Chapter 11

Summary and conclusions

SUMMARY AND CONCLUSIONS

Introduction

Overweight and obesity are currently increasing globally which is attributable to a number of factors including the overall availability of palatable foods and an ongoing trend towards a more sedentary lifestyle. Obesity increases the risk of developing chronic diseases such as type 2 diabetes (T2DM), and obesity *per se* is associated with an increased mortality rate. In the pathogenesis of T2DM, numerous factors and processes lead to increasing tissue insulin resistance and beta-cell failure. These factors include genetic predisposition (1) and an adverse life style and pathophysiological processes such as adipocyte dysfunction causing nutrient spillover and adipokine / cytokine release (2;3), beta-cell degeneration (4), and deterioration of the postprandial incretin effect by gut hormones GLP-1 and GIP (5;6). It is not surprising that, among others, these pathophysiological processes have been the focus of research during the last decades, given the possibility that once important targets are found, these could possibly lay the foundation of new pharmacological interventions (7).

Two major players in the regulation of body weight are the gut and the brain (8). The hypothalamus, the control center of energy homeostasis, senses the presence of short and long term energy supplies: adipocyte-derived leptin conveys information on the available fat stores, whereas the gut hormones Ghrelin and peptide-YY (PYY) induce respectively orexigenic and anorexigenic effects in the brain (9-11). Inadequate gut and brain responses to energy surfeit may add to the pathogenesis of T2DM.

Weight reduction is of primary importance in the treatment of obesity and T2DM (11). Intensive life style interventions and very low calorie diets (VLCD) are widely accepted ways to achieve weight reduction and diminish obesity related co-morbidities (12). The last decade, bariatric surgery, especially the Roux-and-Y gastric bypass (RYGB), has shown to be an effective treatment for obesity and related co-morbidities such as T2DM, cardiovascular disease, cancer and overall mortality (13-15). The potential mechanisms leading to the beneficial effects of RYGB surgery are complex and involve multiple organs and mechanisms such as calorie restriction, rerouting of the gastrointestinal tract and alterations in incretinsignaling (7;16). Moreover, the direct effects of this procedure have led to the hypothesis that several of these effect are independent of weight loss (17).

In this thesis, we evaluated both acute and more long-term effects of weight loss strategies based on calorie restriction, gastric banding and a very low calorie diet, versus weight loss by the drastic yet effective roux-en-y-gastric bypass procedure.

Calorie restriction is a major determinant of the short-term metabolic effects of gastric bypass surgery in obese type 2 diabetic patients

In **chapter 2** we describe the direct effects of VLCD treatment, GB and RYGB surgery on fasting and postprandial glucose metabolism in obese subjects with NGT or with T2DM and examine the suggested additive effects of the gastrointestinal arrangements of RYGB to the effects of caloric restriction *per se*. Of importance, in the "index" paper of this thesis, we describe that the baseline characteristics of our different intervention groups were similar and that acute weight loss after three weeks was also similar in all groups. To mimic the physiological postprandial response, our subjects consumed a mixed meal.

We found that calorie restriction directly improved glucose metabolism and blunted hyperinsulinemia in obese (diabetic) humans. Additional duodenal exclusion through RYGB, however, did not improve postprandial glucose levels any further in NGT or in T2DM subjects, despite an increase in postprandial gut GLP-1 and PYY levels and insulin release.

Apparently, after RYGB, equivalent– but expedited - postprandial glucose levels elicit a more pronounced insulin release, facilitated by accelerated delivery of nutrient-rich chyme which induces an exaggerated GLP-1 response. This suggests that the surgical procedure, on the short term, hampers insulin action, an effect which is offset by the simultaneously increase in incretin levels. Insulin resistance is a well-known consequence of surgical stress, and the intestinal rearrangement after the RYGB procedure might thereby induce insulin resistance and "limit" the effects of this procedure on the short term as compared to calorie restriction per se (18;19).

