Changes in Food Preference and Taste Responses after Roux- en-Y Gastric Bypass

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Dedicated to Mira & Moritz

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Abstract

Currently, the most powerful therapy for obesity is bariatric surgery both in terms of significant weight loss and long-term efficacy. Most surgeons regard the Roux-en-Y gastric bypass (gastric bypass) operation as “gold standard” for obesity treatment. However, underlying mechanisms by which gastric bypass induces and sustains weight loss are not fully understood, but include reduced hunger, increased satiety, increased energy expenditure, altered taste, as well as reduced preference for foods with a high fat and sugar content. In fact, gastric patients often report idiosyncratic changes in taste perception that involves “sweet” taste and a calorie-dense food.

I herein aimed to investigate how gastric bypass reduces intake of and preference for food high in fat and sugar in rats and humans. I found that the proportion of dietary fat in gastric bypass patients was significantly reduced six years after surgery compared with patients after vertical-banded gastroplasty. In addition, gastric bypass patients had an increased sucrose detection sensitivity compared with before surgery and controls, but hedonic taste ratings of sucrose in bypass patients remained unchanged. Rats after gastric bypass exhibit a shift away from high to low fat food. When compared to sham-operated rats, gastric bypass rats did not prefer high sucrose and fat concentrations in a two bottle preference test, but preoperative sucrose exposure reduced this effect. There was no difference in appetitive or consumatory behaviour in the brief access test between the sham-operated and gastric bypass rats. An oral gavage of 1 ml corn oil in gastric bypass rats induced conditioned taste aversion which was also demonstrated after exogenous

administration of the GLP-1 receptor agonist exendin-4 (2 µg/kg intraperitoneal) in unoperated rats.

These findings suggest that an altered food preference may contribute to long-term maintained weight loss after gastric bypass. Postingestive effects resulting in conditioned taste aversion may partially explain this observation.

List of abbreviations (alphabetical)

Ad lib	ad libitum fed
AgRP	Agouti-related peptide
ANOVA	Analysis of Variance
AP	Area postrema
ARC	Arcuate nucleus
BBM	Brush-border membrane
BMI	Body mass index
CART	Cocaine-and amphetamine-regulated transcript
CCK	Cholecystokinine
CD 36	Cluster of Differentiation 36
CRP	C reactive protein
CT	Computer tomography
DRK channels	delayed rectifying potassium channels
EDTA	Ethylenediaminetetraacetic acid
FA	False alarm
fMRI	Functional Magnetic Resonance Imaging
GLP-1	Glucagon like peptide 1
ICT	Integrated chip technology
Kcal	Kilocalories
LiCl	Lithium chloride
mRNA	Messenger Ribonucleic acid
NAcc	Nucleus accumbens
NaCl	Sodium chloride
NPY	Neuropeptide Y
NTS	Nucleus tractus solitarius

OFC	Orbitofrontal Cortex
OLETF rat	Otsuka Long Evans Tokushima Fatty rat
PBN	Pontine parabrachial nucleus
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
POMC	Pro-opiomelanocortin
PYY	Peptide YY
Rpm	rounds per minute
SEM	Standard error of the mean
T1R2	Type 1 Taste Receptor 2
T1R3	Type 1 Taste Receptor 3
VAS	Visual Analogue Scale

Chapter 1: Introduction

The obesity epidemic

Obesity is a currently the largest nutrition related condition affecting not only developed, but increasingly developing countries (World Health Organisation (WHO) 2006). It is a chronic and relapsing disease and is becoming more prevalent in younger patients. Over 400 million people are currently diagnosed as clinically obese with a body mass index (BMI) ≥ 30 kg/m^2 and nearly 1.6 billion are overweight (BMI 25 to 29.9 kg/m^2). Obesity is globally responsible for millions of deaths per year and a huge economic cost to the world health service economy as a result of its associated co-morbidities including type 2 diabetes mellitus, hypertension and hyperlipidaemia. The mechanisms that render a person obese have not yet been fully elucidated. Evidence implicates multiple factors including poor diet, sedentary lifestyle, environmental cues, genetics and disturbed energy balance (James 2008).

Non-surgical treatments modalities

Traditional weight loss strategies involving a healthy diet and increased levels of physical activity are only effective in the short term and deliver up to 7% of body weight loss (Bray 2008). Obesity specialists realized early that the majority of obese patients, especially the ones seeking help, go to extreme efforts to overcome their

disease. They are fighting an evolutionary imposed metabolic pathophysiological process aimed at maintaining weight homeostasis and protecting against the effects of famine (Cummings and Schwartz 2003). The introduction of new anti-obesity medications in the last ten years has been a step in the right direction. However, even the most effective of these drugs offer only 5-10% body weight loss which re-accumulates should the drug be discontinued (Bray 2008;Padwal and Majumdar 2007). Two of the three most successful anti-obesity agents have recently been withdrawn from the market due to serious health and safety concerns: Rimonabant (Accomplia®), an inverse agonist for the cannabinoid receptor CB1, was withdrawn by the European Medicines Agency in January 2009 due to concerns over increased suicidality and depression. Sibutramine (Reductil®), a centrally-acting serotonin-norepinephrine reuptake inhibitor, was also demonetized in Europe secondary to an increased cardiovascular risk. Thus, the only drug currently available in Europe for obesity treatment is the pancreatic lipase inhibitor orlistat. However, its use is limited by the unpleasant adverse effect of anal leakage of oily faeces and the magnitude of weight loss achieved may be insufficient to ameliorate the life-threatening complications of obesity.

Obesity surgery: procedures and weight loss mechanisms

Currently, the most powerful therapeutic modality for obesity is bariatric surgery both in terms of significant body weight loss and long term efficacy (Buchwald et al. 2004;Buchwald et al. 2009). The average weight loss after obesity surgery varies from 15% to 35% depending on the procedure employed (Buchwald, Avidor,

Braunwald, Jensen, Pories, Fahrbach, & Schoelles 2004). It was in the 1950s that these procedures were originally designed and have over the last century undergone numerous technical modifications that have improved both safety and efficacy profiles. Rather unexpectedly, bariatric surgery has been shown to at least ameliorate or even cure Type 2 Diabetes, the metabolic syndrome and have profound effects on the cardiovascular system (Ashrafian et al. 2008;Buchwald, Estok, Fahrbach, Banel, Jensen, Pories, Bantle, & Sledge 2009;Bueter et al. 2009a). These clinically significant changes take place within a few days or weeks post operatively and have lead to the concept of “metabolic surgery”.

The most common obesity surgery operations are gastric banding, Roux-en-Y gastric bypass and biliopancreatic diversion. In gastric banding an expandable silastic band is placed around the proximal stomach creating a gastric pouch. Other variations of this type of surgery include vertical banded gastroplasty in which restriction is achieved with stapling and banding and finally the more recently developed sleeve gastrectomy where a gastric sleeve tube remains after 85% of stomach excision.

The biliopancreatic diversion with or without duodenal switch was designed to limit nutrient absorption. However, with time the gastrointestinal tract (in contact with food) may undergo hypertrophy (Borg et al. 2006) and calorie malabsorption may become less prominent (Pilkington et al. 1976).

The most popular procedure is the Roux-en-Y gastric bypass, an operation some regard as the “gold standard” for the treatment of obesity currently. It incorporates a partial gastrectomy, anastomosis of the stomach pouch to the jejunum and an

entero-entero anastomosis between the excluded biliary alimentary limbs (Olbers et al. 2003).

Patients with a BMI of more than 40 kg/m^2 or with a BMI of more than 35 kg/m^2 and significant co-morbidities may be referred for bariatric surgery based on the most recent international societies/institutes guidelines (i.e. National Institute of Clinical Excellence, National Institutes of Health, The American College of Surgeons, The American Society of Bariatric Surgeons) (The National Institute of Clinical Excellence 2006). These patients should be assessed by an expert multidisciplinary team after behavioral or drug therapies have failed.

The mechanism of weight loss following obesity surgery is still not fully understood. A small stomach may play a role, but changes in eating behavior, decreased appetite and meal frequency (Brown et al. 1982;Halmi et al. 1981;Kenler et al. 1990;Morinigo et al. 2006;Olbers et al. 2006;Sugerman et al. 1987) and paradoxical increases in energy expenditure have been implicated (Bueter et al. 2009b;Stylopoulos et al. 2009). This introduction will concentrate on the most novel weight loss mechanism which postulates alterations in food preference due to changes in taste.

Mechanism of weight loss after bariatric surgery – Gut hormones

Gastric bypass increases postprandial levels of the L-cell hormones glucagon like peptide 1 (GLP-1) and peptide YY (PYY) (le Roux et al. 2006a). These gut hormones are anorexigenic and their administration either peripherally or centrally reduces

hunger and enhances satiation (Baggio and Drucker 2007;Batterham et al. 2003). Studies have demonstrated that postprandial PYY and GLP-1 levels start rising early after the operation and remain elevated for many months after surgery (le Roux et al. 2007). In patients with poor weight loss after gastric bypass, the postprandial PYY and GLP-1 responses are lower compared with patients who lost more weight after gastric bypass (le Roux, Welbourn, Werling, Osborne, Kokkinos, Laurenius, Lonroth, Fandriks, Ghatei, Bloom, & Olbers 2007) supporting the idea of an important role of GLP-1 and PYY in the weight lowering effects of gastric bypass. Moreover, inhibition of the gastrointestinal hormone response with octreotide after gastric bypass increased appetite and food intake (le Roux, Welbourn, Werling, Osborne, Kokkinos, Laurenius, Lonroth, Fandriks, Ghatei, Bloom, & Olbers 2007). The combined effect of having elevated levels of GLP-1 and PYY reduces food intake more than predicted by individual hormone infusions alone (Neary et al. 2005). This combination of gut hormone responses might contribute to the successful weight loss and its maintenance after gastric bypass. Interestingly, recent studies reported increased GLP-1 and PYY levels three months after Sleeve Gastrectomy similarly to gastric bypass (Peterli et al. 2009), but this procedure requires increased study before further insights can be revealed regarding its gut hormonal modulation. In contrast, although an optimally inflated gastric banding also reduces hunger and induces early satiation, changes in appetite are independent of gut hormone changes. Thus, non-hormonal mechanisms have been suggested following gastric banding (Dixon et al. 2005).

Food preferences after obesity surgery

Changes in appetite behavior after obesity surgery were reported in the 1970s. Halmi using structured interviews reported that post gastric bypass patients reached satiety much faster compared to before surgery and the reason for reduced food intake was lack of “desire” (Halmi, Mason, Falk, & Stunkard 1981). More importantly there was a statistically significant reduction in intake for high fat meals and high calorie carbohydrates six months after surgery. At the same time patients found these foods “no longer enjoyable”. In an attempt to explain the changes in high calorie carbohydrate eating, dumping syndrome was implicated even though that was not evaluated further (Halmi, Mason, Falk, & Stunkard 1981).

These findings were replicated by Brown who used food diaries to show that both total fat and carbohydrate intake was significantly lower after gastric bypass. Patients stated that they were “not interested in sweets or deserts after surgery” even though again this was not formally quantified (Brown, Settle, & Van Rij 1982).

Kenler et al were the first to conduct a study comparing gastric bypass and horizontal gastroplasty (Kenler, Brolin, & Cody 1990), as it was recognised very early that the superior weight loss after gastric bypass may be due to changes in taste preference rather than gastric restriction alone (Kenler, Brolin, & Cody 1990). This comparative trial using diet interviews showed that gastric bypass patients consumed 45% less solid sweets and sweet high calorie beverages and 37% less milk or ice cream compared to gastroplasty patients (Kenler, Brolin, & Cody 1990). Milk and ice cream consumption increased postoperatively in the gastroplasty group as these food substances may have been easier to swallow. Dumping syndrome

was suggested (but not proven) to be responsible for the changes in sweet consumption (Kenler, Brolin, & Cody 1990). Some patients reported “losing their taste” for milk and ice cream even without having unpleasant gastrointestinal symptoms. On this basis the authors recommended gastric bypass as a more suitable procedure for sweet and ice cream eaters supporting the findings and recommendations of the Sugerman group (Sugerman, Starkey, & Birkenhauer 1987). Olbers compared patients after gastric bypass and vertical banded gastroplasty in a randomised controlled trial (Olbers, Bjorkman, Lindroos, Maleckas, Lonn, Sjostrom, & Lonroth 2006). The latter group of patients consumed a significantly higher proportion of their total calories as fat and carbohydrates in contrast to the bypass group (Olbers, Bjorkman, Lindroos, Maleckas, Lonn, Sjostrom, & Lonroth 2006). Interestingly post gastric bypass patients preferred fruit and vegetables and consciously avoided fat and reported not feeling well after its consumption, potentially as a result of a dumping phenomenon (Olbers, Bjorkman, Lindroos, Maleckas, Lonn, Sjostrom, & Lonroth 2006).

More recently, Thomas concentrated on the consumption of fatty foods post gastric bypass (Thomas and Marcus 2008). High fat foods were avoided compared to low fat foods after surgery and patients even reported low fat food “intolerance”. Reasons to explain the reduced consumption of high fat foods may include compliance with bariatric dietetic advice or learned behaviours due to negative postingestive or postabsorptive effects. The authors propose that the unexpected low fat food intolerance could potentially be explained by altered intestinal transit and digestion due to changes in texture (Thomas & Marcus 2008).

Based on these findings and the terminology used in the literature of the time (desire, not interested or intolerance) it was realized that obesity surgery and specifically gastric bypass doesn't just reduce the amount that people eat but also changes the perception of food and thus eating behaviour, leading to the concept of behavioral surgery. Is this altered perception a result of changes in taste or post ingestive effects? Can gastric bypass influence fundamental and evolutionary robust physiological circuits like the gustatory system, something that behavioral anti-obesity interventions have not succeeded in achieving? And if so at what level of taste transduction does this manipulation take place – the taste bud, the brain or both? These questions have raised the interest of obesity and behavior researchers in the mid-90s and the last few years and have lead to ground breaking clinical and preclinical experimental work.

Evidence for fat as a distinct taste modality

Taste encompasses the chemical senses of taste and olfaction as well as the oral perception of texture (Drewnowski 1997a). However, it is controversial, whether fat generates a distinct taste quality (Spector and Glendinning 2009). Prior work indicated that fat detection was based on its ability to both alter the tactile food properties and retain food-related odors. There is accumulating evidence, however, supporting the specific involvement of the gustatory system. Mechanisms that have been identified for the initial events in the taste transduction of free fatty acids include the intracellular transport of free fatty acids by the fatty acid transporter CD-36 (Fukuwatari et al. 1997) and an inhibition of delayed rectifying potassium (DRK)

channels (Gilbertson et al. 1997;Gilbertson et al. 1998). The CD36 fatty acid translocater is expressed in murine taste cells, and may serve as a receptor. CD36 is expressed on the apical surface of taste cells (Abumrad 2005). Following interaction of CD36 with fatty acids derived from hydrolysis of triglycerides by lingual lipase, a signal is transduced to nerve fibers, which leads to taste perception and release of bile acids, preparing the digestive system for fat absorption (Abumrad 2005). CD36 binds fatty acids with high affinity and facilitates their transfer into the cell in interaction with other proteins (Laugerette et al. 2005). The CD36 protein is necessary for normal responsiveness to fatty acids at both the cellular and behavioral (i.e. 30 min or 24-hour intake) levels (Gaillard et al. 2008;Laugerette, Passilly-Degrace, Patris, Niot, Febbraio, Montmayeur, & Besnard 2005;Sclafani et al. 2007).

Concomitant with a role for the taste system in nutrient recognition, the specificity of DRK channels in the anterior tongue (fungiform taste buds) is limited to the essential (cis-polyunsaturated) fatty acids (Gilbertson, Fontenot, Liu, Zhang, & Monroe 1997), while the posterior tongue, however, appears to be less specific, and taste cells in the foliate and circumvallate taste buds also responded to the monounsaturated fatty acids, palmitoleic and oleic acid, in a preliminary study (Gilbertson et al. 2005). In one recent study, Gilbertson et al investigated in more detail the role of DRK channels in fat chemoreception in two strains of rats (Gilbertson, Liu, York, & Bray 1998). One strain, the Osborne–Mendel rats may be broadly classified as an obesity-prone, fat-preferring rat while the other, S5B/Pl rats, is obesity-resistant and carbohydrate preferring [16]. Gilbertson et al compared the two strains of rats for their electrophysiological responses in fungiform taste receptor cells to a variety of fatty acids using patch clamp recording, for the ability of fatty acids to alter taste

preference in behavioral assays, and for quantitative expression of DRK channels using quantitative real-time polymerase chain reaction (PCR). It was found that Osborne – Mendel rats exhibited a greater DRK current density and express quantitatively more DRK channels as assayed using quantitative real-time PCR. No differences were found when comparing expression of fatty acid activated two pore domain potassium channels indicating that differences in DRK expression may contribute to the phenotypic differences between Osborne – Mendel and S5B/Pl rats and that these channels may play roles in helping to shape dietary preference and fat intake.

There is also other experimental evidence that rodents and humans can detect long chain fatty acids through oral mechanisms (Chale-Rush et al. 2007;Pittman et al. 2007). In addition, rodents will lick for fats in a concentration-dependent manner (Glendinning et al. 2008).

The sense of Taste – Basic Pathways

Taste encompasses the “chemical senses of taste and olfaction as well as the oral perception of texture” (Drewnowski 1997b). Food preference is influenced by taste with three categories of taste processing (Spector & Glendinning 2009): *Stimulus identification* (sensory) is the detection or discrimination of sensory signals arising from taste cell activation. *Ingestive motivation* (reward) involves processes that promote or discourage ingestion. *Digestive preparation* (physiological changes) refers to feed-forward physiological reflexes that protect oral tissues, aid digestion, and facilitate homeostasis. Behavioural responses to taste stimuli can however also

be influenced by non-gustatory factors, including olfactory, somatosensory, and visceral signals. A comprehensive description of the complex gustatory system in primates is beyond the scope of this chapter. However some basic concepts will be introduced for better understanding of the studies discussed later on.

Taste signals originate from taste receptors located in the mouth and even the small intestine (Jang et al. 2007). The primary taste cortex in the primate anterior insula and adjoining frontal operculum contains not only taste neurons tuned to sweet, salt, bitter, sour, and umami as exemplified by monosodium glutamate (Baylis and Rolls 1991;Rolls et al. 1996;Scott et al. 1986;Yaxley et al. 1990), but also other neurons that encode oral somatosensory stimuli including viscosity, fat texture, temperature and capsaicin (Verhagen et al. 2004). Some neurons in the primary taste cortex respond to particular combinations of taste and oral texture stimuli, but do not respond to olfactory stimuli or visual stimuli such as the sight of food (Verhagen, Kadohisa, & Rolls 2004). Neurons in the primary taste cortex do not represent the reward value of taste, that is, the appetite for a food, in that their firing is not decreased to zero by feeding the taste to satiety (Rolls, Critchley, Wakeman, & Mason 1996;Yaxley et al. 1988).

A secondary cortical taste area in primates was discovered in the caudolateral orbitofrontal cortex (OFC), extending several millimetre in front of the primary taste cortex (Rolls et al. 1990). Neurons in this region respond not only to each of the four classical prototypical tastes sweet, salt, bitter and sour, (Rolls 1997) but also there are many neurons that respond best to umami tastants such as glutamate (which is present in many natural foods such as tomatoes, mushrooms and milk) (Baylis & Rolls 1991) and inosine monophosphate (which is present in meat and some fish

such as tuna) (Rolls, Critchley, Wakeman, & Mason 1996). This evidence, taken together with the identification of glutamate taste receptors (Maruyama et al. 2006;Zhao et al. 2003), leads to the view that there are five prototypical types of taste information channels, with umami contributing, often in combination with corresponding olfactory inputs (McCabe and Rolls 2007;Rolls et al. 1998;Rolls 2009), to the flavour of protein. In addition, other neurons respond to water, and others to somatosensory stimuli including astringency as exemplified by tannic acid (Critchley and Rolls 1996), and capsaicin (Rolls et al. 2003). Taste responses are found in a large mediolateral extent of the OFC (Critchley & Rolls 1996;Kadohisa et al. 2004;Rolls 2008). Texture of fatty foods activates neurons in the OFC, which also receive inputs from the other chemical senses and taste specific neurons. Therefore differences in the “sweetness” and odour of an ice cream for example can influence the activation of the neurons involved with fat perception (Rolls 2007;Spector 2010).

These mechanisms have been studied by using advanced techniques such as functional magnetic resonance imaging (fMRI) and positron emission tomography. Using fMRI, changes in region of interest neuronal activity can be measured through alterations in blood oxygen dependent signal which reflects concentrations of deoxyhaemoglobin a recognised paramagnetic contrast agent (Tataranni and DelParigi 2003).

Functional taste domains

Taste function can be experimentally classified into at least three general domains (Spector & Glendinning 2009):

Stimulus identification is the detection or discrimination of sensory signals arising from taste cell activation in the oral cavity. Stimulus identification involves the discrimination between the sensory signals representing different taste stimuli arising from the interactions of chemical compounds with taste receptors.

Ingestive motivation refers to processes that promote or discourage ingestion of foods and fluids on the basis of taste input. Ingestive motivation can be further divided into two functional subclasses: Appetitive behavior ("wanting") can be defined as actions that lead to contact with the taste stimulus (e.g. searching, foraging, approach to a drinking spout) and reflects how much the stimulus is wanted. Consummatory behavior ("liking") represents the behavior that is elicited during the contact with the taste stimulus (e.g. oral motor responses, swallowing) and reflects how much the stimulus is liked.

Digestive preparation refers to physiological reflexes that fall into a general class referred to as cephalic phase responses, which are internal physiological events triggered by stimulus contact with any sensory receptor of the head (Powley 1977). Cephalic phase responses generally prepare to digest, absorb and then store nutrients that enter the body through feeding. Cephalic phase reflexes can be both intrinsic and learned. For example, most animals readily learn to avoid foods that

render them ill through conditioned taste preferences and conditioned taste aversions and rats can be conditioned to react aversively to sweet solutions by rendering them ill after ingestion. Consequently, they will decrease their intake of the sweet solutions when they are exposed to them again. The vagus nerve is thought to be an important pathway for cephalic phase responses (Rozin 1976).

General procedural requirements in the assessment of taste function

For taste function assessment it is important to circumvent the influence of postingestive factors in both animals and humans. Postingestive effects can be positive (e.g. satiation, fullness) or negative (nausea, visceral pain) and occur after food ingestion. They also include postabsorptive effects. Hence, two important methodological features must be considered for the experimental protocols: First, only small volumes of taste solutions must be used. Second, immediate responses to the taste stimulus should be measured. For animal experiments, the application of these methodological features requires the use of a special stimulus delivery system and a lickometer (Spector 2003).

Procedures for assessing taste function in animals

Animal psychophysical procedures primarily measure sensory and discriminative (Stimulus identification) or hedonic (Ingestive motivation) taste functions. Various procedures have been designed to assess taste sensitivity and discrimination

independent of the motivational properties of the taste stimuli themselves. Most techniques are based on the principles of operant conditioning procedures. Here, a taste stimulus signals another event independent of its affective valence as the motivation to respond is usually provided by an imposed schedule of food or water restriction. This motivates the animal to respond correctly to obtain a food or water reinforcement. Some investigators have used a two-alternative, forced-choice procedure in which two-bottles, one containing a taste solution and the other containing water, were presented, and if the water-deprived animal licked from the "incorrect" solution, it received an electric shock (HARRIMAN and MACLEOD 1953). Others used a yes/no procedure in which a single stimulus is presented on a trial and the animal indicates whether the solution contains the relevant taste stimulus (Morrison and Norrison 1966). Here, food-deprived rats were trained to press a lever if the stimulus was a taste compound and another lever if the stimulus was water; correct responses were rewarded with a food pellet. For my experiments, a two-response operant taste discrimination procedure was used, in which water-deprived rats were trained and tested in a specially designed computer-controlled gustometer as decribed below (Eylam and Spector 2002;Spector et al. 1990).

The hedonic taste responsiveness of animals can be effectively evaluated with a brief-access test. Here, very small samples of a taste stimulus are presented for very brief duration (e.g. 10 sec) and the animal's unconditioned licking responses are measured (Smith 2001;Spector & Glendinning 2009). Using Davis-Rig Lickometers (Davis MS-160, DiLog Instruments, Tallahassee, Florida, USA), this test allows minimizing any postingestive effects of any substance tested as only small amounts are ingested. Several concentrations can be presented in a random order and a

concentration-response function can be derived in a single session. The brief access test measures both components of the hedonic motivation to ingest a specific stimulus: Firstly, the animal's approach and contact with the stimulus which is known as the appetitive component ("Wanting" – e.g. how often does the rat go to the spout and starts drinking?) and secondly, the oral motor responses generated once the stimulus is ingested which is called the consummatory component ("Liking" – e.g. how fast does the rat lick?).

Procedures for assessing taste function in humans

So far, most studies in humans have assessed food preference after bariatric surgery using either survey or scaling methods. These methods are subject to the inaccuracy of verbal report and may not reflect the actual "affective" value of the stimulus. To the best of our knowledge, psychophysical methods as described previously have not been imposed for human studies yet; at least not in the context of bariatric surgery.

Bariatric surgery and taste function

Stimulus identification after bariatric surgery

So far, only the effects of bariatric surgery on sweet, but not on fat taste sensitivity have been studied. Although different methodologies were used, most studies

demonstrated a selectively decreased sweet taste detection threshold after gastric bypass (Burge et al. 1995;Scruggs et al. 1994) suggesting an altered sweet taste preference after the operation. However, there might also be an increased fat detection sensitivity after gastric bypass potentially explaining a reduced preference for high fat foods in patients after gastric bypass as it may translate into a more intense sensation and result in reduced consumption of high calorie food. However, taste detection thresholds are only one basic aspect of taste function in general and have been shown to vary as a function of genetics, pharmacological treatment, and neural manipulations (Spector 2003) . It also remains unclear how differences in fat taste sensitivity correlate with suprathreshold sensitivity and whether they accurately reflect the hedonic evaluation of higher concentrations of fat stimuli (Bartoshuk 1978).

Ingestive motivation and obesity

The concepts of taste hedonics and alterations in reward responses have not been fully explored as a potential mechanism for the development of obesity. Research into eating control has recently focused on the role of dopamine in reward-based behaviors. Obese individuals rate fatty food more rewarding and work harder for the delivery of more food (Epstein et al. 2007). The reward-related dopamine release in mesolimbic brain structures caused by the ingestion of highly palatable foods can also be elicited by cues that predict food availability in the absence of actual food stimuli (Stoeckel et al. 2008). Furthermore, the central reward pathways of obese subjects show greater activation in functional magnetic resonance imaging when

presented with pictures of high calorie foods compared to lean controls (Wang et al. 2001). Remarkably, the density of dopamine type 2 receptors in mesolimbic brain areas has been found to be significantly reduced in severly obese patients (Volkow et al. 2002). Thus, there might be a decreased reward sensing in obese patients leading to a compensatory behavior of increased food and in particular fat intake. In other words, some forms of obesity may partly result from overeating in an attempt to compensate for reduced dopaminergic activity in the mesolimbic system. This state might be similar to that of a reward deficiency syndrome (Corwin and Grigson 2009) as the same mesolimbic brain areas are activated by recreational drugs. Some authors have suggested obesity as an addiction state (Steele et al. 2010).

Ingestive motivation after bariatric surgery

The effect of bariatric surgery on complex central reward circuits has not been fully elucidated yet. Two recent studies investigated the dopamine type 2 receptor density in reward-processing brain areas after gastric bypass using positron-emission computed tomography, but findings were controversial: Steele et al described an increased dopamine type 2 receptor availability in the brain following RYGB (Dunn et al. 2010), while Dunn et al found the opposite (le Roux, Aylwin, Batterham, Borg, Coyle, Prasad, Shurey, Ghatei, Patel, & Bloom 2006a).

Digestive preparation after bariatric surgery

The effect of bariatric surgery on cephalic phase reflexes has not been studied.

Taste and gut hormones

Metabolic mechanisms may facilitate the effect of gastric bypass on taste pathways. As described earlier, GLP-1 and PYY are gut hormones co-secreted by the L cells in response to a meal, and their postprandial levels are increased after gastric bypass (Baggio & Drucker 2007;Batterham, Cohen, Ellis, le Roux, Withers, Frost, Ghatei, & Bloom 2003). In addition to their anorexigenic functions (Batterham et al. 2007), high PYY levels activate brain regions related to food reward including the ventral striatum, OFC and insular cortex (Grill et al. 2007), and GLP-1 receptors have been isolated in brain reward areas (Shin et al. 2008). GLP-1 and its receptor have also been isolated from taste cells of taste buds and adjacent intragemnal afferent fibres respectively, interacting in a paracrine manner (Shin, Martin, Golden, Dotson, Maudsley, Kim, Jang, Mattson, Drucker, Egan, & Munger 2008). Importantly, mice lacking the GLP-1 receptor show decreased behavioral responsiveness to sucrose (Kokrashvili et al. 2009b). Furthermore, recognition and transduction of sweet-tasting compounds has been shown to involve in part α-gustducin and the sugar binding receptor subunit T1R3 (Jang, Kokrashvili, Theodorakis, Carlson, Kim, Zhou, Kim, Xu, Chan, Juhaszova, Bernier, Mosinger, Margolskee, & Egan 2007). These molecules also partly mediate the glucose-dependent GLP-1 secretion from enteroendocrine L cells of the gut (Jang, Kokrashvili, Theodorakis, Carlson, Kim, Zhou, Kim, Xu, Chan,

Juhaszova, Bernier, Mosinger, Margolskee, & Egan 2007). The molecular similarities between enteroendocrine L cells of the gut and taste receptor cells of the oral cavity suggest that GLP-1 could play a role in gustatory function (Halmi, Mason, Falk, & Stunkard 1981). For example, stimulation of sweet taste receptors might lead to GLP-1 release which then in turn may act to enhance or maintain sweet taste sensitivity through a positive feedback mechanism. Thus, GLP-1 and PYY input to the taste signal pathways at multiple levels and sufficiently high plasma levels of both hormones may affect peripheral and central taste signalling after gastric bypass. The reductions in preference for sucrose and potentially for high fat food reported after gastric bypass are consistent with this possibility.

Rationale for use of a Roux-en-Y gastric bypass rat model

It has been previously reported that subjective taste changes occur after gastric bypass surgery (Brolin et al. 1994;Brown, Settle, & Van Rij 1982;Olbers, Bjorkman, Lindroos, Maleckas, Lonn, Sjostrom, & Lonroth 2006). The proportion to which these changes are psychologically versus physiologically based is unknown. The realm of psychological issues and pathology contributing to obesity is vast. However, if the physiological basis of taste changes could be defined, this could be the target of new treatments to pharmacologically alter food taste making it less appealing.

To study the physiologic aspects of taste change in the absence of individual psychological issues requires an animal model. Although little is known about the mechanisms underlying taste change after gastric bypass in humans, it is clear that an animal model providing uniform eating experiences and removing the

environmental background associated with human weight loss could be helpful in determining some of these mechanisms.

It is therefore important that the rat gastric bypass model is physiologically as close as possible to human bypass patients. This includes not only technical aspects (e.g. size of the gastric pouch), but also physiological (e.g. postprandial levels of gastrointestinal hormones such as GLP-1 and PYY) and behavioral aspects (e.g. reduced preference of caloric dense food).

Surgical technique of Roux-en-Y gastric bypass in rats

Rats were food deprived for 12 hours overnight, but water was available ad libitum. Before surgery, rats were weighed, and then anesthetized with isofluorane (4% for induction, 3% for maintenance). Preoperatively, gentamicin 8 mg/kg and carprofen 0.01 ml were administered intraperitoneally (ip) as prophylaxis for postoperative infection and pain relief. Surgery was performed on a heating pad to avoid decrease of body temperature during the procedure. Prior to a midline laparotomy, the abdomen was shaved and disinfected with surgical scrub. In the sham group a 7 mm gastrotomy on the anterior wall of the stomach with subsequent closure (interrupted prolene 5-0 sutures) and a 7 mm jejunotomy with subsequent closure (running prolene 6-0 suture) was performed. In the gastric bypass group, the proximal jejunum was divided 15 cm distal to the pylorus to create a biliopancreatic limb. After identification of the caecum, the ileum was then followed proximally to create a common channel of 25 cm. Here, a 7 mm side-to-side Jejuno-Jejunostomy (running prolene 7-0 suture) between the biliopancreatic limb and the common channel was

performed. Figure 1 shows a schematic illustration of the pre- and postoperative anatomy.

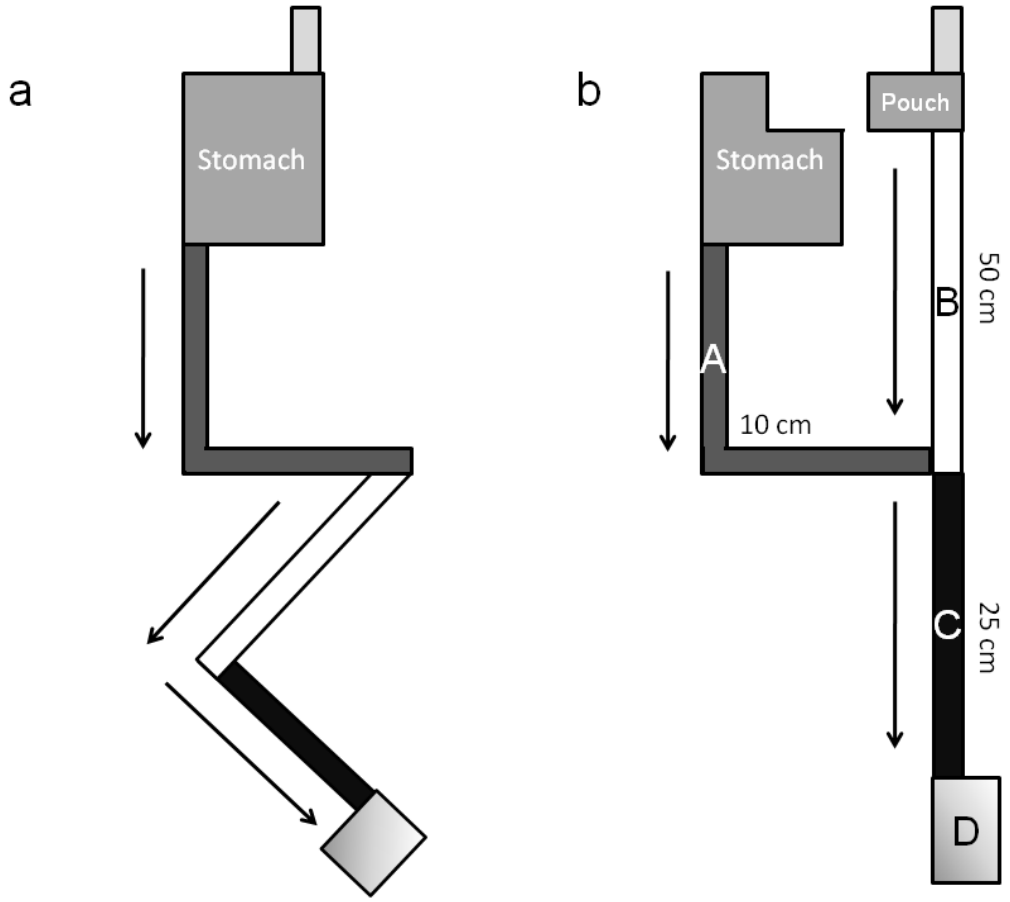


Figure 1: Diagrammatic representation of the gastrointestinal anatomy before (a) and after (b) the gastric bypass operation.(A) Biliopancreatic limb (~ 10 cm), (B) Alimentary limb (~50 cm), (C) Common channel (~25 cm), (D) Caecum.

