or an increased metabolic efficiency to adapt to the negative energy balance in order to limit loss of body weight. Interestingly, as mentioned above, an increased energy expenditure in obese type 2 diabetes patients who were treated with exenatide or liraglutide for 1 year has been reported previously[17] and we can speculate on a potential underlying mechanism. It is well-known that after prolonged treatment for approximately 1 year, GLP-1 receptor agonist-induced weight loss reaches a plateau phase[10,52]. Theoretically, when this phase is reached and energy balance is restored, the stimulating effect on energy expenditure by GLP-1 is unmasked. However, since body weight remains stable from that time, the clinical relevance of such a potentially increased energy expenditure induced by GLP-1 receptor agonism may be limited.

Furthermore, we investigated the effect of liraglutide on BAT. Activated BAT can combust free fatty acids and (to a lesser extent) glucose, resulting in increased energy expenditure[53]. Studies in rodents have shown that BAT activity is increased after intracerebroventricular injection of the GLP-1 receptor agonists liraglutide[17] and exendin-4[46]. As a consequence of combustion of intracellular triglycerides in brown adipocytes, BAT activation results in reduction of the fat fraction of BAT depots, which can be assessed using MRI[54]. In this thesis we investigated the effect of treatment of Western European patients with type 2 diabetes with liraglutide for 26 weeks on BAT and showed that treatment

does not affect the fat fraction in the supraclavicular BAT depot. No other studies have reported on effects of GLP-1 receptor agonists on BAT in patients with type 2 diabetes. Nonetheless, a lack of effect on the MRI-measured fat fraction was recently also shown in another trial with healthy non-obese subjects treated with exenatide for 12 weeks by Janssen et al[51]. Interestingly, in that study, an increased BAT volume and glucose uptake as measured by ^{18}F-fluorodeoxyglucose (FDG) positron emission tomography (PET) imaging was observed. This suggests that in healthy non-obese subjects treatment with exenatide and potentially other GLP-1 receptor agonists, has potential to increase metabolic activity of BAT, with increased uptake of glucose to replenish triglyceride stores via induction of lipogenesis. Such a lipogenic effect might counteract potential effects on the MRI-measured fat fraction of BAT, especially when BAT activation is only modest. The proposed effects of liraglutide on energy metabolism is schematically depicted in **Figure 2**.

