Energy balance in obesity

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General introduction

Obesity is a global public health issue and rising at an alarming rate. According to the World Health Organization (WHO), there are 1.3 billion overweight and 600 million obese individuals. Obesity increases the risk of type 2 diabetes (T2D), stroke, cardiovascular diseases and kidney diseases. Eventually this will lead to an increased risk of mortality [1]. Recently it has been hypothesized that the pathogenesis of obesity and T2D involves shared defects in brain systems controlling both body fat and blood glucose homeostasis. Indeed, obesity is often associated with disturbances in glucose homeostasis and most obese animal models are characterized by hyperinsulinemia [2,3]. This hyperinsulinemic response combined with insulin resistance is often associated with hypertension, dyslipemia, visceral adiposity and low grade inflammation [4,5].

Under healthy conditions, energy intake equals energy expenditure, which renders body weight relatively stable. The general hypothesis states that there is a certain set point or settling point for body weight or a so-called body weight homeostasis, rendering a constant body weight over prolonged periods despite considerable temporary fluctuations in food intake and physical activity levels. Evidence for this comes from the observation that when animals are involuntarily overfed or deprived from food and consequently gain respectively lose weight, they compensate for this until they reach the body weight of the controls again [6].

In contrast, accumulation of body fat is fundamentally a reflection of positive energy balance, where energy consumed exceeds that expended through metabolism, thermogenesis and physical activity. Although energy balance has a high degree of precision in its regulation, this regulation is perturbed in favor of caloric over-consumption, which is generally considered an inherited trait from our ancestors for whom this evolutionary conserved strategy was advantageous to secure energy reserves in times of scarcity or unpredictability [7]. Thus, the system controlling energy balance is very sensitive to a negative state but relatively tolerant to a positive state.

THE CONTROL OF ENERGY BALANCE

Once food is consumed, nutrients are detected in the gastrointestinal (GI) tract which trigger mechanoreceptors in the stomach (as a consequence of gastric distension) and stomach contents are emptied into the duodenum and small intestine where they can release gut hormones [8] involved in termination of meals (box 1). In short, gut hormones within the GI tract are connected within the brain via the

Box 1 | Gut hormones

Gut hormones are secreted by enteroendocrine cells (e.g. I, K and L-cells) in the gastrointestinal (GI) tract and are crucial in the gut-brain system to control eating behavior. There are two main groups of gut hormones 1) Appetite stimulators (orexigens/orexigenic hormones), such as ghrelin and 2) satiety stimulators (anorexigens/anorexigic hormones), such as cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1), and peptide tyrosine tyrosine (PYY). Another important hormone (produced primarily by adipose tissue, but also to a lesser extent the stomach) is leptin, which in states of increased leptin-signaling reduces food intake and increases energy expenditure which promotes leanness. Next to these hormones with a direct effect on eating behavior, glucagon, and glucose-dependent insulinotropic peptide (GIP), play a major role in metabolism and consequently eating behavior and therefore should be taken into account in energy balance as well. Gut hormones within the digestive tract are connected with the brain via the vagus nerve or enter the systemic circulation and subsequently the brain via the blood-brain barrier and transmit signals for satiety and hunger to the brain. Hormones secreted from the GI tract play crucial roles in prandial and postprandial metabolic homeostasis, while these signals play lesser roles in the basal state, when insulin-independent processes predominate. Basal-state energy homeostasis depends on input from leptin, which, unlike other gut hormones, circulates at levels that vary little on a meal-to-meal basis, but instead flows in parallel with changes of energy balance. The state- and time-dependent release of gut hormones indicate that there is an essential balance in hormonal food intake regulation [9,20–25].