Chapter 2: Role of the vagus for body weight loss in a rodent model of Roux-en-Y Gastric Bypass

Introduction

Traditionally, the vagal nerve is thought to have an important role in the regulation of food intake and body weight, but only a few reports examined whether vagal preservation is effective or necessary in weight control after bariatric surgery (Perathoner et al. 2009;Sundbom et al. 2007;Wang and Liu 2009). There is considerable controversy and confusion about the relative importance of the nerve as gut hormones released from enteroendocrine cells in the distal ileum like Glucagon-like peptide (GLP-1) and peptide tyrosine-tyrosine (PYY) can inform the brain either through the circulation or via afferent vagal fibres or both (Berthoud 2008). Often various routes are described for same physiological effects, particularly for food intake (Berthoud 2008).

In this chapter, I describe variations in the technique for gastric bypass surgery in rats in the area of the gastro-jejunostomy. Here, the para-esophageal bundle can be found which contains the left gastric vessels and the dorsal vagal trunk that consists of about 4/5 right vagal fibres and about 1/5 left vagal fibres (Niederhausern W.v. 1953). The aim of this study was to assess whether preservation of the vagal fibres in the para-oesophageal bundle has an impact on body weight and food intake after gastric bypass in rats.

Material and Methods

Animals

Male Wistar rats used were individually housed under a 12-hour / 12-hour light-dark cycle and at a room temperature of 21 ± 2 ºC. Water and standard chow were available ad libitum, unless otherwise stated. All experiments were approved by the Veterinary Office of the Canton Zurich, Switzerland. Experiments were performed at the Institute of Veterinary Physiology and Zürich Centre for Integrative Human Physiology, Vetsuisse Faculty University of Zurich in Zurich, Switzerland. Body weight and food intake were measured daily in study 1 and 2 for a postoperative period of 60 days and in study 3 for 75 days.

Surgery

Gastric bypass surgery was performed as described on page 32. Two different techniques were used to handle the vagal fibres in the para-oesophageal bundle in the area of the gastric pouch. All groups were operated in chronological order. In a first group, 25 rats (Body weight 348 ± 3.9g) were randomized for gastric bypass (n = 17) or sham operation (n = 8). In this group the vagal fibres were not preserved in the gastric bypass rats as the para-esophageal neurovascular bundle was completely ligated (Study 1). In a subsequent group, 18 rats (332 ± 2.4g) were randomized to gastric bypass (n = 10) or sham operation (n = 8). Here, the vagal fibres were preserved as the left gastric vessels were separated and selectively ligated in all gastric bypass rats (Study 2). Significant differences in body weight and

energy intake were observed in these two groups. As it was unclear whether these differences were related to the different techniques of vagal preservation, a third group (Study 3) of 39 rats (471 ± 4.3g) was randomized for gastric bypass without vagal preservation (n = 14) or gastric bypass with vagal preservation (n = 14) or sham operation (n = 11). Figure 2 gives a schematic illustration of the different techniques used.

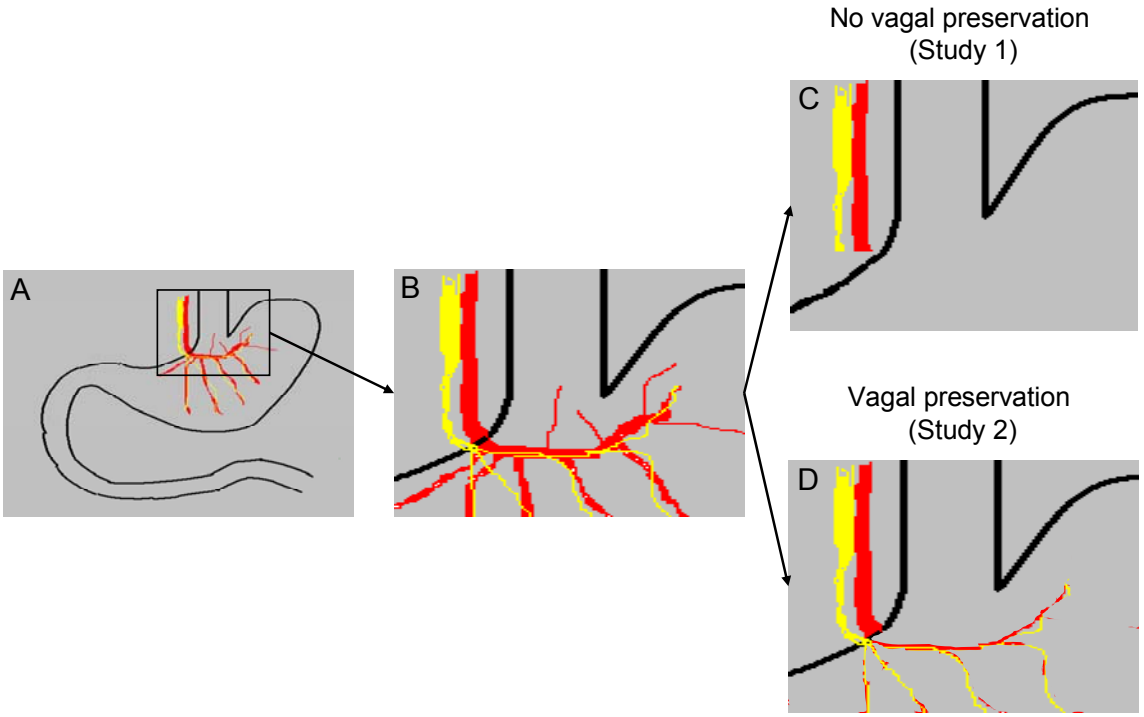


Figure 2: Schematic illustration of the two different techniques to handle the vagal fibres in the para-oesophageal neurovascular bundle in the area of the gastric pouch with vagal fibres (yellow) and the left gastric vessel (red). (A) preoperative anatomy, (B) magnification, (C) complete ligation of the para-oesophageal bundle with no preservation of the vagal fibres and (D) selective ligation of the left gastric vessel with preservation of the vagal fibres.

Hormone assay

Animals from study 3 were fasted for 12 hours from the beginning of the light cycle on postoperative day 50. At the onset of the dark cycle animals were offered 5g of standard chow all of which was consumed within half an hour by the animals. Approximately 200 µl of blood was obtained by puncture of a sublingual vein under brief isoflurane anesthesia from sham-operated controls, gastric bypass with and without vagal preservation (each n=6). Blood was collected into EDTA-rinsed tubes and, immediately centrifuged at 3000 rpm for 10 min at 4°C. The supernatant was stored at -80°C until further analysis. Concentrations of active GLP-1 and PYY were analyzed using a rat endocrine lincoplex kit (RENDO-85K, Labodia SA, Yens, Switzerland).

Measurement of size of the gastrojejunostomy

To exclude that the differences in body weight between bypass rats were due to different levels of restriction and subsequent differences in food intake, sizes of the gastro-jejunostomy were measured during necropsy in all gastric bypass rats of study 3.

CRP analysis

Blood was obtained from all animals of study 3 by puncture of a sublingual vein under brief isoflurane anesthesia. Blood was collected into EDTA-rinsed tubes and

immediately centrifuged at 3000 rpm for 10 min at 4°C. Plasma was stored at -80°C before analysis for C-reactive protein (Abbott, UK) to assess inflammation.

Faecal analysis

To evaluate nutrient malabsorption, faeces were collected over 24-hours on postoperative days 15 and 59 from all animals in study 3. Faeces were dried in an oven and weighed; calorie content was measured using a ballistic bomb calorimeter (Jackson et al. 1977).

Statistics

All data were normally distributed and are expressed as mean ± SEM. Student's t-test for independent samples and one-way ANOVA with repeated measures and post-hoc Bonferroni test for each time point were used to test for significant differences. $P < 0.05$ was considered significant.

Results

Mortality

Overall surgical mortality was 13.4% (11/82). Gastric bypass-related mortality was 14.5% (8/55), while mortality after sham-operation was 11.1% (3/27, p=0.67). There was no mortality difference between bypass rats with complete ligation and with preservation of the para-esophageal bundle. All eight bypass rats showed signs of respiratory distress along with hypersalivation and dysphagia within the first two postoperative days after the operation and were euthanized immediately after onset of symptoms. Necropsy revealed that these symptoms originated at the level of the gastro-jejunostomy where food did not pass through and was retained in the oesophagus. Whether this was due to inflammatory swelling following anastomotic leakage or due to anastomotic constriction remains unclear. The three sham-operated rats died without prior noticeable symptoms. Necropsy revealed in two cases a small bowel ileus presumably due to a volvulus after inappropriate repositioning of the viscera into the abdominal cavity at the end of the operation. In one case a leak at the site of the gastrotomy was found.

Energy intake

In study 1 there was no difference in average daily energy intake between gastric bypass rats and sham-operated rats over a period of 60 days (sham: 97.4±2.5 kcal vs. bypass: 89.3±4.7 kcal, p=0.30). In contrast, gastric bypass rats of study 2 ate significantly less than the sham-operated rats (sham: 76.7±2.2 kcal vs. bypass

52.5±4.8 kcal, p<0.001). In study 3, there was no difference in average energy intake between bypass rats without vagal preservation and sham-operated rats over a period of 75 days, while bypass rats with vagal preservation ate significantly less than sham-operated rats and rats without vagal preservation (sham: 118.7±3.9 kcal vs. Bypass with vagal preservation: 84.4±3.3 kcal vs. Bypass without vagal preservation: 102.8±7.5 kcal, p<0.001). The average daily energy intake is shown for all three groups in figure 3.

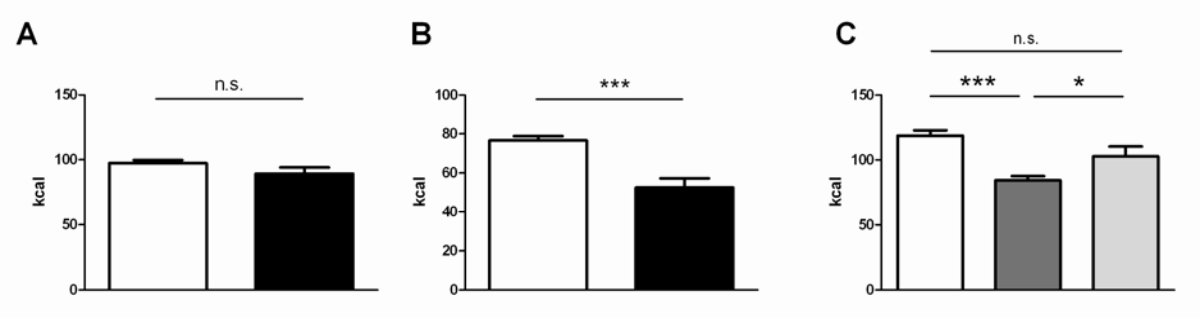


Figure 3: Average daily energy intake during study 1 (A) over 60 days for sham-operated ad libitum fed rats (n=7, white column) and for gastric bypass rats (n=14, black column). Average daily energy intake during study 2 (B) over 60 days for sham-operated ad libitum fed rats (n=8, white column) and for gastric bypass rats (n=8, black column). Average daily energy intake of study 3 (C) over 75 days for sham-operated ad libitum fed rats (n=10, white column) and for gastric bypass rats with vagal preservation (n=11, dark grey) or without vagal preservation (n=10, light grey). All data are shown as mean values ± SEM. Post-hoc differences between the three groups are indicated (*** = p<0.001 and * = p<0.05).

Body weight

In all three studies gastric bypass rats had a significant lower body weight than sham-operated rats from day 5 after surgery throughout the rest of the observation period. After a short period of post surgical weight loss, sham-operated rats in all three studies constantly gained weight for the rest of the study. In study 1 gastric bypass rats started to regain weight around postoperative day 25 and there was no

difference between their body weight before surgery and after surgery at the end of the observation period (day 0: 457.0±7.4 g vs. day 60: 468.0±9.3 g, p=0.36). In study 2, gastric bypass animals lost about 20% of their preoperative weight by postoperative day 25 and their body weight then plateaued around 260 g (day 0: 330.8±5.8 g vs. day 60: 259.1±16.3 g, p=0.001). In study 3, there was no difference in body weight between bypass rats without vagal preservation and bypass rats with vagal preservation until postoperative day 40 (day 40: bypass with vagal preservation: 408.3±11.2 g vs. bypass without vagal preservation: 414.4±11.2 g, p=0.70). However, thereafter bypass rats without vagal preservation started to regain weight for the rest of the observation period, while bypass rats with preserved vagal fibres maintained their low body weight (day 75: bypass with selective ligation: 365.8±14.6 g vs. bypass with complete ligation: 468.0±9.3 g, p<0.001). The development of body weight after surgery is shown for all groups in figures 4.

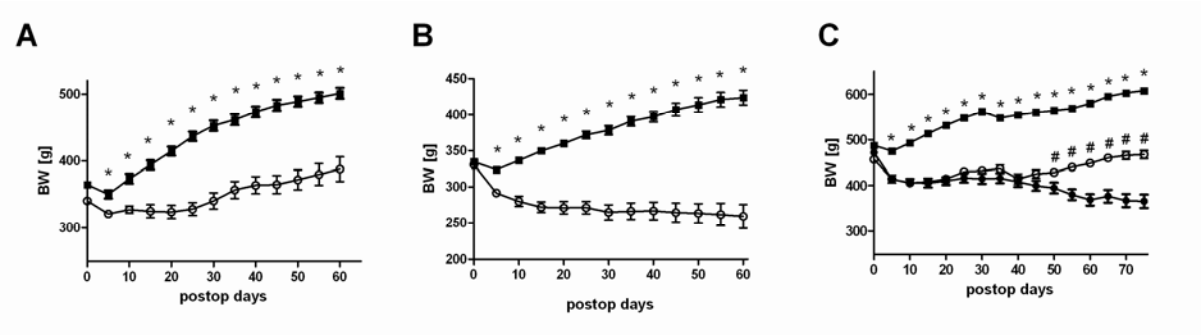


Figure 4: Body weight change in study 1 (A) for the gastric bypass (-o-) (n=14) and sham-operated rats (-■-)(n=7), in study 2 (B) for the gastric bypass (-o-) (n=8) and sham-operated rats (-■-)(n=8) and in study 3 (C) for the gastric bypass rats without vagal preservation (-o-) (n=10) and gastric bypass rats with vagal preservation (-●-) (n=11) and sham-operated rats (-■-)(n=10). Data are shown as mean values ± SEM (* = p<0.05 for sham vs. bypass; # = p<0.05 for bypass without vagal preservation vs. bypass with vagal preservation).

Postprandial plasma levels of PYY and active GLP-1

One-way ANOVA revealed significant differences for levels of PYY and active GLP-1 after gastric bypass with and without vagal preservation in comparison to sham-operated controls of study 3 (PYY: sham: 29.5±7.1 pg/ml vs. bypass with vagal preservation: 70.4±8.8 pg/ml vs. bypass without vagal preservation: 83.2±14.3 pg/ml, $p<0.01$; GLP-1: sham: 85.8±2.1 pg/ml vs. bypass with vagal preservation: 146.9±23.7 pg/ml vs. bypass without vagal preservation: 155.4±24.1 pg/ml, $p<0.05$). However, post-hoc Bonferroni testing showed no significant difference for PYY and GLP-1 levels between gastric bypass rats with or without vagal preservation (figure 5).

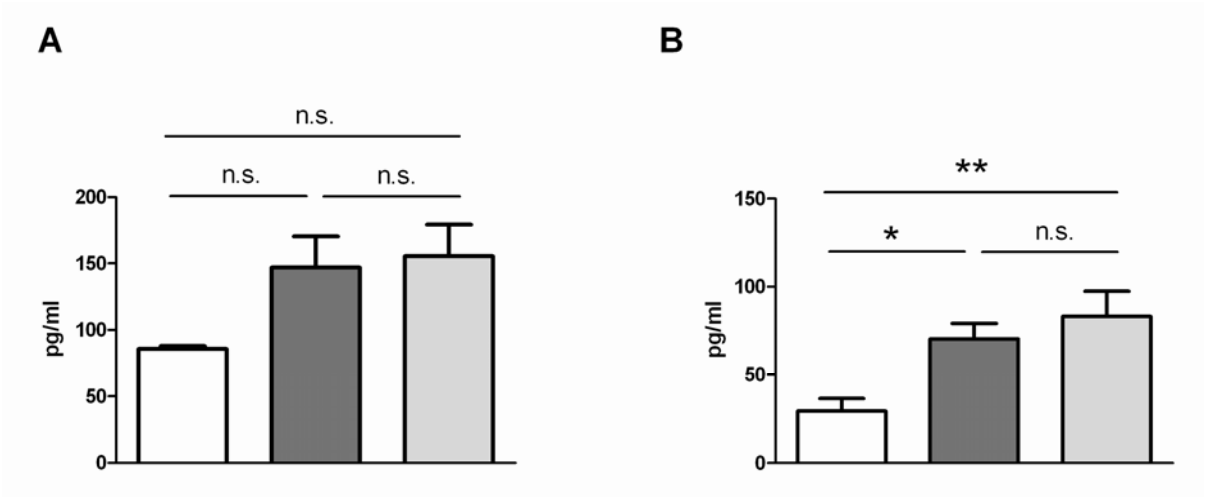


Figure 5: Levels of active GLP-1 (A) and PYY (B) for sham-operated ad libitum fed rats (n=6, white column) and for gastric bypass rats with vagal preservation (n=6, dark grey) or without vagal preservation (n=6, light grey). Data are shown as mean values ± SEM. Post-hoc differences between the three groups are indicated (** = $p<0.01$ and * = $p<0.05$).

Size of the gastro-jejunostomy

There was no gastrogastric fistula in any of the gastric bypass rats of study 3. The overall size of the gastro-jejunostomy in all gastric bypass rats was 15.4±0.4 mm. There was no difference in size of the anastomosis between rats in which the complete para-esophageal bundle was ligated and rats in which the left gastric vessels were separated and selectively ligated (bypass with selective ligation: 15.2±0.4 mm vs. 15.6±0.7 mm, p=0.69).

CRP analysis

C-reactive protein levels were below 2mg/L in all animals of study 3 indicating that there was no postsurgical infection or inflammation 28 days after surgery.

Faecal analysis

There was no increase in either fresh faecal mass (sham: 8.4±0.5 g vs. Bypass with vagal preservation: 7.5±0.6 g vs. Bypass without vagal preservation: 7.2±0.6 g, p=0.31) or faecal calorie content (sham: 3.56±0.04 kcal vs. Bypass with vagal preservation: 3.43±0.05 kcal vs. Bypass without vagal preservation: 3.65±0.06 kcal, p=0.24) in the gastric bypass animals compared to the sham-operated rats in study 3.

Discussion

In this study body weight and food intake after gastric bypass were related to whether the vagal fibres within the para-oesophageal bundle were preserved or not while there were no differences in levels of GLP-1 and PYY between these two groups. This finding highlights the important role of the vagal nerve for mediating the inhibitory effects of gut hormones such as PYY and GLP-1 on food intake and body weight after gastric bypass surgery in rats. It is further supported by previous reports describing that ablation of the vagal-brainstem-hypothalamic pathway attenuates the inhibitory effects of PYY and GLP-1 on food intake (Abbott et al. 2005). This is an important observation as only a few reports examined whether vagal preservation is effective or necessary in weight control after bariatric surgery (Perathoner, Weiss, Santner, Brandacher, Laimer, Holler, Aigner, & Klaus 2009;Sundbom, Holdstock, Engstrom, & Karlsson 2007;Wang & Liu 2009).

The size of the gastric pouch and the lengths of the different limbs used in this study have been proven to effectively induce weight loss (Bueter et al. 2009c). An increasing body of evidence in humans indicates that up to certain limits the size of the gastric pouch and length of the different limbs is of less importance for the outcome of gastric bypass (Muller et al. 2008). In support of this observation, I demonstrated that the level of restriction measured by the size of the gastro-jejunostomy has no impact on different levels of weight loss and food intake after gastric bypass in rats.

In conclusion, my gastric bypass technique induces reliable weight loss in rats with an acceptable mortality. I propose that vagal nerve fibres should be preserved during gastric bypass in rats. Restriction at the gastro-jejunal anastomosis does not seem to

be critical for the weight loss. Although the mechanisms have not yet been fully elucidated, vagal preservation may play an important role in inducing and maintaining weight loss after gastric bypass in humans and rats.

Chapter 3: Changes in energy expenditure after Roux-en-Y Gastric Bypass in rats

Introduction

One of the proposed mechanisms for reduced food intake after bypass surgery is the secretory stimulus to L-cells in the distal gut, resulting in increased levels of gastrointestinal satiation hormones such as peptide YY (PYY) and peptides of the enteroglucagon family (Borg, le Roux, Ghatei, Bloom, Patel, & Aylwin 2006;Korner et al. 2005;le Roux, Welbourn, Werling, Osborne, Kokkinos, Laurenius, Lonroth, Fandriks, Ghatei, Bloom, & Olbers 2007;Nadreau et al. 2006;Rubino 2008). These hormones stimulate anorectic pathways in the hypothalamus and brainstem leading to reduced food intake (Murphy and Bloom 2006) and may also influence energy expenditure (Badman and Flier 2005).

Gastric bypass surgery has been successfully modeled in rat experiments. The body weight loss after gastric bypass in rats is not only due to decreased food intake, as sham-operated pair-fed controls weigh more than gastric bypass rats (Guijarro et al. 2008;le Roux, Aylwin, Batterham, Borg, Coyle, Prasad, Shurey, Ghatei, Patel, & Bloom 2006a;Nadreau, Baraboi, Samson, Blouin, Hould, Marceau, Biron, & Richard 2006;Sclafani et al. 1978;Sclafani 1987). Possible explanations such as malabsorption and inflammation have been excluded (le Roux, Aylwin, Batterham, Borg, Coyle, Prasad, Shurey, Ghatei, Patel, & Bloom 2006a), thus the weight difference despite similar food intake raises the possibility of enhanced energy expenditure (le Roux, Aylwin, Batterham, Borg, Coyle, Prasad, Shurey, Ghatei, Patel, & Bloom 2006a) as previously speculated (de Castro et al. 2008;Furnes et al.

2008). I therefore tested the hypothesis that energy expenditure would be higher after bypass surgery.

Material and Methods

Animals and housing

Thirty adult diet-induced obese male Wistar rats weighing 480 – 500 g were used for energy expenditure experiments, and sixteen adult male Wistar rats weighing 330-350 g were used for morphometric gut analysis. All animals were individually housed under artificial 12 hour / 12 hour light-dark cycle and at a room temperature of 21±2ºC unless otherwise stated. Water and standard chow were available ad libitum. All experiments were approved by the Veterinary Office of the Canton Zurich, Switzerland. Experiments were performed at the Institute of Veterinary Physiology and Zürich Centre for Integrative Human Physiology, Vetsuisse Faculty University of Zurich in Zurich, Switzerland.

Surgery

Surgery was performed according to an established protocol with preservation of the vagal fibres in the para-oesophageal bundle as previously described (page 35).

Indirect calorimetry

Rats were individually housed in Plexiglas air-tight metabolic cages (41x41x31 cm) on a layer of wood shavings under the same light and temperature conditions as described above. Water and standard powder chow (GLP3433, Provimi Kliba Ag, Switzerland) were available ad libitum, unless otherwise stated. Food intake and

water intake were measured continuously. Physical activity was monitored by a 3-dimensional array of infrared light beams and sensors. Thus, the activity data provided represent a relative measure of locomotor activity of the rats. The activity data do not relate to an absolute measurement of distance moved or to a spatial position. Measurements were conducted in an open circuit calorimetry system (AccuScan Inc., USA) (Wielinga et al. 2007). Energy expenditure was calculated for each 2 min sample according to Weir (WEIR 1949) using the following equitation: total energy expenditure (kcal/h) = $3.9xV(O_2)L/h+1.1xV(CO_2)L/h$. The respiratory quotient was defined as the quotient of CO_2 production and O_2 consumption.

Experimental design

The thirty diet-induced obese rats used in the energy expenditure experiments were randomized to gastric bypass (n=14) or sham operation (n=16). After a recovery period of 7 days sham-operated animals were randomly divided into two groups of 8 rats each: shams with no dietary manipulation (ad libitum fed shams weighing 488.8±3.9 g) and food-restricted shams whose postoperative weight was matched to the weight of bypass animals (body weight-matched shams weighing 474.3±4.2 g). Starting on day 7 after gastric bypass surgery, the body weight-matched shams received as much food daily as was necessary for them to maintain a similar body weight to the bypass rats. Based on experiences from previous studies, rats were given 10 g of standard chow in the beginning of food restriction. This amount of food was offered at dark onset and readjusted every third day depending on the body weight. Sixteen metabolic cages were used and measurements were conducted in the following order on three consecutive days: bypass (n=8) vs. sham ad libitum fed

(n=8) (40 days after surgery) and bypass (n=6) vs. shams body weight-matched (n=8) (75 days after surgery). Diet-induced thermogenesis was measured in rats that were fasted for 12 hour from the beginning of the light cycle and received a 5 g meal at subsequent dark onset. Diet-induced thermogenesis was calculated as the cumulative increase in energy expenditure after a 5 g test meal compared to fasting values before the test meal (expressed as percentage of the energy content of the test meal: 17.6 kcal).

Faecal analysis

To evaluate nutrient absorption, faeces were collected over 24 h on postoperative days 15 and 59 from all animals. Faeces were dried in an oven and weighed; calorie content was measured using a ballistic bomb calorimeter (Jackson, Davis, & Macdonald 1977).

Blood analysis

Blood was obtained by puncture of a sublingual vein under brief isoflurane anesthesia on postoperative day 80. Approximately 200 µl blood was collected into EDTA-rinsed tubes and immediately centrifuged at 3000 rpm for 10 min at 4°C. Plasma was stored at -80°C before analysis. Measurements of C-reactive protein (Abbott, UK) were made to assess inflammation.

Measurement of Body composition

Adipose tissue mass was measured using a rodent CT scanner (Latheta, Aloka, Japan). Rats were anesthetized with isoflurane and the area between vertebrae L1 and L5 was scanned using an X-ray source tube voltage of 50 kV, current of 1 mA, pitch size of 2 mm, and a speed of 4.5 sec per image (roughly 25 images per rat). Aloka© software was used to estimate volumes of adipose tissue and non-adipose tissue using differences in X-ray density. Adipose tissue weights were computed using the density factor of 0.92 g/cm^3. Scanning was undertaken seventy days after surgery.

Gut morphometry

For the study of gut morphometry 16 male Wistar rats were randomized to gastric bypass (n=8) or sham operation (n=8). All rats were ad libitum fed throughout the complete observation period of 60 days. Rats were fasted for 24 hours before being killed to ensure the small bowel was free of chow residue. The entire small bowel from the duodenum to the ileocaecal valve was collected. Total wet weight and length of the small bowel were measured in the sham-operated rats, whilst in gastric bypass rats the weight and length of the three limbs (alimentary, biliopancreatic and common channel) were measured separately and then added.

For analysis of gut morphometry, two centimeter segments of the alimentary, biliopancreatic limb and common channel from bypass operated rats and corresponding segments of jejunum, duodenum, and ileum of sham-operated rats were opened on the mesenteric border and fixed overnight at 4°C in Zamboni's

fixative (2% paraformaldehyde, 15% picric acid, pH 7.4). Transverse segments from each segment were incubated in 20% sucrose in phosphate-buffered saline (PBS) overnight at 4°C and then embedded in OCT compound. Sections of intestine (12 µm) were cut on a cryostat, thaw-mounted onto slides coated with poly-D-lysine and stored at −20°C until use. Sections were then processed for hematoxylin and eosin staining. Sections were washed 3 times at 10 minute intervals in PBS containing 0.1% Triton X-100 and then rinsed in distilled water. Sections were immersed in Ehrlich's Alum Hematoxylin for 4 minutes and then rinsed in distilled water. Sections were then dipped 2-3 times in 0.5% acid alcohol and rinsed in distilled water. Sections were soaked in Scott's Blueing for 30 seconds before being rinsed in distilled water for 30 seconds. Next, the sections were dipped once in Eosin Y acid washed stain and again rinsed in distilled water. Slides were then coverslipped with bicarbonate-buffered glycerol and sections were examined for morphometric analysis. Muscle thickness (circular + longitudinal muscle), mucosal height (villus height + crypt depth), villus height and crypt depth were measured in well-orientated sections under a Zeiss Axioplan microscope fitted with an eyepiece graticule by an observer blinded to the group. Three measurements per tissue were taken and an average was obtained.

Statistical analysis

All data were normally distributed and are expressed as mean ± SEM. Student's t-test for independent samples and one-way ANOVA with repeated measures and post-hoc Bonferroni test for each time point were used to test for significant differences. $P<0.05$ was considered significant. For all analyses data from the two

gastric bypass groups were pooled, because data did not differ between the two time points (day 40 and day 75 after surgery).

Results

Body weight

Figure 6 shows the body weight changes for both groups. For the energy expenditure experiments (figure 6a), body weight was significantly lower in gastric bypass rats compared to the sham-operated ad libitum fed group from day 5 after surgery. On postoperative day 70, the difference in weight was almost 200 g (sham ad lib: 603.2±6.6 g vs. bypass: 414.3±13.8 g, $p<0.001$). After a short period of post surgical weight loss, shams ad libitum fed constantly gained weight for the rest of the study. In contrast, gastric bypass animals lost 11.2±1.4% of their preoperative weight by postoperative day 10; body weight then plateaued around 415 g.

Food restriction started one week after surgery for the body weight-matched shams (n=8). There was no significant difference in body weight between the gastric bypass group and the food restricted body weight-matched rats on and after day 55 (sham body weight-matched: 412.2±3.0 g vs. bypass: 408.7±9.4 g, p=0.78).

There was no increase in either fresh faecal mass (sham ad lib: 8.4±0.5 g vs. sham body weight-matched: 6.6±0.6 g vs. bypass: 7.3±0.4 g, p=n.s.) or faecal calorie content (sham ad lib: 3.56±0.04 kcal/g vs. sham body weight-matched: 3.51±0.04 kcal/g vs. bypass: 3.65 ± 0.04 kcal/g, p=n.s.) in the gastric bypass animals compared to the control groups. C-reactive protein levels were below the detection limit of the assay (<2mg/L) in all animals suggesting no postsurgical infection or inflammation 28 days after surgery.

In the gut morphometry experiments, body weight was significantly lower in gastric bypass rats compared to the sham-operated group from day 5 after surgery (figure

6b); sham-operated rats gained weight for the rest of the study, while gastric bypass animals lost 15.4±1.1% of their preoperative weight by postoperative day 10 and then plateaued around 260 g. The difference in body weight on day 60 was 164 g (sham ad lib: 423.6±10.2 g vs. bypass: 259.1±16.3 g, p<0.001).

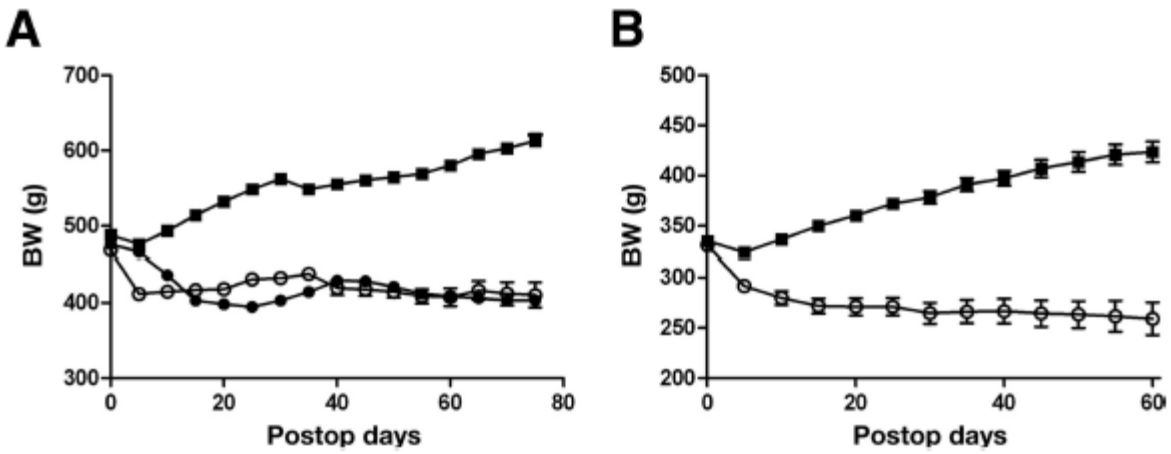


Figure 6: Body weight change for the gastric bypass (-o-) (n=14) and sham-operated rats ad libitum fed (-■-)(n=8) and sham-operated body weight-matched (-●-)(n=8) used for energy expenditure measurements (a) and for gastric bypass (-o-) (n=8) and sham- operated rats ad libitum fed (-■-)(n=8) used for gut morphometry analysis (b). Data are shown as mean values ± SEM.

Body Composition

Adipose tissue mass between vertebrae L1 and L5 in gastric bypass was lower than in sham-operated ad libitum fed rats, but similar to body weight-matched shams (sham ad lib: 27.6±2.7 g vs. sham body weight-matched: 5.3±0.9 g vs. bypass: 11.6±1.3 g, p<0.001). Non-adipose tissue in gastric bypass was lower than in sham ad libitum fed rats, but higher than in body weight-matched shams (sham ad lib: 107.1±2.9 g vs. sham body weight-matched: 71.0±1.1 g vs. bypass: 80.9±2.4 g, p<0.001).

Food intake outside metabolic cages

Food intake followed similar patterns as body weight. Figure 7a shows the average daily food intake for rats of the energy expenditure experiments (postoperative day 1-70). Daily food intake was consistently lower after gastric bypass (sham ad lib: 34.0±1.2 g vs. bypass: 27.5±0.8 g, $p<0.001$). Body weight-matched shams required significantly less food than gastric bypass animals to maintain the same level of body weight (sham body weight-matched: 16.2±0.5 g vs. bypass: 27.5±0.8 g, $p<0.001$). Gastric bypass rats used for the analysis of gut morphometry also ate significantly less than their sham-operated counterparts (sham: 32.5±0.4 g vs. bypass: 26.0±0.5 g, $p<0.001$).