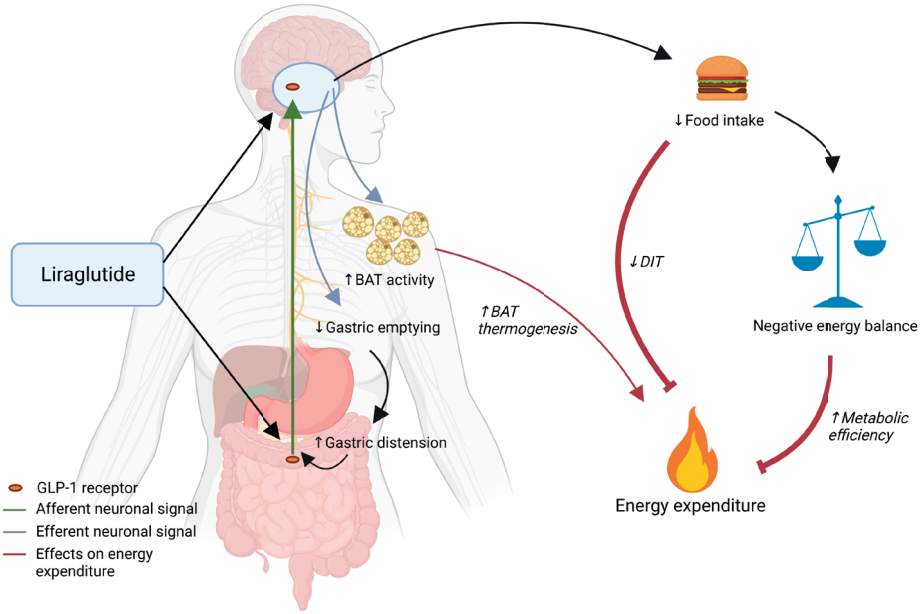

Figure 2. Schematic depiction of the proposed effects of liraglutide on energy metabolism. Liraglutide may exert effects on the central nervous system either directly, via activation of central GLP-1 receptors or indirectly, via activation of afferent fibers of the vagal nerve. This can immediately result in effects on the regulation of appetite and satiety or act via reduction of gastric emptying, which results in gastric distension and subsequently induction of satiation. Consequently, food intake is decreased, resulting in decreased diet-induced thermogenesis and promoting a negative energy balance. To counteract the negative energy balance, we propose that metabolic efficiency might be increased. In contrast, energy expenditure may be stimulated as a consequence of efferent neuronal signaling towards BAT, increasing BAT thermogenesis. The fact that the stimulating effect on energy expenditure by GLP-1 via BAT thermogenesis is surpassed by the effects caused by reduced food intake (as shown in the figure using wider arrows), might explain our observation of reduced energy expenditure upon treatment with liraglutide. BAT, brown adipose tissue; DIT, diet-induced thermogenesis; GLP-1, glucagon-like peptide-1 . Created with BioRender.com.

Of note, when comparing our results of effects of liraglutide on the MRI-measured fat fraction to other studies measuring effects of interventions on BAT, a few things are of importance. ^{18}F-FDG PET is the current gold standard for assessing BAT volume and activity and measurement of the fat fraction with MRI has been shown to correlate well to the glucose uptake rate measured using ^{18}F-FDG PET in healthy subjects, at least upon activation of BAT by cold exposure[55]. It should be realized though that cold exposure is potentially the most effective trigger to activate BAT and reduce its fat fraction. In addition, the uptake of glucose by BAT is greatly decreased in patients with type 2 diabetes[56], making it difficult to compare our results measured with MRI in subjects with type 2 diabetes to studies performed with ^{18}F-FDG PET in healthy subjects with normal insulin sensitivity. Furthermore, we must take caution in directly comparing effects of different GLP-1 receptor agonists. For example, exenatide is based on a natural occurring peptide bearing a 53% homology to human GLP-1 while liraglutide is an analogue of human GLP-1 which is modified to slow down its renal clearance[57]. In addition, we can speculate on the difference in observations between humans and rodents, the latter showing relatively large effects of GLP-1 receptor agonism on BAT. Rodents have a relatively high thermoneutral temperature point with increased cold-induced thermogenesis below a temperature of 29-33°C[58] while humans have a thermoneutral point of 21-23°C (lightly clothed)[59]. Therefore, in rodents a large relative amount of BAT and therefore energy combustion by BAT likely contributes to a larger extent to total resting energy expenditure than in humans, which may increase the potential of activation of BAT by GLP-1. Experiments investigating GLP-1 receptor agonism in rodents at thermoneutral temperature, which would reduce cold-induced thermogenesis by BAT, or in humans at low temperatures, which could promote this, could provide more information on the effectiveness of GLP-1 receptor agonists to activate BAT under different ambient temperatures. Finally, the effects in rodents were observed after intracerebroventricular injection of medication[17,46], resulting in high local concentrations to stimulate sympathetic outflow to BAT. Indeed, it has been shown that transfer of liraglutide to cerebrospinal fluid is minimal[60]. Therefore, although GLP-1 receptor agonists could theoretically affect the central nervous system via peripheral effects on the vagal nerve, this may limit therapeutic potential of the current generation of (peripherally administered) GLP-1 receptor agonists since effects on energy expenditure and BAT are largely mediated via central nervous system activation[61].

To conclude, although studies in rodents support a role for (central) GLP-1 receptor agonism in energy expenditure and BAT activity, liraglutide induces weight loss in humans by decreasing energy intake rather than by activating BAT or increasing energy expenditure. However, there seems to be potential for exenatide and possibly other

GLP-1 receptor agonists to activate BAT. Therefore, further development of GLP-1 receptor agonists targeting BAT activation could yield clinical benefit.