vagus nerve or enter the systemic circulation and subsequently the brain via the blood-brain barrier and then transmit signals for satiety and hunger. One of the brain areas that is profoundly involved in the regulation of eating behavior and glucose homeostasis is the hypothalamus [9]. Within the hypothalamus, neuronal circuits spanning several nuclei (i.e., the arcuate nucleus (ARC), the lateral hypothalamic nucleus (LHN), paraventricular nucleus (PVN) and the ventromedial nucleus (VMH)) express and release numerous factors (including neuropeptides) which regulate eating behavior, but also neuroendocrine and autonomic nervous system activity in various peripheral organs. In addition to the hypothalamus, hindbrain circuits, the dorsal vagal complex and the brainstem (NTS; nucleus tractus solitarius) are involved in energy homeostasis as well, often in integral fashion to the hypothalamic circuits [10]. However, it is increasingly apparent that the hedonic/reward brain systems can override the hypothalamic/brain stem systems that regulate energy balance and that the variety of energy dense, high sugar and high fat foods that the western society is exposed to is hyper-stimulating these systems [11 12,13]. This scenario can drive food consumption beyond homeostatic needs, which in combination with a low level of physical activity (i.e., many individuals do not reach the minimum of 30 min of daily physical activity) that is seen in current daily life, is likely to provide an explanation for the high obesity prevalence [14–16].

When weight loss after obesity is successful, metabolic rate will often be regulated to a lower level as part of a homeostatic system aimed at attaining the setpoint/ settling point. This evolutionary conserved compensation to spare energy and increase feelings of hunger and suppress satiety, makes it even more difficult to sustain a lowered body weight [17]. Consequently, adherence to dietary advice is difficult and health outcomes after lifestyle changes are often disappointing[18]. To tackle this problem, the search for a pharmacotherapeutic 'magic' pill, which could reduce body weight – specifically body fat –, started already near the end of the 19th century [19]. Although several discoveries yielded a potential treatment (e.g. based on the supplementation of gut hormones), such strategies did not translate into pharmacotherapy capable of efficiently decreasing body fat for a long period. Only recently, the pharmacotherapy by GLP-1 analogs have been demonstrated to effectively reduce body weight, improve glucose regulation and lower mortality. While pharmacotherapy is now able to result in substantial weight loss (i.e. > 10%) and even better medication is coming relatively fast, the best results are achieved by bariatric surgery (also known as metabolic surgery due to its profound effects on e.g. glucose, lipids and blood pressure) leading to an average weight loss of 30% which is sustained over more than a decade.

BARIATRIC SURGERY, MECHANISMS OF ACTION

Bariatric surgery has been shown to not only effectively lower (excess) body weight, but also correct many of the associated comorbidities such as T2D, heart disease and dyslipidaemia [26]. A joint statement by international diabetes organizations refined the treatment algorithm of T2D to regard bariatric surgery as alternative option to pharmacotherapy, since clinical evidence from numerous (randomized) clinical trials showed effective and long-term glucose lowering effects after bariatric surgery, which even starts before major weight loss [27]. The surgical treatment is (highly) recommended for individuals with type 2 diabetes and body mass index (BMI) of at least 35 kg/m^2, but is also suggested for patients with a BMI > 30 kg/m^2 in whom optimal glycemic regulation is difficult to achieve. Although in general the health outcomes are outstanding, some negative side-effects such as dumping syndrome, vitamin and mineral deficiencies, and a small but increased prevalence of alcoholism and suicide (attempts) need to be mentioned [28].

Bariatric surgery comprises several surgical procedures aimed at sustained weight loss. Historically bariatric surgery is divided into two classes: (1) mechanical reduction of the volume capacity of the stomach (gastric banding and sleeve

gastrectomy) and (2) partial selective malabsorptive procedures (Roux-en-Y gastric bypass (RYGB) surgery) [29,30]. Laporoscopic adjustable gastric banding was the first bariatric surgery that was thought to be a simple and effective method to decrease energy intake and achieve weight loss. However, reports show that laporoscopic adjustable gastric banding does not always result in long-term weight loss [18,31]. In addition, this type of bariatric surgery is not efficient for altering gut hormones (no changes in GLP-1, and GIP) and thereby improving glucose homeostasis (no effects on circulating glucose levels; summarized in table 1) [26,31–34]. There is increasing evidence that certain physiological/endocrine/autonomic mechanisms are more important drivers of therapeutic success than volume restriction or malabsorption. For example, the changed intestinal anatomy (i.e., where the distal parts of the intestines are exposed to undigested nutrients, while at the same time excluding the more proximal intestinal parts) can lead to a change in the concentrations of gut hormones, which subsequently lead to alterations in energy balance parameters. Therefore, RYGB surgery is now seen as the golden standard for bariatric surgery. During this procedure a small pouch remains of the stomach and the duodenum and proximal jejenum is excluded from the passage of food. These anatomical changes lead to increased gastric emptying, which result in the