Food intake in metabolic cages

Meal patterns were different between the three groups in the energy expenditure experiment. In the dark phase gastric bypass and sham-operated ad libitum fed rats ate more than in the light phase. Dark phase food intake in gastric bypass rats was lower than in sham ad libitum fed rats (sham ad lib: 26.6±1.1 g vs. bypass: 17.0±1.5 g, $p<0.001$), while they ate more during the light phase (sham ad lib: 2.7±0.5 g vs. bypass: 4.5±0.7 g, $p<0.05$, Figure 7b). Sham-operated body weight-matched rats consumed all their food during the first half of the dark phase and are therefore not represented in figure 7b.

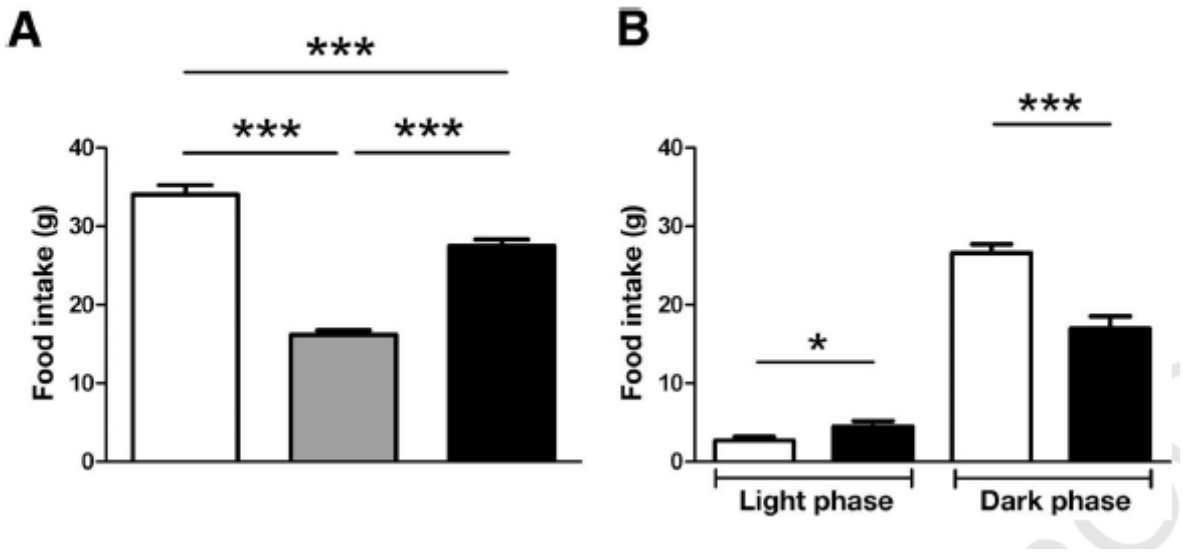


Figure 7: (A) Average daily food intake over 70 days for sham-operated ad libitum fed rats (n=8, white column), for sham-operated body weight-matched rats (n=8, grey column) and for gastric bypass rats (n=14, black column). Data are shown as mean values ± SEM (*** = p<0.001). (B) Average food intake during dark and light phase for sham-operated ad libitum fed (n=8, white columns) and gastric bypass rats (n=8, black columns). Data are shown as mean values ± SEM (* = p<0.05, *** = p<0.001).

Energy Expenditure

Twenty four hour energy expenditure was increased after gastric bypass compared to sham-operated ad libitum fed rats and sham-operated body weight-matched controls (sham ad lib: 4.29±0.08 kcal/kg/h vs. sham body weight-matched: 3.98±0.10 kcal/kg/h vs. bypass: 4.50±0.04 kcal/kg/h, p<0.001). Sham body weight-matched rats had lower total energy expenditure than sham-operated ad libitum fed rats (p<0.05). When analyzing the two phases of the light dark-cycle separately, it was obvious that during the light phase, when overall activity is typically low, energy expenditure in gastric bypass rats was significantly higher than in sham-operated ad libitum fed animals and body weight-matched shams (sham ad lib: 3.63±0.04 kcal/kg/h vs. sham body weight-matched: 3.42±0.05 kcal/kg/h vs. bypass: 4.12±0.03 kcal/kg/h, p<0.001). In the dark phase, when overall activity is typically higher, there was no

difference in energy expenditure between gastric bypass and sham-operated ad libitum fed rats, but energy expenditure in bypass rats was higher than in body weight-matched shams (sham ad lib: 4.81±0.06 kcal/kg/h vs. sham body weight-matched: 4.46±0.15 kcal/kg/h vs. bypass: 4.81±0.04 kcal/kg/h, $p<0.01$). Figure 8a shows average 24 hour, light phase and dark phase energy expenditure for all groups.

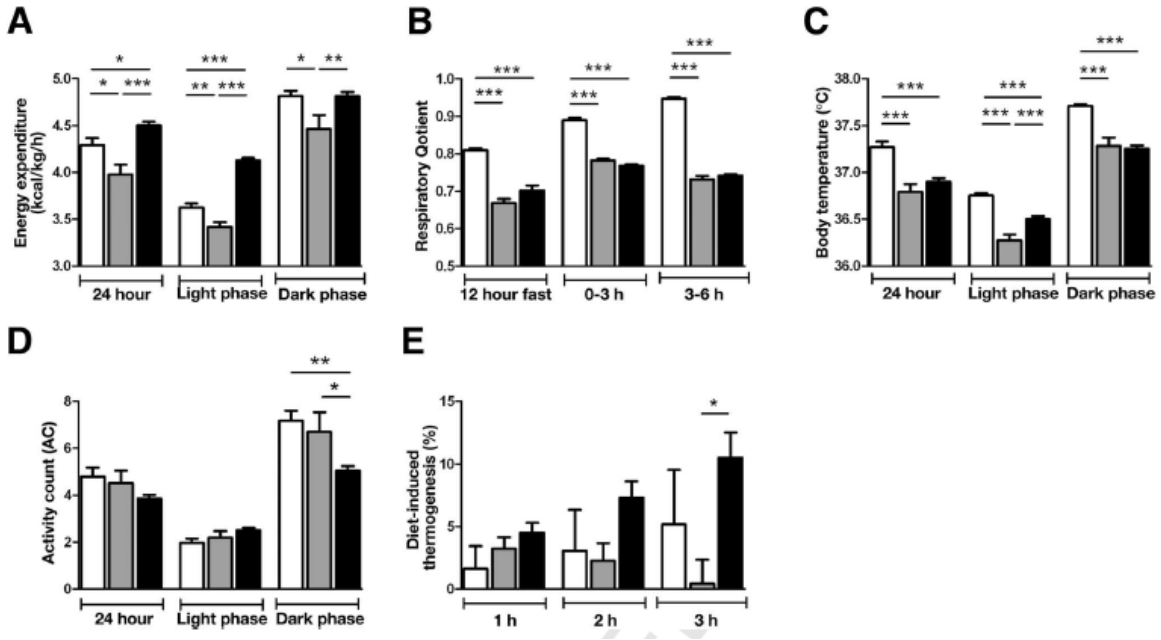


Figure 8: Differences in maintenance energy expenditure (A), respiratory quotients (B), average body temperature (C), activity (D) and diet-induced thermogenesis (E) for sham-operated ad libitum fed (n=8, white columns), for sham-operated body weight-matched (n=8, grey columns) and for gastric bypass rats (n=14, black columns). While data for energy expenditure, body temperature and activity are shown during 24 hour, the light and dark phase, respiratory quotients are shown during 12 hour fasting and within the first six hours after a 5g test meal. Data for diet-induced thermogenesis are expressed as a percentage of the energy content of a 5g test meal and shown at 1h, 2h and 3h after re-feeding with the test meal after a 12 hour fasting period. All data are shown as mean values ± SEM (* = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$).

Respiratory Quotient

Respiratory quotients were examined during 12 hours of fasting and for the subsequent 6 hours after offering a fixed test meal of 5 g. Respiratory Quotients as measured during the light and dark phase are shown in Figure 8b. During fasting gastric bypass rats had a lower respiratory quotient than sham-operated ad libitum fed rats, but there was no difference to sham-operated body weight-matched rats. The pattern was similar for the 0-3 hour observation period after the test meal for gastric bypass, sham ad libitum fed and sham body weight-matched rats (sham ad lib: 0.89±0.01 vs. sham body weight-matched: 0.78±0.01 vs. bypass: 0.77±0.01, $p<0.001$) and the 3–6 hour observation period after the test meal (sham ad lib: 0.95±0.01 vs. sham body weight-matched 0.73±0.01 vs. bypass: 0.74±0.01, $p<0.001$). Respiratory quotient between gastric bypass and sham body weight-matched rats was not different during fasting or the six hours after the test meal.

Body Temperature

Body temperature as measured during the light and dark phase is shown in Figure 8c. Body temperature in gastric bypass rats was lower than in sham-operated ad libitum fed rats, but higher compared to body weight-matched sham rats during the light phase (sham ad lib: 36.8±0.02°C vs. sham body weight-matched: 36.3±0.06°C vs. bypass: 36.5±0.03°C, $p<0.001$). During the dark phase, average body temperature in gastric bypass rats was lower than in sham-operated ad libitum fed rats, but no different compared to body weight-matched sham rats (sham ad lib:

37.7±0.02°C vs. sham body weight-matched: 37.3±0.09°C vs. bypass: 37.3±0.03°C, $p<0.001$).

Physical activity

A dissociation between total energy expenditure and body temperature was observed and thus, physical activity was analyzed (Figure 8d). No difference in activity over 24 hour or during the light phase was seen among all three groups. During the dark phase, however, gastric bypass rats were less active than sham-operated ad libitum fed rats and sham-operated body weight-matched rats (sham ad lib: 7.19±0.4 activity counts vs. sham body weight-matched: 6.70±0.8 activity counts vs. bypass: 5.04±0.2 activity counts, $p<0.001$).

Diet-Induced Thermogenesis

Diet-induced thermogenesis was measured over three hours after a 5 g standard test meal after a 12h fast. The sham-operated ad libitum fed and the sham-operated body weight-matched groups consumed all 5 g within 20 minutes, the gastric bypass animals required 30 minutes. Figure 8e shows the diet-induced thermogenesis for all groups for the first three hours after the test meal. Three hours after the 5 g test meal, gastric bypass rats had a significantly greater diet-induced thermogenesis than the body weight-matched controls, but bypass was not different from the sham-operated ad libitum fed rats (sham ad lib: 5.2±4.4% vs. sham-body weight-matched: 0.41±1.9% vs. bypass: 10.5±2.0%, $p<0.05$).

Gut morphometry

Differences in gut morphometry are summarized in figure 9. There was no difference in total length of the complete small bowel between sham-operated and gastric bypass rats (sham ad lib: 108.6±1.7 cm vs. bypass: 110±2.2 cm, p=0.82). In contrast, the wet weight of the small bowel was 72.1% higher after gastric bypass than after sham-operations (sham ad lib: 12.2±0.6 g vs. bypass: 21.0±1.2 g, p<0.001). Average weight of the alimentary limb was 10.6±0.8 g, of the biliopancreatic limb 2.7±0.2 g and of the common channel 7.8±0.6 g. Muscle thickness (sham ad lib: 95.0±8.7 µm vs. bypass: 247.9±32.5 µm, p<0.001), mucosal height (sham ad lib: 530.8±19.1 µm vs. bypass: 969±58.2 µm, p<0.001), villus height (sham ad lib: 390.4±21.7 µm vs. bypass: 673.6±63.8 µm, p<0.001) and crypt depth (sham ad lib: 140.4±8.0 µm vs. bypass: 295.4±20.6 µm, p<0.001) were significantly increased in the alimentary limb after gastric bypass in comparison to the corresponding section of the jejunum of the sham-operated controls. Gastric bypass rats had a significantly greater villus height of the common channel than sham-operated animals (sham ad lib: 287.1±18.1 µm vs. bypass: 464.6±73.9 µm, p<0.05). There was a trend towards an increase in mucosal height (sham ad lib: 490.4±29.6 µm vs. bypass: 673.8±99.7 µm, p=0.09) and muscle thickness (sham ad lib: 490.4±29.6 µm vs. bypass: 673.8±99.8 µm, p=0.09) in the common channel.

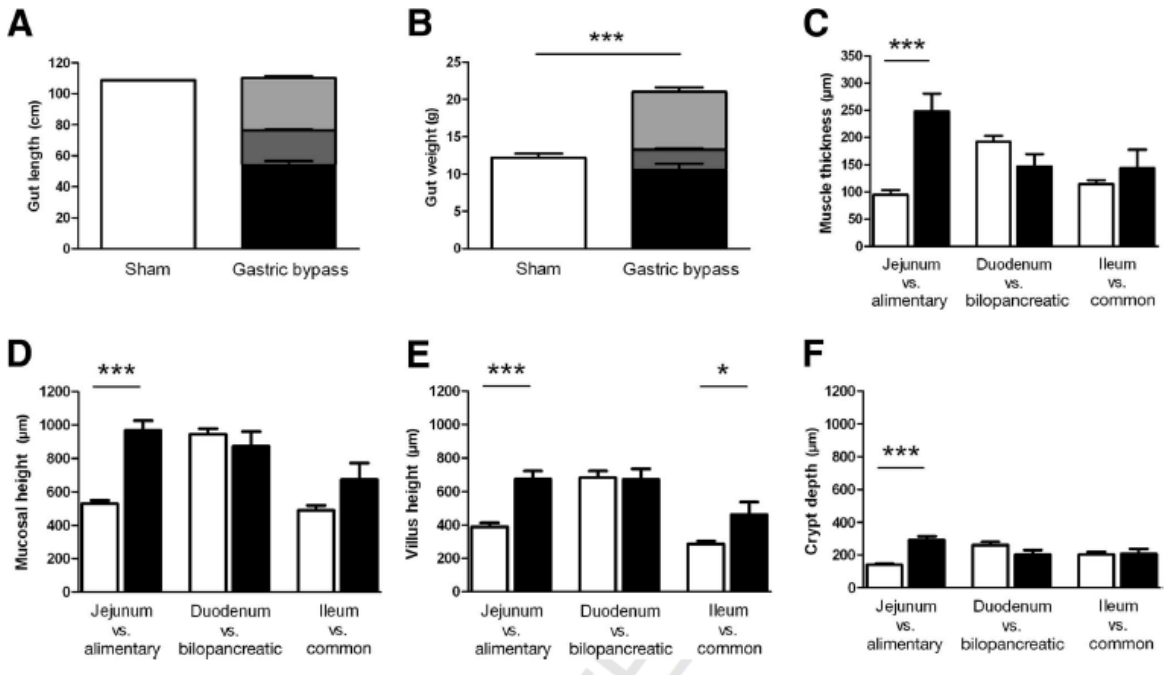


Figure 9: Length (A) and weight (B) of the entire small bowel and differences in gut morphometry in rats 60 days after gastric bypass (n=8) and sham operation (n=8). Differences in muscle thickness (C), mucosal height (D), villus height (E) and crypt depth (F) are shown for the alimentary limb, the biliopancreatic limb and the common channel after gastric bypass in comparison to the corresponding parts of jejunum, duodenum and ileum after sham-operation. Data are shown as mean values ± SEM (*** = $p<0.001$, * = $p<0.05$).

Discussion

I demonstrate a higher total energy expenditure in rats after gastric bypass compared to ad libitum fed and body weight-matched sham groups which is in accordance with some, but not all previous reports of energy expenditure in humans (Carrasco et al. 2007;Das et al. 2003;Flancbaum et al. 1997). Differences in energy expenditure were mainly due to changes during the light phase when physical activity is typically low. Gastric bypass surgery did not only prevent the expected decrease in energy expenditure subsequent to body weight loss, but actually increased 24 hour and in particular light phase energy expenditure in comparison to the control groups.

My data suggest that gastric bypass induces profound changes in food intake, energy expenditure and the mechanisms by which the body controls energy expenditure. Gastric bypass increases postprandial levels of PYY and GLP-1 (Korner, Bessler, Cirilo, Conwell, Daud, Restuccia, & Wardlaw 2005;le Roux, Aylwin, Batterham, Borg, Coyle, Prasad, Shurey, Ghatei, Patel, & Bloom 2006a), which are satiating inducing gut hormones and hence favour an anorectic state and facilitate body weight loss through modulation of the hypothalamus and brainstem (Abbott, Monteiro, Small, Sajedi, Smith, Parkinson, Ghatei, & Bloom 2005;Larsen et al. 1997), also being involved in the control of energy expenditure (Murphy & Bloom 2006). In fact, PYY has been shown to activate anorectic POMC expressing neurons in the ARC (Batterham et al. 2002) and to inhibit NPY neurons (cuna-Goycolea and van den Pol 2005), suggesting a potential to increase energy expenditure.

In summary, not only did gastric bypass surgery prevent the expected decrease in energy expenditure subsequent to body weight loss in this diet-induced obese rat

model, but 24 hour and in particular light phase energy expenditure were higher than in sham controls. Diet-induced thermogenesis was also higher after gastric bypass surgery compared to body weight-matched controls. Increased energy expenditure may offer an additional explanation why gastric bypass surgery is superior to dieting for successfully maintaining long-term body weight loss.

Chapter 4: Sodium and water handling after Roux-en-Y Gastric Bypass in rats

Introduction

Hypertension is associated with central adiposity and insulin resistance (Chen et al. 2009;Kannel et al. 1967;Stamler et al. 1978), but the pathophysiological mechanism remains unclear. There are several plausible hypotheses, including insulin resistance (DeFronzo et al. 1975;Natali et al. 1993), aldosterone and so-called aldosterone releasing factors (Connell and Davies 2005;Laragh 2001), as well as hyperleptinemia (Galletti et al. 2008;Rahmouni et al. 2005), leading to sodium retention, increased blood volume and finally elevated blood pressure. Alternatively, it has been suggested that increased aldosterone levels might be secondary to increased intra-abdominal pressure (Sugerman et al. 1997;Sugerman et al. 1998). A proposed mechanism is that increased intra-abdominal pressure raises the diaphragm, which increases pleural pressure, decreasing venous return to the heart. Increased intra-abdominal pressure would also increase inferior vena cava pressure, resulting in increased renal venous pressure and a decrease renal perfusion. Both mechanisms would activate the renin-angiotensin-aldosterone system, leading to increased renal sodium and water retention (Sugerman, Windsor, Bessos, & Wolfe 1997;Sugerman, Windsor, Bessos, Kellum, Reines, & DeMaria 1998). However, reduction of visceral fat mass and decrease in sympathetic nerve activity and/or sodium retention do not occur immediately after gastric bypass surgery, and they do not explain the early reductions in blood pressure reported by Ahmed at al (Ahmed et al. 2008). Therefore, I hypothesized that renal sodium and water handling may be

altered by bypass surgery, and that this might contribute to the early improvement in blood pressure control that occurs.

Thus, the aim in this study was to evaluate water intake, urine output, and renal sodium excretion in rats before and shortly after gastric bypass surgery in response to an acute oral sodium challenge.

Material and Methods

Animals

Twenty one male wistar rats (body weight 348±19g) were randomized to have either a gastric bypass (n=14) or sham operation (n=7). The work was performed under UK Home Office licence (PL 70-5569), and all animals were kept in identical environmental conditions (temperature 24ºC, humidity 60%, light cycle 7.00 – 19.00) with normal chow (RM1 diet, Special Diet Services Ltd, UK) and tap water *ad libitum* unless otherwise stated. Body weight was measured daily.

Metabolic cage experiments

Urine output, water intake and sodium excretion were measured at three different time points. Firstly, before surgery and following a previous oral sodium load; secondly, after surgery, but without an oral sodium load (baseline measurements); thirdly, after surgery and following an oral sodium load. Prior to each experiment, animals were maintained on a low sodium diet and given deionized water *ad libitum* for one week to establish a stable urinary excretion rate for sodium, and to enhance endogenous mechanisms for sodium retention (Mu et al. 1995). The low sodium diet was identical to normal chow, except for its sodium content (D02051701, Research Diets Inc., New Brunswick, NJ, USA; sodium content 102.6 ppm). For measurements after an oral sodium load, sodium (1.5 mmol Na/ kg body weight) was given intragastrically by oral gavage over 10s as hyperosmolar NaCl solution (616 mM) at the beginning of the light phase (7.00 am). Animals were then placed in individual

metabolic cages for urine collection, and to record water intake over 8 hours. For baseline measurements animals were placed in metabolic cages without having received oral sodium load. In all experiments urine was collected in pre-weighed plastic tubes. Water was given in pre-weighed plastic bottles that were also re-weighed at the end of the experiment. The cages were cleaned and rinsed with deionized water after each experiment.

Surgery

Surgery was performed according to an established protocol with preservation of the vagal fibres in the para-oesophageal bundle as previously described (page 35).

Measurement of urinary sodium

Urine sodium concentration was measured by Integrated Chip Technology (ICT) using the Architect ci16200 (Abbott, Illinois, USA). It obtains millivolt readings, and then converts them to assay-specific analyte conversion units. The measurement of ICT reference solution and ICT samples are used to calculate the assay results.

Statistical analysis

Data is presented as mean ± SEM. Data were compared with the use of 2-tailed, paired Student t tests (Graphpad Prism, USA). $P<0.05$ was considered significant.

Body weight

Body weight was significantly lower in gastric bypass rats compared to the sham-operated group from day 5 after surgery (sham: 349.9 ± 6.1 g vs. bypass: 313.6 ± 6.4 g, $p<0.01$); sham-operated rats gained weight for the rest of the study. The difference in body weight on day 60 was 165 g (sham: 501 ± 8 g vs. bypass: 346 ± 21 g, $p<0.001$). Figure 10 shows the percentage of initial body weight for all bypass (n=14) and sham-operated rats (n=7).

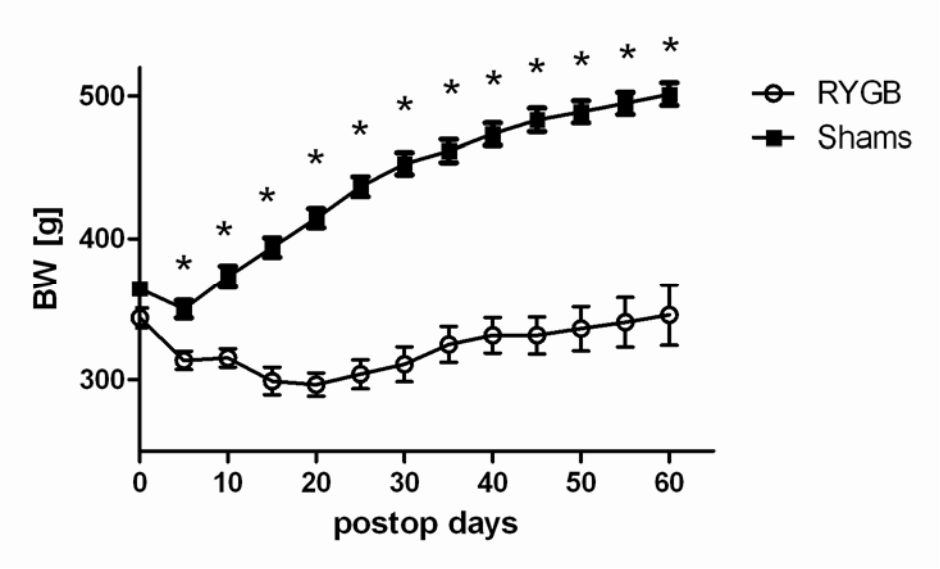


Figure 10: Body weight change in gastric bypass (-o-) (n=14) and sham-operated rats (-■-) (n=7). Data are shown as mean values ± SEM (* = $p<0.05$).

Urine output (volume)

In gastric bypass rats, sodium loading after surgery led to a greater increase in urine output when compared with urine output following the same sodium load before surgery (pre-op: 0.015 ± 0.002 ml/g body weight vs. post-op: 0.034 ± 0.007 ml/g

body weight, p= 0.03). There was no change in urine output in the sham-operated group after the sodium load when compared with their pre-operative response after sodium loading (pre-op: 0.011 ± 0.001 ml/g body weight vs. post-op: 0.010 ± 0.002 ml/g body weight, p= 0.44). Gastric bypass rats produced significantly more urine than sham-operated rats after sodium loading (sham: 0.010 ± 0.002 vs. bypass: 0.034 ± 0.007 ml/g body weight, p= 0.04). There was no difference in baseline urine production between these groups after surgery (sham: 0.011 ± 0.001 ml/g body weight vs. bypass: 0.015 ± 0.002 ml/g body weight, p= 0.12). Figure 11 summarizes data for urine output.

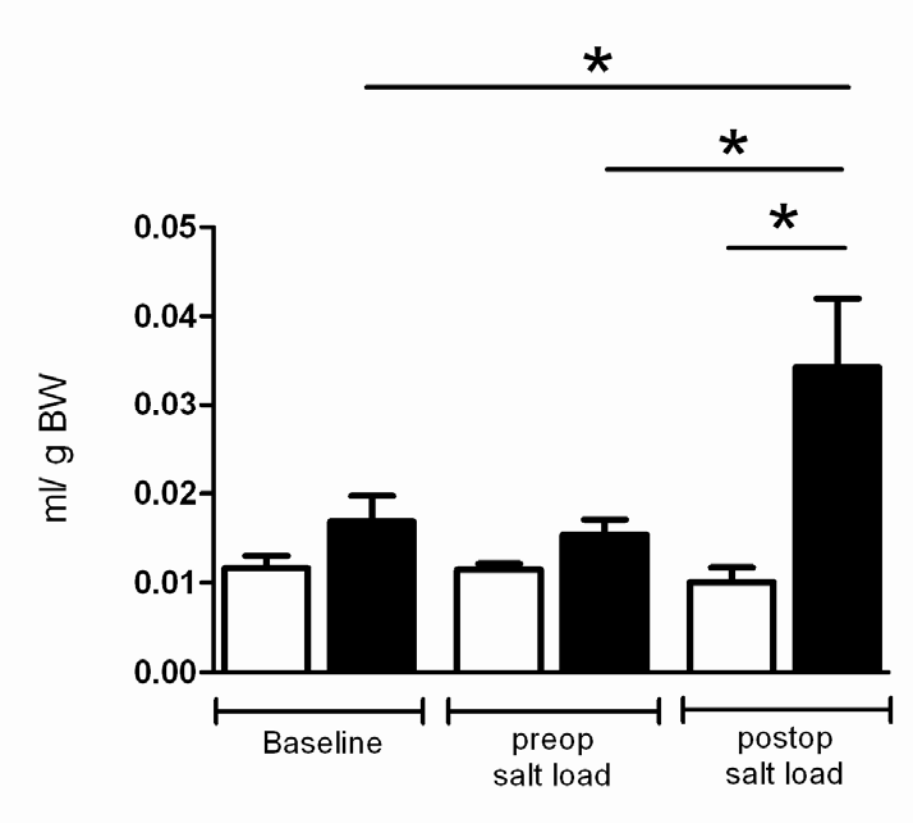


Figure 11: Urine production of bypass-operated (black columns, n=14) and sham-operated rats (white columns, n=7) after oral sodium loading (1.5 mmol Na/ kg body weight of a 616 mM NaCl solution) pre-operatively and on post-operative day 30. Baseline urine output was measured without oral sodium loading. Data are shown as mean values ± SEM. p>0.05 was considered significant (*).

Water intake

Data for water intake are summarized in Figure 12. Gastric bypass rats consumed significantly more water after the sodium load compared with before surgery (pre-op: 0.033 ± 0.006 ml/g body weight vs. postop: 0.065 ± 0.012 ml/g body weight, p= 0.02). No changes were observed for water intake pre- and post-sham surgery after the sodium load (pre-op: 0.029 ± 0.006 ml/g body weight vs. post-op: 0.021 ± 0.002 ml/g body weight, p=0.31). Bypass rats also drank significantly more water than sham-operated rats (sham: 0.021 ± 0.002 ml/g body weight vs. bypass: 0.065 ± 0.012 ml/g body weight, p= 0.02) after the sodium load. There was no difference in baseline water intake between the groups (sham: 0.029 ± 0.006 ml/g body weight vs. bypass: 0.033 ± 0.006 ml/g body weight, p=0.68).

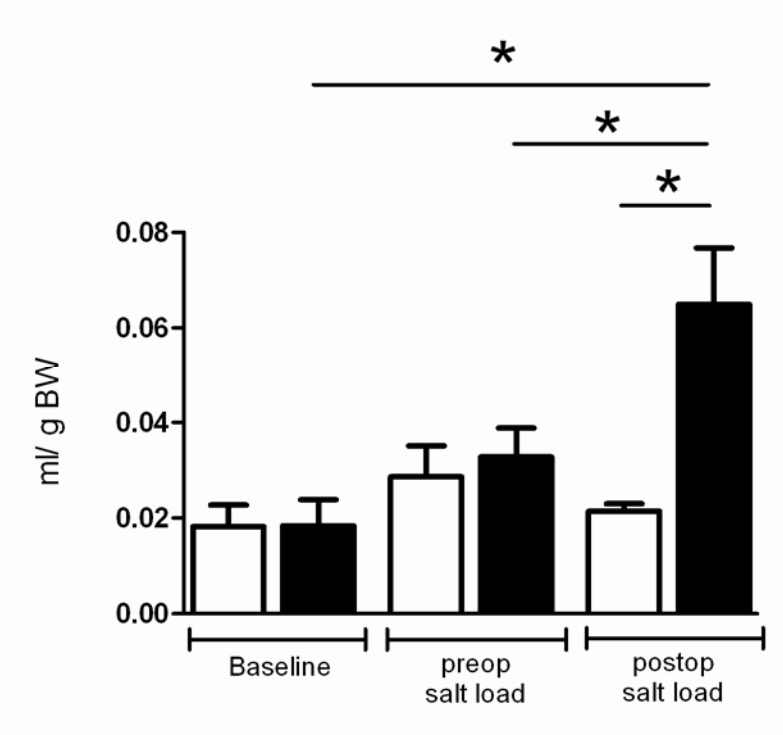


Figure 12: Water intake of bypass-operated (black columns, n=14) and sham-operated rats (white columns, n=7) after oral sodium loading (1.5 mmol Na/ kg body weight of a 616 mM NaCl solution)

pre-operatively and on post-operative day 30. Baseline water intake was measured without oral sodium loading. Data are shown as mean values ± SEM. p>0.05 was considered significant (*).

Sodium excretion

Post-operative sodium loading led to a greater increase in cumulative sodium excretion in the gastric bypass rats compared with their pre-operative response (pre-op: 31.7 ± 8.7 µmol vs. post-op: 65.9 ± 10.7 µmol, p= 0.02). No changes in sodium excretion were observed pre- or post-sham surgery after oral salt loading (pre-op: 40.9 ± 16.0 µmol vs. post-op: 36.2 ± 10.7 µmol, p= 0.81). Gastric bypass rats had a greater sodium excretion in bypass rats compared with their sham-operated counterparts after salt loading (sham: 36.2 ± 10.7 µmol vs. bypass: 80.9 ± 14.4 µmol, p=0.03). There was no significant difference between baseline sodium excretion between gastric bypass rats and sham-operated rats (sham: 40.9 ± 16.0 µmol vs. bypass: 31.7 ± 8.7 µmol, p= 0.59). Data are summarized in Figure 13.

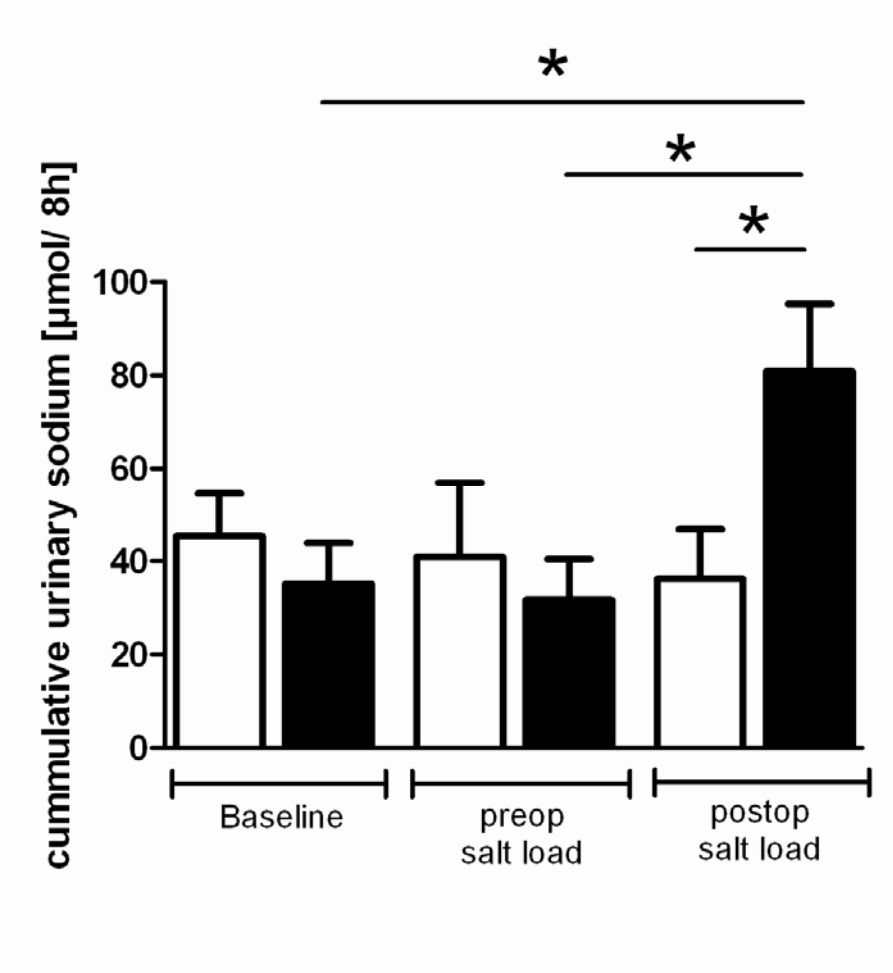


Figure 13: Cumulative sodium excretion of bypass-operated (black columns, n=14) and sham-operated rats (white columns, n=7) after oral sodium loading (1.5 mmol Na/ kg body weight of a 616 mM NaCl solution) preoperatively and on postoperative day 30. Baseline sodium excretion was measured without oral sodium loading. Data are shown as mean values ± SEM. $p>0.05$ was considered significant (*).

Discussion

The beneficial effect of gastric bypass surgery on arterial hypertension is well documented (Adams et al. 2007;Buchwald 2005). The reduction of visceral fat mass, and subsequent decrease in sympathetic activation and sodium retention, is not immediate and does not explain the early reduction in blood pressure observed after gastric bypass described by Ahmed at al (Ahmed, Rickards, Coniglio, Xia, Johnson, Boss, & O'Malley 2008). Thus, I reasoned that other mechanisms might be involved in the early resolution of hypertension after gastric bypass, and that alteration of renal sodium and water handling could be one of them.