Our results therefore imply that calorie restriction is the major determinant of the *short-term* metabolic effects of gastric bypass surgery in obese NGT and T2DM patients. Indeed, it has been shown that administration of the "post-RYGB" diet only or gastric banding improves glucose metabolism to a similar extent (20;21). Recent evidence suggests that reduction of nutrient entry (i.e. after RYGB surgery or placement of endoluminal sleeves) into the proximal gut may be involved in this process (22). Upper intestinal lipids suppress glucose production in rats, and this mechanism is desensitized

by high fat feeding (23). Conversely, calorie restriction may restore proper functioning of this mechanism to reduce endogenous glucose production in response to a meal.

On the long term, however, the potential of RYGB to increase satiety by its effect on gut hormone release facilitates sustained weight loss (24). This is supported by evidence that, on the long term, resolution of T2DM after RYGB is dependent on the amount of weight lost (25;26). More recent reports, concerning long term results, show that insulin sensitivity improves in proportion to the decrease in BMI post-surgery whereas diabetes remission is dependent on the GLP-1 response and pre-surgery beta-cell insulin secretion capacity (27;28). However, increased GLP-1 levels may not be crucial for the control of glucose metabolism, since it has been shown that infusion of a GLP-1 receptor antagonist does not deteriorate glucose tolerance in subjects after RYGB (29). In conclusion, whereas on the short term calorie restriction may seem the most important factor to improve glucose metabolism after RYGB, the long term effects depend on its potency to induce GLP-1 secretion and thereby, induce prevailing satiety, to enable sustainable weight loss resulting in diabetes remission.

Roux-en-Y gastric bypass, but not calorie restriction, increases postprandial glucagon release in obese patients with or without type 2 diabetes

In **chapter 3** we describe glucagon responses in obese T2DM subjects and the effect of the weight loss interventions on postprandial glucagon release. We hypothesized that, given the fact that glucagon is co-regulated with insulin and gut hormones, its postprandial response would also be altered by RYGB. We found that parallel to increased postprandial insulin and GLP-1 levels, postprandial glucagon is increased after RYGB, but not after calorie restriction. Moreover, we found evidence for a strong correlation between GLP-1 and glucagon release after RYGB. These findings are particularly interesting, since an increased glucagon response seems contra-intuitive given the physiological effects of GLP-1 and insulin to inhibit glucagon release. Indeed, the correlation between glucagon and GLP-1 levels suggest that similar to GLP-1, the glucagon response to nutrient ingestion is stimulated by the gastro-intestinal rearrangements of RYGB. Recent evidence is in concordance with this: 1 year after RYGB, meal stimulated glucagon was increased significantly and moreover, was associated with hepatic insulin resistance and higher rates of endogenous glucose production during a test meal (28)**.**

There is no clear explanation for the increase in postprandial glucagon levels after RYGB, however several mechanisms could induce this paradoxical effect. Possibly, rapid glucose fluxes from the gut after RYGB are transported directly to the portohepatic

blood, activating portal glucose sensors and initiating neural reflexes. For example, portal hyperglycemia shifts glucose uptake towards the liver and away from other peripheral tissues such as adipose tissue and muscle (30), thereby inducing glucagon secretion by alpha cells (28)**.** Increased glucagon release by the gut in response to food intake, in concert with GLP-1, may be an alternative explanation for postprandial hyperglucagonemia after RYGB. Both glucagon and GLP-1 share the same prohormone, proglucagon, which is processed to GLP-1 and glucagon by prohormoneconvertase (PC) 1 and 2 respectively. PC1 and PC2 are expressed in the proximal gut (31), and therefore might be affected by altered gut stimulation after RYGB. The exact mechanism that induces the glucagon release post RYGB remains to be determined, but seems an interesting challenge given the multiple effects of glucagon that have drawn increasing attention during the last couple of years.

More functions have recently been attributed to glucagon than its classical role to stimulate endogenous glucose production. For example, glucagon inhibits food intake, stimulates energy expenditure and fatty acid oxidation and lowers circulating lipids (as reviewed (32)). In view of these functions, glucagon could add to the beneficial effects of RYGB, whereas GLP-1 could outweigh the effect of glucagon to increase glucose production. A dual glucagon/GLP-1 receptor agonist was reported to have superior lipid- and bodyweight-lowering effects as compared to a selective GLP-1 receptor agonist in diet induced obese mice (33). A similar effect is induced by administration of oxyntomodulin, a natural agonist of both GLP-1 and glucagon (34), which decreases food intake and stimulates weight loss. Therefore, the simultaneous administration of GLP-1 and glucagon in obese (diabetic) subjects could be an interesting new pharmacological option, and focus of new research.