3. Effects of GLP-1 receptor agonists on cardiometabolic health

3.1 The role of visceral adipose tissue and ectopic fat in metabolic health

The main cause of type 2 diabetes is insulin resistance of various metabolic organs in combination with insufficient insulin secretory capacity of the pancreas to overcome this resistance. Although several factors can contribute to the development of insulin resistance, central obesity with concomitant accumulation of VAT, and increased fat accumulation in non-adipose tissue are major risk factors[3,4,62-65]. Under physiological conditions subcutaneous adipose tissue (SAT) functions as a reservoir for energy storage. However, when its storage capacity is exceeded, necrosis of adipocytes attracts immune cells resulting in induction of insulin resistance. The VAT contains more hypertrophic adipocytes than SAT and shows more macrophage infiltration and inflammation[66]. Furthermore, an increased rate of basal lipolysis in VAT compared to SAT results in increased release of free fatty acids (FFAs) to the portal circulation[67]. As a result of this, ectopic fat accumulation occurs in different organs, leading to disrupted function of those organs[3]. Hepatic steatosis results in insulin resistance of hepatocytes leading to impaired insulin-induced suppression of glycogenolysis and gluconeogenesis[68]. Furthermore, hepatic insulin resistance results in overproduction of VLDL, accelerating dyslipidemia[69]. In skeletal muscle cells, increased intramyocellular lipid accumulation results in accumulation of toxic lipid intermediates. This leads to defects in insulin signaling and subsequently decreased glucose transporter 4 (GLUT4) regulated glucose transport over the cell membrane and consequently insulin resistance[70,71]. In addition, excess of myocardial and epicardial fat may contribute to remodelling of the heart and contractile dysfunction[72]. Lastly, ectopic renal fat accumulation can cause podocyte lipotoxicity contributing to initiation and progression of albuminuria and chronic kidney disease[73,74].

As might be expected, in line with the above, a structured weight loss programme induced greater improvement of metabolic parameters in subjects with mainly reduction of VAT than in subjects with mainly reduction of SAT[75], and diet-induced weight loss in obese adolescents with excessive accumulation of hepatic triglyceride and normal glucose tolerance improved hepatic and skeletal muscle insulin sensitivity[76]. In **Chapter 6** we showed that treatment of obese patients with cardiovascular disease and type 2 diabetes with a 16-week very low calorie diet (VLCD) of 450-1000 kcal/day, besides reducing SAT volume, reduced VAT volume and ectopic fat accumulation in all compartments that we measured. Furthermore, this resulted in greatly improved glycemic control and im-

provement of dyslipidemia. Of note, these improvements were paralleled by decreased levels of the endocannabinoid anandamide (AEA). The endocannabinoid system (ECS) consists of endocannabinoids, their receptors and associated metabolic enzymes and increased ECS activity is associated with obesity and type 2 diabetes[77,78]. Indeed, treatment of obese subjects with rimonabant, an inverse agonist for the CB_1 receptor, induced marked weight loss and improved lipid metabolism, but increased the risk of psychiatric adverse events by binding to CB_1 receptors in the central nervous system, resulting in discontinuation of its clinical use[79-81]. Recently, it was shown that insulin resistance correlates with increased expression of the cannabinoid receptor type 1 (CB_1) in both SAT and VAT[82]. Furthermore, in the same study, *ex vivo* CB_1 inhibition with the antagonist AM281 resulted in reduction of lipolysis rates. Also, recently, high circulating levels of the endocannabinoid 2-arachidonoylglycerol (2-AG), but not AEA, were shown in obese and overweight patients with nonalcoholic fatty liver disease (NAFLD)[83]. We anticipate that in the study in which patients with type 2 diabetes were exposed to a VLCD, the reduction of activity of the endocannabinoid system as measured by a reduction of AEA, may have contributed to the improved metabolic phenotype induced by weight loss. Together, these findings underline the role of the ECS in metabolism, making it a promising target to improve metabolic health. Future studies can focus on nutritional interventions, which can affect the availability of biosynthetic precursors[84], or pharmacological agents targeting the ECS. Since the CB_1 receptor inverse agonist rimonabant failed because of psychiatric side effects[81], targeting the ECS should probably be strictly peripheral though. Recently published results on treatment with JM-00266, a non-brain penetrant CB_1 receptor inverse agonist, showing an improved glucose tolerance and insulin sensitivity in wild-type mice, but not in CB_1 receptor knockout mice, are very promising[85].