I have demonstrated a significant increase in urine output, water intake and sodium excretion after gastric bypass surgery compared with pre-operative measurements. Sham-operated animals show no changes in water intake, urine production or sodium excretion after surgery.

My data suggest that gastric bypass induces profound changes in sodium and water handling. As gastric bypass significantly rearranges the gastrointestinal anatomy, I suggest that gastrointestinal and central neuroendocrine signaling contribute to increased sodium and water excretion (Lowell and Spiegelman 2000). Potential mediators between the gut and the kidney include both, Peptide YY (PYY) (Playford et al. 1995) and glucagon-like peptide (GLP)-1, which have been shown to have diuretic and natriuretic properties (Michell et al. 2008). Thus, it is reasonable to speculate that GLP-1 and PYY could mediate a link between the gastrointestinal tract and kidney in terms of sodium and water excretion (Gutzwiller et al. 2004;Gutzwiller et al. 2006;Michell, Debnam, & Unwin 2008).

In conclusion, gastric bypass surgery in humans, and in the rat, provides us with a valuable model in which to explore the role of the gastrointestinal tract in sodium and water homeostasis, and other electrolytes, and perhaps also in salt-sensitive hypertension.

Chapter 5: Analysis of Fat Preference after Roux-en-Y Gastric Bypass in humans

Introduction

Patients reach satiety earlier after gastric bypass surgery (Brolin, Robertson, Kenler, & Cody 1994;Brown, Settle, & Van Rij 1982;Halmi, Mason, Falk, & Stunkard 1981) and report a reduced desire to consume fatty food as they no longer found it enjoyable (Brolin, Robertson, Kenler, & Cody 1994;Brown, Settle, & Van Rij 1982;Halmi, Mason, Falk, & Stunkard 1981). Total fat intake is lower after gastric bypass partly because of a reported disinterest in desserts and ice cream (Brolin, Robertson, Kenler, & Cody 1994;Brown, Settle, & Van Rij 1982). A randomised controlled trial comparing gastric bypass and vertical-banded gastroplasty confirmed a reduced intake of high fat foods one year after gastric bypass (Olbers et al. 2005).

In this study, we tested how gastric bypass changes fat preference and intake of fat in humans. We used data from a randomised controlled trial between gastric bypass and vertical-banded gastroplasty (Olbers, Fagevik-Olsen, Maleckas, & Lonroth 2005) to establish the importance of the phenomenon in humans. It was the aim of this study to evaluate human patients six years after being randomised to gastric bypass or vertical-banded gastroplasty.

Material and Methods

In this study, 16 patients (11 female) were included from a prospective clinical trial which randomized patients to gastric bypass and vertical-banded gastroplasty during 2000-2001 (Olbers, Fagevik-Olsen, Maleckas, & Lonroth 2005). I was involved in the design and analysis of this study. Between 12/2006 and 06/2007, nine gastric bypass and seven vertical-banded gastroplasty patients were included at an average of six years after surgery (range 5.8-6.8 years). The study protocol was approved by the local ethics committee (Reference number 359-09) and the study was conducted according to the principles of the Helsinki declaration.

Both operations were performed laparoscopically as described previously (Olbers, Fagevik-Olsen, Maleckas, & Lonroth 2005). The validated Swedish Obese Subjects study questionnaire was used for dietary assessment (Lindroos et al. 1993). The questionnaires included 49 questions on ordinary food consumption patterns during the past 3 months, with the emphasis on portion size and day of week. Amounts of snacks and sweets were quantified using sizes for preconfectioned packages as sold in Sweden. Bread-type, thickness, and contents of sandwiches were described in detail, owing to the large contribution of sandwiches in the Swedish diet. The amounts of food reported by the subjects were converted into grams, from which daily intake of energy and 29 different nutrients were computed. In addition, a short questionnaire was used to explore whether the patient avoided certain foods. Included were direct questions (e.g., Do you eat whole meat?) and an open question (e.g., Do you avoid eating any foods? Why?).

Results

Gastric bypass patients and vertical gastroplasty patients reduced their body mass index by 26.5±2.9 % and 17.8±2.5 % respectively six years after surgery. There were however no statistically significant changes in reported energy intake six years after surgery (preop: gastroplasty: 3050.0±354.5 kcal vs. bypass: 2552.3±219.5 kcal, p=0.26 and postop: gastroplasty 2854.6±258.4 kcal vs. bypass: 2322.9±183.6 kcal, p=0.11). Proportions of total energy intake from protein, fat, and carbohydrates six years after surgery are shown in Figure 14A. Bypass patients reported lower proportion of calories ingested as fat compared to patients after vertical-banded gastroplasty (p=0.046). There was no difference in the proportion of calories from carbohydrates (p=0.09) or proteins (p=0.48) as compared with vertical-banded gastroplasty patients. As shown in Figure 14B, gastroplasty patients reported a higher proportion of their total energy intake from foods high in fat, e.g. cheese and sausages (p=0.041) and desserts (p=0.007) than bypass patients, who instead reported a higher relative intake from fruits and vegetables (p=0.004).

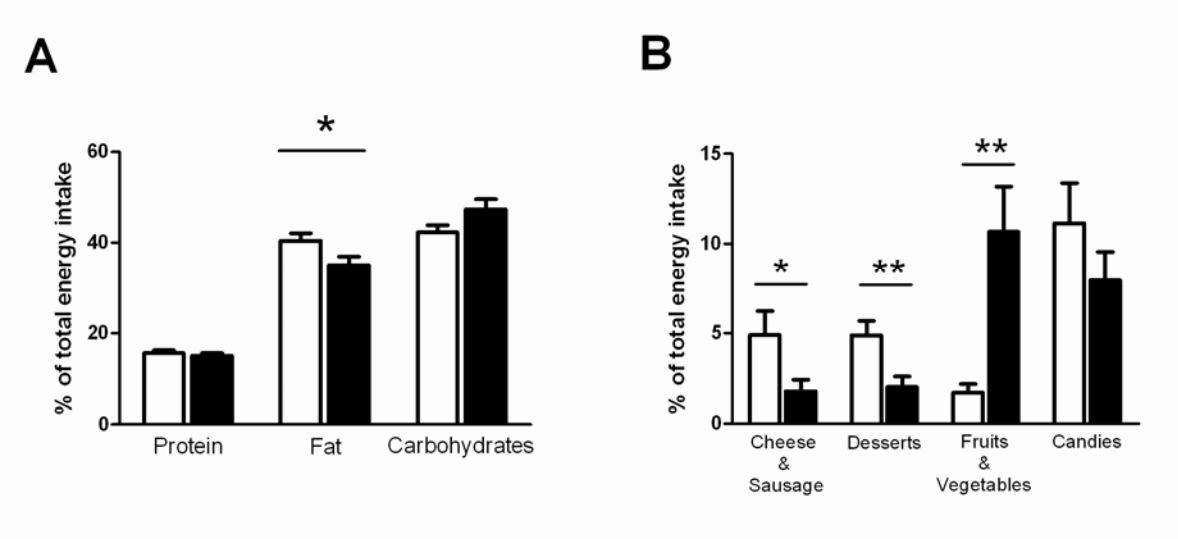


Figure 14: The proportion of total energy intake from protein, fat, and carbohydrates (A) and from various food groups (B) 6 years after laparoscopic gastric bypass (black columns) and laparoscopic vertical-banded gastroplasty (white columns). Data are shown as mean values ± SEM (* = $p<0.05$, ** = $p<0.01$).

Discussion

Patients randomised to gastric bypass six years earlier decreased their liking for fat compared to pre surgery, but the same is not the case after vertical-banded gastroplasty. This long-term reduction in dietary fat following gastric bypass was found to be the single most pronounced differing factor in the dietary composition between the two groups six years after the operation. As part of general lifestyle advice (Blundell and MacDiarmid 1997) to achieve adequate and sustained weight loss a reduction in total energy intake by reducing dietary fat is recommended.

My findings add to previous reports in humans which have shown a reduced dietary fat intake one year after gastric bypass surgery (Olbers, Bjorkman, Lindroos, Maleckas, Lonn, Sjostrom, & Lonroth 2006). Interestingly, the reduced preference for fat was absent or at least less pronounced in patients six years after vertical-banded gastroplasty in which the anatomical rearrangement of the small bowel is not part of the operation and which is known not to induce changes in postprandial gut hormone levels (Valverde et al. 2005). Thus, changes in fat preference might be at least partly mediated by alterations in gastrointestinal and central neuroendocrine signalling (Korner, Bessler, Cirilo, Conwell, Daud, Restuccia, & Wardlaw 2005;le Roux, Aylwin, Batterham, Borg, Coyle, Prasad, Shurey, Ghatei, Patel, & Bloom 2006a;Stylopoulos, Hoppin, & Kaplan 2009). Indeed, gastric bypass increases postprandial levels of PYY and GLP-1 (Korner, Bessler, Cirilo, Conwell, Daud, Restuccia, & Wardlaw 2005;le Roux, Aylwin, Batterham, Borg, Coyle, Prasad, Shurey, Ghatei, Patel, & Bloom 2006a), which are satiating inducing gut hormones and hence favour an anorectic state and facilitate body weight loss through modulation of the hypothalamus and brainstem (Abbott, Monteiro, Small, Sajedi, Smith, Parkinson,

Ghatei, & Bloom 2005;Larsen, Tang-Christensen, & Jessop 1997), In addition, GLP-1 or PYY may also influence fatty acid detection or perception and there may be parallels with the recognition of sweet stimuli. Mice lacking the GLP-1 receptor show decreased behavioural responsiveness to sucrose. This receptor has been shown to be expressed on taste afferent fibers, and GLP-1 is expressed in taste buds cells (Feng et al. 2008;Shin, Martin, Golden, Dotson, Maudsley, Kim, Jang, Mattson, Drucker, Egan, & Munger 2008).

Chapter 6: Analysis of Fat Preference after Roux-en-Y Gastric Bypass in rats

Introduction

A limitation of human studies investigating changes in fat preference after gastric bypass surgery includes the use of survey or scaling. Complementary use of an animal model can add new insights as it circumvents some of the problems of verbal report that may interfere with the assessment of the actual “affective” value of the stimulus. As food preference is influenced by taste, the gustatory system is a prime candidate to explain the effects outlined above.

In this study, I tested how gastric bypass changes fat preference and intake of fat in rats. I used my rat model to assess fat preference because it allows greater latitude in behavioural, endocrine, and molecular measurements while providing a logical bridge with reports of changes in human taste preference following surgery. Thus, the aims of this study were to use a rat model to further investigate the underlying mechanisms of : a) preference for solid high fat versus low fat chow, b) preference for increasing fat concentrations in a liquid preparation early and late after gastric bypass, c) licking responses to increasing fat concentrations in a liquid preparation in a brief access test that minimises postingestive consequences and d) whether reduced preference for fat may be due to induction of conditioned taste aversion perhaps mediated through increased endogenous levels of GLP-1.

Material and Methods

Animals

Obese male Wistar rats were individually housed under a 12 hour /12 hour light-dark cycle at a room temperature of 21±2 ºC. Water and standard chow were available *ad libitum*, unless otherwise stated. All experiments were performed under a license issued by the Home Office UK (PL70-6669) or approved by the Veterinary Office of the Canton Zurich, Switzerland.

Surgery

After 1 week of acclimatization, the obese rats were randomized to gastric bypass or sham operation. Surgery was performed according to an established protocol with preservation of the vagal fibres in the para-oesophageal bundle as previously described (page 35).

Hormone assay

Rats used in the late two bottle preference test were fasted for 12 hours from the beginning of the light cycle. At the onset of the dark cycle animals were offered 5g of standard chow all of which was consumed within half an hour by the animals. Blood was then obtained by puncture of a sublingual vein under brief isoflurane anesthesia from sham-operated controls (n=9) and gastric bypass rats (n=9). Blood was collected into EDTA-rinsed tubes and immediately centrifuged at 3000 rpm for 10 min at 4°C. The supernatant was stored at -80°C until further analysis.

Concentrations of active GLP-1 and PYY were analyzed using a rat endocrine lincoplex kit (RENDO-85K, Labodia SA, Yens, Switzerland).

Food preference

A food preference test was presurgically conducted with 26 obese male Wistar rats. Food was offered in three equal compartments which were filled with 30 g of the following three food choices: 60% fat diet (Research Diets, D12492, energy content: 23.9 kilojoule per gram (kJ/g)), a 60% fat diet with added Bisto® (gravy type flavour) and normal chow with 2% fat (RM1 diet, Special Diet Services Ltd, UK, 14.7 kJ/g). Bisto® was added to one section of the high fat chow for the rats to differentiate it from the other high fat chow in the next section. The three diet options thus contained three distinct flavours and two different calorie densities. Food intake was recorded after 24-hour intervals over two days by weighing the food at the end of the dark cycle. Rats were then randomised to bypass (n=13) or sham operations (n=13) for baseline measurements; the effect of surgery on high fat versus low fat intake was tested about ten days after surgery in the same animals.

Two bottle preference test

Intralipid® (Fresenius Kabi, UK) is a fat emulsion used for parenteral nutrition in malnourished patients. The emulsion consists of soy bean oil, egg phospholipids, glycerin, omega-6 essential fatty acids, alpha-linoleic acid and linolenic acid. We diluted the standard 20% Intralipid® solution with distilled water to provide seven concentrations (0.005%, 0.01%, 0.05%, 0.1%, 0.5%, 1%, 5%) for this study.

The obese rats were presented with two pre-weighed bottles, one of which contained distilled water and the other of which contained Intralipid® solution in ascending concentrations. The volume of the bottles containing distilled water or Intralipid® was made up to 200 ml every day. Readings were recorded at the start of the light phase by re-weighing the bottles. The positions of the bottles were switched each day to preclude the development of a side preference. To control for spillage during the manipulation of the bottles, two additional bottles were placed in cages without animals, and daily measurements were obtained. The average amount of spillage (0.69±0.04 ml) was subtracted from measured volumes of distilled water and Intralipid® intake before further analysis.

Each animal was tested for 14 days (7 x 2-day periods). Intralipid® preference for each 24-h period was defined as: [Intake of Intralipid (in ml) ／ Total Fluid Intake (ml)] x 100. With this type of preference experiment, a score of 50% conventionally represents neutrality, equality of preference, indifference, or inability to distinguish; 0% to 49% indicates rejection, aversion, or refusal; and 51% to 100% represents varying degrees of preference and avidity.

Two bottle preference tests were performed early after surgery (10 days) and late after surgery (200 days). In the early experiment 10 days after surgery, three groups of fat taste naive rats were used. Twelve sham-operated controls, eighteen gastric bypass rats and six unoperated controls were subjected to the two bottle preference test as described above. Preference (%), acceptance (Intralipid® intake in ml), food (chow) intake (g) and total energy intake (Intralipid plus chow; kJ) were measured daily. In the late experiment 200 days after surgery, ten sham-operated controls and

ten gastric bypass rats were subjected to the same two bottle preference test as described above.

Brief Access Tests

Sixteen obese male Wistar rats aged 10 weeks that were naive to the taste of Intralipid® were tested in a lickometer (Davis MS-160, DiLog Instruments, Tallahassee, FL) after being randomized to sham- or gastric bypass operation (each n=8). The brief access test procedure was conducted as previously described (Smith 2001). Briefly, a rat was placed in the test chamber of the apparatus. A motorized shutter opened allowing the rat access to a single sipper tube containing Intralipid®. A small fan, positioned above the sample slot directed a current of air past the drinking spout to minimize potential olfactory cues from the Intralipid®. Rats initiated a trial by licking the spout. Each trial was 10 s, followed by a 7.5-s intertrial interval during which time the tube was changed via a motorized block for the next trial. A concentration-response/ licking function was derived in three test sessions of 30 minutes each during which rats were able to initiate as many trials as possible. The briefness of the test as suggested by its name minimises any postingestive effects of the substance tested as only small amounts are ingested. Before being tested for Intralipid® all rats underwent 4 days of water training as described previously (Spector & Glendinning 2009). The same seven Intralipid® concentrations as in the two bottle preference tests (0.005%, 0.01%, 0.05%, 0.1%, 0.5%, 1%, 5%) as well as distilled water were used and presented in randomized order (without replacement) in blocks of trials. Rats were tested on a 23-h restricted water-access schedule as

well as with water available ad libitum for three daily sessions every other day in two subsequent weeks.

Conditioned taste aversion for corn oil

Fat taste naive obese male Wistar rats were used. Sixteen gastric bypass and 22 sham-operated rats were individually housed for one week with *ad libitum* access to food and water before they underwent the conditioned taste aversion experiment. Rats were slightly sedated by brief exposure to isoflurane before the oral gavage with corn oil or saline. The five groups included gastric bypass rats receiving saline (n=8), gastric bypass rats receiving corn oil (n=8), sham-operated rats receiving saline (n=8), sham-operated rats receiving corn oil (n=8) and sham-operated rats receiving intraperitoneal lithium chloride as a positive control (i.p. LiCl, 76.2 mg/kg body weight) (n=6). Lithium chloride has been previously shown to induce visceral malaise when injected intraperitoneally and is therefore commonly used as an illness-inducing control in a conditioned taste aversion paradigm (Gu et al. 1993;Lamprecht and Dudai 1995;Yamamoto et al. 1992).

At the beginning of the experiment water was withdrawn from all animals at the start of the dark phase (day 0). In order to acclimatise rats to weighing and timing of distilled water presentation, animals were presented with two water bottles (volume 100 ml) at the onset of the light phase from day one until day four for 30-min and four hours later for another 45-min period. At the end of presentation, water bottles and rats were weighed. Individual water consumption from each bottle was measured for each rat every day. On day 5 each rat was given 30 min access to the conditioned stimulus (novel flavour of 0.3% solution of saccharine sodium salt hydrate) contained in both bottles at the onset of the light phase. Immediately following the access to

that novel flavour, rats were weighed and received either an oral gavage of 1 ml corn oil, 1 ml sterile isotonic saline or an intraperitoneal injection of LiCl. The small volume of 1ml for oral gavage was chosen to minimize potential side effects by the administered volume per se, considering the altered anatomy of the stomach in gastric bypass rats. Rats were offered water four hours after light onset for a 45-min period.

The same protocol was repeated on day 8 and day 11. On all other days rats were given access to water for 30 min at the onset of the light phase and four hours later for another 45 min as described above (wash-out period). On day 14 each rat was presented with two bottles, one containing water and the other containing 0.3% saccharine solution in counterbalanced fashion and the respective consumption was measured for 30 minutes at light onset.

Conditioned taste aversion against GLP-1 receptor agonist exendin-4

Unoperated obese rats (400±15 g) received one hour daily access to water for 7 days to ensure stable fluid intake during the one hour period. On day 8, rats were given one hour access to a novel flavour of 0.3% solution of saccharine sodium salt hydrate solution (rather than water) followed immediately by treatment. As positive control for the formation of a conditioned taste aversion, one group of rats (n=7) was given 76.2 mg/kg lithium chloride intraperitoneally. Another group (n=7) received intraperitoneal sterile isotonic saline as control. To test the ability of exendin-4 to induce a conditioned taste aversion, another group of rats (n=7) received 2 µg/kg body weight exendin-4 intraperitoneally. To serve as further control for the anorexic

effects of exendin-4, another group of seven rats received intraperitoneal amylin (20µg/kg). This dose of amylin produces a similar reduction in one hour food intake tests as exendin-4, but amylin does not induce conditioned taste aversion (Lutz et al. 1995;Mack et al. 2007a).

All groups consumed similar amounts of saccharin solution (12-13 ml) during the one hour access prior to the various treatments. After 2 intervening days of one hour access to water, rats were tested for the acquisition of a conditioned taste aversion. In a two-bottle intake test, rats were first given 5 seconds access to water and saccharin separately (in counterbalanced order) to ensure that each rat sampled both solutions. The rats were then simultaneously presented with water and saccharin for one hour. The same test was repeated on the following day.

Tissue dopamine assay

Sixty days after surgery and after one week of *ad libitum* access to normal chow (RM1 diet, Special Diet Services Ltd, UK), nine gastric bypass rats and six sham-operated controls were briefly anesthetized with 4% isofluorane and then decapitated. Brains were removed, snap-frozen and immediately stored at -80°C. For further analysis, a three mm portion of the striatum was measured by utilizing a flexible measuring tape and then dissected. The caudate nucleus, putamen and nucleus accumbens were extracted from the striatum section by using tailored hole punches referring to exact coordinates according to the Paxinos rat brain atlas. The individual tissue samples were then weighed and placed in ice-cold phosphate buffer solution (PBS) and homogenized. All homegenates were split into two equal

portions, with one half of each treated with 0.2 M perchloric acid (1:10, w/v) containing ascorbic acid (0.2 µM) and EDTA (0.2 µM), to precipitate cell debris. These were centrifuged at 9000 × g for 15 minutes at 4°C, supernatants passed through a syringe filter (10 µm pore size) and whole tissue dopamine levels estimated using HPLC with electrochemical detection (Biggs et al. 1992). Dopamine peak areas were converted to dopamine amounts using an external standard method and expressed as amount of dopamine in picograms (pgs) per gram of striatal cortical tissue.

Statistical analysis

All data were normally distributed and expressed as mean ± SEM. Student's t-test for independent samples was used to test for significant differences. Preference, acceptance, food intake and energy intake in the two bottle preference tests were analyzed with a two-way group (between subjects) x concentration (within subjects) analysis of variance (ANOVA). A one-way ANOVA followed by Bonferroni post hoc tests for each concentration was applied when there was a significant group x concentration interaction. In the brief access test, the mean number of licks at each concentration per trial was collapsed across the three test sessions. For each rat, the mean number of licks to water was subtracted from the mean number of licks at each concentration, yielding a Licks-to-Intralipid® / Licks-to-Water value. This measure has also been successfully used in previous studies (Jiang et al. 2008;Spector et al. 1996b) to produce concentration-response curves that are relative to a water baseline. The lick response (adjusted for water) for each

concentration of a stimulus was compared using ANOVAs. The statistical rejection criterion of 0.05 was used for all analyses.

Results

Food preference

Average body weight of the obese rats before surgery was 385±7.8 g. After a short period of post surgical weight loss, body weight increased in sham-operated rats to 427.8±12.1 g on postoperative day 10 and it increased further for the rest of the observation period. In contrast, gastric bypass animals lost 13.8±3.0% of their preoperative weight by postoperative day 10 (318.7±8.2 g); body weight then leveled off around 320 g. Figure 15 shows intake of the three types of diet before and after surgery. There was no difference in total 48 hour energy intake before and after sham-operation (1277±115 kJ vs. 1318±102 kJ, p=0.35); gastric bypass rats significantly reduced their 48h energy intake after surgery (1297±92 kJ vs. 813±202 kJ, p<0.001). Sham-operated rats consumed similar proportions of the three food choices before and after surgery (high fat: 609±82 kJ before vs. 621±89 kJ after surgery, p=0.72; high fat plus Bisto®: 633±91 kJ vs. 658±93 kJ, p=0.48; normal chow: 36±27 g vs. 39±26 kJ, p=0.75). Gastric bypass rats significantly reduced their energy intake of the two high fat diets (high fat: 607.4±62.1 kJ vs. 344±89 kJ, p<0.001; high fat plus Bisto®: 649±105 kJ vs. 352±108 kJ, p<0.001), while they significantly increased their intake of the normal chow (normal chow: 41±26 kJ vs. 117±63 kJ, p<0.001). In view of the 40% reduction of total energy intake after surgery, gastric bypass increased normal chow intake from 3.2±2.1 % to 14.0±6.6 % (p<0.001) of total energy intake.

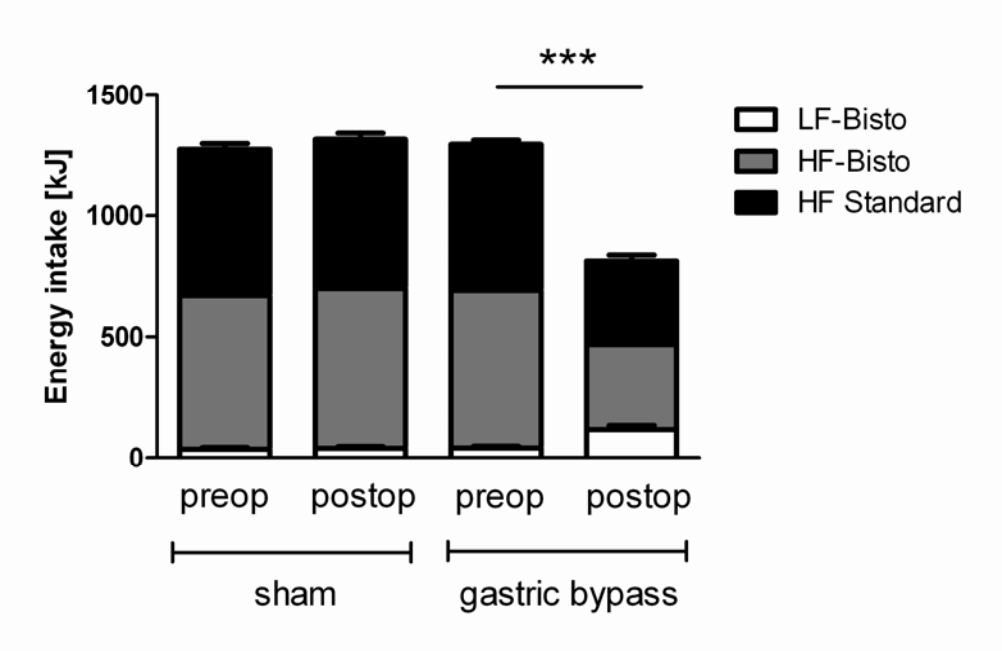


Figure 15: Energy (kJ) from 60% high fat (HF) diet, of 60% HF diet plus Bisto® (gravy type flavour) and normal chow (LF) in gastric bypass rats (n=13) and sham-operated rats (n=13) before and after surgery over a 48 hour period. Data are shown as mean values ± SEM (*** = p<0.001: total energy intake preoperative vs. postoperative after gastric bypass).

Two bottle preference test

Body weight

The average presurgical body weight of the obese rats used for the early weight stabilisation phase experiment was 368.3±2.0 g. Ten days after surgery the sham-operated controls weighed 403.5±6.4 g and the gastric bypass rats weighed 352.8±6.4 g (p<0.001). The average presurgical body weight of the rats used for the late weight stabilisation phase was 476.8±4.1 g; body weight 200 days after surgery increased to 712.5±10.8 g in the sham-operated rats and was 455.9±14.3 g in the gastric bypass rats (p<0.001). Figure 16 shows the body weight changes for both groups.

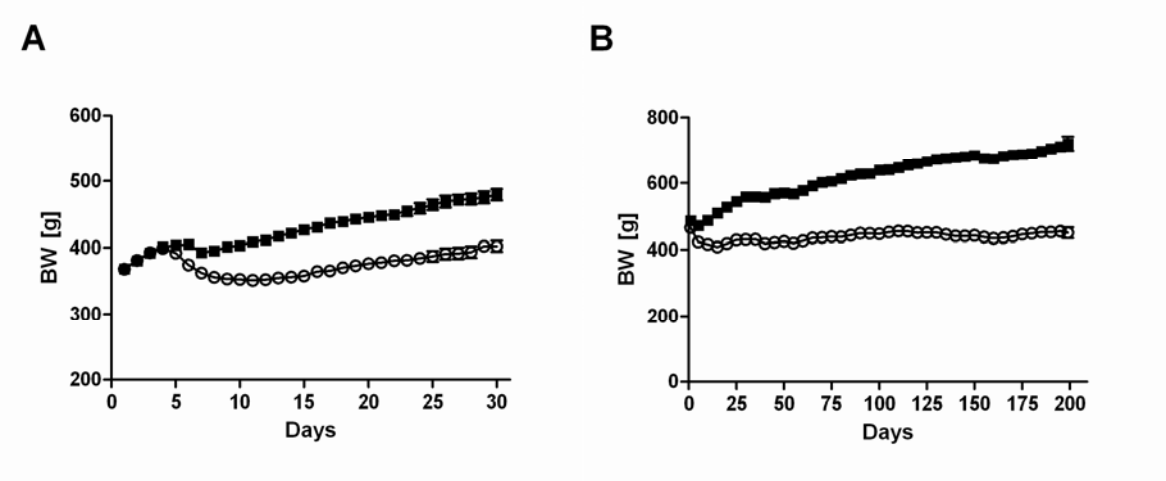


Figure 16: Body weight change for the gastric bypass (-o-) and sham-operated rats ad libitum fed (-■-) used for the two bottle preference test in the early phase (A) and in the late phase (B) after surgery. Data are shown as mean values ± SEM.

Postprandial plasma levels of PYY and active GLP-1

Gastric bypass rats had significantly higher plasma active GLP-1 and PYY levels compared to sham-operated controls measured 30 minutes after the 5g test meal (Figure 17).

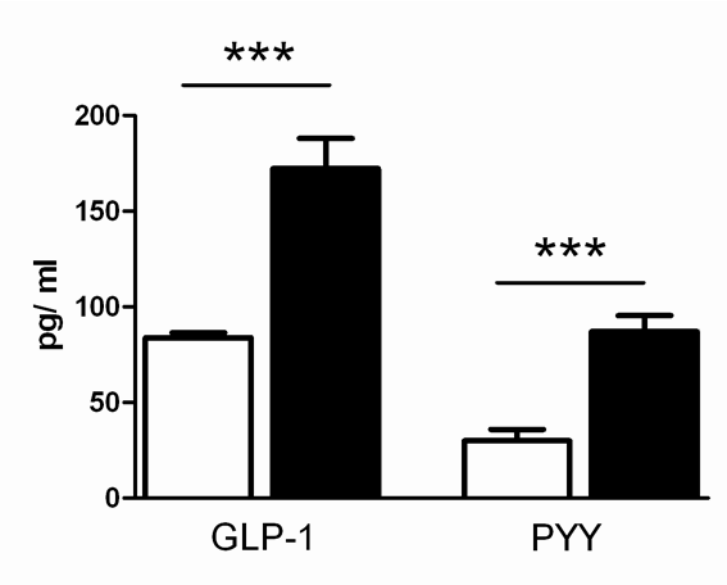


Figure 17: PYY- and GLP-1 level for the gastric bypass (n=9, black) and sham-operated rats ad libitum fed (n=10, white used for the two bottle preference test in the late phase after surgery. Data are shown as mean values ± SEM (*** = p<0.001).

Preference

Two-way ANOVA revealed a significant main effect of Intralipid® concentration ($F(6,482)=33.3$; $p<0.001$), but not of surgical group ($F(2,482)=2.11$; $p=0.12$). However, the group x concentration interaction was also significant ($F(12,482)=4.48$; $p<0.001$). Ten days after surgery both unoperated and sham-operated rats showed a similar increase in preference (Intralipid versus total intake) for Intralipid® at concentrations above 0.1%. In contrast, gastric bypass rats did not show a clear preference for the Intralipid® solutions (Figure 18A, 0.5% Intralipid®: unoperated: 92.8±0.9 % vs. sham: 82.4±3.8 % vs. bypass: 66.5±5.5 %, $p<0.01$; 1% Intralipid®: unoperated: 96.2±0.6 % vs. sham: 90.7±2.8 % vs. bypass: 74.1±4.5 %, $p<0.01$; 5% Intralipid®: unoperated: 96.4±0.5 % vs. sham: 92.8±1.7 % vs. bypass: 63.2±5.5 %, $p<0.001$).

Observations were similar in the late phase of weight stabilisation study. The two-way ANOVA showed a significant main effect of Intralipid® concentration ($F(6,266)=7.73$; $p<0.001$), but not of surgical group ($F(1,266)=2.80$; $p=0.12$), while the group x concentration interaction was also significant ($F(6,266)=9.93$; $p<0.001$). On postoperative day 200 sham-operated rats had a higher preference for Intralipid® concentrations above 0.1%, while gastric bypass rats showed no preference for Intralipid® (Figure 19A).

Acceptance

A significant main effect of Intralipid® concentration ($F(6,482)=90.17$; $p<0.001$) and of surgical group ($F(2,482)=107.0$; $p<0.001$) was found in the two-way ANOVA. The group x concentration interaction was also significant ($F(12,482)=21.35$; $p<0.001$). During the early weight stabilisation phase unoperated rats and sham-operated rats showed increased acceptance of Intralipid® intake (in ml) at concentrations above 0.1%, whilst acceptance did not increase in gastric bypass rats with higher concentrations (Figure 18B, 0.5% Intralipid®: unoperated: 83.8±8.6 ml vs. sham: 58.2±4.6 ml vs. bypass: 28.1±2.9 ml, $p<0.001$; 1% Intralipid®: unoperated: 96.3±10.6 ml vs. sham: 60.2±6.1 ml vs. bypass: 30.0±2.4 ml, $p<0.001$; 5% Intralipid®: unoperated: 112.9±10.5 ml vs. sham: 81.5±7.7 ml vs. bypass: 21.5±2.0 ml, $p<0.001$). In the late phase of weight stabilisation two-way ANOVA revealed a significant main effect of Intralipid® concentration ($F(6,266)=36.31$; $p<0.001$) and of surgical group ($F(1,266)=158.89$; $p<0.001$). The interaction was also significant ($F(6,266)=41.60$; $p<0.001$). Gastric bypass rats also had a lower acceptance for Intralipid®

concentrations above 0.1 % compared to their sham-operated counterparts (Figure 19B).

Food intake

Two-way ANOVA revealed a significant main effect of surgical group ($F(2,482)=9.45$; $p<0.001$), but not of Intralipid® concentration ($F(6,482)=1.34$; $p=0.24$). There was no group x concentration interaction ($F(12,482)=0.88$; $p=0.56$). Average daily food intake for all three groups of the early weight stabilisation phase throughout the two bottle experiment was 29.3±0.3 g for unoperated rats, 31.3±0.3 g for sham-operated rats and 29.9±0.3 g for gastric bypass rats ($p<0.001$). In this group, food intake did not change with increasing Intralipid® concentrations throughout the two bottle experiment (Figure 18C). In the late phase of weight stabilisation, the main effects of Intralipid® concentration ($F(6,266)=4.91$; $p<0.001$) and of surgical group ($F(1,266)=30.89$; $p<0.001$) were significant in the two-way ANOVA. However, there was no significant group x concentration interaction ($F(6,266)=0.43$; $p=0.85$). Gastric bypass rats ate significantly less per day than the sham-operated controls throughout the two bottle experiment (sham: 34.0±1.2 g vs. bypass: 27.5±0.8 g, $p<0.001$). As seen during the early phase experiment, food intake did not change with increasing Intralipid® concentrations (Figure 19C).