Calorie restriction and Roux-en-Y gastric bypass have opposing effects on circulating FGF21 in morbidly obese subjects

Chapter 4 describes the effects of the different weight loss interventions on relatively novel metabolic regulators: bile salts and fibroblast growth factors 19 and 21. Both FGF19 and FGF21 expression is stimulated after postprandial activation of bile salt signalling pathways. FGF19, expressed in the distal small intestine, is important for hepatic protein and glycogen synthesis. FGF21 is expressed in liver and adipose tissue and stimulates glucose uptake, fatty acid oxidation and energy expenditure. Gastrointestinal arrangements after RYGB are suggested to affect intestinal bile acid metabolism, therefore we hypothesized that FGF levels in turn would be differently affected by RYGB as compared to calorie restriction.

We observed that FGF21 levels are increased in obese NGT and T2DM subjects at baseline. Weight loss per se was associated with reduced bile salt and FGF21 levels. RYGB however, induced an increase of both postprandial bile salts and FGF21. Apparently, after RYGB, the effect of weight loss on bile salts and FGF21 levels is overruled by yet unknown metabolic stimuli that result in elevation of both bile salts and FGF21. Perhaps, similar to the stimulation of glucagon release after RYGB, intestinal glucose fluxes after RYGB stimulate a sensing mechanism in the portal circulation, thereby inducing hepatic FGF21 expression. Intriguingly, recent publications show evidence of FGF21 as regulator of the effects of glucagon (35-37).

Given the above mentioned evidence, and the recent finding that treatment with the FGF21 analogue LY2405319 improves dyslipidemia, decreases insulin levels, lowers body weight and increased adiponectin levels (37;38) makes FGF21 a promising farmacological target. Moreover, it suggests that this gut-liver-pancreas axis may be one of the important mediators of the effects or the RYGB procedure.

Roux-en-Y Gastric bypass and calorie restriction induce comparable time dependent effects on thyroid hormone function tests in obese female subjects

In Chapter 5 the effect of the different weight loss interventions on the pituitary-thyroid-axis is described. During weight loss, the body decreases its energy expenditure, in order to minimise the "fuel" lost, among other factors, by decreased activity of the pituitary-thyroid-axis. We hypothesized that differences in transient systemic inflammation and the catabolic state between the intervention types could lead to differential effects on central and peripheral thyroid hormone physiology.

We found that weight loss directly influences thyroid hormone regulation, independently of the weight loss strategy that is used. Indeed, we statistically proved that the effects induced by calorie restriction versus RYGB are the same. Overall, we found a decrease in TSH and T3 levels, both acutely and three months after the interventions, whereas we noticed a transient increase in the levels of T4 and rT3.

These effects may be explained by a combination of decreased leptin, decreased TSH levels, and transient changes in peripheral thyroid hormone metabolism. Indeed, the thyroid hormone changes in our subjects, specifically the increase in rT3 and decrease in T3, resemble changes in thyroid hormone levels seen in the non-thyroidal illness syndrome, which is described during critical illness and after surgical procedures. The correlation between rT3 levels and levels of inflammatory parameters IL-6 and

IL-8 further adds to the suggestion that a certain degree of non-thyroidal illness has affected peripheral deiodinase activity thereby stimulating the synthesis of rT3 instead of T3 from T4 levels.

Whatever the mechanism, changes in rT3 levels were transient and the decreases of TSH and T3 levels remained within the normal range. This suggests that these effects may induce a slight decrease in energy expenditure with weight loss, which however is likely not of clinical relevance.

Autonomic nervous system activity in diabetic and healthy obese and the effect of distinct weight loss strategies

Chapter 6 describes the effects of weight loss on (the recovery of) the enhanced sympathetic nervous tone seen in obesity. First we evaluated the differences in sympathetic nervous system activity between Lean, Obese NGT subjects and Obese T2DM subjects. Because calorie restriction and perioperative stress and anaesthetics may aggravate sympathetic nervous tone as well, we hypothesized that the effects of restriction and RYGB on the autonomic nervous system would be different between three weeks and three months after intervention.