Table 1 | Physiological change after bariatric surgery seen in animal models, 1) Ileal transposition: lower part of ileum transposed to an upper part; 2) Laparoscopic adjustable gastric banding: reduced gastric volume; 3) Roux-en-Y gastric bypass surgery; Reduction gastric volume and bypass of duodenal and upper jejunal part of small intestine; 4) Vertical sleeve gastrectomy: reduced gastric volume. Energy expenditure consists of, resting metabolic rate (RMR), non-exercise activity thermogenesis (NEAT) and/or ingestion-related energy expenditure (IEE). Arrows (↑↓) indicate an increase or decrease, an equal sign (=) has no change, and a dash (-) indicates data were not assessed.

	Energy balance parameters		**Gut hormone response**				**Ref.**
Laparoscopic adjustable gastric banding	Body weight	↓ =	CCK	-	Glucose	=	[26,31–34]
	Energy expenditure	↓	GLP-1	=	GIP	=	
	Energy intake	↓ =	Leptin	↓	Insulin	↓	
	Physical activity	↑	PYY	↑	Ghrelin	↑	
Roux-en-Y gastric bypass	Body weight	↓	CCK	↑	Glucose	↓	[31,33–39]
	Energy expenditure	↓↑	GLP-1	↑	GIP	↓ ↑	
	Energy intake	↓	Leptin	↓	Insulin	↑	
	Physical activity	↑	PYY	↑	Ghrelin	↑	
Vertical Sleeve Gastrectomy	Body weight	↓	CCK	↑	Glucose	↓	[34,40–44]
	Energy expenditure	↓	GLP-1	↑	GIP	=	
	Energy intake	↓	Leptin	↓	Insulin	↑	
	Physical activity	↑	PYY	↑	Ghrelin	↓ ↑	
Ileal transposition	Body weight	= ↓	CCK	-	Glucose	↓	[29,87–91]
	Energy expenditure	↓	GLP-1	↑	GIP	↓	
	Energy intake	=	Leptin	↓	Insulin	↓	
	Physical activity	↑	PYY	↑	Ghrelin	↑	

delivery of incomplete digested nutrients to the small intestine. Subsequent intestinal adaptation occurs that facilitates hypersecretion of gut hormones (i.e., CCK, Ghrelin, GLP-1 and PYY) that are involved in appetite and glucose homeostasis. These changes in gut hormone secretion results in a decrease in energy intake and an increased physical activity/energy expenditure (i.e., resting metabolic rate (RMR), non-exercise activity thermogenesis (NEAT) and/or ingestion-related energy expenditure (IEE)). In addition, glucose homeostasis is improved due to a decrease in hepatic glucose production and increase in insulin levels (summarized in table 1) [31,33–39]. This is an important reason that bariatric surgery is now regarded as a highly valuable treatment option against T2D. Over the last years sleeve gastrectomy (SG), in which roughly 80% of the stomach along the greater curvature is removed, has gained popularity because its relative simplicity [28]. Although RYGB surgery shows a superior weight loss, most changes in energy balance parameters (i.e., decreased energy intake and increased physical activity/energy expenditure) and glucose homeostasis (increased secretion CCK, GLP-1, PYY resulting in increased insulin and decreased glucose levels) are similar in both procedures (summarized in table 1) [34,40–44].

Another bariatric surgical method is the ileal transposition (IT) procedure, which involves merely transplantation of a distal ileal gut segment to region just below the duodenum. It is an interesting method to investigate which of the physiological alterations of the gut, and thereby gut hormones (an increase in circulating GLP-1, PYY and ghrelin levels and a decrease in circulating leptin and GIP levels), are responsible for the effects seen after RYGB on glucose homeostasis and energy balance parameters (i.e. energy intake and energy expenditure; see summary in table 1) [29].