Calorie intake

Total calorie intake was the sum of calories consumed as food (14.74 kJ/g) and Intralipid® (energy content of the standard 20% solution: 42.0 kJ/ml). There was a

significant main effect of Intralipid® concentration ($F(6,482)=24.75$; $p<0.001$), but not of surgical group ($F(2,482)=1.04$; $p=0.3549$) in the two-way ANOVA. The group x concentration interaction was also significant ($F(12,482)=5.94$; $p<0.001$). During the early weight stabilisation phase both unoperated and sham-operated rats increased their calorie intake when exposed to the 5% Intralipid® solution, but gastric bypass rats did not (Figure 18D, unoperated: 668.1±28.1 kJ vs. sham: 633.0±16.5 kJ vs. bypass: 507.1±12.2 kJ, $p<0.001$). During the late weight stabilisation phase, the main effects of Intralipid® concentration ($F(6,266)=5.79$; $p<0.001$) and of surgical group ($F(1,266)=75.53$; $p<0.001$) were significant in the two-way ANOVA. The group x concentration interaction was also significant ($F(6,266)=5.91$; $p<0.001$). Sham-operated rats increased their energy intake when exposed to the 0.5%, 1% and 5% Intralipid® solutions compared to gastric bypass rats, which showed no increase in energy intake even with the highest Intralipid® concentration (5%) (Figure 19D, 0.5% Intralipid®: sham: 400.5±11.3 kJ vs. bypass: 326.0±14.9 kJ, $p<0.001$, 1% Intralipid®: sham: 419.8±8.6 kJ vs. bypass: 356.3±17.2 kJ, $p<0.01$, 5% Intralipid®: sham: 478.1±12.2 kJ vs. bypass: 321.4±13.9 kJ, $p<0.001$).

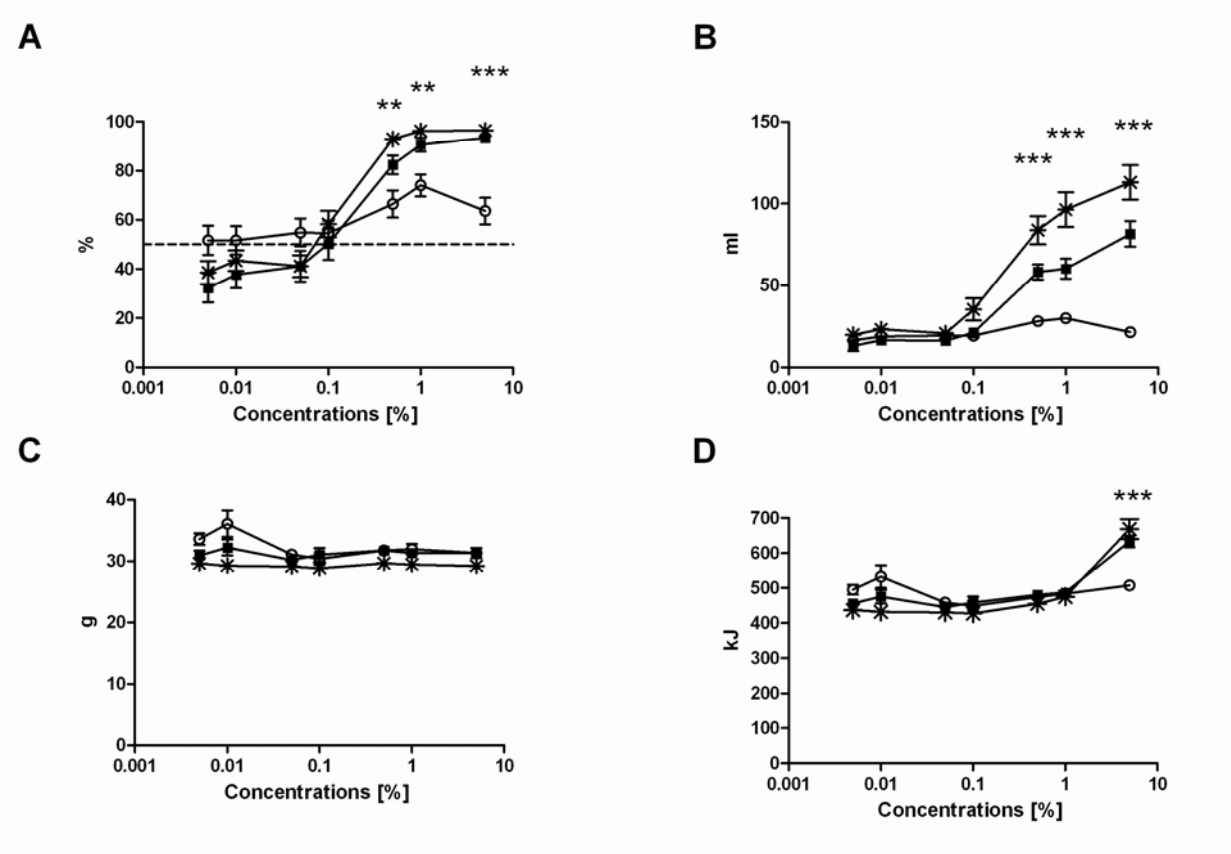


Figure 18: Two bottle preference test in gastric bypass rats (n=18, -O-), in sham-operated rats (n=12, -■-) and unoperated rats (n=6, -X-) during the early weight stabilisation phase; Seven Intralipid® concentrations were used in ascending order: 0.005%, 0.01%, 0.05%, 0.1%, 0.5%, 1%, 5%; A Preference; B Acceptance; C Food intake; D Total calorie intake. Data are shown as mean values ± SEM (** = p<0.01, *** = p<0.001 using one-way ANOVA for concentration to concentration analysis between all three groups indicate difference between gastric bypass rats from sham and unoperated rats). X-axes are displayed on a log10 scale.

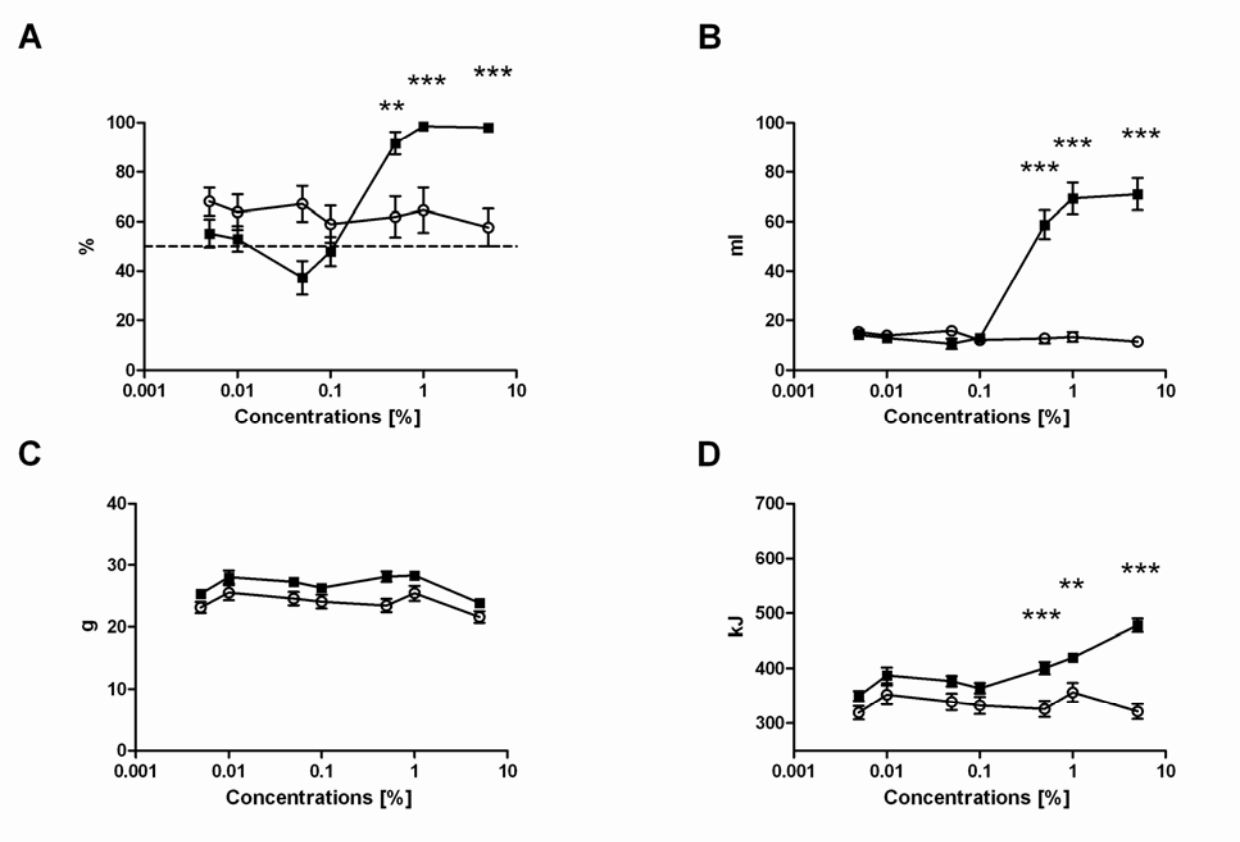


Figure 19: Two bottle preference test in gastric bypass (n=10, -O-) and sham-operated rats (n=10, -■-) during the late weight stabilisation phase (postoperative day 200); Seven Intralipid® concentrations were used in ascending order: 0.005%, 0.01%, 0.05%, 0.1%, 0.5%, 1%, 5%; A Preference; B Acceptance; C Food intake; D Total calorie intake. Data are shown as mean values ± SEM (** = $p<0.01$, *** = $p<0.001$ using one-way ANOVA for concentration to concentration analysis between the two groups indicate difference between gastric bypass rats and sham-operated rats). X-axes are displayed on a log10 scale.

Brief Access Tests

Body weight

The average presurgical body weight of the obese rats used for the brief access test was 434±6 g. From postoperative day 5 the sham-operated controls weighed significantly more compared to the gastric bypass rats (postop day 5: sham: 430±8 g

vs. bypass: 377±7 g, $p<0.001$). Body weight changes for both groups are shown in figure 20A.

Licking response

Two-way ANOVA revealed no significant difference between the licking response of sham-operated and gastric bypass operated rats after surgery with or without water restriction. When water was available ad libitum prior to the test, there was a significant main effect of Intralipid® concentration ($F(6,54)=15.16$; $p<0.001$), but not of surgical group ($F(1,54)=1.52$; $p=0.25$) in the two-way ANOVA. The group x concentration interaction was also not significant ($F(6,54)=1.20$; $p=0.32$). When water was restricted, there was a significant main effect of Intralipid® concentration ($F(6,60)=5.16$; $p<0.001$), but not of surgical group ($F(1,60)=0.00$; $p=0.99$) in the two-way ANOVA. The group x concentration interaction was also not significant ($F(6,60)=0.61$; $p=0.72$). The Intralipid® concentration-response functions (i.e. the number of licks to Intralipid adjusted to water baseline) for the two test conditions are shown in figure 20B and C.

Number of trials

Two-way ANOVA revealed no differences between gastric bypass rats and sham-operated controls in the absolute number of trails initiated to the Intralipid® concentrations with or without water restriction. When water was available ad libitum prior to the test, there was a significant main effect of Intralipid® concentration ($F(6,54)=5.85$; $p<0.001$), but not of surgical group ($F(1,54)=4.86$; $p=0.055$) in the

two-way ANOVA. The group x concentration interaction was also not significant ($F(6,54)=2.04$; $p=0.076$). When water was restricted, there was a significant main effect of Intralipid® concentration ($F(6,60)=6.08$; $p<0.001$), but not of surgical group ($F(1,60)=0.41$; $p=0.54$) in the two-way ANOVA. The group x concentration interaction was also not significant ($F(6,60)=1.73$; $p=0.13$). The number of initiated trials for each Intralipid® concentration during the two test conditions is shown in figure 20D and E.

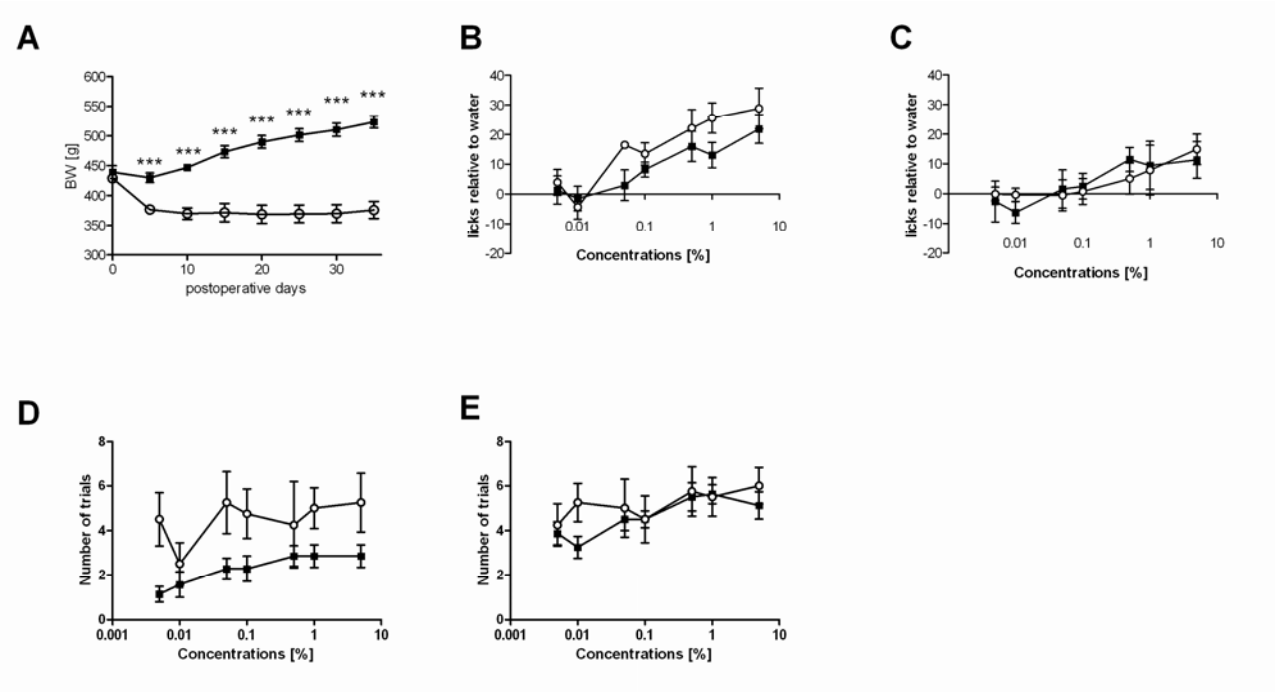


Figure 20: Body weight changes for the gastric bypass (n=8, -o-) and sham-operated rats ad libitum fed (n=8, -■-) used for the brief access test (A). Postoperative Intralipid® concentration-response functions relative to a water baseline are shown without (B) and with 23h water restriction (C). The absolute number of initiated trials for each Intralipid® concentration are shown without (D) and with (E) water restriction. Data are shown as mean values ± SEM (*** = $p<0.001$). X-axes are displayed on a log10 scale.

Conditioned taste aversion for corn oil

Mean saccharine and water intake were significantly different between all groups ($p<0.001$). There was no difference in saccharine intake between sham-operated rats that were exposed to gavage with sterile isotonic saline or corn oil on the final test day (saline: 13.8±1.5 ml vs. corn oil: 10.1±1.8 ml, $p=0.13$); both groups showed a significantly higher saccharine intake when compared to water intake (saline gavage: saccharine: 13.8±1.5 ml vs. water: 1.0±0.5 ml, $p<0.001$ and corn oil gavage: saccharine: 10.1±1.8 ml vs. water: 1.2±0.6 ml, $p<0.001$). Saccharine intake of sham-operated rats was significantly reduced after intraperitoneal injection of the positive control LiCl when compared to rats that received oral saline or corn oil gavage (saline: 13.8±1.5 ml vs. corn oil: 10.1±1.8 ml vs. LiCl: 0.32±0.1 ml, $p<0.001$). In contrast to sham operated rats, gastric bypass rats reduced their saccharine intake significantly after corn oil gavage when compared to saline gavage (saline: 10.8±1.5 ml vs. corn oil: 4.1±1.5 ml, $p<0.01$). Gastric bypass rats preferred saccharine over water after saline gavage (saccharine: 10.8±1.5 ml vs. water: 1.1±0.6 ml, $p<0.001$), but there was no preference after corn oil gavage (saccharine: 4.1±1.5 ml vs. water: 4.9±1.4 ml, $p=0.68$). Saccharine and water intake for all groups are shown in Figure 21A. Apart from the positive control group, saccharine intake expressed as percentage of total fluid intake was significantly reduced in gastric bypass rats after corn oil gavage in comparison to all other groups (Figure 21B, sham saline: 93.1±3.6 % vs. sham corn oil: 87.8±6.1 % vs. bypass saline: 90.1±4.0 % vs. bypass corn oil: 43.6±14.5 %, $p<0.001$).

Conditioned taste aversion for the GLP-1 receptor agonist exendin-4

Saccharine intake was significantly reduced in rats that had received the positive control lithium chloride when compared to rats that received saline or amylin (saline: 47.7±7.8 ml vs. amylin: 37.4±6.9 ml vs. lithium chloride: 12.4±4.9 ml, $p<0.001$). There was no difference in saccharine intake between rats that were exposed to sterile isotonic saline or amylin on the final test day ($p=0.58$); both groups showed a significantly higher saccharine intake when compared to water intake (saline: saccharine: 47.7±7.8 ml vs. water: 3.4±0.5 ml, $p<0.001$ and amylin: saccharine: 37.4±6.9 ml vs. water: 3.5±1.0 ml, $p<0.01$). Furthermore, rats reduced their saccharine intake significantly after receiving exendine-4 when compared to saline administration (saline: 47.7±7.8 ml vs. exendine-4: 18.2±8.5 ml, $p<0.01$). The rats showed no preference after exendine-4 injection for saccharine (saccharine: 18.2±8.5 ml vs. water: 10.0±2.0 ml, $p=0.32$). Saccharine and water intake for all groups are shown in figure 21C.

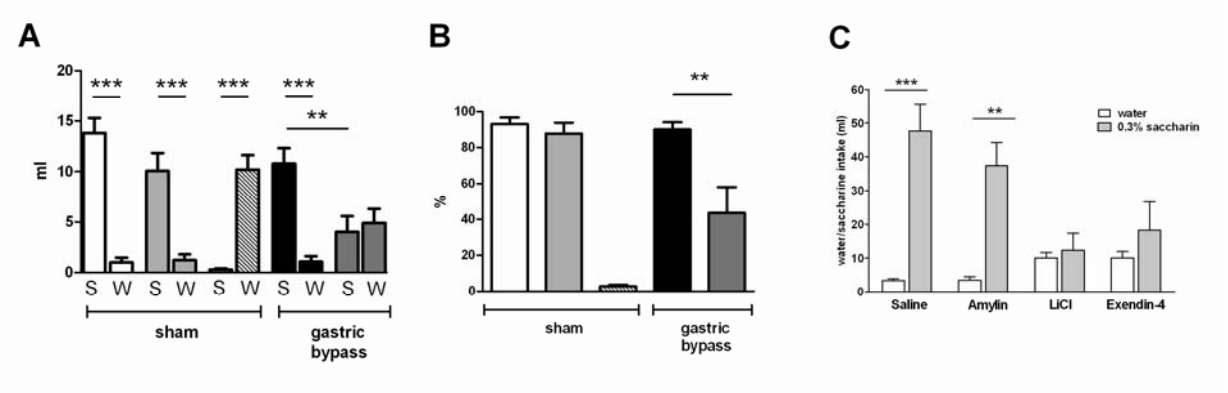


Figure 21: Conditioned taste aversion; A Saccharine (S) and water (W) intake in sham-operated rats after oral gavage with 1ml of sterile isotonic saline (n=8, white), 1ml of corn oil (n=8, light grey), i.p. injection of 76.2 mg/kg body weight LiCl (striped) and in gastric bypass rats after oral gavage with sterile isotonic saline (n=8, black) and corn oil (n=8, dark grey); B Saccharine intake expressed as percentage of total fluid intake in sham-operated rats after oral gavage with sterile isotonic saline (n=8, white), corn oil (n=8, light grey), i.p. injection of 76.2 mg/kg body weight LiCl (striped) and in gastric bypass rats after oral gavage with sterile isotonic saline (n=8, black) and corn oil (n=8, dark grey). **C** Water and saccharine intake in rats after intraperitoneal administration of sterile isotonic saline (n=7), amylin (20 µg/kg, n=8), lithium chloride (76.2 mg/kg, n=7) and exendin-4 (2 µg/kg, n=7). Data are shown as mean values ± SEM (** = p<0.01, *** = p<0.001; saccharine versus water).

Tissue dopamine assay

As shown in Figure 22, dopamine concentrations in the striatal tissue and in the nucleus accumbens did not substantially differ between gastric bypass rats and sham-operated counterparts when both groups were fed *ad libitum* with normal chow (striatum: sham: 22.0±5 pmol/g vs. bypass: 19.7±2.3 pmol/g, p=0.65 and nucleus accumbens: sham: 15.5±5.6 pmol/g vs. bypass: 9.5±0.9 pmol/g, p=0.21).

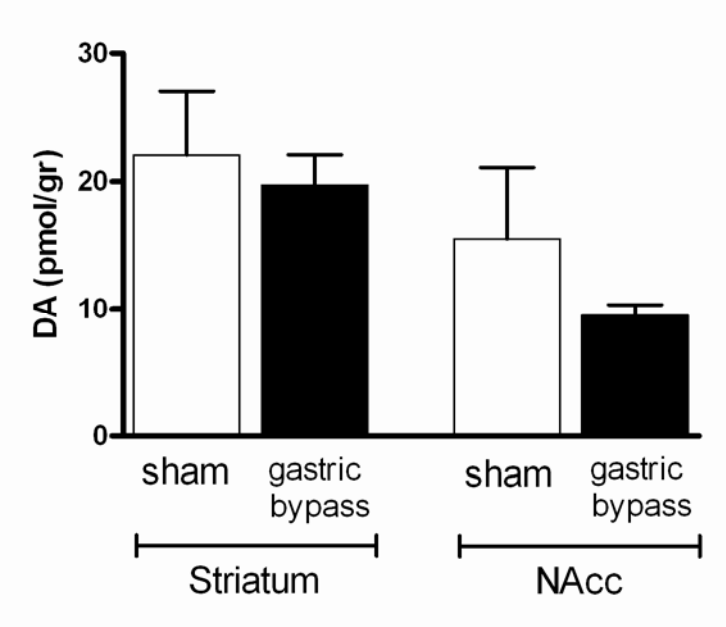


Figure 22: Tissue dopamine levels in the Striatum and Nucleus Accumbens (NAcc) of gastric bypass and sham-operated rats when being ad libitum fed. Data are shown as mean values ± SEM.

Discussion

I demonstrated a reduced preference of rats for concentrations of Intralipid® of 0.5% and above when offered during a two bottle preference test, but not in a brief access test. Because rats ingest significantly more Intralipid® during the two bottle preference test than during the brief access test where postingestive effects are minimal, I concluded that possible mechanisms may include postingestive factors such as the induction of an aversive effect. I further investigated this possibility and found that gastric bypass rats treated with 1ml of corn oil by gastric gavage showed a marked reduction in their preference for saccharine solution that is normally highly preferred by rats. Interestingly, the conditioned taste aversion seen after gavage of a small volume of corn oil in our study was of a similar magnitude compared with the conditioned taste aversion produced by peripheral administration of the GLP-1 receptor agonist exendin-4. Thus, it seems plausible to suggest that alterations in fat preference after gastric bypass may result in part from the induction of an aversive response mediated by increased levels of GLP-1.

My data in the rat gastric bypass model are consistent with previous human findings that gastric bypass does not only reduce food intake (Korner, Bessler, Cirilo, Conwell, Daud, Restuccia, & Wardlaw 2005;le Roux, Aylwin, Batterham, Borg, Coyle, Prasad, Shurey, Ghatei, Patel, & Bloom 2006a;le Roux, Welbourn, Werling, Osborne, Kokkinos, Laurenius, Lonroth, Fandriks, Ghatei, Bloom, & Olbers 2007), but also preference for food high in fat (Brolin, Robertson, Kenler, & Cody 1994;Brown, Settle, & Van Rij 1982;Halmi, Mason, Falk, & Stunkard 1981;Olbers, Bjorkman, Lindroos, Maleckas, Lonn, Sjostrom, & Lonroth 2006).

In conclusion, gastric bypass in rats reduces preference for high fat food and high concentrations of Intralipid® solution. Postingestive effects and conditioned taste aversion may partly explain my findings. By elucidating the mechanisms by which obesity surgery reduces consumption of high fat foods, new surgical and non-surgical therapies could be developed that mimic these mechanisms and so promote safe and effective weight loss.

Chapter 7: Analysis of Sweet Taste Sensitivity and Hedonic rating of sucrose after Roux-en-Y Gastric Bypass in humans

Introduction

Patients after gastric bypass often report idiosyncratic changes in taste perception that involve “sweet” taste and a selective reduction in food with high carbohydrate content (Brolin, Robertson, Kenler, & Cody 1994;Brown, Settle, & Van Rij 1982;Halmi, Mason, Falk, & Stunkard 1981;Olbers, Fagevik-Olsen, Maleckas, & Lonroth 2005;Scruggs, Buffington, & Cowan, Jr. 1994;Tichansky et al. 2006). The gustatory system is a prime candidate as a contributor to the observed effects. It remains unclear, however, whether such changes in preferences, even if taste-related, are attributable to changes in the intensity of the sensory signals generated by food or by their altered evaluation in so called “reward” circuits in the brain, or both (Hajnal et al. 2010a;Shin et al. 2010;Tichansky et al. 2011;Zheng et al. 2009).

Against this background I hypothesized that gastric bypass surgery alters sweet taste function associated with sweeteners and affects the preference for sucrose in humans. I therefore examined oral-sensory sucrose taste detection thresholds of patients and controls before and after gastric bypass by asking patients to taste, but not to swallow sucrose solutions. Taste detection thresholds can be considered as an effective way to assess the functional status of oral-sensory receptors and the sensitivity of downstream gustatory circuits (Hajnal, Kovacs, Ahmed, Meirelles, Lynch, & Cooney 2010a;Spector 2000;Spector & Glendinning 2009). Although taste sensitivity has been shown to vary as a function of genetics (Lyall et al. 2004), pharmacological treatment (Spector et al. 1996a), and neural manipulations

(Pittman, Crawley, Corbin, & Smith 2007), it does not necessarily directly relate to perceived suprathreshold insensitivity or hedonic responsiveness (Bartoshuk 1978;Spector 2000). I therefore used a hedonic visual analogue scale (VAS) to test what sucrose concentrations gastric bypass patients find “just about right” when they don’t swallow the solution (Conner and Booth 1988;Drewnowski et al. 1985;Frijters and Rasmussen-Conrad 1982).

Material and Methods

Subjects

All human studies were performed according to the principles of the Declaration of Helsinki. The Research and Ethics committee at Charing Cross Hospital, London, approved the study (REC reference number: 08/H0711/122). Exclusion criteria included presence of type 2 diabetes mellitus, pregnancy, breast feeding, substance abuse, more than three alcoholic drinks per day, psychiatric illness and chronic medical conditions that would make it unsafe to have a general anaesthetic. Written informed consent was obtained from all participants. Nine obese subjects were investigated for sucrose sensitivity one week before and six weeks after gastric bypass surgery. Nine lean control subjects were also tested at similar time intervals.

Surgery

Laparoscopic Roux-en-Y gastric bypass was performed as described before (Olbers, Lonroth, Fagevik-Olsen, & Lundell 2003). Briefly, the patients were positioned in supine position with extended legs placed on a footrest with head up tilt. The surgeon stood on the patient's right side and the assistant on the left. Seven ports were positioned and the left liver lobe was lifted with a self-retraining retractor, and a 30° angle laparoscope was used. The dissection started with incision of the phrenico-gastric peritoneal reflection at the angle of His. Starting 4 cm from the gastroesophageal junction at the lesser curvature, dissection was performed medial to the nerve of Latarjet to reach the bursa omentalis. Repeated firing of a 45-60 mm linear cutting stapler made an oblique partition of the stomach from the lesser

curvature up to the angle of His. This created a gastric pouch with an estimated size of 15-25 ml. All patients have been operated upon with an antecolic and antegastric Roux-en-Y loop, with the greater omentum and gastro-colic ligament completely divided caudo-cranially using ultrasound scissors, to shorten the distance for jejunum to gastric pouch.

The anastomotic technique was as follows:

1. The gastro-jejunostomy was created by stapling the jejunum to the posterior wall of the gastric pouch using a 45 mm 3.5-mm-staple linear cutting stapler. The remaining defect was sutured with two running resorbable 3/0 sutures anchored at both staple-line edges.
2. The entero-entero-anastomosis was created in a similar manner as a side-to-side anastomosis with 45-60 mm 3.5-mm stapler. The length of the Roux limb was selectively chosen to be between 60 and 150 cm.
3. To complete the Roux-en-Y construction, the loop was divided with a linear cutting stapler between the anastomosis

Sucrose detection sensitivity

Nine obese subjects (8 female, 1 male) were investigated for sucrose sensitivity one week before and two months after gastric bypass surgery. Nine normal weight control subjects (7 female, 2 male) were also tested at similar time intervals. Detection tests for sucrose were all performed in the morning after an overnight fast starting before 23:00. Room temperature was kept constant at 21°C for all test sessions. All solutions were prepared daily using the same still natural mineral water (Caledonian Still Natural Mineral Water, Sainsbury's Supermarkets Ltd., London, UK:

pH 7.4, Calcium 27 mg/l, Chloride 6.4 mg/l, Bicarbonate 103 mg/l, Magnesium 6.9 mg/l, Sodium 6.6 mg/l, Sulfates 10.6 mg/l) and presented at room temperature. Seven sucrose (Sigma Aldrich, Dorset, UK) concentrations were used in this study: 2.1, 6.25, 12.5, 25, 50, 100 and 300 mM. Concentrations were tested in eight blocks with each block consisting of seven sucrose and seven water stimuli. Sucrose and water stimuli were presented in random order without replacement. Thus, each of the seven sucrose concentrations was presented once within a block. Water and sucrose stimuli (15 ml) were offered in polystyrene cups that were filled immediately before the test began. The subjects were given a period of five seconds to sample the stimulus in the mouth. Subjects then expectorated the sample and were given another five seconds to indicate whether the stimulus was water or not. If a subject reported that the stimulus was not water they were asked to describe the quality of the taste as sweet, sour, salty or bitter. Each stimulus was followed by a thorough ten second water rinse (30 ml) which was expelled before the next stimulus was offered. After four blocks, the assessment was interrupted with a 10 minutes rest period. To help ensure maintained vigilance, the patients were rewarded for correct responses with the presentation of a token and penalized by loss of a token for incorrect responses.

Hedonic visual analogue scale to test the concentration reported as "just about right"

Ten obese subjects (8 female, 2 male) were investigated with a VAS to test the concentration of sucrose that was "just about right" one week before and two months after gastric bypass surgery. Nine lean control subjects (7 female, 2 male) meeting the same exclusion criteria were also tested at similar time intervals. All tests were

performed in the morning after an overnight fast starting before 23.00 h. Room temperature was kept constant at 21°C for all test sessions. All solutions were prepared daily using the same still natural mineral water as in the detection study and presented at room temperature. Seven sucrose (Sigma Aldrich, Dorset, UK) concentrations were used in this study: 0, 12.5, 25, 50, 100, 200 and 400 mM. Concentrations were tested in three blocks with each block consisting of seven sucrose samples presented in random order without replacement. Sucrose samples (15 ml) were offered in polystyrene cups that were filled immediately before the test began. Between sucrose concentrations subjects rinsed their oral cavity with 15 ml water. Subjects were given a period of five seconds to sample the stimulus in the mouth. Subjects then expectorated the sample and were given another five seconds to indicate the acceptability of the sample on the VAS which was a bipolar scale, 200 mm long with the ends anchored by the phrases “Far too sweet – I would never drink it” and “Far too little sweet- I would never drink it” whilst the midpoint anchoring was “Just About Right – My ideal sweetness in a soft drink”.

Statistical analysis

A hit was defined as when the subject correctly reported that the stimulus was different from water. A false alarm (FA) was defined as when the subject incorrectly reported that the stimulus was different from water. The hit rate for a given sucrose concentration was adjusted for the false alarm rate to derive a corrected hit rate using equation 1:

$$Corrected\ Hit\ Rate = \frac{P(hit) - P(FA)}{1.0 - P(FA)}$$

Equation 1

where P(hit) = the proportion of trials of a given concentration that were hits, P(FA) = the proportion of water trials that were false alarms. Thus, when the uncorrected hit rate is equal to the false alarm rate, the corrected hit rate = 0. The corrected hit rate values were subjected to various two-way analyses of variance (ANOVAs). Because there was very little or no variance around the sample means for the highest three concentrations for the groups both preoperatively and postoperatively, only the scores for the lower 4 concentrations, representing the dynamic range of performance, were used in the ANOVAs. In addition, concentration-response curves were fit to the corrected hit rate values for each subject preoperatively and postoperatively to derive a family of individual psychometric functions using equation 2:

$$f(x) = \frac{a}{1+10^{((\log_{10}(x)-c)*b)}}$$

Equation 2

where log10(x) = log10 concentration, a = the upper asymptote of performance, b = slope, and c = the log10 concentration at 1/2a performance (i.e. EC50). We defined the c-parameter as the threshold because it represents the inflection point of the psychometric function and is thus optimally represents lateral shifts in sensitivity. The shifts in c-parameters were analyzed in a one-way ANOVA. Although all control subjects and patients were included in the analyses of corrected hit rate described

above, one control subject and two patients had to be discarded from the c-value analysis because either their preoperative or postoperative curve fits accounted for only 77% or less of the variance. All other subjects had curve fits that accounted for at least 85% of the variance (mean = 96.7%, se = ±0.7%). A p-value below 0.05 was considered statistically significant.

The ratings on the “Just About Right” Scale were analyzed with a two-way group (between subjects) x concentration (within subjects) analysis of variance (ANOVA). A p-value ≤0.05 was considered statistically significant.