We found that SNS over-activity is more pronounced in obese T2DM subjects as compared to NGT subjects. Reduction of SNS over-activity coincides with weight loss with the time-course of reduction dependent on the type of intervention, however a certain degree of reduction is seen after three months in RYGB and calorie restriction patients. Surgery or calorie restriction may transiently induce SNS over-activity but do not prevent a direct restoration of sympathovagal balance. Reduction of sympathetic overactivity in obesity is of importance, since sympathetic overactivity is an important cause of heart failure on the long term (39).

The effects of Roux-en-Y gastric bypass surgery on the plasma lipidome largely overlap with the effects of calorie restriction

With the advent of metabolomics technologies, it is now possible to determine a broad array of circulating lipids, lipid derivatives and numerous metabolites all at the same time. We used this approach to quantify differences in the circulating lipid pool between obese NGT and T2DM subjects. Furthermore, we aimed to analyse the direct effect of the different weight loss strategies on circulating lipids and lipid derivatives, which we describe in chapter 7.

Our data indicate that the lipidome of obese NGT and obese T2DM differ significantly from Lean, predominantly in TG species between T2DM and Lean. The changes in the lipidome after GB, RYGB and VLCD demonstrate a significant overlap and implicate that the common denominator of all interventions, calorie restriction, underlies the majority of the observed effects on the lipidome.

Because in the current study it was impossible to measure the expression levels and/or activity of proteins implicated in elongation and desaturation of fatty acids, which are supposed to be affected in obesity and insulin resistance, we used product:precursor ratios as a proxy for enzymatic activity. This showed a significant increase in the ratios, used as proxys for FADS1, Δ5-desaturase and SCD-1 after RYGB. Additional experiments are required to validate that the expression levels of these desaturases and elongases in adipose or hepatic tissue samples are influenced by weight loss. From the current available data, it cannot be determined whether the observed changes in the lipidome are the cause of or the consequence of the altered metabolic state in obesity and diabetes. The improving metabolomics technologies may be applied in the near future to distinguish this cause or effect relationship between the lipidome and the metabolic footprint of obesity and type 2 diabetes mellitus.

Roux and Y gastric bypass surgery, but not calorie restriction, reduces plasma branched chain amino acids in obese subjects independent of weight loss or the presence of type 2 diabetes mellitus

In chapter 8, we define the differences between lean, Obese NGT and obese T2DM subjects in circulating branched chain amino acids (BCAAs), given their suggested involvement in the pathogenesis of insulin resistance. We also determine the expression levels of genes involved in the branched chain amino acid (BCAA) degradation pathway. Finally, we analysed whether weight loss would cause a decrease in BCAAs, and whether this effect would depend on intervention type.

We show that BCAA levels are increased in subjects with obesity, and even more increased in subjects with T2DM, in parallel with insulin resistance and a downregulation of gene expression in the BCAA catabolic pathway in adipose tissue. This suggests a role for deregulated BCAA metabolism in the pathogenesis of insulin resistance and glucose intolerance. It has been suggested that BCAAs interfere with insulin signaling via the mTOR pathway (40). We could not conclude this from this study, but in further research it would be interesting to study the mTOR pathway in skeletal muscle of NGT and T2DM subjects and its relation to branched chain amino acids.

BCAA declined after RYGB, but not in response to calorie restriction alone. Moreover, we did not find any correlation between metabolic parameters and BCAA levels. As such, the RYGB procedure affects BCAA metabolism independently of weight loss or its metabolic effects. More importantly, our data show that calorie restriction had a similar effect on insulin sensitivity without affecting plasma BCAA concentrations, which implies that there may be no decisive role for BCAAs in the overall metabolic effect of the RYGB procedure. It is currently unknown what causes the increase in BCAA levels in obesity and T2DM, and moreover, what causes the decrease after RYGB. Several explanations could be suggested, for example the proven down regulation of BCAA catabolic genes in expanding adipose tissue in human and in rodents (41), the way of food processing or the gut microbiome, all of which may be affected by RYGB and need careful analyses.