THE VAGUS NERVE AS TARGET TO STUDY ENERGY BALANCE REGULATION

The vagus nerve consists of three different fiber types defined by Erlanger and Gasser. (1) Large myelinated A-fibres carry mostly somatic afferent and efferent information, and small myelinated A-fibres primarily transmit visceral afferent information; (2) B-fibres provide efferent sympathetic and parasympathetic preganglionic innervation; and (3) small unmyelinated C-fibres primarily carry afferent visceral information [45]. The vagus nerve connects the brain regions related to glucose sensing with the gastro-intestinal tract and integrates this mechanism in metabolic regulation, (neuro)endocrine activity and behavior [46, 47].

Abdominal organs involved in food intake and glucose homeostasis, including liver, pancreas and the intestines are profoundly innervated by efferent fibers within the three abdominal (hepatic, gastric, and celiac) branches of the vagus nerve from ventral roots arising on either side of the hindbrain [48]. In addition, the two dorsal vagus trunks that arise on either side of the hindbrain largely contain afferent fibers from organs to the brain that signal various aspects of nutritional and gastrointestinal processes [20]. For example, glucose is detected by portal sensors, which initiate a signal to the brain via afferents conveyed within the vagus nerve, which promotes a decrease in food intake and alteration in autonomic output that suppresses hepatic glucose production [20]. Digestion and absorption are controlled by efferent innervation of the gut. The vagus nerve has inherent plasticity, changing its sensitivity to gut hormones in response to nutritional status [49]. In short, this regulation via the vagus nerve is aimed at preserving energy balance and preventing fluctuations in body weight and glucose homeostasis [50]. Thus, the vagus nerve is an important factor showing contrasting physiological effects of its afferent and efferent fibers [4,5,21].

Vagus nerve signal transduction and activity can also be different depending upon the model or situation. In lean rats, vagus nerve activity show an increase in the expression of anorexigenic receptors and neuropeptides in the fed state, and an increase in orexigenic receptor expression following prolonged food withdrawal [58]. In obesity, chronic high fat feeding increases energy intake and therefore increases gastric capacity. This increase in gastric capacity alters the mechano-stimulated signaling, which is of importance in the gut hormone response and thereby the vagus feedback loop. This results in a dysregulated signal transduction from the abdominal organs to the brain. In short, the sensitivity of the vagus nerve depends on the combination of gut hormones, feeding and metabolic state [4, 59]. Insulin resistance, without the confounding factors of obesity, diabetes, and significant hypertension, is usually associated with a reduction in vagal activity, which occurs via attenuation in reflex activity [4,5]. Moreover, this vagus nerve sensitivity is decreased in obese subjects, and therefore, the threshold of vagus nerve signaling is increased [4,5] A recent study reported that vagal neurons are critically implicated in the antidiabetic actions of bariatric surgery in rodents [51]. Moreover, during bariatric surgery procedures, often a partial and unintentional vagotomy occurs.

These observations, in combination with the extensive and accessible connections between the gastro-intestinal tract and the central nervous system, sparked interest in modulation of vagus nerve activity. The aim of modulation of vagus nerve

activity is to establish sustained health promoting effects in individuals with the metabolic syndrome similar as seen after different types of bariatric surgery. Strategies to understand the physiological role of the vagus nerve seen after bariatric surgery are (selective) vagotomy, vagal blocking and increase signal transduction. With (selective) vagotomy, signals carried by the vagus nerve are interrupted with a surgical intervention (similar as seen after bariatric surgery). With vagal blocking, signals carried by the vagus nerve are interrupted with the use of high frequency electric pulses. Both procedures lead to a decrease in energy intake and to weight loss (however contrasting effects are observed with vagotomy; see summary in table 2). No effects on gut hormones or glucose homeostasis parameters are seen after vagal blocking [52–57]. Some reports show an increase in anorexic gut hormones (CCK and GIP) and a decrease in orexigenic hormones (ghrelin), but also a decrease in insulin levels without changes in glucose levels after vagotomy (see summary in table 2). This indicates that both procedures may be interesting against obesity, however limited as therapy against 2 diabetes. Increasing the signal transduction by electrical stimulation of the vagus nerve is hypothesized to not only induce weight loss, but also improve glucose homeostasis.