Results

Demographic data

Table 1 shows the demographics for the nine obese patients (8 female, 1 male) and the nine lean control subjects (7 female, 2 male).

				preop		**Postop**	
No	**Initials**	**Sex**	**Operation**	**Weight [kg]**	**BMI [kg/m2]**	**Weight [kg]**	**BMI [kg/m2]**
Pat1	JHM	F	Gastric bypass	132.7	49.3	106.1	39.4
Pat2	BM	M	Gastric bypass	132.1	35.8	102.2	27.7
Pat3	JO	F	Gastric bypass	132.7	51.2	115.6	44.6
Pat5	JL	F	Gastric bypass	123.8	46.6	107.9	40.6
Pat6	VG	F	Gastric bypass	92.0	36.9	85.2	34.0
Pat7	AL	F	Gastric bypass	132.7	47.0	106.1	37.6
Pat8	AG	F	Gastric bypass	128.3	46.6	111.1	40.3
Pat9	AS	F	Gastric bypass	96.7	43.0	90.2	40.1
Pat10	VQ	F	Gastric bypass	114	46.8	101.1	41.5
Average				**120.6**	**44.8**	**102.8**	**33.8**
SEM				**5.4**	**1.8**	**3.3**	**4.5**
Co1	MG	F	none	59.0	19.9	59.1	19.9
Co2	MB	M	none	91.0	24.7	92.2	25.0
Co3	SK	F	none	49.5	19.8	49.5	19.8
Co4	KoD	F	none	70.0	23.9	70.4	23.9
Co5	CleR	M	none	96.0	27.7	96.1	27.7
Co8	LC	F	none	54.6	18.2	54.6	18.2
Co9	KF	F	none	56.0	22.7	56.3	22.7
Co10	SB	F	none	57.0	21.2	57.8	21.2
Co11	DoR	F	none	65.0	22.2	65.3	22.2
Average				**66.5**	**22.3**	**66.6**	**22.3**
SEM				**5.5**	**1.0**	**5.6**	**1.0**
p-value				**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**

Table 1: Demographic data of obese patients (n=9) and lean control subjects (n=9).

Body weight

The nine patients after gastric bypass reduced their mean body weight from 120.6±5.4 kg to 102.8±3.3 kg within six weeks ($p<0.001$) resulting in a BMI reduction from 44.8±1.8 to 38.4±1.6 kg/m^2 ($p<0.001$). In contrast, the nine normal weight controls with a BMI of 22.0±1.0 kg/m^2 kept a stable body weight (66.5±5.5 kg vs. 66.6±5.6 kg, $p=0.99$). For the visual analogue scaling study, the ten patients after gastric bypass had a mean body weight reduction from 117.4±7.2 kg to 103.4±6.5 kg ($p<0.001$) between the two test time points resulting in a BMI reduction from 42.7±1.7 to 37.7±1.7 kg/m^2 ($p<0.001$). In contrast, the nine normal weight controls with a BMI of 22.4±0.9 kg/m^2 had a similar body weight at the two tests (65.5±4.6 kg vs. 65.8±4.8 kg, $p=0.33$).

Corrected Hit Rate Analysis for sucrose taste detection

The mean corrected hit rates (proportion of sucrose trials correctly adjusted for false alarm rate) for control subjects and patients pre- and postoperatively are displayed in Figure 23. Table 2 summarizes the two-way ANOVA values for comparison of corrected hit rates pre- and postoperatively between controls and patients. Preoperatively, there was a significant main effect of concentration ($p<0.001$), but no significant difference in corrected hit rates between controls and patients ($p=0.71$). There was also no statistically significant interaction between surgical group and concentration ($p=0.28$). Postoperatively, gastric bypass patients had significantly higher corrected hit rates to the lowest 4 sucrose concentrations compared with controls ($p=0.046$). There was also a significant main effect of concentration ($p<0.001$), but no significant group x concentration interaction ($p=0.37$).

Two-way ANOVA values of a within group comparison of performance as a function of concentration and time are shown in Table 3. Postoperatively, patients performed significantly better than preoperatively (p=0.048). There was also a significant main effect of concentration (p<0.001), but the interaction was not significant (p=0.64). In contrast, the performance of controls, did not change postoperatively compared with the preoperative values (p=0.33). There was a main effect of concentration (p<0.001), but no significant interaction (p=0.73).

	Concentration	**Surgical group**	**Concentration** X **Surgical group**
preoperative	F(3,48)=59.5, p<0.001	F(1,16)=0.14, p=0.71	F(3,48)=1.31, p=0.28
postoperative	F(3,48)=60.3, p<0.001	F(1,16)=4.679, p=0.046	F(3,48)=1.07, p=0.37

Table 2: Two-way ANOVA values for comparison of corrected hit rates pre- and postoperatively between controls and patients as a function of concentration and surgical group

	Concentration	**Time**	**Concentration** X **time**
controls	F(3,24)=188.5, p<0.001	F(1,8)=1.07, p=0.33	F(3,24)=0.442, p=0.73
patients	F(3,24)=44.43, p<0.001	F(1,8)=5.42, p=0.048	F(3,24)=0.56, p=0.64

Table 3: Two-way ANOVA values for comparison of corrected hit rates for controls and patients as a function of concentration and time

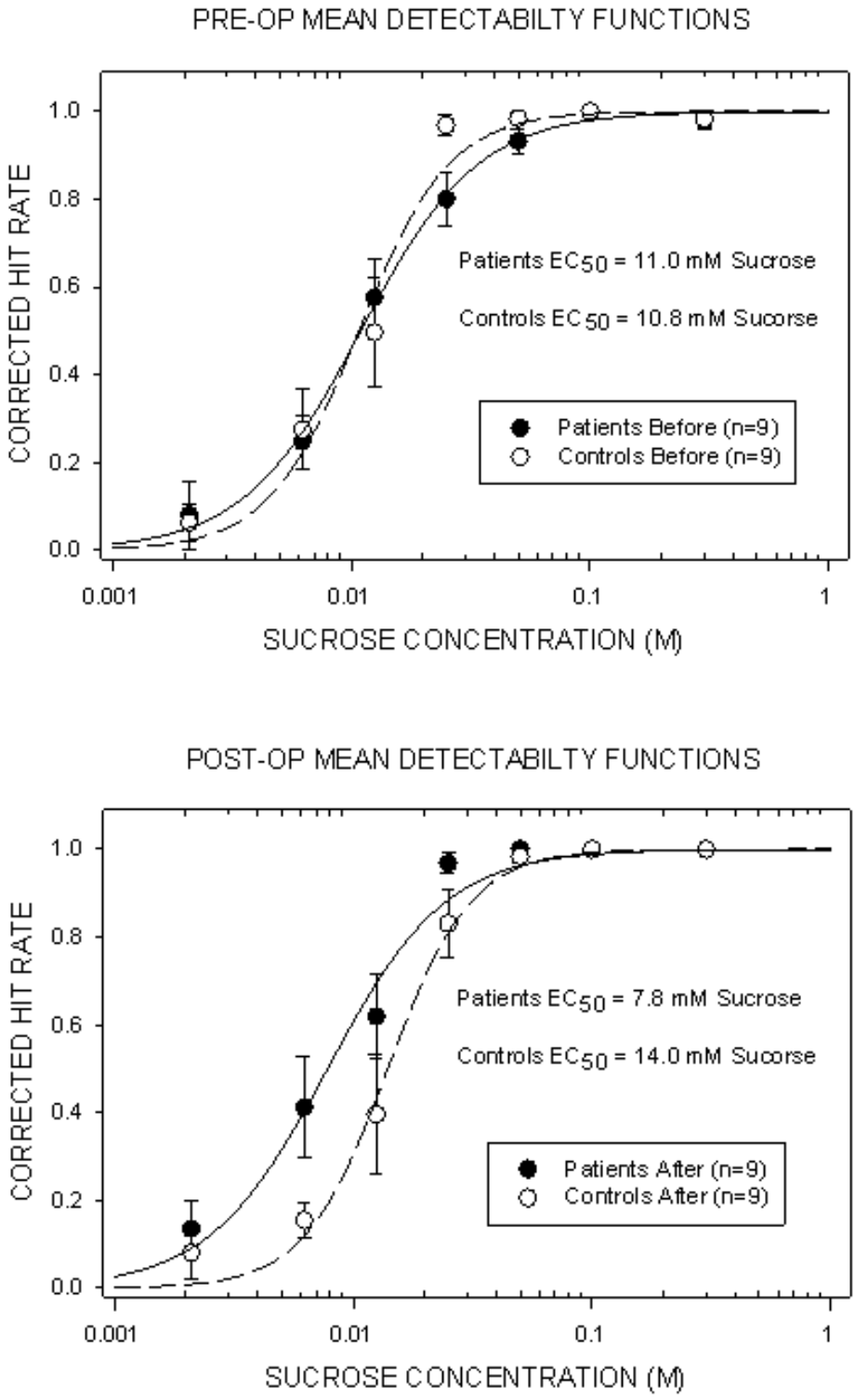


Figure 23: Mean (±se) corrected hit rate for patients (filled circles) and controls (open circles) preoperatively (top) and postoperatively (bottom) as a function of sucrose concentration. Curves were fit to the mean data points using equation 2 in text. The EC50 was derived from the c-parameter in the curve fit and represents the concentration at which the corrected hit rate is ½ of the maximum asymptote. X-axes are displayed on a log10 scale.

C-Parameter Analysis

Preoperatively, curves fit to the mean corrected hit rates for controls and patients produced remarkably similar c-values (EC50) of 10.8 mM sucrose for controls and 11.0 mM sucrose for patients. Postoperatively, the c-values based on the curve fits for the mean corrected hit rates were: controls = 14.0 mM sucrose and gastric bypass patients = 7.8 mM, suggesting that controls had ”thresholds” that were 1.8-times higher than patients. The c-value of the curve fit of the mean corrected hit rate measured before surgery decreased (indicating greater sensitivity) by 1.4 times after surgery.

The preoperative to postoperative shift in the c-values representing the EC50 of the individual curve fits for the subjects who had fits that accounted for at least 85% of the variance was compared between the two groups (Figure 24). Although the 0.21 $\log_{10}$ increase in the EC50 in controls ($F((1,7)=5.014$, $p=0.061$) and the 0.194 decrease in the EC50 in patients ($F(1,6)=5.54$, $p=0.057$) relative to their preoperative values just missed the statistical rejection criterion, there was a clear difference between the relative shifts in the EC50 between the two groups ($F(1,13)=10.15$, $p=0.007$).

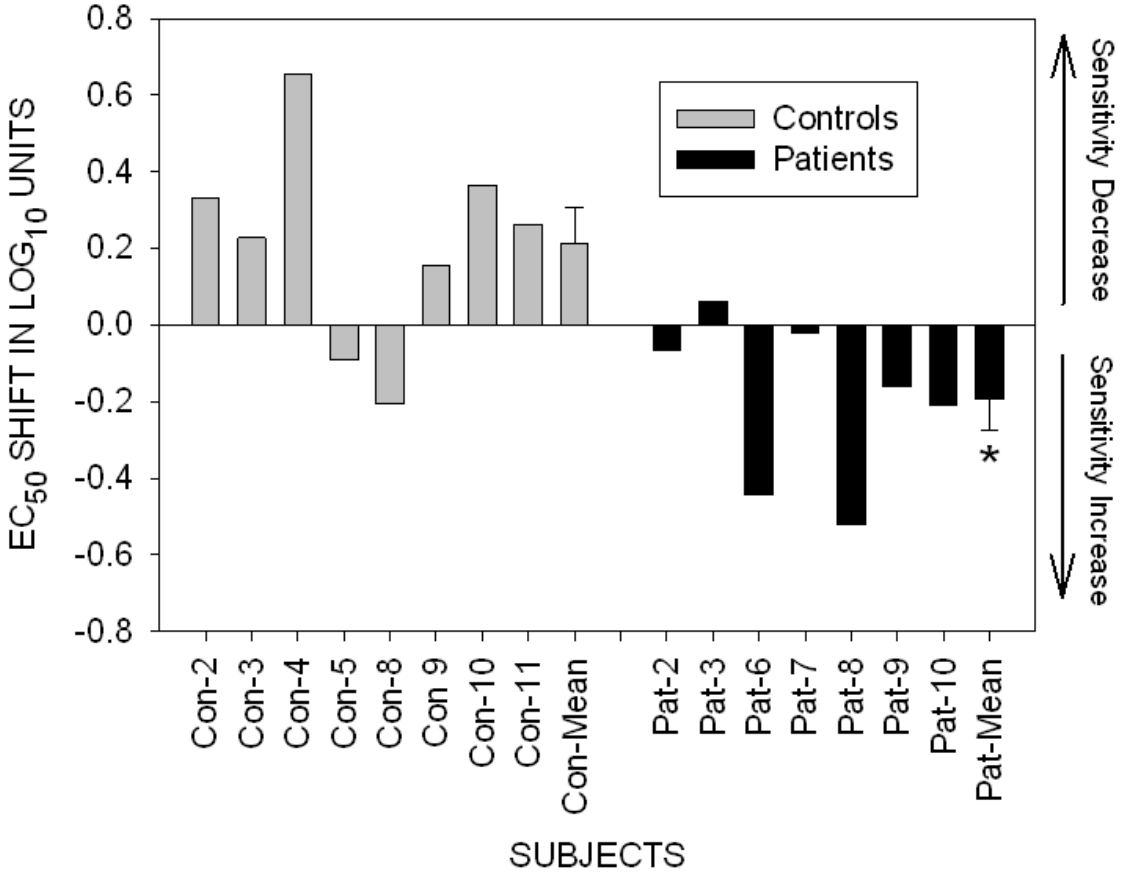


Figure 24: Shifts in the EC_{50} preoperatively vs. postoperatively for individual patients (black) and control subjects (gray) and their respective means (±se). Bars going up represent rightward shifts in the detectability function indicating a decrease in sensitivity. Bars going down represent leftward shifts in the detectability function indicating an increase in sensitivity. Asterisk represents significant difference compared with control shift (p=0.007).

Hedonic visual analogue scale to determine the sucrose concentration which was "just about right"

The taste acceptability ratings for control subjects and patients pre- and postoperatively are displayed in Figure 25. Table 4 summarizes the two-way ANOVA values for the hedonic ratings pre- and postoperatively between controls and patients as a function of surgical group and concentration. Before surgery, there was a significant main effect of concentration ($p<0.001$), but no significant difference in

hedonic ratings between controls and patients (Figure 28 top) (p=0.63). There was also no statistically significant interaction between surgical group and concentration (p=0.93). After surgery, there was also a significant main effect of sucrose concentration (p<0.001), but no difference in hedonic ratings between controls and patients (p=0.59). There was also no significant interaction between surgical group and concentration (p=0.81).

Two-way ANOVA values of a within group comparison of hedonic ratings as a function of concentration and time are shown in Table 5. Patients showed no difference in their taste acceptability ratings of the seven sucrose concentrations pre- and postoperatively (p=0.20). There was a significant main effect of sucrose concentration (p<0.001), but no significant time x concentration interaction (p=0.85). Hedonic ratings did also not differ between the two assessments of the normal weight group confirming the reliability of the visual analogue scale (Figure 25 bottom) (p=0.06). Here, there was a significant main effect of sucrose concentration (p<0.001), but no significant time x concentration interaction (p=0.99).

	Concentration	**Surgical group**	**Concentration X Surgical group**
preoperative	F(6,102)=78.11; p<0.001	F(1,102)=0.24; p=0.63	F(6,102)=0.32; p=0.93
postoperative	F(6,102)=105.3; p<0.001	F(1,102)=0.30; p=0.59	F(6,102)=0.49; p=0.81

Table 4: Two-way ANOVA values for comparison of hedonic ratings pre- and postoperatively for patients and controls as a function of concentration and surgical group.

	Concentration	**Time**	**Concentration** X **time**
controls	F(6,56)=39.21; p<0.001	F(1,56)=3.74; p=0.06	F(6,56)=0.10; p=0.99
patients	F(6,63)=41.60; p<0.001	F(1,63)=1.69; p=0.20	F(6,63)=0.44; p=0.85

Table 5: Two-way ANOVA values for comparison of hedonic ratings for patients and controls between as a function of concentration and time.

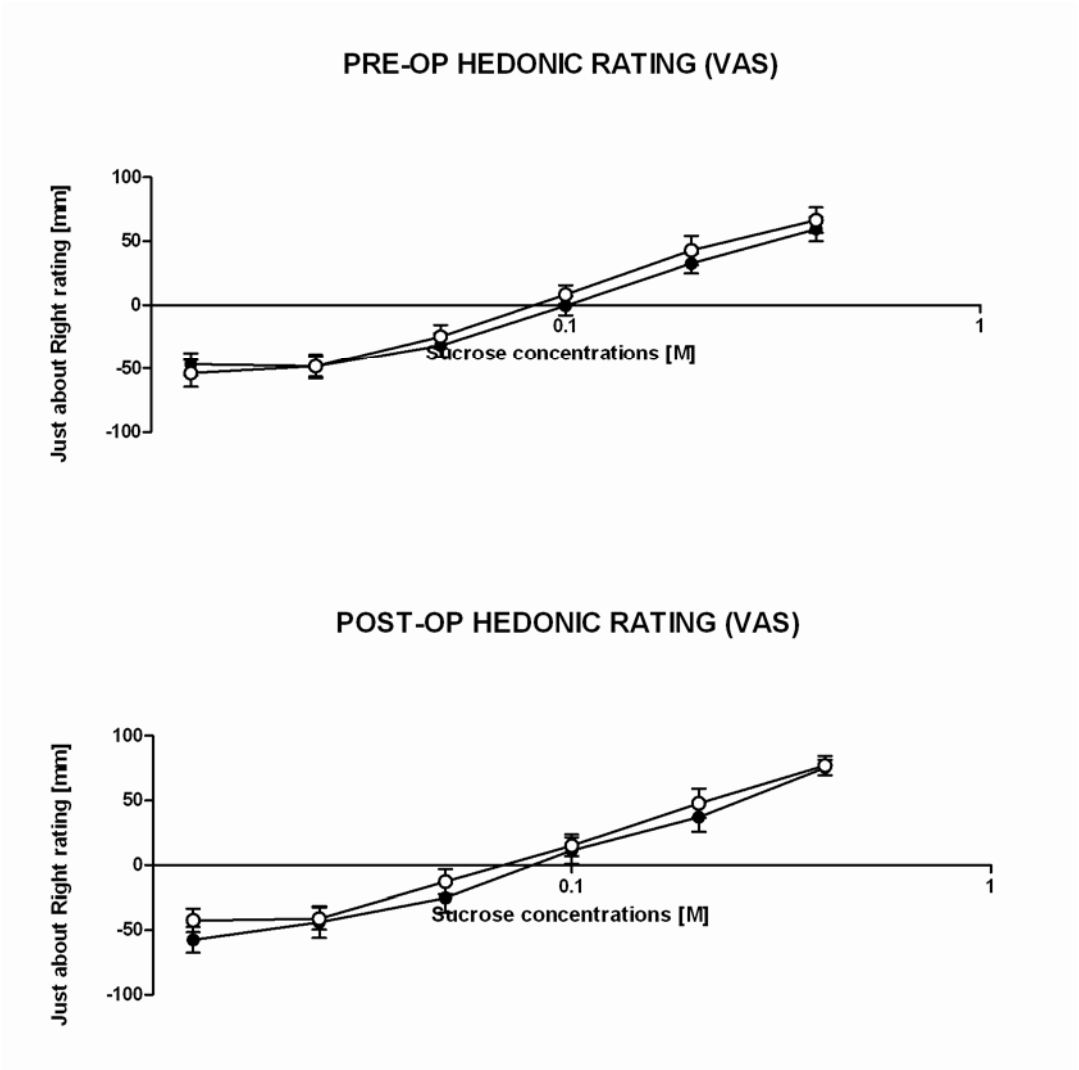


Figure 25: Ratings on a hedonic visual analogue scale to determine the sucrose concentration which was considered "Just-about-Right" for patients (filled circles, n=10) and controls (open circles, n=9) preoperatively (top) and postoperatively (bottom) as a function of sucrose concentration. The ideal sweet concentration was defined as the point at which the plotted line intersects the x axis. X-axes are displayed on a log10 scale. Water ratings were as follows: Preoperative: patients: -59.7±10.6 mm vs. controls: -55.6±10.7 mm, p=0.78; patients: -49.1±11.1 mm vs. controls: -53.1±10.7 mm, p=0.80).

Discussion

I demonstrated that gastric bypass patients can detect lower concentrations of sucrose when compared to normal weight controls. This has been reported before (Brolin, Robertson, Kenler, & Cody 1994;Brown, Settle, & Van Rij 1982;Halmi, Mason, Falk, & Stunkard 1981), but in contrast to previous experiments I used the method of constant stimuli in which taste stimuli are presented randomly and performance is assessed across a set of concentrations allowing for the derivation of a psychometric function. Threshold measures do not necessarily correlate with suprathreshold sensitivity (Bartoshuk 1978) and thus may not accurately reflect the hedonic evaluation of higher concentrations of taste stimuli. Accordingly, I complemented our measures of sucrose taste sensitivity with a visual analogue scale that is designed to estimate the sucrose concentration that is “just about right” (Conner & Booth 1988;Drewnowski, Brunzell, Sande, Iverius, & Greenwood 1985;Frijters & Rasmussen-Conrad 1982). Despite an increased sensitivity to detect sucrose in lower concentrations, surprisingly I found that there was no difference in the hedonic ratings of sucrose solutions by patients before compared with after gastric bypass. This discrepancy could be due to a potential lack of correspondence between sucrose detection thresholds on one hand and the perceived intensity of suprathreshold sucrose concentrations on the other hand (Bartoshuk 1978). I also cannot dismiss the possibility that other scaling procedures for measuring the hedonic value of taste stimuli might reveal effects of gastric bypass on sucrose acceptability (Bartoshuk et al. 2006), but at least with the scale employed here there was no evidence of a postoperative change. While this deserves further attention in future experiments, my results suggest that the changes in food preference observed

after gastric bypass might not represent a fundamental shift in the hedonic evaluation of the food, but may be more related to other factors such as postingestive events and learning.

Chapter 8: Analysis of Sucrose Preference and Sweet Taste after Roux-en-Y Gastric Bypass in rats

Introduction

Potential mechanisms to explain an altered sweet preference, regardless of whether central or peripheral in origin, include changes in the T1R2 and T1R3 taste receptors, which bind with natural and artifical sweeteners, in the gut (Stearns et al. 2010) and/or the gut hormones associated with gastric bypass such as glucagon-like-peptide 1 (GLP-1) and peptide YY (PYY), which can potentially influence appetite (Lenard and Berthoud 2008). Mice lacking the GLP-1 receptor show decreased behavioural responsiveness to sucrose (Shin, Martin, Golden, Dotson, Maudsley, Kim, Jang, Mattson, Drucker, Egan, & Munger 2008), but it remains to be fully elucidated whether circulating GLP-1 modulates peripheral (e.g., taste buds) or central (brain) gustatory function (Jang, Kokrashvili, Theodorakis, Carlson, Kim, Zhou, Kim, Xu, Chan, Juhaszova, Bernier, Mosinger, Margolskee, & Egan 2007). However, PYY and GLP-1 administration in rodents has been shown to induce conditioned taste aversion by activation of brainstem neurons that mediate effects of aversive stimuli (Halatchev and Cone 2005;Seeley et al. 2000;Thiele et al. 1997). There is also electrophysiological and behavioral evidence demonstrating that leptin decreases responsiveness specifically to sweeteners (Horio et al. 2010;Jyotaki et al. 2010;Shigemura et al. 2004). Given that gastric bypass surgery dramatically decreases adipose mass and, in turn, circulating levels of leptin (Beckman et al. 2010), selective increases in taste sensitivity to sweeteners would be expected.

Against this background we hypothesized that gastric bypass surgery alters sweet taste function associated with sweeteners and affects the preference for sucrose in rats. We used a rat model to specifically assess the 24h preference in a standard two-bottle preference test to investigate how gastric bypass rats treat different taste stimuli in the context of natural feeding and drinking; this test allowed us greater latitude in behavioural, endocrine, and molecular measurements while providing a logical bridge with reports of changes in human taste acceptability following surgery (Spector & Glendinning 2009). In this study we used taste compounds representing four of the commonly accepted taste qualities, sucrose (sweet), sodium chloride (salty), quinine hydrochloride (bitter) and citric acid (sour). However, a possible confounding factor is that rats tend to consume less of a novel food or flavour than of familiar food, a phenomenon called neophobia (Barker et al. 1977). Moreover, it has been shown that neophobia can be enhanced in the context of recent visceral malaise (Barker, Best, & Domjan 1977). We therefore investigated the potential relevance of preoperative sucrose experience for a postoperatively reduced sucrose preference and tested sucrose preference in rats before and after gastric bypass surgery.

Animals

Diet-induced obese male Wistar rats were individually housed in polycarbonate cages in a room with automatically controlled temperature of 21 ± 2 ºC and a 12h / 12h light-dark cycle. Water and standard chow (RM1 diet, Special Diet Services Ltd, UK) were available ad libitum, unless otherwise stated. All experiments were performed under a license issued by the Home Office UK (PL 70-6669).

Surgery

Surgery was performed according to an established protocol with preservation of the vagal fibres in the para-oesophageal bundle as previously described (page 35).

Two bottle preference test

Two groups of ten sham-operated controls and ten RYGB rats were subjected to standard two-bottle preference tests as described below. The first group was tested initially for sucrose and then for sodium chloride, the second group was tested first for quinine hydrochloride and then for citric acid. Another group of seven sham-operated rats and seven RYGB rats were subjected to a two-bottle preference test for sucrose before and after surgery. All two-bottle preference tests were started 10 days after surgery when the weight loss had plateaued. Food (g) and fluid (ml) intake and solution preference (%) were measured daily. For the two-bottle test involving sucrose, we calculated total energy intake as the sum of calories consumed as

normal chow (3.5 kcal/g) and sucrose solution (4.1 kcal/g). All solutions were prepared daily with deionized water and presented at room temperature. Test stimuli consisted of seven concentrations of sucrose (1, 3, 10, 30, 100, 300, 1000 mM), sodium chloride (15, 35, 73, 150, 300, 600, 1200 mM), quinine hydrochloride (0.003, 0.01, 0.03, 0.1, 0.3, 1, 3 mM) and citric acid (0.1, 0.3, 1, 10, 30, 100 mM; all Sigma Aldrich, Dorset, UK). The rats were presented with two pre-weighed bottles, one of which contained deionized water and the other of which contained the test solutions in ascending concentrations. Positions and content of the bottles were changed one hour after the start of the light phase and bottles were then weighed 24 hours thereafter. Rats were given access to the same concentration for two days and the positions of the bottles were switched each day to preclude the development of a side preference.

Preference was defined as: [intake of test solution (in ml)／total fluid intake (ml)] x 100. With this type of preference experiment, a score of 50% conventionally represents indifference between the two stimulus options (water and sucrose or sodium chloride or quinine hydrochloride or citric acid).

Preference, acceptance, food intake and energy intake during the two-bottle preference tests were analyzed with a two-way group (between subjects) x concentration (within subjects) analysis of variance (ANOVA). Post-hoc Bonferroni tests for each concentration were applied when there was a significant group x concentration interaction. The conventional $p \leq 0.05$ was used as the statistical rejection criterion.

Blood collection and Hormone assay

Blood from nine ad libitum fed gastric bypass rats and nine sham-operated controls was collected on postoperative day 60 and plasma GLP-1 and PYY were measured as described before (Kreymann et al. 1987;le Roux et al. 2006b).

Measurement of T1R2 and T1R3 mRNA and protein expression in the small intestine brush-border membrane (BBM) of rats

Intestinal segments were opened longitudinally, and the mucosa scraped off using glass slides and the resulting mucosa snap-frozen and stored at -80°C. Intestinal BBM vesicles were subsequently prepared as previously described (Marks et al. 2006). RNA was extracted from tissue, using Trizol, according to manufacturer's instructions (Invitrogen, Paisley, UK). RNA was reverse transcribed and T1R transcripts were measured as previously described (Marks, Srai, Biber, Murer, Unwin, & Debnam 2006), with β-actin (GenBank accession number NM031144; forward position 937–955, reverse position 1223–1208) used as the house-keeping gene. Expression of the *Tasr2* and *Tasr3*, the protein products of which form a functional hetrodimer binding with sweetener compounds, was measured, using specific intron-spanning primers designed from the published sequences from rat; T1R2 (GenBank accession number XM_00107479.1; forward primer position 3759-3778, reverse position 3923-3904) and T1R3 (GenBank accession number NM_130818.1; forward primer position 2107-2126, reverse position 2327-2308).

The concentration of protein in the BBM vesicles was determined using the Bradford method (Bradford 1976). For Western blotting, BBM samples (20-30 μg of protein)

were prepared as previously described (Marks, Srai, Biber, Murer, Unwin, & Debnam 2006), using Rabbit polyclonal antibodies against T1R2 and 3 (Santa Cruz Biotechnology, USA). Mouse mAb for β-actin were used as a loading control (Abcam, Cambridge, UK). The comparative delta Ct method was used to calculate the gene expression, relative to β-actin, using Kruskal-Wallis. T1R2 and T1R3 protein expression values were calculated relative to β-actin and expressed as a ratio of control average (%), using Student's unpaired t test with p considered significant at ≤ 0.05.

Statistical analysis

Data were normally distributed and expressed as mean ± SEM. Preference, acceptance, food intake and energy intake in the two bottle preference tests were analyzed with a two-way group (between subjects) x concentration (within subjects) analysis of variance (ANOVA). A one-way ANOVA followed by Bonferroni post hoc tests for each concentration was applied when there was a significant group x concentration interaction. Student's t-test for independent samples was used to test for significant differences. A p-value <0.05 was considered statistically significant.

Results

Body weight and food intake

Data for body weight development and average daily food intake after surgery were pooled for all groups of rats, because data did not differ between the groups. Figure 26A shows that the preoperative body weight of all gastric bypass rats (441±8 g) and sham-operated rats (425± 9 g) was similar ($p=0.17$). After postoperative day 15 the body weight (381±8 g) of the gastric bypass rats was significantly lower than that of the sham-operated group (426±8 g) ($p<0.001$). After a short period of post-surgical weight loss, subsequent weight gain was constant and similar among sham-operated rats. After postoperative day 15 until the end of the experiment on day 60 there was a difference in body weight between the two groups (day 60: sham: 476±10 g vs. bypass: 372±11 g, $p<0.001$) that was associated with a lower food intake for gastric bypass (21.7±0.5 g) rats relative to sham-operated rats (28.2± 0.2 g, $p<0.001$) (Figure 26B).

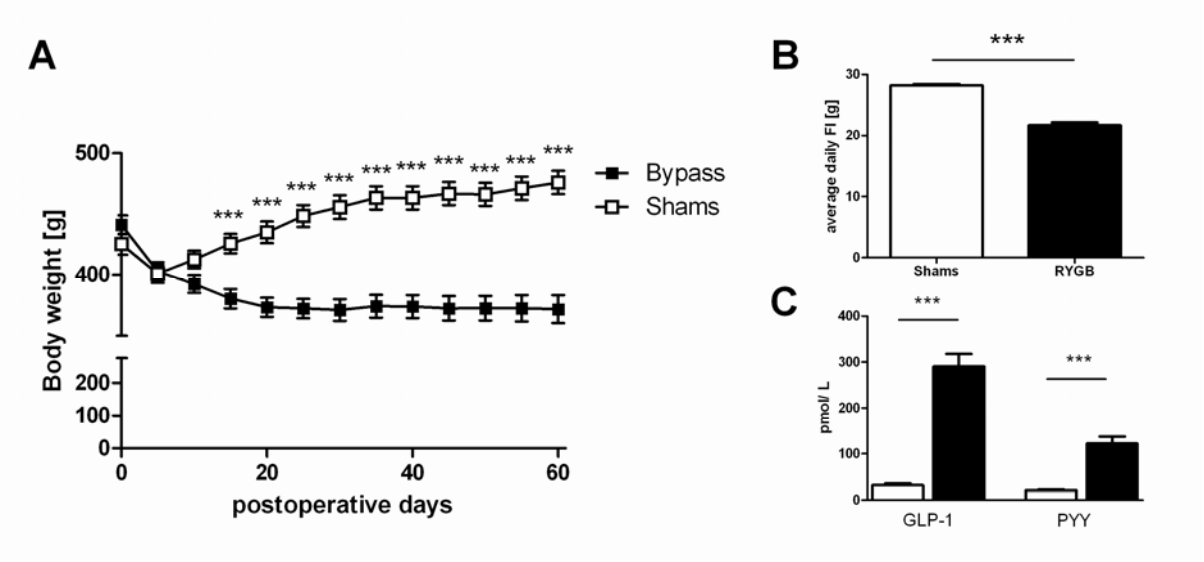


Figure 26: (A) Body weight change for all gastric bypass (-o-) (n=20) and sham-operated rats (-■-) (n=20) throughout the complete observation period of 60 days. (B) Average daily food intake of sham-operated rats (n=20, white) and gastric bypass rats (n=20, black) throughout the entire observation period of 60 days. (C) GLP-1 and PYY level for nine sham-operated rats (white) and nine gastric bypass rats (black). Data are shown as mean values ± SEM (*** = p<0.001).

Postprandial plasma levels of active GLP-1 and PYY

Figure 26C demonstrates that gastric bypass rats had higher levels of plasma GLP-1 ($p<0.001$) and PYY ($p<0.001$) in comparison to sham-operated controls.

Two-bottle preference test in naïve rats after gastric bypass

Preference

Twenty-four hour preferences for all four test solutions are shown in the left column of Figure 27. The values of the two-way ANOVAs for preference for all four taste stimuli are summarized in Table 6. There was a significant main effect of sucrose

concentration ($p<0.001$) and of surgical group ($p<0.001$) as well as a significant group x concentration interaction ($p<0.001$). Sham-operated rats showed an increase in 24h preference ((sucrose intake / total intake)*100) for sucrose at concentrations above 10 mM. In contrast, gastric bypass rats showed a much lower preference for sucrose concentrations above 10 mM (Figure 27A). There was no difference in 24h preference or intake for sodium chloride (Figure 27C), for quinine hydrochloride (Figure 27E) and for citric acid (Figure 27G and Table 6).

	Concentration	Surgical group	Concentration X Surgical group
Sucrose	F(6,276)=10.28; p<0.001	F(1,276)=62.10; p<0.001	F(6,276)=6.65; p<0.001
Sodium Chloride	F(6,266)=100.45; p<0.001	F(1,266)=0.12; p=0.73	F(6,266)=0.31; p=0.93
Quinine hydrochloride	F(6,251)=49.08; p<0.001	F(1,251)=2.05; p=0.15	F(6,251)=0.35; p=0.91
Citric Acid	F(6,236)=18.77; p<0.001	F(1,236)=0.11; p=0.74	F(6;236)=1.94; p=0.08

Table 6: Two-way ANOVA values for preference during the two-bottle preference tests in naïve rats as a function of concentration and surgical group for all four taste stimuli

Intake

Twenty-four hour intakes for all four test solutions are shown in right column of figure 27. The values of the two-way ANOVAs for intake for all four taste stimuli are summarized in Table 7. There was a significant main effect of sucrose concentration ($p<0.001$) and of surgical group ($p<0.001$) as well as a significant group x concentration interaction ($p<0.001$). However, sham-operated and gastric bypass rats significantly increased their sucrose intake (in ml) within the 24h period at concentrations above 10 mM (Repeated Measures ANOVA: $p<0.001$ and $p=0.036$, respectively; Figure 27B). There were no differences in intake between sham-operated rats and gastric bypass rats for sodium chloride (Figure 27D), for quinine hydrochloride (Figure 27F) and for citric acid (Figure 27H and Table 7).