3.2 Effects of GLP-1 receptor agonism on VAT and ectopic fat

As has been discussed in section 3.1, interventions specifically targeting ectopic fat are likely a very effective therapeutic strategy to reduce insulin resistance and improve metabolic health in patients with type 2 diabetes. The effects of GLP-1 receptor agonism on VAT and ectopic fat will be discussed in this section. In **Chapter 3** we described the effects of treatment with liraglutide on ectopic fat accumulation in patients with type 2 diabetes from South Asian descent. South Asians develop type 2 diabetes more often and at a lower BMI than Western Europeans, which is related to a relatively high amount of VAT[86-88]. GLP-1 receptor agonism reduces body weight, and reductions of SAT[89], VAT[90-92], epicardial fat volume[93,94] and hepatic triglyceride content[30,95] have been reported in study populations of different compositions. We were the first to investigate the effects of liraglutide on different fat depots and ectopic fat in a group of South Asian patients with type 2 diabetes. In our per-protocol analysis we showed that liraglutide

compared to placebo added to standard care decreases the VAT volume, without significantly decreasing the volume of SAT and epicardial fat and the myocardial and hepatic triglyceride content. When comparing the effects on SAT and VAT to other studies in which subjects were treated with liraglutide, our results are mostly in line, showing a decrease of VAT[90-92]. However, Suzuki et al[89], using a relatively low dose of 0.9 mg/day liraglutide and studying Japanese type 2 diabetes patients, reported a decrease of SAT without an effect on VAT. And similarly, when comparing the South Asian to the Western European subjects of our MAGNA VICTORIA-trial, it must be noted that in the Western European treatment group, despite decreasing SAT volume, no effects on VAT or ectopic fat accumulation were observed[96]. The reason for the discrepancy in effect on different adipose tissue depots between the populations is unclear but an ethnic-specific effect of liraglutide or of weight loss itself might play a role. There are indications for an ethnic-specific effect from a study comparing the effect of VLCD in overweight non-diabetic South Asians to Western Europeans[97]. Although the VLCD equally reduced body weight in both groups, the diet reduced pericardial adipose tissue in the Western Europeans only. Importantly, the reduction of VAT in the populations of South Asians we studied was accompanied by and associated with a decrease in HbA1c, indicating improved glycemic control. This demonstrates that treatment with liraglutide and possibly other GLP-1 receptor agonists could be an effective treatment option for South Asian patients with type 2 diabetes and possibly in other populations with increased VAT accumulation.

We can only speculate on the mechanism behind GLP-1 induced reduction of VAT. GLP-1 may directly activate the GLP-1 receptor on adipocytes, although this is unlikely since most studies show no presence of the GLP-1 receptor on primary adipocytes[98-100]. Furthermore, improved perfusion of adipose tissue as a consequence of vasodilatation by GLP-1[101,102] can possibly affect its metabolic regulatory function. Whether GLP-1 may differently affect perfusion between VAT and other adipose tissues including SAT remains to be investigated. Another potential mechanism by which GLP-1 receptor agonism could specifically reduce VAT may relate to the autonomic nervous system. Mouse studies have shown that activation of central GLP-1 receptors improves insulin sensitivity as related to an increased sympathetic outflow[46,103]. Since sympathetic innervation of adipose tissue is an important activator of lipolysis and innervation of VAT and SAT is partially separated[104,105], this could theoretically result in specifically enhanced lipolysis in VAT as compared to SAT.