Table 2 | Changes in energy balance parameters and gut hormones seen in animal models after 1) Vagotomy: transection of subdiaphragmatic vagus nerve; 2) Vagal blocking: use of high frequency electrical pulse to disrupt the vagus nerve; 3) Vagus nerve stimulation: delivery of electrical signals from an implanted pulse generator to an electrode around the vagus nerve; 4) Capsaicin treatment: ablation of small unmyelinated fibers of the afferent vagal nerves. Arrows (↑↓) indicate an increase or decrease, an equal sign (=) has no change, and a dash (-) indicates data were not assessed.

	Energy balance parameters		**Gut hormone response**				**Ref.**
Vagotomy	Body weight	↓ =	CCK	↑	Glucose	=	[23,92–96]
	Energy expenditure	-	GLP-1	=	GIP	= ↑	
	Energy intake	↓↑	Leptin	=	Insulin	↓	
	Physical activity	-	PYY	-	Ghrelin	↓	
Vagal blocking	Body weight	↓	CCK	=	Glucose	=	[91–95]
	Energy expenditure	=	GLP-1	=	GIP	=	
	Energy intake	↓	Leptin	-	Insulin	=	
	Physical activity	-	PYY	=	Ghrelin	-	
Vagus nerve stimulation	Body weight	↓ =	CCK	=	Glucose	↓ ↑	[5]
	Energy expenditure	↑	GLP-1	= ↑	GIP	-	[84–93]
	Energy intake	↓	Leptin	=	Insulin	↓ ↑	[57,75–80]
	Physical activity	-	PYY	↑	Ghrelin	=	
Capsaicin treatment	Body weight	=	CCK	-	Glucose	↓ =	[81–86]
	Energy expenditure	= ↑	GLP-1	= ↑	GIP	-	
	Energy intake	↓	Leptin	↓	Insulin	↓ ↑	
	Physical activity	-	PYY	=	Ghrelin	=	

Vagus nerve stimulation (VNS) is approved by the Food and Drug Administration (FDA) since 2015 as treatment option for obesity, and is getting more attention as potential new treatment against type 2 diabetes. VNS has an effect on both arms of energy balance, as it increases energy expenditure while simultaneously decreasing energy intake resulting in weight loss (see summary in table 2). The decrease in energy intake can be attributed to a increase in GLP-1 and PYY. However, mixed results are seen when looking at glucose homeostasis parameters (↓ ↑ glucose levels, ↓ ↑ insulin levels; see summary in table 2) [84–93] [57,75–80].

The typical range of vagal nerve stimulation are relatively straightforward for both clinical studies (30-40 Hz, 130-500 μs, 1.5-2.25 mA, 10% active cycle (30 sec ON + 5 min OFF) with asymmetric biphasic pulses) and preclinical studies (0.05-40 Hz, 0.1-500 ms, 0.25-6 mA, active cycle 12/h or 30 sec ON + 5 min OFF with monophasic rectangular pulses). This variability is also necessary to target the different fibers of the vagus nerve as substantially higher activation (and block) thresholds for myelinated vs unmyelinated fibers are needed (see table 3 for an overview) [52]. Thus, selective activation or blockade of specific vagal fibers to specific organs has been shown to impact different metabolic processes resulting in different (glycaemic) outcomes. Capsaicin is an interesting technique to study effects of blockade of vagal fibers. It specifically target afferent nerves and small unmyelinated fibres, resulting in a decreased (afferent) signal transduction from the periphery to the brian. Reports show some effects on energy balance parameters, gut hormones and glucose homeostasis parameters, however for all mixed results are seen (see summary in table 2) [81–86]. Although our understanding of the role of vagus nerve in regulating food intake and appetite is increasing, studies investigating the detailed effect of vagus nerve stimulation on energy balance parameters are scarce. Important for consideration is that outcomes of VNS are highly variable. This could be attributed to stimulation at different locations along the vagal tracks, and the use of different electrical stimulation settings affecting different fibers (see table 3 for an overview).

Table 3 | Location and electrical parameters of different studies about vagus nerve stimulation (VNS). aVNS/eVNS is specific stimulation of the afferent (aVNS) or efferent (eVNS) vagal nerve branches. *Only studies focused on metabolic effects of VNS are taken into account.