Overall caloric intake was only analysed for rats that were in the sucrose study, as neither sodium chloride nor quinine hydrochloride nor citric acid contain calories. There was a significant main effect of sucrose concentration ($F(6,276)=14.11$;

p<0.001) and of surgical group (F(1,276)=347.07; p<0.001) as well as a significant interaction (F(6,276)=16.12; p<0.001) for total caloric intake, which represented the sum of calories consumed as food (3.5 kcal/g) and sucrose (energy content of a 1000 mM sucrose solution: 1.4 kcal/ml). Despite a decrease in chow intake, the sham-operated rats increased their total caloric intake when exposed to concentrations of the sucrose solutions above 30 mM. In contrast, gastric bypass rats showed no significant change in total energy intake which is accounted for by their relatively low sucrose intake (One-way ANOVA: p=0.34; data not shown, p < 0.05).

	Concentration	**Surgical group**	**Concentration** X **Surgical group**
Sucrose	F(6,276)=38.06; p<0.001	F(1,276)=189.05; p<0.001	F(6,276)=18.59; p<0.001
Sodium Chloride	F(6,266)=43.78; p<0.001	F(1,266)=1.89; p=0.17	F(6,266)=0.54; p=0.78
Quinine hydrochloride	F(6,251)=34.50; p<0.001	F(1,251)=0.97; p=0.33	F(6,251)=1.31; p=0.26
Citric Acid	F(6,236)=35.22; p<0.001	F(1,236)=1.89; p=0.17	F(6;236)=1.62; p=0.14

Table 7: Two-way ANOVA values for intake during the two-bottle preference tests in naïve rats as a function of concentration and surgical group for all four taste stimuli

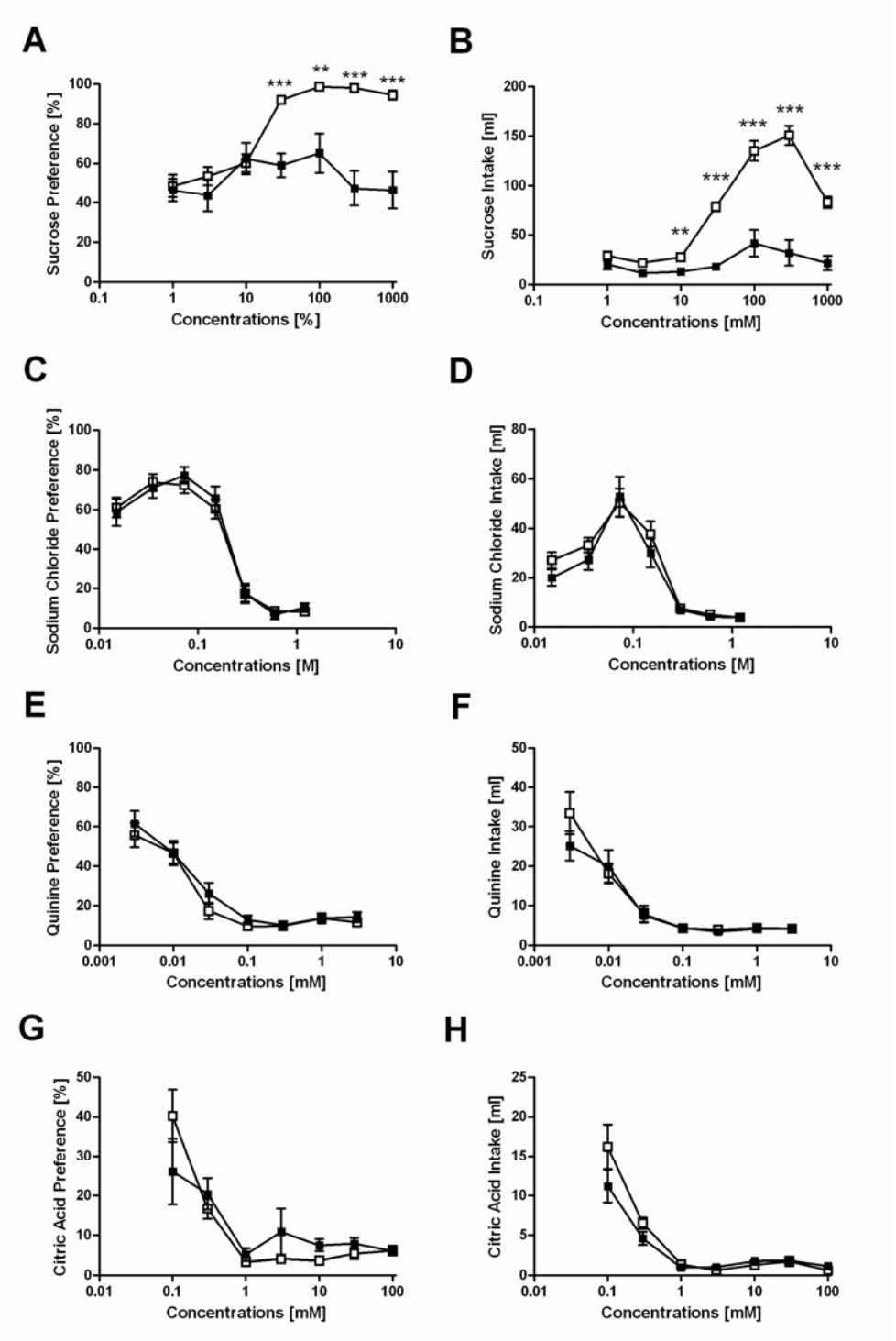


Figure 27: Two-bottle preference test in gastric bypass rats (n=10, -■-) and sham-operated rats (n=11, -□-); (A) 24 hour sucrose preference and (B) intake; (C) 24 hour sodium chloride preference and (D) intake; (E) 24 hour quinine hydrochloride preference and (F) intake; (G) 24 hour citric acid preference and (H) intake. Data are shown as mean values ± SEM with differences between gastric bypass rats and sham-operated rats. When two-way ANOVA revealed a significant group x concentration interaction , post-hoc Bonferroni test was used for concentration to concentration analysis between the two groups (** = $p<0.01$, *** = $p<0.001$). X-axes are displayed on a log10 scale.

Two-bottle preference test for sucrose in gastric bypass rats with preoperative sucrose experience

Preference

Twenty-four hour preferences for sucrose before and after gastric bypass and sham-operation are shown in the left column of figure 28 and two-way ANOVA values for sucrose preference pre- and postoperatively are summarized in table 8. Preoperatively, there was no difference in sucrose preference between the two surgical groups (p=0.73; Figure 28A). After surgery, there was a significant main effect of surgical group (p=0.039) and a significant group x concentration interaction (p=0.011). However, there was no main effect for sucrose concentration (p=0.061; Figure 28C). A repeated measures ANOVA for each group showed that there was a significant sucrose concentration effect for gastric bypass rats (p=0.002), but not for sham-operated rats (p=0.21).

	Concentration	**Surgical group**	**Concentration** X **Surgical group**
Preoperative	F(6,168)=38.58; p<0.001	F(1,168)=0.12; p=0.73	F(6,168)=0.41; p=0.87
Postoperative	F(6,144)=2.07; p=0.06	F(1,144)=4.77; p=0.039	F(6,144)=2.54; p=0.011

Table 8: Two-way ANOVA values for pre- and postoperative sucrose preference during the two-bottle preference tests as a function of concentration and surgical group

Intake

Twenty-four hour sucrose intakes are shown in the right column of Figure 28 before and after gastric bypass and sham-operations. Table 9 summarizes the two-way ANOVA values for sucrose intake pre- and postoperatively. Preoperatively, there

was no difference in sucrose intake between the two surgical groups (p=0.27; Figure 28B). Postoperatively, the two-way ANOVA indicated a significant main effect of surgical group (p<0.001) as well as for sucrose concentration (p<0.001). There was also a significant group x concentration interaction (p<0.001; Figure 28D).

	Concentration	**Surgical group**	**Concentration X Surgical group**
preoperative	F(6,168)=45.99; p<0.001	F(1,168)=1.28; p=0.27	F(6,168)=0.49; p=0.82
postoperative	F(6,144)=17.11 p<0.001	F(1,144)=28.90; p<0.001	F(6,144)=6.82; p<0.001

Table 9: Two-way ANOVA values for pre- and postoperative sucrose intake during the two-bottle preference tests as a function of concentration and surgical group

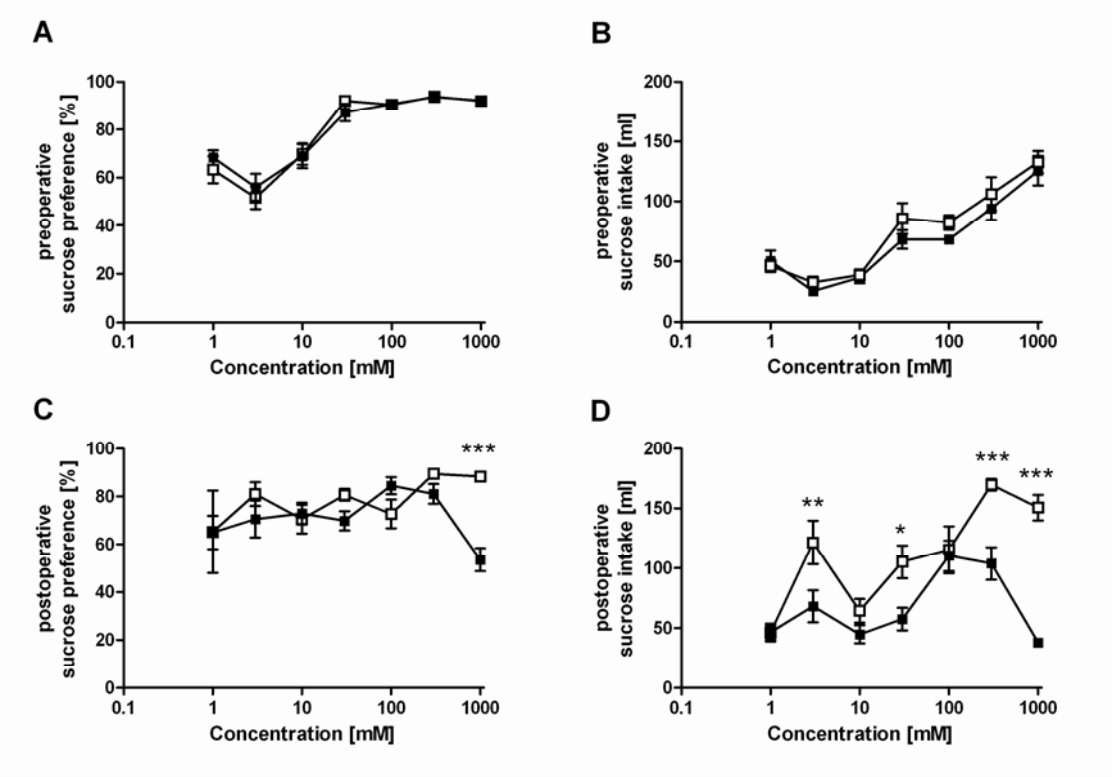


Figure 28: Two-bottle preference test in gastric bypass rats (n=7, -■-) and sham-operated rats (n=7, -□-) before and after gastric bypass and sham surgery; (A) preoperative 24 hour sucrose preference and (B) intake; (C) postoperative 24 hour sucrose preference and (D) intake; Data are shown as mean values ± SEM with differences between gastric bypass rats and sham-operated rats. When two-way ANOVA revealed a significant group x concentration interaction, post-hoc Bonferroni test was used for concentration to concentration analysis between the two groups (** = $p<0.01$, *** = $p<0.001$). X-axes are displayed on a log10 scale.

Intestinal mRNA and brush-border membrane protein levels of sweet taste receptor subunits T1R2 and T1R3

Figure 29 shows the mucosal levels of mRNA (A-C) and protein (D-F) for the two taste receptor proteins, T1R2 and T1R3, in the corresponding duodenal, jejunal and ileal segments of sham- and bypass-operated rats. T1R2 mRNA expression was

significantly lower in the biliopancreatic limb after gastric bypass in comparison with the duodenum of sham-operated rats ($p<0.001$). In contrast, there was no difference in T1R2 mRNA expression in the alimentary limb and common channel of bypass rats compared with the proximal jejunum and terminal ileum in sham-operated rats. T1R3 mRNA expression was similar in gastric bypass rats and sham-operated rats in all examined parts of the small intestine. Consistent with the mRNA expression, there was a significant decrease in brush-border membrane protein expression of T1R2 in the biliopancreatic limb after gastric bypass when compared with the duodenum of sham-operated rats. Furthermore, there was a significant decrease in both T1R2 and T1R3 protein levels in the alimentary limb after gastric bypass. There was no difference in T1R2/3 protein levels between the common channel of bypass rats and the ileum of sham-operated rats.

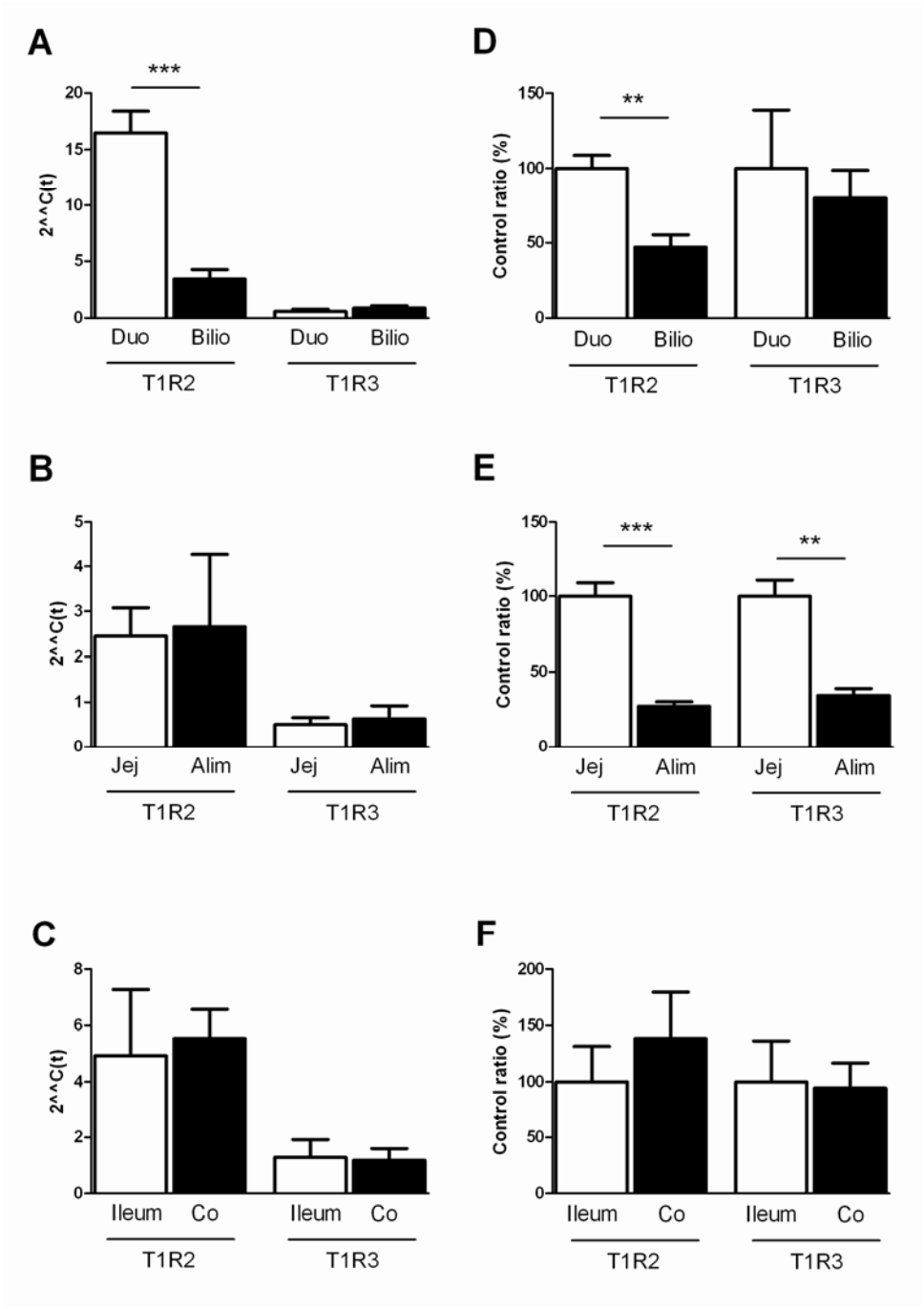


Figure 29: T1R2 and T1R3 mRNA expression (A-C) and brush-border membrane protein levels (D-F) in the biliopancreatic (Bilio), alimentary (Alim) and common channel (Co) of **gastric bypass** rats (black) in comparison with correspondent section of duodenum (Duo), jejunum (Jej) and ileum (Ileum) of sham-operated rats (white) (n=4-6 per group). Relative gene expression is represented as delta delta Ct mean values ± SEM (*** = $p<0.001$).

Discussion

I demonstrated that gastric bypass reduces the preference for sucrose in rats, although preoperative sucrose exposure attenuated this effect. I confirmed that gastric bypass in rats leads to increased postprandial levels of the satiety gut hormones GLP-1 and PYY (Bueter et al. 2010a;le Roux, Aylwin, Batterham, Borg, Coyle, Prasad, Shurey, Ghatei, Patel, & Bloom 2006a;le Roux, Welbourn, Werling, Osborne, Kokkinos, Laurenius, Lonroth, Fandriks, Ghatei, Bloom, & Olbers 2007). The changes in both mRNA and tissue protein levels of the taste receptor proteins T1R2 and T1R3, which form a heterodimer that binds with sweeteners, in the small bowel may contribute to the postingestive effects that could influence sucrose preference.

Several potential mechanisms that could underlie the selective effects of gastric bypass on sucrose preference in rats and these are not necessarily mutually exclusive. For example my observations might be explined by alterations in peripheral or central gustatory processes, Another possibility is that the lower preference of gastric bypass rats for higher sucrose solutions may be induced by postingestive consequences producing visceral malaise (Kyriazakis et al. 1999). Learning processes affecting sucrose preference and intake after gastric bypass may also have contributed to the observed effects.

It will be important for future work to examine whether gastric bypass alters taste detection thresholds in the rat model, which allows for more systematic and targeted manipulations aimed at revealing mechanisms. Further elucidation of the mechanisms by which gastric bypass reduces consumption of high-caloric foods may help in the development of novel surgical and non-surgical therapeutic interventions that will promote safer and more effective weight loss.

Chapter 9: Final Discussion

Role of the vagus for body weight loss in rodent model of Roux-en-Y gastric bypass

My data in the rat model for gastric bypass are consistent with previous findings that gastric bypass surgery can effectively induce food intake and body weight reduction (Adams, Gress, Smith, Halverson, Simper, Rosamond, Lamonte, Stroup, & Hunt 2007;Korner, Bessler, Cirilo, Conwell, Daud, Restuccia, & Wardlaw 2005;le Roux, Aylwin, Batterham, Borg, Coyle, Prasad, Shurey, Ghatei, Patel, & Bloom 2006a). In this randomized study the weight loss and food intake outcome of gastric bypass surgery was dependent on whether vagal fibres were preserved or not during the formation of the gastric pouch. Rats in which the para-oesophageal bundle including the vagal fibres was completely ligated started to regain body weight up to preoperative levels and showed no difference in average daily energy intake compared to their sham-operated counterparts. In contrast, rats in which the para-esophageal bundle including the vagal fibres was preserved and in which the left gastric vessels were selectively ligated, maintained the reduced body weight and ate significantly less than the sham-operated controls throughout the entire study period. Gastric bypass rats had higher postprandial GLP-1 and PYY levels compared to sham-operated controls, but there were no differences in GLP-1 and PYY levels between gastric bypass rats with our without preserved vagal fibres. Furthermore, differences in food intake and body weight were not related to the size of the gastro-jejunostomy in gastric bypass rats and there were no signs of malabsorption or inflammation after gastric bypass in any of the groups.

My data confirm previous findings that gastric bypass in rats increases postprandial levels of peptide YY (PYY) and glucagon-like peptide-1 (GLP-1), which are satiation inducing gut hormones and hence favour an anorectic state and facilitate body weight loss (Borg, le Roux, Ghatei, Bloom, Patel, & Aylwin 2006;Korner, Bessler, Cirilo, Conwell, Daud, Restuccia, & Wardlaw 2005). Both hormones are thought to activate anorectic neurons in the hypothalamic arcuate nucleus (ARC) which promote weight loss (Batterham, Cowley, Small, Herzog, Cohen, Dakin, Wren, Brynes, Low, Ghatei, Cone, & Bloom 2002;Batterham, Cohen, Ellis, le Roux, Withers, Frost, Ghatei, & Bloom 2003;Cone et al. 2001;Larsen, Tang-Christensen, & Jessop 1997). Gut hormones released from enteroendocrine cells in the distal ileum like GLP-1 and PYY can inform the brain either through the circulation or via afferent vagal neurons or both, and there is considerable controversy about the relative importance of these routes (Berthoud 2008). It is possible that a given hormone could use different routes to produce different physiological effects such as changes in eating behavior (Berthoud 2008).

In this study body weight and food intake after gastric bypass were related to whether the vagal fibres within the para-oesophageal bundle were preserved or not while there were no differences in levels of GLP-1 and PYY between these two groups. This finding highlights the important role of the vagal nerve for mediating the inhibitory effects of gut hormones such as PYY and GLP-1 on food intake and body weight after gastric bypass surgery in rats. It is further supported by previous reports describing that ablation of the vagal-brainstem-hypothalamic pathway attenuates the inhibitory effects of PYY and GLP-1 on food intake (Abbott, Monteiro, Small, Sajedi, Smith, Parkinson, Ghatei, & Bloom 2005). This is an important observation as only a few reports examined whether vagal preservation is effective or necessary in weight

control after bariatric surgery (Perathoner, Weiss, Santner, Brandacher, Laimer, Holler, Aigner, & Klaus 2009;Sundbom, Holdstock, Engstrom, & Karlsson 2007;Wang & Liu 2009).

In contrast to my observation, Wang et al. described a greater weight loss after total vagal dissection along with a gastric bypass operation in rats (Wang & Liu 2009). This effect was only present 20 days after surgery and there was no difference in food intake and body weight between bypass rats with or without vagal dissection on postoperative day 100. In this study the bypassed jejunum was about 10 cm in length which is much less in comparison to our technique as described above. This variation in length of the bypassed jejunum may result in differences in postprandial GLP-1 and PYY levels which may result in altered long-term body weight loss. However, the comparability of both studies is limited as Wang et al. used the bypass operation to prevent obesity in rats weighing 180-200g while I performed surgery to treat obesity in obese rats (Wang & Liu 2009).

Weight loss after a gastric bypass operation might also be due to nutrient malabsorption or postoperative inflammation. However, I found no evidence for an increase in either fecal mass or fecal calorie content in the gastric bypass animals with or without vagal preservation. Moreover I did not detect any evidence of increased inflammation in animals with or without vagal ligation post surgery.

The size of the gastric pouch and the lengths of the different limbs used in this study have been proven to effectively induce weight loss (Bueter, Lowenstein, Olbers, Wang, Cluny, Bloom, Sharkey, Lutz, & le Roux 2009c). An increasing body of evidence in humans indicates that up to certain limits the size of the gastric pouch and length of the different limbs is of less importance for the outcome of gastric

bypass (Muller, Rader, Wildi, Hauser, Clavien, & Weber 2008). In support of this observation, I demonstrated that the level of restriction measured by the size of the gastro-jejunostomy has no impact on different levels of weight loss and food intake after gastric bypass in rats.

There are three major limitations of my study. Firstly, I cannot exclude the possibility that the ligation of the paraoesophageal bundle is functionally equivalent only to a partial dissection of the vagal nerve. In addition, I did not perform a secretin test or histological analysis to collect further informations on vagal function to confirm whether the complete ligation of the para-esophageal bundle produced a total or partial vagotomy. Secondly, greater body weight loss after gastric bypass might also be due to a greater total surgical trauma as bypass operations took longer and might have been more stressful for the rats. Finally, it remains unclear whether my results can be translated into humans. Most bariatric surgeons usually aim to preserve the anterior and posterior vagal trunk during formation of the gastric pouch, although there is a lack of supporting data indicating that this approach has beneficial effects. In a recent study, Perathoner et al investigate 40 morbidly obese patients undergoing gastric bypass dividing them into two groups according to vagal nerve preservation (Group 1, n = 25) or vagal nerve dissection (Group 2, n = 22) and found that the dissection of the anterior vagal trunk during pouch formation had no effect on clinical, functional and laboratory results of a gastric bypass operation (Perathoner, Weiss, Santner, Brandacher, Laimer, Holler, Aigner, & Klaus 2009).

In conclusion, my gastric bypass technique induces reliable weight loss in rats with an acceptable mortality. I propose that vagal nerve fibres should be preserved during gastric bypass in rats. Restriction at the gastro-jejunal anastomosis does not seem to be critical for the weight loss. Although the mechanisms have not yet been fully

elucidated, vagal preservation may play an important role in inducing and maintaining weight loss after gastric bypass in humans and rats.

Changes in energy expenditure after Roux-en-Y gastric bypass in rats

I confirmed that body weight loss after gastric bypass was associated with a significant loss of fat mass and to a lesser degree of non-adipose body mass (Guijarro et al. 2007;Stenstrom et al. 2006). Food intake was reduced in gastric bypass rats which may be partly explained by hormonally mediated mechanisms (Atkinson and Brent 1982;Korner, Bessler, Cirilo, Conwell, Daud, Restuccia, & Wardlaw 2005;le Roux, Welbourn, Werling, Osborne, Kokkinos, Laurenius, Lonroth, Fandriks, Ghatei, Bloom, & Olbers 2007). Importantly, the lower food intake after gastric bypass compared with sham-operated ad libitum fed rats only partly explains body weight loss, because the sham-operated body weight-matched group required on average 40% less food than the bypass group to maintain the same level of body weight. Consequently, reduced calorie consumption is important but not the sole cause of weight loss after gastric bypass. I found no increased fecal mass, fecal calorie content or inflammation in the gastric bypass animals; therefore nutrient malabsorption or inflammation are unlikely to play a major role in this weight loss (le Roux, Aylwin, Batterham, Borg, Coyle, Prasad, Shurey, Ghatei, Patel, & Bloom 2006a).

I demonstrate a higher total energy expenditure in rats after gastric bypass compared to ad libitum fed and body weight-matched sham groups which is in accordance with some, but not all previous reports of energy expenditure in humans (Carrasco, Papapietro, Csendes, Salazar, Echenique, Lisboa, Diaz, & Rojas

2007;Das, Roberts, McCrory, Hsu, Shikora, Kehayias, Dallal, & Saltzman 2003;Flancbaum, Choban, Bradley, & Burge 1997). Differences in energy expenditure were mainly due to changes during the light phase when physical activity is typically low. Gastric bypass surgery did not only prevent the expected decrease in energy expenditure subsequent to body weight loss, but actually increased 24 hour and in particular light phase energy expenditure in comparison to the control groups.

Higher energy expenditure after gastric bypass was associated with lower respiratory quotients suggesting that fat rather than carbohydrates was burnt to sustain higher energy expenditure. However, food restricted body weight-matched controls showed similar respiratory quotient levels to the gastric bypass group suggesting that body weight loss rather than a specific effect by the gastric bypass procedure was an important determinant for the observed decrease in respiratory quotient.

As higher levels of total energy expenditure usually result either from greater heat generation or increased physical activity (Lowell & Spiegelman 2000), some of our findings remain unexplained. Firstly, bypass rats were not more physically active than the control groups. The bypass rats showed no difference in spontaneous activity during the light phase to indicate reduced sleep time, but I have not formally evaluated sleep patterns. In fact, at least during the dark phase, when spontaneous activity is usually high, physical activity was lower in the bypass rats than in the sham controls. As gastric bypass induces an increase in postprandial levels of PYY and GLP-1 (Borg, le Roux, Ghatei, Bloom, Patel, & Aylwin 2006) which reduce food intake, the reduced dark phase physical activity may possibly indicate reduced appetite and hence less foraging or food seeking behaviour. The second unexpected finding was the lower body temperature in gastric bypass rats compared to ad libitum

fed sham controls. This was observed throughout the light-dark cycle. However, during the light phase the body temperature of the gastric bypass rats was higher than in the body weight-matched controls despite no difference in physical activity. It must be emphasized that during the light phase gastric bypass rats continued to consume some food, whilst the body weight-matched shams consumed all food during the first half of the dark cycle. Thus, differences in light phase body temperature might be related to food intake and subsequently diet-induced thermogenesis (Shibata and Bukowiecki 1987;Sims and Danforth E Jr 1987).

After a 5 g test meal gastric bypass rats had greater diet-induced thermogenesis than body weight-matched controls, but no difference was observed between gastric bypass rats and the ad libitum fed sham group.

My data suggest that gastric bypass induces profound changes in food intake, energy expenditure and the mechanisms by which the body controls energy expenditure. As gastric bypass significantly rearranges the gastrointestinal anatomy, I suggest that gastrointestinal and central neuroendocrine signaling contribute to increased energy expenditure (Lowell & Spiegelman 2000). Neurons in the hypothalamic arcuate nucleus (ARC) co-express neuropeptide Y (NPY) and agouti-related peptide, which stimulate food intake and weight gain (Schwartz et al. 2000). Another population of ARC neurons co-express pro-opiomelanocortin (POMC) and cocaine-and-amphetamine-regulated transcript (CART), which both promote weight loss (Cone, Cowley, Butler, Fan, Marks, & Low 2001). The balance between NPY and POMC is critical for the maintenance of body weight (Cone, Cowley, Butler, Fan, Marks, & Low 2001;Flier 2004;Schwartz, Woods, Porte, Jr., Seeley, & Baskin 2000). Gastric bypass increases postprandial levels of PYY and GLP-1 (Korner, Bessler, Cirilo, Conwell, Daud, Restuccia, & Wardlaw 2005;le Roux, Aylwin, Batterham, Borg,

Coyle, Prasad, Shurey, Ghatei, Patel, & Bloom 2006a), which are satiating inducing gut hormones and hence favour an anorectic state and facilitate body weight loss through modulation of the hypothalamus and brainstem (Abbott, Monteiro, Small, Sajedi, Smith, Parkinson, Ghatei, & Bloom 2005;Larsen, Tang-Christensen, & Jessop 1997), also being involved in the control of energy expenditure (Murphy & Bloom 2006). In fact, PYY has been shown to activate anorectic POMC expressing neurons in the ARC (Batterham, Cowley, Small, Herzog, Cohen, Dakin, Wren, Brynes, Low, Ghatei, Cone, & Bloom 2002) and to inhibit NPY neurons (cuna-Goycolea & van den Pol 2005), suggesting a potential to increase energy expenditure.

Gastrointestinal effects of GLP-1 and PYY can be resolved by ablation of vagus–brainstem–hypothalamus pathways (Abbott, Monteiro, Small, Sajedi, Smith, Parkinson, Ghatei, & Bloom 2005) indicating a role for the vagus in mediating effects on food intake and potentially energy expenditure. However, it was beyond the scope of this study to assess the potential role of vagal or visceral neural afferent information to the central nervous system.

GLP-1 increases endogenous amylin levels (Lutz 2006). Amylin may be another potential candidate decreasing food intake and increasing energy expenditure (Wielinga, Alder, & Lutz 2007). Of note, the reduced food intake after amylin is independent of GLP-1 and vice versa (Lutz TA, unpublished data). Nonetheless, chronic amylin administration reduces food intake (Osto et al. 2007) and it prevents the decrease in energy expenditure that would typically result from lower food intake and body weight loss (Mack et al. 2007b;Roth et al. 2006) (Lutz TA, unpublished data).

The increase in total energy expenditure might also represent a higher energy requirement after bypass surgery. I also demonstrated significant morphometric changes of the small intestine after gastric bypass surgery (Borg, le Roux, Ghatei, Bloom, Patel, & Aylwin 2006;Nadreau, Baraboi, Samson, Blouin, Hould, Marceau, Biron, & Richard 2006). The observed increase in muscle thickness and mucosal mass after gastric bypass resulted in a 72% increase of the total small bowel weight. The gut is metabolically very active and the mean *in vitro* rates of oxygen consumption in gastrointestinal tissues in rats have been reported to be 15-22% of total oxygen consumption (Cant et al. 1996;Pine et al. 1994). Thus, gut hypertrophy may at least in part explain the higher maintenance energy requirement that contribute to body weight loss.

Postoperative inflammation secondary to infection can lead to a higher energy demands, but I found no evidence of an inflammatory response in my study. Other mechanisms that should be considered but may be less likely include decreased leptin after gastric bypass. Usually high leptin and not low leptin contributes to increased energy expenditure (Trakhtenbroit et al. 2009). Although low leptin levels may explain the lower body temperature in bypass rats than in ad libitum fed controls, it does not explain the observed difference in body temperature between bypass and body weight-matched rats.

This study does not explain why average body temperature was reduced while total energy expenditure was higher after gastric bypass. One possible explanation is that more heat was dissipated to the immediate environment of the rats especially since gastric bypass rats had significantly less body fat and hence less thermal isolation. I did not assess cutaneous vasodilation to further explore potential mechanisms. Another explanation may be an up regulated activity of brown adipose tissue, but the

measuring system did not allow the separate assessment of brown adipose tissue and tail temperature.

In summary, not only did gastric bypass surgery prevent the expected decrease in energy expenditure subsequent to body weight loss in this diet-induced obese rat model, but 24 hour and in particular light phase energy expenditure were higher than in sham controls. Diet-induced thermogenesis was also higher after gastric bypass surgery compared to body weight-matched controls. Increased energy expenditure may offer an additional explanation why gastric bypass surgery is superior to dieting for successfully maintaining long-term body weight loss.

Sodium and water handling after gastric bypass surgery in a rat model

Both central and peripheral abnormalities account for the development and maintenance of high arterial pressure in obesity (Hall 2003). Visceral obesity is considered an important risk factor for hypertension and cardiovascular disease (Sironi et al. 2004); it is linked to hyperinsulinemia, hyperleptinemia and increased levels of aldosterone, and so-called aldosterone releasing factors, all of which lead to activation of the sympathetic and renin-angiotensin-aldosterone systems (El-Atat et al. 2004;Rahmouni et al. 2003;Rahmouni et al. 2004). In addition, increased aldosterone levels might also be the result of an increased intra-abdominal pressure activating the renin-angiotensin-aldosterone system, leading to increased sodium and water reabsorption (Bloomfield et al. 1997;Sugerman, Windsor, Bessos, & Wolfe 1997).

The beneficial effect of gastric bypass surgery on arterial hypertension is well documented (Adams, Gress, Smith, Halverson, Simper, Rosamond, Lamonte, Stroup, & Hunt 2007;Buchwald 2005). The reduction of visceral fat mass, and subsequent decrease in sympathetic activation and sodium retention, is not immediate and does not explain the early reduction in blood pressure observed after gastric bypass described by Ahmed at al (Ahmed, Rickards, Coniglio, Xia, Johnson, Boss, & O'Malley 2008). Thus, I reasoned that other mechanisms might be involved in the early resolution of hypertension after gastric bypass, and that alteration of renal sodium and water handling could be one of them.