As mentioned earlier, hepatic steatosis is an important risk factor for disruption of glucose and lipid metabolism. South Asians have been shown to have a higher hepatic triglyceride content, even at young age and without overweight or obesity (BMI <25 kg/m^2)[15,106]. We investigated the effects of liraglutide on hepatic triglyceride content in

patients with type 2 diabetes of South Asian (**Chapter 2**) and Western European descent (**Chapter 5**). In the population of Western Europeans we studied, hepatic triglyceride content was decreased after treatment with liraglutide, but also after treatment with placebo in addition to standard care. This is in line with two other trials comparing effects of liraglutide vs insulin[107] and exenatide vs insulin or pioglitazone[108], showing equally reduced hepatic triglyceride content in all treatment groups. In contrast to our results, however, several trials in patients with NAFLD and type 2 diabetes under inadequate glycemic control on oral glucose-lowering drugs did show reductions of hepatic steatosis upon treatment with exenatide compared to insulin[95,109] and also in patients with NASH, treatment with liraglutide compared to placebo led to histological resolution of NASH with no worsening of fibrosis[30]. Importantly, none of these trials included patients already on insulin therapy, which proves a preserved β-cell function in these patients and might have affected results, since GLP-1 receptor agonism efficacy is affected by residual β-cell function[110] and, therefore, the effects on insulin secretion in patients with deteriorated β-cell function might be smaller. The observation that placebo in addition to standard care decreased hepatic triglyceride content in the Western European patients in our study similarly to liraglutide, might be explained by improved glycemic control after intensified treatment with glucose-lowering drugs in the placebo-group.

In the South Asian patients with type 2 diabetes no decrease of hepatic triglyceride content was observed after treatment with liraglutide or after intensified treatment, although weight loss was similar to the Western European group. We were the first to report on effects of GLP-1 receptor agonism on hepatic steatosis in this specific population. However, surprisingly and unexplained, the hepatic triglyceride content in the South Asian patients with type 2 diabetes we studied was already relatively low at baseline compared to the Western European patients, with mean hepatic triglyceride contents of 10% and 18%, respectively. Also, in other trials that showed reduction of hepatic triglyceride content upon treatment with GLP-1 receptor agonists as mentioned above, baseline hepatic triglyceride levels were considerably higher, indicating that the potency of liraglutide to reduce hepatic steatosis may be higher with increased hepatic steatosis. Studying of effects of GLP-1 receptor agonism on hepatic steatosis in subjects with more pronounced hepatic triglyceride content could provide further insight. Furthermore, an ethnic-specific effect, with impaired potential to reduce hepatic steatosis in the South Asian population, cannot be excluded. To conclude, although liraglutide does not improve hepatic steatosis in South Asian patients with type 2 diabetes, the reduction of VAT and improved glycemic control demonstrate that GLP-1 receptor agonism can be an effective treatment option.

3.3 The relationship between hepatic steatosis and CETP

In addition to effects of GLP-1 receptor agonism on hepatic triglyceride content, we investigated the possible relationship between hepatic triglyceride content and CETP, as described in **Chapter 5** of this thesis. CETP facilitates the transfer of triglycerides from VLDL to high-density lipoprotein (HDL), coupled to transfer of cholesteryl esters from HDL towards VLDL resulting in cholesterol enrichment of atherogenic VLDL particles and decreased HDL-cholesterol levels and may contribute to a pro-atherogenic profile[111,112]. Logically, pharmacological CETP inhibitors have been developed, but unfortunately most trials failed to show a beneficial cardiovascular effect[113-115]. Anacetrapib was shown to reduce the incidence of major coronary events by 9% compared to placebo[116], but the manufacturer decided not to pursue commercialization. Interestingly, recently, results were published on long-term safety and efficacy of anacetrapib after cessation of study treatment, showing that beneficial effects on major coronary events increased in follow-up, likely due to the accumulation of anacetrapib in adipose tissue resulting in a pharmacological action long after cessation of therapy[117]. Furthermore, the recently published data on the CETP inhibitor obicetrapib, a highly potent CETP inhibitor, showed very promising results by decreasing ApoB and reducing LDL-cholesterol concentration by up to 51%[118]. Although these observations refuelled interest in CETP and its role in dyslipidemia and atherosclerotic cardiovascular disease, regulation of the production of CETP is still not fully elucidated.

The circulating CETP concentration is mainly determined by Kupffer cells[119,120], suggesting a link between hepatic inflammation/steatosis and CETP production. To investigate a potential relationship between hepatic triglyceride content and circulating CETP, we studied a large population-based cohort with overweight subjects and did not observe an association between hepatic triglyceride content and circulating CETP. However, the mean hepatic triglyceride content in this population was low in both men (3.4%) and women (1.7%). Therefore, although a relationship between hepatic triglyceride content and CETP is not present in overweight subjects without hepatic steatosis, we cannot exclude a relationship in subjects with NAFLD. Further studies in subjects with different stages of NASH could help clarify this relationship.