Obese females show enhanced functional connectivity of brain regions involved in cognitive control, motivation and reward

The brain is crucial for the control of food intake, food reward and energy homeostasis. We hypothesized that brain circuits involved in default mode activity, homeostatic energy control and emotion and reward would show different functional connectivity patterns in obese subjects with NGT and with T2DM as compared to lean in the fasting state and in response to food intake.

We found that functional connectivity in several brain networks, in particular the central orexigenic (appetite stimulating) network and the reward network, is altered in obese subjects as compared to lean, whereas no differences exist between NGT and T2DM obese subjects. Obesity enhanced hypothalamic functional connectivity of brain areas implicated in cognitive and emotional control, such as the PFC, insular cortex and dorsal striatum, in the fasted state, possibly reflecting an enhanced craving for food. Strikingly, whereas food intake rendered positive functional connectivity between hypothalamus and inferior frontal cortex and insula in lean, it elicited no response in these regions in obese. These responses in lean subjects reflect an ability to "evaluate" the perception of hunger in the fasted state in lean subjects, and a "satiation" of this signal after food intake, whereas in obesity there is certain insensitivity to these food related cues.

It has been suggested that obesity is associated with an imbalance between brain circuits promoting reward seeking and those governing cognitive control. Obese subjects show enhanced responsiveness to food related cues, possibly through abnormal high inputs

from the amygdala and insula, and dysfunctional inhibitory control by the prefrontal regions. Our data add to this hypothesis since we show enhanced FC strength of the frontal cortices in the fasted state and no response upon food intake in reward areas in the obese subjects.

Restrictive and metabolic weight loss strategies in obese females alter functional connectivity network activity in the fasting and postprandial state

In view of the differences in brain functional connectivity in obese as compared to lean controls at baseline, and given the suggested effect of several gut peptides on the brain's homeostatic control centres, we evaluated the response to food intake on functional brain connectivity after RYGB and restrictive weight loss.

We show preliminary data concerning the response to food intake in functional connectivity brain networks, in particular the central orexigenic network and the reward network, after weight loss by calorie restriction only or RYGB. We did not find statistically different effects between the interventions; however, both interventions altered the response to food intake as compared to baseline. Weight loss induced by GB and VLCD was associated with increased functional connectivity in brain areas important for food motivation and reward in the fasted state (hypothalamus-putamen connectivity). RYGB was associated with an increased postprandial connectivity in areas implicated in satiety and gustation (insula, parietal operculum), possibly reflecting the increase in circulating satiety factors and increased gustatory perception after this procedure.

These data need to be replicated in additional studies, and during weight stabilization and maintenance of the reduced body weight. However, our results do suggest alterations in brain functional connectivity in response to different weight loss strategies, which may have implications for the long-term success or failure of RYGB versus restrictive weight loss interventions.

Overall conclusion

In this thesis, we evaluated the acute and more long-term effects of different weight loss strategies; pure calorie restriction by very low calorie diet and gastric banding, versus the drastic surgical procedure Roux-en-Y gastric bypass. Moreover, we found differences between NGT and T2DM subjects at baseline, which enable us to better be able to dissect the subsequent effects of the procedures.

To our surprise, and in contrast to previous studies, we observed no additional effect of the RYGB as compared to calorie restriction, on our main outcome parameters: postprandial glucose, insulin and the gut peptide levels three weeks after surgery. Furthermore, both restrictive and RYGB induced weight loss resulted in comparable effects on the lipidome, circulating thyroid hormone levels and the autonomic nervous system. For these outcome parameters, it seems that calorie restriction is the common denominator of the effect of the different weight loss strategies on the short term.

Clearly distinct effects of RYGB, however, were seen on bile salt, FGF21 and glucagon levels in response to food intake. Although neither the exact mechanisms, nor the eventual metabolic effect are as yet clear, the gut-liver-pancreas axis may be an important mediator of the effect of the RYGB, and perhaps more important, suggests some interesting targets for (future) pharmacological interventions. Furthermore, although the insulin secretagogues GLP-1 and GIP and satiety hormone PYY, do not influence postprandial glucose level directly, they may be an important additional effect of the bypass procedure, resulting in decreased food intake in obese subjects by inducing satiety, which no pharmacological intervention has succeeded in as yet.

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