Species/ model	Stimulation parameters	Stimulation site	Main results (metabolic parameters)	Ref
Dog/normal	10 Hz, 13.5mA, 5 ms	Anterior and posterior thoracic vagus nerve	↑ Secretion pancreatic polypeptide and somatostatin	[76]
Dog/normal	10 Hz, 3 mA, 5 V, 8 ms.	Bilateral vagus nerve	↑ Circulating insulin levels	[97]
Rat/normal	30 Hz, 1.5 mA, 500 ms	Left cervical vagus nerve	↓ Body weight gain and mesenteric adipose tissue	[77]
Rat/normal	30 Hz, 50 μA, 0.2 ms	Dorsal motor nucleus	↑ Circulating insulin levels	[78]
Rat/normal	10 Hz, 10 V, 1 ms	Subdiaphragmatic anterior and posterior vagus nerves	↑ Secretion insulin and glucagon; ↓ secretion somatostatin	[79]
Rat/normal	10-20 Hz, 6 V, 1.0 ms	Pancreatic vagus nerve	↑ Circulating insulin levels	[80]
Rat/normal	15 Hz, VNS aVNS eVNS 40 kHz 4 kHz	Subdiaphragmatic anterior vagus nerve	↑ Circulating glucose and glucagon levels	[57]
Rat/normal	5 Hz, 3 V, 1 ms	Right or left cervical vagus nerve (afferent/efferent)	VNS ↑ Circulating glucose and glucagon levels; aVNS ↑ Circulating glucose levels; eVNS ↑ Circulating insulin levels	[60]
Rat/normal	5 Hz, 25 V, 0.5 ms	Left cervical vagus nerve	↑ Circulating glucose levels and insulin immunoreactivity	[61]
Rat/normal	5 Hz, 3 V, 1 ms	Right cervical vagus nerve	↑ Circulating glucose levels; ↓ circulating insulin levels	[67]
Rats/normal and T2DM	5 Hz, 2 mA, 0.3 ms	Subdiaphragmatic anterior vagus nerve	↓ Circulating glucose levels, this effect is inhibited by the addition of exendin (GLP-1 antagonist)	[69]
Rat/diet-induced obesity	20 Hz, 0.5-0.75 mA	Left cervical vagus nerve	↓ Circulating insulin levels, HOMA index, total cholesterol, triglyceride, LDL and visceral fat.	[70]
Rats/diet-induced obesity	10 Hz, 200 mV, 10 ms	Subdiaphragmatic anterior vagus nerve	↓ Food intake, body weight gain, circulating leptin levels; ↑ circulating ghrelin levels	[71]
Rat/diet-induced obesity	0.05 Hz, 200 mV, 10 ms	Subdiaphragmatic anterior vagus nerve	↓ meal size, body weight gain	[72]
Rats/diet-induced obesity	40 Hz, 6 mA	Subdiaphragmatic anterior and posterior vagus nerve	↓ Food intake, body weight gain; ↑ circulating GLP-1, PYY and pancreatic polypeptide	[5]

Table 3 | *Continued*

Species/ model	Stimulation parameters	Stimulation site	Main results (metabolic parameters)	Ref
Rats/diet-induced obesity	1 Hz, 200 mV, 10 ms	Subdiaphragmatic anterior vagus nerve	↓ Food intake, body weight gain, total cholesterol concentration, triglyceride and leptin levels; ↑ nesfatin-1	[73]
Min pigs	34 Hz, 4 V, 0.5 ms	Subdiaphragmatic anterior vagus nerve	↓ Body weight gain, back fat and intramuscular fat gain	[74]
Mini pigs/ obese	30 Hz, 2.5 mA, 500 µs	Bilateral vagus nerves	↓ Body weight gain, food intake and sweet cravings	[62]
Mini pig/ obese	30 Hz, 1-5 mA	Subdiaphragmatic anterior and posterior vagus nerve	↓ Food intake	[63,64]
Human/ obese	40 Hz, 10 mA, 0.208 ms	Anterior gastric wall	↓ Excess BMI	[65]
Human	30 Hz, 0.25-1.5 mA, 500 µs	Left vagus nerve (chest)	↑ Energy expenditure	[66]
Human/ obese depressed	30 Hz, 0.25-1.5 mA, 250-500 µs	Left cervical vagus nerve	↑ Weight loss	[75]