Previous human studies with pioglitazone[121] and prolonged caloric restriction[122] have shown reduction of hepatic triglyceride content as well as plasma CETP concentration. Since GLP-1 receptor agonists also have previously been shown to reduce hepatic triglyceride content[30,95,109], we investigated the relationship between hepatic triglyceride content and CETP in patients with type 2 diabetes of Western European descent treated with liraglutide. In the trial we performed, the decrease in hepatic triglyceride content did not coincide with a decrease of circulating CETP concentration. It must be noted, as

mentioned in section 3.2, that in this trial the group treated with placebo in addition to standard care showed a similar decrease of hepatic steatosis. Importantly, in the LEAN-trial[30], although liraglutide induced histological resolution of NASH by liraglutide, no effects were observed on lobular inflammation. Combined with our results, this suggests that liraglutide fails to affect CETP production by Kupffer cells. This may seem surprising given that both pioglitazone[123] and GLP-1[124] have been shown to affect expression of the transcription factor liver X receptor alpha (LXRα), which is crucial in regulation of CETP expression[125,126]. However, in macrophages LXRα is mainly activated by oxidized sterols and the endogenous ligand desmosterol[127,128], while the effects of GLP-1 receptor agonism on these sterol derivatives has not been studied as yet. Studies comparing the effects of GLP-1 receptor agonists in human CETP transgenic mice on LXRα activation and its target genes including CETP could provide further insight in whether GLP-1 receptor activation may regulate CETP production by Kupffer cells.

3.4 Effects of GLP-1 receptor agonism on cardiovascular function

Type 2 diabetes is associated with a 4 to 5-fold increased risk to develop congestive heart failure, even without presence of prior coronary heart disease[129]. Although heart failure may result from coronary artery disease, in many patients with diabetes the cause of heart failure is diabetic cardiomyopathy, which is typically non-ischemic and preceded by diastolic dysfunction. Most patients with diabetes subsequently develop heart failure with preserved ejection fraction (HFpEF). The pathogenic process is multifactorial comprising disturbance of insulin signaling, oxidative stress, inflammation, autonomic dysfunction and formation of advanced glycation end-products (AGEs) as a consequence of hyperglycemia. Furthermore, diabetes is associated with increased myocardial triglyceride accumulation, which adversely affects cardiomyocyte function. Together, these processes induce increased cardiac stiffness and myocardial dysfunction[130,131]. Unfortunately, although the process of diabetic cardiomyopathy is increasingly unraveled, pharmacological options to treat or prevent development of heart failure remain limited.

In **Chapter 3** of this thesis, we describe effects of GLP-1 receptor agonists on cardiac function, based on the notion that direct actions of GLP-1 on the myocardium may improve cardiac function[132], and indirect effects of improved glycemic control and weight loss with concomitant reduction of myocardial steatosis can improve diastolic function[133,134]. Specifically, we evaluated effects of liraglutide on cardiac function in a randomized controlled trial in patients with type 2 diabetes of South Asian descent. While liraglutide improved VAT volume and glycemic control, liraglutide did not beneficially modulate diastolic function and myocardial tissue characteristics. These results are in line with evidence from other recent trials included in a recent meta-analysis,

showing that liraglutide does not improve left ventricular systolic and diastolic function[135]. Recently, similar results were reported for treatment with albiglutide[136], which makes us conclude that current-generation GLP-1 receptor agonists do not directly improve diabetic cardiomyopathy. This is in contrast to the relatively new therapeutic modality of sodium-glucose cotransporter 2 (SGLT2) inhibitors, which have recently been shown to reduce the risk of hospitalization for heart failure in patients with type 2 diabetes. Furthermore, SGLT2 inhibitors reduce progression of kidney disease and renal death[137-139]. These results have resulted in a recent change of international treatment guidelines, now providing a strong recommendation to initiate SGLT2 inhibitors early in patients with type 2 diabetes and heart failure and/or chronic kidney disease[140].