GENERAL CONSIDERATIONS FOR THIS THESIS

Many animal species consume food in discrete bouts, or meals. While this arrangement has the benefit of liberating the animal from the need to continuously seek and consume food, it also imposes the need to ingest and assimilate comparatively large amounts of nutrients in a single sitting [7,25]. An alteration in the regulation in feeding behavior by diet appears to play a role in obesity development and maintenance [98]. Meal-related stimuli, such as mechanical distension, chemical properties of the luminal contents, gut hormones and neurotransmitters can be sensed by vagal afferents and play a direct role in reducing meal size. Current literature shows a successful decrease in energy intake after different types of bariatric surgery and after manipulation of the vagus nerve (with the exception of vagotomy), although the latter is not always accompanied with weight loss (summarized in table 2). Furthermore, efforts that tried to establish a relevant role for single factors in RYGB-mediated effects failed [29-33]. Moreover, what should be taken into account is that during the surgical procedures, often a partial and unintentional vagotomy occurs. Indeed, it is likely that the construction of a gastric pouch inevitably results in vagus nerve damage [20,51,103–105]. Hence, RYGB surgery has a direct impact on the innervation of the stomach and further down the digestive system via the vagus nerve, which in turn may actively play a role in the metabolic actions

and side effects of gastric bypass [106]. In addition, IT is an interesting method to investigate which of the physiological alterations of the gut after RYGB surgery are responsible for the effects seen on energy balance (i.e., energy intake and energy expenditure) and glucose homeostasis [29]. Hence, the decrease in energy intake, increase in energy expenditure and improvement in glucose homeostasis after RYGB surgery could be the results of the hyper-secretion of gut hormones, damage to the vagus nerve or both [49]. Thus, investigating RYGB surgery, IT and vagus nerve stimulation in different body phenotypes (i.e. healthy, obese, or T2D) is a crucial step to understand how RYGB surgery successfully alters both arms of the energy balance scale, i.e. energy intake and expenditure and glucose homeostasis.

Another factor that should be taken into consideration is that different types of bariatric surgery trigger specific alterations in energy balance and variation herein, which may contribute to the observed large inter-individual differences in weight loss after these procedures [26,107]. This in combination with a lack of objective measurements of meal related parameters makes it also difficult to interpret the effect of bariatric surgery on body weight homeostasis.

To avoid this caveat the studies on the effect bariatric surgery and vagus nerve stimulation presented in this thesis focuses specifically on eating behavior using an automated method and glucose homeostasis using stable isotope labelled glucose or a mixed-meal tolerance test. These methods could help improve preclinical research into energy balance regulation.

AIM AND OUTLINE OF THE THESIS

The general aim of this thesis is to study the effects of Roux-en-Y gastric bypass (RYGB) surgery, ileal transposition (IT) and vagus nerve stimulation (VNS) on energy balance parameters and glucose homeostasis. This is investigated in healthy rats after IT, a diet-induced obese rat model after RYGB and after VNS in type 2 diabetic rats.

Part I – Effects of bariatric surgery on energy balance

In **chapter 2** the effects of continued feeding a high fat diet with added sucrose (HF/S) on RYGB-induced body weight loss is explored. In **chapter 3** detailed meal related parameters were investigated and coupled to glycemic control after RYGB-induced body weight loss. In **chapter 4** a detailed description of the ileal transposition as bariatric surgery procedure is described, and its effects on different energy balance parameters are studied.

Part II – Effects of vagus nerve stimulation on energy balance

Chapter 5 uncovers the acute effect of vagus nerve stimulation (VNS) on glucose homeostasis using labelled glucose. It gives a detailed description of how glucose is processed by the pancreas, liver and peripheral tissue during VNS without the effect of weight change. In **chapter 6** the effects of VNS on eating behavior and glycemic control are studied in a type 2 diabetic rat model over a three week period. The main findings of this thesis are summarized and discussed in **chapter 7**.

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Part I

Effects of bariatric surgery on energy balance