However, although GLP-1 receptor agonists do not improve diabetic cardiomyopathy, over the past years, several trials have shown the effectiveness of GLP-1 receptor agonists in reducing atherosclerotic myocardial infarction, stroke, and cardiovascular death (MACE) in patients with and without established atherosclerotic cardiovascular disease (ASCVD)[141]. This reduction of major cardiovascular events in patients with established ASCVD of approximately 14% is similar to the effect of SGLT2 inhibitors[139]. However, reduction of MACE in patients *without* established ASCVD upon treatment with SGLT2 inhibitors has not been shown. This might be explained by the fact that most trials primarily enrolled patients with high cardiovascular risk, resulting in underpowered analyses of patients without established ASCVD, although a more pronounced anti-atherosclerotic effect of GLP-1 receptor agonism is probably also important. The reduction of cardiovascular events by GLP-1 receptor agonism can largely be explained by the fact that GLP-1 receptor agonists can improve several risk factors for cardiovascular disease, including glycemic control, body weight, dyslipidemia and blood pressure[142]. Furthermore, the observation that the GLP-1 receptor is present on endothelial cells, monocytes, macrophages, and vascular smooth muscle cells suggests that GLP-1 can beneficially interfere with the atherogenesis process[143]. Indeed, in rodents, GLP-1 receptor agonism results in decreased formation, expansion, and vulnerability of atherosclerotic lesions[144]. These are important findings, since South Asians are at increased risk to develop atherosclerotic cardiovascular disease and even asymptomatic South Asians with type 2 diabetes show a higher prevalence and extent of coronary artery disease compared to matched Western European subjects[145]. Therefore, GLP-1 receptor agonists should be considered as treatment strategy in patients with high atherogenic cardiovascular risk such as the South Asian population.

3.5 A role for novel dual GLP-1/GIP receptor agonists

Although treatment with GLP-1 receptor agonists nowadays is widely recommended for treatment of obesity and type 2 diabetes, many patients unfortunately do not achieve

treatment goals. Therefore, optimization of pharmacological agents is very important. A possible way to improve therapy could be by using dual GLP-1/GIP receptor agonists. Indeed, already in 1993 it was shown that combined infusion of GLP-1 and GIP in healthy volunteers resulted in augmented β-cell responses compared to either hormone alone[146]. Recently, tirzepatide, a long-acting dual GLP-1/GIP receptor agonist has been developed and, interestingly, treatment of type 2 diabetes patients for 26 weeks improved markers for insulin sensitivity and β-cell function to a greater extent than dulaglutide, a GLP-1 receptor agonist[147]. Also, tirzepatide has proven to be superior to semaglutide with respect to improvement of glycemic control and reduction of body weight[148]. Furthermore, tirzepatide has recently shown spectacular effects in treatment of obesity[149] and NASH[150]. In fact, 72 weeks of treatment with tirzepatide (15 mg/week) decreased body weight of obese subjects with a mean body weight of 104.8 kg at baseline by an unprecedented 20.9%, which approximates the effect of bariatric surgery[149].

Dual agonists thus greatly enhance the therapeutic efficacy of GLP-1 receptor agonism, and several mechanisms may be involved. The GIP receptor is present on adipocytes and it is hypothesized that agonism can improve the lipid-buffering capacity of white adipose tissue by increasing adipose tissue perfusion, recruiting lipoprotein lipase (LPL) and enhancing insulin-stimulated glucose uptake[151]. This can subsequently result in reduction of ectopic fat accumulation. In addition, dual receptor agonism in mice has shown to cause more robust anorexigenic effects, possibly because, centrally, distinct neurons are activated and inhibiting food intake[152,153]. Interestingly, recent results from our group show that combined GIP/GLP-1 receptor agonism in mice, compared to single GLP-1 or GIP receptor agonism, diminishes inflammation and increases VLDL turnover, thereby attenuating atherosclerosis severity (Van Eenige et al, submitted). This may prove a useful therapeutic strategy to further decrease atherosclerotic cardiovascular risk in the South Asian population and other populations compared to GLP-1 receptor agonism alone, which warrants further investigation. Furthermore, very recently it was shown in mice that LY3437943, a triple agonist for the glucagon, GIP, and GLP-1 receptors is on equimolar basis even more effective than tirzepatide with respect to inducing a greater body weight reduction and increasing energy expenditure[154]. Although effects in humans are still to be studied, these results are a very promising basis for the future development of triple agonists in the treatment of obesity and type 2 diabetes.

CONCLUDING REMARKS AND FUTURE PERSPECTIVES

The prevalence of type 2 diabetes keeps rising and type 2 diabetes is one of the largest health problems in the world. VAT and ectopic fat accumulation play a crucial role in the

pathogenesis of type 2 diabetes, and GLP-1 receptor agonists have potential to reduce body weight and ectopic fat accumulation and improve cardiometabolic health. In this thesis we provide insight in the mechanism behind liraglutide-induced weight reduction, showing that liraglutide reduces body weight by reducing energy intake, rather than by activating BAT or increasing energy expenditure. Furthermore, we studied effects of liraglutide on the various adipose tissue depots and ectopic fat deposition and demonstrated a specific reduction of VAT in South Asian patients with type 2 diabetes. This reduction was accompanied by a significant improvement of glycemic control. Lastly, we provided evidence that liraglutide does not improve cardiac function and myocardial tissue characteristics and thus does not improve diabetic cardiomyopathy.

The South Asian population is at high risk to develop cardiovascular disease and type 2 diabetes, which is incompletely understood but can partly be explained by pronounced VAT and ectopic fat deposition. Based on results of this thesis, we propose that GLP-1 receptor agonists might be useful therapeutic means to improve glycemic control by reducing volume of VAT, which has been causally linked to insulin resistance and has been shown to be a major contributor to metabolic risk, in South Asian type 2 diabetes patients. Possibly, this can also be applied to other patients with type 2 diabetes in combination with pronounced VAT accumulation. Furthermore, our results implicate that also patients with overweight (BMI between 25 and 30 kg/m^2), and not only with obesity (BMI ≥30 kg/m^2) can greatly benefit from treatment with GLP-1 receptor agonists, which is important since currently the lower BMI limit for reimbursement by health care providers lies at 30 kg/m^2. In addition, although SGLT2 inhibitors, with proven beneficial effects on heart failure and progression of kidney disease, might be a better therapeutic option for treatment of type 2 diabetes in most patients, GLP-1 receptor agonists, due to their anti-atherosclerotic effects, might be the best current option in patients with high risk for atherosclerotic complications, such as the South Asian population. We expect that long-term treatment with GLP-1 receptor agonists will further reveal its potential in decreasing atherosclerotic cardiovascular disease and overall improving cardiometabolic health in the forthcoming years. Until then, patient-centered decision making with careful identification of risk factors for heart failure and atherosclerosis is warranted.

Over the past years, GLP-1 receptor agonists have proven to be an effective treatment option for obesity and type 2 diabetes. Their successors that are currently being clinically developed, including tirzepatide, a dual GLP-1/GIP receptor agonist, and LY3437943, a next-generation triple GLP-1/GIP/glucagon receptor agonist, appear to be even more effective in reduction of body weight and improvement of glycemic control[149,154]. Interestingly, GLP-1/GIP/glucagon receptor triple agonism in mice increases energy expenditure, which is likely caused by additionally increasing hepatic fatty

acid oxidation as compared to dual GLP-1/GIP receptor agonism. Since dual GLP-1/GIP receptor agonists have been shown to cross the blood-brain barrier[155], and LY3437943 is possibly also able to do that, dual and triple agonism may also be more effective in activating BAT by enhancing sympathetic outflow from the hypothalamus compared to liraglutide. Taken together, if dual and triple agonists demonstrate not to cause more adverse reactions compared to GLP-1 receptor agonists, they may play a key role in the battle against reducing weight loss in overweight and obesity and improve associated cardiometabolic diseases including type 2 diabetes, NAFLD/NASH and atherosclerotic cardiovascular disease in the next decades.

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