I have demonstrated a significant increase in urine output, water intake and sodium excretion after gastric bypass surgery compared with pre-operative measurements. Sham-operated animals show no changes in water intake, urine production or sodium excretion after surgery.

In many groups of patients with hypertension there is disturbed sodium balance (Postnov 1990) attributed to impaired renal sodium excretion. However, only a few studies have focused on the possible role of the gastrointestinal tract in the control of sodium balance, and thus systemic blood pressure. The concept that dietary intake and composition can affect renal function is perhaps self-evident, but a detailed characterization of this relationship is still lacking. Several physiological mechanisms are involved in controlling sodium balance, in particular the hormones aldosterone, angiotensin II (Bouhnik et al. 1992), and atrial natriuretic peptide (Sterzel et al. 1987); but there is some evidence supporting involvement of the gastrointestinal tract. Analogous to the ‘incretin effect’, characterized by an exaggerated plasma insulin response to an oral glucose load compared with the same amount of glucose given intravenously, oral ingestion of sodium chloride has a greater natriuretic effect

than when the same amount is given intravenously to subjects on a low sodium diet (Lennane et al. 1975). This effect has been shown to be independent of changes in aldosterone and atrial natriuretic peptide (Lennane, Carey, Goodwin, & Peart 1975). In the case of insulin release, the important incretin gut hormone has been shown to be GLP-1, which has since been developed into a successful treatment for type 2 diabetes (Baggio & Drucker 2007). Although the mechanism for the analogous effect on sodium excretion, and potentially blood pressure control, has yet to be identified; the GLP-1 response after bypass remains a candidate, as it is a known natriuretic (Gutzwiller, Tschopp, Bock, Zehnder, Huber, Kreyenbuehl, Gutmann, Drewe, Henzen, Goeke, & Beglinger 2004;Holst 2004).

Animal studies provide some evidence that the gastrointestinal tract can exert a direct influence on renal function. Morgan et al observed that salt-sensitive Harlan Sprague Dawley (HSD) rats (SS/Jr) with transplanted kidneys from salt-resistant HSD rats (SR/Jr) developed significant salt-induced hypertension, suggesting that extra-renal factors also contribute to hypertension in this model of hypertension (Morgan et al. 1990). These findings were not accounted for by any changes in established hormones known to control renal sodium excretion, including aldosterone, renin and angiotensin II (Bouhnik, Richoux, Huang, Savoie, Baussant, henc-Gelas, & Corvol 1992), or atrial natriuretic peptide (Sterzel, Luft, Gao, Lang, Ruskoaho, & Ganten 1987). Hence, the presence of an intestinal natriuretic factor renal sodium excretion was proposed (Morgan, DiBona, & Mark 1990).

My data suggest that gastric bypass induces profound changes in sodium and water handling. As gastric bypass significantly rearranges the gastrointestinal anatomy, I suggest that gastrointestinal and central neuroendocrine signaling contribute to increased sodium and water excretion (Lowell & Spiegelman 2000). Potential

mediators between the gut and the kidney include both, Peptide YY (PYY) (Playford, Mehta, Upton, Rentch, Moss, Calam, Bloom, Payne, Ghatei, Edwards, & . 1995) and glucagon-like peptide (GLP)-1, which have been shown to have diuretic and natriuretic properties (Michell, Debnam, & Unwin 2008). Thus, it is reasonable to speculate that GLP-1 and PYY could mediate a link between the gastrointestinal tract and kidney in terms of sodium and water excretion (Gutzwiller, Tschopp, Bock, Zehnder, Huber, Kreyenbuehl, Gutmann, Drewe, Henzen, Goeke, & Beglinger 2004;Gutzwiller, Hruz, Huber, Hamel, Zehnder, Drewe, Gutmann, Stanga, Vogel, & Beglinger 2006;Michell, Debnam, & Unwin 2008).

However, this study cannot distinguish between a direct effect of hypertonic saline to stimulate thirst, and increase water intake after gastric bypass, and an indirect effect of increased renal sodium excretion to stimulate thirst and offset salt and water loss. Also, a non hypertensive rat strain was used, and blood pressure was not measured to determine if the observed increase in sodium excretion lead to any change in blood pressure.

In conclusion, gastric bypass surgery in humans, and in the rat, provides us with a valuable model in which to explore the role of the gastrointestinal tract in sodium and water homeostasis, and other electrolytes, and perhaps also in salt-sensitive hypertension. Gastric bypass results in a greater urine output, water intake and sodium excretion in salt-restricted rats following an oral sodium load. This observation may provide an insight into the mechanism of the early improvement in arterial hypertension seen in patients after gastric bypass surgery.

Patients randomised to gastric bypass six years earlier decreased their liking for fat compared to pre surgery, but the same is not the case after vertical-banded gastroplasty. The results of my rat experiments after gastric bypass show a shift away from solid high fat to solid low fat food. We confirmed previous findings demonstrating that gastric bypass leads to increased postprandial levels of plasma GLP-1 and PYY when compared to sham-operated control rats (Korner, Bessler, Cirilo, Conwell, Daud, Restuccia, & Wardlaw 2005;le Roux, Aylwin, Batterham, Borg, Coyle, Prasad, Shurey, Ghatei, Patel, & Bloom 2006a). We also demonstrated a reduced preference of rats for concentrations of Intralipid® of 0.5% and above when offered during a two bottle preference test, but not in a brief access test. Because rats ingest significantly more Intralipid® during the two bottle preference test than during the brief access test where postingestive effects are minimal, I concluded that possible mechanisms may include postingestive factors such as the induction of an aversive effect. In line with this I observed that exogenous administration of the GLP-1 receptor agonist exendin-4 induced a conditioned taste aversion of similar magnitude as an oral gavage of a small volume of corn oil; this suggests that exaggerated postprandial GLP-1 responses might play a role in mediating the decreased fat preference after gastric bypass.

In humans a long-term reduction in dietary fat following gastric bypass was found to be the single most pronounced differing factor in the dietary composition between the two groups six years after the operation. As part of general lifestyle advice (Blundell & MacDiarmid 1997) to achieve adequate and sustained weight loss a reduction in total energy intake by reducing dietary fat is recommended.

My findings add to previous reports in humans which have shown a reduced dietary fat intake one year after gastric bypass surgery (Olbers, Bjorkman, Lindroos, Maleckas, Lonn, Sjostrom, & Lonroth 2006) ; this set the stage for use of the rat as an animal model to pursue the physiological, endocrine, and molecular mechanisms underlying the effects of gastric bypass surgery on food preference. In this regard, I demonstrated that rats after gastric bypass decrease their total energy intake from pelleted food by 37%, and specifically decrease their preference for high fat, while actually increasing low fat chow consumption. The relative contribution of normal chow to energy intake increased four-fold, while the contribution of high fat chow decreased by 11% after gastric bypass; this reflects the direction of the preference shift from high toward low fat chow. Nevertheless, it should be noted that in this model, rats still ingested more calories from high fat than from low fat chow.

Rats after gastric bypass when given a choice over 48 hours between distilled water and Intralipid® solutions had a lower preference for the higher Intralipid® concentrations compared to sham-operated rats; the latter showed a clear preference for high concentrations (above 0.1%) of Intralipid®. Sham-operated rats consumed up to 100 ml per day of Intralipid® – a volume equivalent to 20% of their body weight. After bypass, the reduced preference for Intralipid® was present early and persisted for at least 200 days.

In contrast, I observed no difference in the preference for Intralipid® between bypass and control rats in a brief access test which is specifically designed for rats to ingest only very small amounts of Intralipid® during the 30 minute test sessions; hence, the total amount of Intralipid® ingested is much smaller than during the 48 hours of exposure in the two bottle preference tests (Smith 2001;Spector & Glendinning 2009). Consequently, associations of particular Intralipid® concentrations with

postingestive effects such as satiety or visceral malaise are minimized in the brief access test (Smith 2001;Spector & Glendinning 2009).

I therefore concluded that the reduced preference for high fat food seen in rats after gastric bypass may in part be due to negative postingestive effects including conditioned taste aversion against high concentrations of fat. I further investigated this possibility and found that gastric bypass rats treated with 1ml of corn oil by gastric gavage showed a marked reduction in their preference for saccharine solution that is normally highly preferred by rats. However, the taste aversion was not as strong as in the case of the positive control lithium chloride because gastric bypass rats continued to consume at least 50% (versus about 5% in lithium chloride treated rats) of their fluid intake as saccharine solution; further, in the two bottle tests comparing Intralipid® and distilled water intake, Intralipid still made up about 50% of total liquid intake. The lithium chloride group was simply included as a basic positive control in the taste aversion experiment, without having a priori knowledge of whether corn oil would serve as an effective unconditioned stimulus; hence I did not make an effort to match the aversive potency of the treatments. Accordingly, this can potentially explain the disparity in the magnitude of the aversion between the two groups.

Interestingly, the conditioned taste aversion seen after gavage of a small volume of corn oil in our study was of a similar magnitude compared with the conditioned taste aversion produced by peripheral administration of the GLP-1 receptor agonist exendin-4. Thus, it seems plausible to suggest that alterations in fat preference after gastric bypass may result in part from the induction of an aversive response mediated by increased levels of GLP-1, but it was beyond the scope of my studies to

assess neuronal activity in specific brain areas like the area postrema or the solitary tract.

Whether gastric bypass also induces an aversion against the ingestion of highly concentrated sugar solutions is unknown. Comparing fat aversion after gastric bypass to aversion to sucrose or a combination of fat and sucrose may yield more insight. Smith et al. reported that rats conditioned with corn oil show a more profound aversion to the sucrose/corn oil mixture than rats conditioned with sucrose, suggesting that the salient feature of the sucrose/corn oil mixture is the oil (Smith et al. 2000). It would be instructive to compare the relative effectiveness of corn oil versus mineral oil infusions to induce a conditioned taste aversion to determine whether it is the nutritive or non nutritive properties of the fluid that are critical.

My data in the rat gastric bypass model are consistent with previous human findings that gastric bypass does not only reduce food intake (Korner, Bessler, Cirilo, Conwell, Daud, Restuccia, & Wardlaw 2005;le Roux, Aylwin, Batterham, Borg, Coyle, Prasad, Shurey, Ghatei, Patel, & Bloom 2006a;le Roux, Welbourn, Werling, Osborne, Kokkinos, Laurenius, Lonroth, Fandriks, Ghatei, Bloom, & Olbers 2007), but also preference for food high in fat (Brolin, Robertson, Kenler, & Cody 1994;Brown, Settle, & Van Rij 1982;Halmi, Mason, Falk, & Stunkard 1981;Olbers, Bjorkman, Lindroos, Maleckas, Lonn, Sjostrom, & Lonroth 2006). The reduced preference for fat was absent or at least less pronounced in patients six years after vertical-banded gastroplasty in which the anatomical rearrangement of the small bowel is not part of the operation and which is known not to induce changes in postprandial gut hormone levels (Valverde, Puente, Martin-Duce, Molina, Lozano, Sancho, Malaisse, & Villanueva-Penacarrillo 2005). However, gastric bypass rats still showed a substantial preference for the solid high fat diet which made up 86% of

total energy intake. In contrast, preference for the liquid Intralipid® concentrations was reduced up to 50%.

Gastric bypass leads to reduced hunger (Borg, le Roux, Ghatei, Bloom, Patel, & Aylwin 2006), increased satiation (Borg, le Roux, Ghatei, Bloom, Patel, & Aylwin 2006) and increased energy expenditure (Bueter et al. 2010b;Stylopoulos, Hoppin, & Kaplan 2009), all of which are at least partly mediated by alterations in gastrointestinal and central neuroendocrine signalling (Korner, Bessler, Cirilo, Conwell, Daud, Restuccia, & Wardlaw 2005;le Roux, Aylwin, Batterham, Borg, Coyle, Prasad, Shurey, Ghatei, Patel, & Bloom 2006a;Stylopoulos, Hoppin, & Kaplan 2009). Indeed, I confirmed previous findings demonstrating that gastric bypass leads to increased postprandial levels of GLP-1 and PYY when compared to sham-operated control rats (Korner, Bessler, Cirilo, Conwell, Daud, Restuccia, & Wardlaw 2005;le Roux, Aylwin, Batterham, Borg, Coyle, Prasad, Shurey, Ghatei, Patel, & Bloom 2006a)'(Baggio & Drucker 2007;Karra et al. 2009). In addition, GLP-1 or PYY may also influence fatty acid detection or perception and there may be parallels with the recognition of sweet stimuli. Mice lacking the GLP-1 receptor show decreased behavioural responsiveness to sucrose. This receptor has been shown to be expressed on taste afferent fibers, and GLP-1 is expressed in taste buds cells (Feng, Liu, Zhou, Wang, & Liu 2008;Shin, Martin, Golden, Dotson, Maudsley, Kim, Jang, Mattson, Drucker, Egan, & Munger 2008).

Part of the lower preference for high fat food may be induced by postingestive consequences that produce visceral illness. This is supported by our observation that rats did not show a reduced preference for concentrations of Intralipid® of 0.5% or higher when they were only allowed to ingest very small amounts during a brief

access test. Consistent with my previous finding, the conditioned taste aversion experiment showed that corn oil administered by gavage (thus excluding a direct effect of corn oil within the oral cavity) produced signs of conditioned taste aversion, although the effects were less intense than after lithium chloride. This is consistent with my findings that gastric bypass rats still ingested half of their total fluid intake from Intralipid® during the two bottle preference test. Hence, postingestive aversive consequences may be one, but not the only factor to explain reduced fat preference after gastric bypass.

In conclusion, gastric bypass in humans and rats reduces preference for high fat food and high concentrations of Intralipid® solution. Postingestive effects and conditioned taste aversion may partly explain my findings. By elucidating the mechanisms by which obesity surgery reduces consumption of high fat foods, new surgical and non-surgical therapies could be developed that mimic these mechanisms and so promote safe and effective weight loss.

Sweet taste and preference after gastric bypass

I demonstrated that gastric bypass reduces the preference for sucrose in rats, although preoperative sucrose exposure attenuated this effect. In contrast, there was no change in salt, bitter and sour preferences after gastric bypass. My findings are consistent with previous reports in humans (Olbers, Bjorkman, Lindroos, Maleckas, Lonn, Sjostrom, & Lonroth 2006) suggesting the rat model is reliable to pursue the physiological, endocrine, and molecular mechanisms underlying the effects of gastric bypass on taste and food preference. In this regard, I confirmed that gastric bypass in rats, as in humans, leads to increased postprandial levels of the satiety gut hormones GLP-1 and PYY (Bueter, Lowenstein, Ashrafian, Hillebrand, Bloom, Olbers, Lutz, & le Roux 2010a;le Roux, Aylwin, Batterham, Borg, Coyle, Prasad, Shurey, Ghatei, Patel, & Bloom 2006a;le Roux, Welbourn, Werling, Osborne, Kokkinos, Laurenius, Lonroth, Fandriks, Ghatei, Bloom, & Olbers 2007). The changes in both mRNA and tissue protein levels of the taste receptor proteins T1R2 and T1R3, which form a heterodimer that binds with sweeteners, in the small bowel may contribute to the postingestive effects that could influence sucrose preference.

I also investigated changes in sucrose taste detection thresholds in humans as one basic aspect of taste function in general and found that gastric bypass patients can detect lower concentrations of sucrose when compared to normal weight controls. This has been reported before (Brolin, Robertson, Kenler, & Cody 1994;Brown, Settle, & Van Rij 1982;Halmi, Mason, Falk, & Stunkard 1981), but in contrast to previous experiments I used the method of constant stimuli in which taste stimuli are presented randomly and performance is assessed across a set of concentrations

allowing for the derivation of a psychometric function. Moreover, subjects obtained feedback by receiving tokens for correct responses and losing tokens for incorrect responses which appeared to keep subjects vigilant and motivated in this game-like competitive setting. Using this novel approach, I confirmed that patients, after gastric bypass, can detect lower sucrose concentrations compared with both their preoperative performance and that of lean control subjects.

I confirmed that gastric bypass increases postprandial plasma levels of the L-cell hormones GLP-1 and PYY which have been shown to reduce hunger and enhance satiety (Baggio & Drucker 2007;Batterham, Cohen, Ellis, le Roux, Withers, Frost, Ghatei, & Bloom 2003). In addition, increased plasma GLP-1 levels may be consistent with my observation of a reduction in sucrose taste thresholds after gastric bypass. GLP-1 is expressed in murine taste bud cells and is considered as a potential paracrine modulator of the peripheral gustatory apparatus, as GLP-1 receptors are found on intragemmal taste afferent nerve fibers (Feng, Liu, Zhou, Wang, & Liu 2008;Shin, Martin, Golden, Dotson, Maudsley, Kim, Jang, Mattson, Drucker, Egan, & Munger 2008). Sufficiently high plasma levels of GLP-1 may affect peripheral taste signalling. Reception and transduction of sweet-tasting compounds has been shown to involve, in part, α-gustducin and the sugar binding receptor subunit T1R3 (Kokrashvili et al. 2009a;Kokrashvili, Mosinger, & Margolskee 2009b;Zhao, Zhang, Hoon, Chandrashekar, Erlenbach, Ryba, & Zuker 2003), but these proteins also partly mediate the glucose-dependent GLP-1 secretion from enteroendocrine L cells of the gut (Jang, Kokrashvili, Theodorakis, Carlson, Kim, Zhou, Kim, Xu, Chan, Juhaszova, Bernier, Mosinger, Margolskee, & Egan 2007). The close cellular and functional relationship of GLP-1, T1R3, and α-gustducin may allow the elevated levels of GLP-1 seen after gastric bypass to influence the taste

signal pathways at multiple levels. The decreased detection thresholds seen after gastric bypass are consistent with this possibility.

A reduction in sweet taste sensitivity is also in agreement with recent findings demonstrating that some taste receptor cells are target of the adipose-derived hormone leptin. Kawai et al. showed that intraperitoneal leptin injections in lean mice suppressed responses of peripheral taste nerves to sucrose and saccharine without affecting other taste qualities (Kawai et al. 2000). This effect was absent in db/db mice which have no leptin receptors (Kawai, Sugimoto, Nakashima, Miura, & Ninomiya 2000). Furthermore, taste bud cells of lean mice expressed mRNA of the leptin receptor (Shigemura et al. 2003) suggesting that leptin may not only be a regulator of food intake and energy expenditure, but also a modulator of taste sensing of sweeteners (Shigemura, Ohta, Kusakabe, Miura, Hino, Koyano, Nakashima, & Ninomiya 2004). As gastric bypass dramatically decreases white adipose tissue, the main source of leptin (Beckman, Beckman, & Earthman 2010), one would expect selective increases in taste sensitivity to sweeteners after this type of operation, which I found here.

Threshold measures do not necessarily correlate with suprathreshold sensitivity (Bartoshuk 1978) and thus may not accurately reflect the hedonic evaluation of higher concentrations of taste stimuli. Accordingly, I complemented our measures of sucrose taste sensitivity with a visual analogue scale that is designed to estimate the sucrose concentration that is “just about right” (Conner & Booth 1988;Drewnowski, Brunzell, Sande, Iverius, & Greenwood 1985;Frijters & Rasmussen-Conrad 1982). This scale allowed us to assess the relative taste acceptability of a wide range of suprathreshold sucrose concentrations, while keeping potential confounding postingestive factors to a minimum as all samples only contacted the oral cavity and

were not swallowed. The “just about right” VAS has been used extensively in sensory consumer testing and marketing research because it effectively links taste compound concentration with acceptance and thus provides information on the affective value of the stimulus (Conner & Booth 1988;Drewnowski, Brunzell, Sande, Iverius, & Greenwood 1985;Frijters & Rasmussen-Conrad 1982). Despite an increased sensitivity to detect sucrose in lower concentrations, surprisingly I found that there was no difference in the hedonic ratings of sucrose solutions by patients before compared with after gastric bypass. This discrepancy could be due to a potential lack of correspondence between sucrose detection thresholds on one hand and the perceived intensity of suprathreshold sucrose concentrations on the other hand (Bartoshuk 1978). I also cannot dismiss the possibility that other scaling procedures for measuring the hedonic value of taste stimuli might reveal effects of gastric bypass on sucrose acceptability (Bartoshuk, Duffy, Hayes, Moskowitz, & Snyder 2006), but at least with the scale employed here there was no evidence of a postoperative change. While this deserves further attention in future experiments, my results suggest that the changes in food preference observed after gastric bypass might not represent a fundamental shift in the hedonic evaluation of the food, but may be more related to other factors such as postingestive events and learning.

The rats in our study displayed a decreased sucrose preference after gastric bypass, although this effect was attenuated in rats that had preoperative sucrose experience. In contrast, there was no change in NaCl, quinine, and citric acid preferences after gastric bypass. This is in line with findings of Hajnal et al showing decreased taste preference for 0.3 and 1.0 M sucrose solutions following gastric bypass in obese CCK-1 receptor deficient OLETF rats compared with intact controls (Hajnal et al. 2010b).

There are several potential mechanisms that could underlie the selective effects of gastric bypass on sucrose preference in obese rats and these are not necessarily mutually exclusive. First, the reduced sucrose preference after gastric bypass could be related to alterations in peripheral or central gustatory processes. For example, Hajnal et al (Hajnal, Kovacs, Ahmed, Meirelles, Lynch, & Cooney 2010b) performed extracellular single neuron recordings in the pontine parabrachial nucleus (PBN), which is the second central relay in the ascending gustatory system of rodents (Norgren and Pfaffmann 1975). Obese CCK-1 receptor-deficient OLETF rats had neural concentration-response functions to oral sucrose stimulation that were shifted to the right compared with lean controls, but this was reversed by gastric bypass such that the curves between the two groups were similar. Although the origin of this effect could still be peripheral, these results demonstrate that the consequences of gastric bypass can be seen in a critical nucleus of the central taste pathway. (Hajnal, Kovacs, Ahmed, Meirelles, Lynch, & Cooney 2010b). The data from my human experiments suggest that sucrose taste sensitivity is enhanced after gastric bypass, but I did not explicitly measure this in rats. Nevertheless, even if the changes in threshold translated into more intense sensations at higher sucrose concentrations, it is unclear why this would result in decreased preference. In fact, one would expect to see a greater preference for the lower sucrose concentrations and this did not occur. Thus, despite the fact that the perceived taste intensity of sucrose might actually be greater in gastric bypass rats, it appears likely that additional factors are at play. Another possibility is that the lower preference of gastric bypass rats for higher sucrose solutions may be induced by postingestive consequences producing visceral malaise (Kyriazakis, Tolkamp, & Emmans 1999). Such negative postingestive effects might be mediated by increased postprandial levels of GLP-1

and PYY as both hormones activate neurons in the area postrema (AP) and the intermediate nucleus tractus solitarius (NTS) (Halatchev & Cone 2005); brainstem areas that are known to mediate effects of certain aversive stimuli (Halatchev & Cone 2005). In addition, peripheral administration of PYY (Halatchev & Cone 2005) and at least central administration of GLP-1 (Seeley, Blake, Rushing, Benoit, Eng, Woods, & D'Alessio 2000;Thiele, Van, Campfield, Smith, Burn, Woods, Bernstein, & Seeley 1997) have been shown to cause conditioned taste aversion in mice and rats, respectively. However, it was beyond the scope of my study to assess neuronal activity in specific brain areas like the AP and the NTS. Nevertheless, gastric bypass rats with no preoperative sucrose experience still ingested approximately half of their total fluid intake from sucrose when exposed to the highest concentration and presurgical sucrose exposure appeared to attenuate this effect. Thus, severely aversive consequences cannot be the sole explanation for the reduced sucrose preference after gastric bypass. Alternatively, perhaps the normal satiating potency of sucrose is enhanced by gastric bypass. It is tempting to speculate that the altered expression of intestinal T1R2 and T1R3 receptor proteins after gastric bypass affected nutrient sensing in the gut with associated consequences on satiety processes. However, the role of intestinal T1R2 and T1R3 receptor proteins in sugar preference remains debatable. For example, one recent study reported that T1R3 knockout mice develop strong preferences for flavors paired with intragastric sugar infusions suggesting that sugar binding gut receptors do not directly influence sugar intake and preference (Sclafani et al. 2010).

Finally, the potential contribution of learning processes affecting sucrose preference and intake after gastric bypass should not be ignored. In my study, the rats that had presurgical sucrose experience appeared to be somewhat refractory to the

suppressive effects of gastric bypass on preferences for low and midrange concentrations of sucrose in a two bottle test suggesting that some form of learning may have influenced the behaviour. It has been shown that rats and other animals can initially display reduced intake of and preference for novel tasting foods and fluids. This phenomenon is referred to as neophobia (BARNETT 1958). Neophobia can also be enhanced if the rat has encountered a recent experience of visceral malaise (Domjan 1974). In addition to neophobia, it is well documented that rats can learn, in a single trial, to avoid consumption of novel tasting foods and fluids when ingestion is followed by certain types of visceral distress such as that caused by administration of agents known to elicit nausea in humans (Barker, Best, & Domjan 1977;Garcia et al. 1955). This can occur even when there is up to a 12 hour delay between the ingestion and the illness and in many cases requires only a single pairing (Barker, Best, & Domjan 1977;Carroll and Smith 1974). However, taste novelty is a key component; it is much more difficult to condition a taste aversion to familiar tasting substances (Barker, Best, & Domjan 1977;Siegel 1974). The adaptive significance of these processes requires little defence especially in a species such as the rat which is incapable of vomiting (BORISON and WANG 1953). Given that taste novelty is a critical feature in the demonstration of neophobia and conditioned taste aversion, it is possible that either of these processes, or even both, underlie the differences in the effects of gastric bypass on sucrose preference seen in animals with vs. without presurgical experience with this taste stimulus (Barker, Best, & Domjan 1977). My results do however caution against some of the initial conclusions regarding sucrose preferences in recent publications (Hajnal, Kovacs, Ahmed, Meirelles, Lynch, & Cooney 2010b), especially as very few humans would be naïve to the taste of sucrose.

In conclusion, gastric bypass increases the oral sensitivity to detect sucrose in humans and reduces sucrose preference in rats. These findings are associated with changes in sugar-binding taste receptors in the gut and increased levels of GLP-1 after gastric bypass. Postingestive factors together with increased sensitivity to detect lower concentrations of sucrose in the mouth as well as learning may explain the changes in food preference seen after gastric bypass. It will be important for future work to examine whether gastric bypass alters taste detection thresholds in the rat model, which allows for more systematic and targeted manipulations aimed at revealing mechanisms. Further elucidation of the mechanisms by which gastric bypass reduces consumption of high-caloric foods may help in the development of novel surgical and non-surgical therapeutic interventions that will promote safer and more effective weight loss.

Chapter 10: Summary

Patients after gastric bypass reported a lack of desire to consume fatty food as they no longer found it enjoyable (Halmi, Mason, Falk, & Stunkard 1981). Total fat intake is lower after gastric bypass, because of a reported disinterest in sweets or deserts after surgery (Brolin, Robertson, Kenler, & Cody 1994;Brown, Settle, & Van Rij 1982;Olbers, Bjorkman, Lindroos, Maleckas, Lonn, Sjostrom, & Lonroth 2006). The gustatory system is a prime candidate to contribute to the observed effects after gastric bypass and changes might be mediated by altered gut hormone levels (Shin, Martin, Golden, Dotson, Maudsley, Kim, Jang, Mattson, Drucker, Egan, & Munger 2008). It remains however unclear, whether such changes in preferences, even if taste-related, are attributable to changes in the intensity of the sensory signals generated by food (stimulus identification) or by their altered evaluation in so called "reward" circuits in the brain (ingestive motivation), or both. Finally, gastric bypass may also induce negative postingestive consequences via cephalic phase reflexes that lead to changes in food preference (digestive preparation).

In conclusion, I have demonstrated that gastric bypass in humans and rats reduces the preference for high fat food and high concentrations of Intralipid® solution. Postingestive effects and conditioned taste aversion may partly explain my findings. Furthermore, gastric bypass increases the oral sensitivity to detect sucrose in humans and reduces sucrose preference in rats. My findings are associated with changes in sugar-binding taste receptors in the gut and increased levels of GLP-1 after gastric bypass. Postingestive factors together with increased sensitivity to detect lower concentrations of sucrose in the mouth as well as learning may explain

the changes in food preference seen after gastric bypass. It will be important for future work to examine whether gastric bypass alters taste detection thresholds in the rat model, which allows for more systematic and targeted manipulations aimed at revealing mechanisms.

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Appendix - Published papers

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ANIMAL RESEARCH

Vagal Sparing Surgical Technique but Not Stoma Size Affects Body Weight Loss in Rodent Model of Gastric Bypass

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Abstract
Background The aim of this study was to evaluate whether gastric bypass with or without vagal preservation resulted in a different outcome.
Methods Body weight, food intake and postprandial peptide YY (PYY) and glucagon-like peptide (GLP-1) levels were compared between gastric bypass (n=55) and sham-operated rats (n=27) in three groups. In group 1 (n=17), the vagal nerve was not preserved, while in group 2 the vagal nerve was preserved during gastric bypass (n=10). In group 3, gastric bypass rats (n=28) were randomised for either one of the two techniques.
Results Rats in which the vagal nerve was preserved during gastric bypass showed a lower body weight (p<0.001) and reduced food intake (p<0.001) compared to rats in which the vagal nerve was not preserved during the gastric bypass operation. Levels of PYY and GLP-1 were significantly increased after gastric bypass compared to sham-operated controls (p<0.05), but there was no difference between gastric bypass rats with and without vagal preservation. Differences in food intake and body weight were not related to the size of the gastro-jejunostomy in gastric bypass rats. There were no signs of malabsorption or inflammation after gastric bypass.
Conclusion We propose that the vagal nerve should be preserved during the gastric bypass operation as this might play an important role for the mechanisms that induce weight loss and reduce food intake in rats. In contrast, the gastro-jejunal stoma size was found to be of minor relevance.

Keywords Gastric bypass · Rats · Para-oesophageal bundle · Vagal nerve · Left gastric vessels · Weight loss

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Introduction

Bariatric surgery has been proven to be the most effective treatment for severe obesity and its inherent co-morbidities resulting in significant and sustained weight loss with a proven mortality benefit [1, 2]. At present, the Roux-en-Y gastric bypass procedure (gastric bypass) provides reliable and sustainable weight loss. Given the rapid increase in gastric bypass procedures, it is important to understand the underlying mechanisms by which gastric bypass induces and sustains weight loss [3, 4]. The use of animal models for gastric bypass surgery is a valuable tool and has been shown to be a valid model to mimic human weight loss after gastric bypass [5–7]. However, there is significant variation in techniques used in humans and rodent models. The results for weight loss, food intake and mortality rates are heterogeneous [5–15].

The vagal nerve is thought to have an important role in the regulation of food intake and body weight, but only a

few reports examined whether vagal preservation is effective or necessary in weight control after bariatric surgery [15–17]. Gut hormones released from enteroendocrine cells in the distal ileum like glucagon-like peptide (GLP-1) and peptide YY (PYY) can signal either through the circulation or via afferent vagal fibres [18].

In this study, we describe variations in the technique for gastric bypass surgery in rats in the area of the gastrojejunostomy. Here, the para-oesophageal bundle can be found which contains the left gastric vessels and the dorsal vagal trunk that contains 4/5 of the right vagal fibres and a 1/5 of the left vagal fibres [19]. The aim of our study was to assess whether preservation of the vagal fibres in the para-oesophageal bundle impacts on body weight and food intake after gastric bypass in rats.

Material and Methods

Animals

Male Wistar rats used were individually housed under a 12/12 h light–dark cycle and at a room temperature of 21±2°C. Water and standard chow were available ad libitum, unless otherwise stated. All experiments were performed under a licence issued by the Home Office UK (PL 70-5569) or approved by the Veterinary Office of the Canton Zurich, Switzerland. Body weight and food intake were measured daily in groups 1 and 2 for a postoperative period of 60 days and in group 3 for 75 days.

Surgery

All operations reported in this study were performed by one surgeon (MB). After 1 week of acclimatisation, rats were randomised to gastric bypass or sham operation. Rats were food deprived for 12 h overnight, but water was available ad libitum. Before surgery, rats were weighed, and then anaesthetised with isofluorane (4% for induction, 3% for maintenance). Preoperatively, gentamicin 8 mg/kg and carprofen 0.01 ml were administered intraperitoneally (ip) as prophylaxis for postoperative infection and pain relief. Surgery was performed on a heating pad to avoid decrease of body temperature during the procedure. Prior to a midline laparotomy, the abdomen was shaved and disinfected with surgical scrub. In the sham group, a 7 mm gastrotomy on the anterior wall of the stomach with subsequent closure (interrupted prolene 5-0 sutures) and a 7 mm jejunotomy with subsequent closure (running prolene 6-0 suture) was performed. In the gastric bypass group, the proximal jejunum was divided 15 cm distal to the pylorus to create a biliopancreatic limb. After identification of the caecum, the ileum was then followed proximally to create a common channel of 25 cm. Here, a 7 mm side-to-side jejunojejunostomy (running prolene 7-0 suture) between the biliopancreatic limb and the common channel was performed.

The two techniques described below in this paper relate to how the stomach was transected close to the gastro-oesophageal junction to create a small gastric pouch with no more than 3 mm of gastric mucosa left. The gastric pouch and alimentary limb was anastomosed end-to-side using a running prolene 7-0 suture. The gastric remnant was closed with interrupted prolene 5-0 sutures. The complete bypass procedure lasted approximately 60 min and the abdominal wall was closed in layers using 4-0 and 5-0 prolene sutures. Approximately 20 min before the anticipated end of general anaesthesia, all rats were injected with 0.1 ml of 0.3% buprenorphine subcutaneously to minimise postoperative discomfort. Immediately after abdominal closure, all rats were injected subcutaneously with 5 ml of normal saline to compensate for intraoperative fluid loss. After 24 h of wet diet (normal chow soaked in tap water), regular chow was offered on postoperative day 2.

Experimental Design

The vagal fibres in the para-oesophageal bundle in the area of the gastric pouch were subjected to two different techniques. All groups were operated in chronological order. In a first group, 25 obese rats (body weight (BW) 348±3.9 g) were randomised for gastric bypass (n=17) or sham operation (n=8). In this group the vagal fibres were not preserved in the gastric bypass rats as the para-oesophageal neurovascular bundle was completely ligated (group 1). In a subsequent group, 18 obese rats (332±2.4 g) were randomised to gastric bypass (n=10) or sham operation (n=8). Here, the vagal fibres were preserved as the left gastric vessels were separated and selectively ligated in all gastric bypass rats (group 2). Significant differences in body weight and energy intake were observed in these two groups. As it was unclear whether these differences were related to the different techniques of vagal preservation, a third group (group 3) of 39 obese rats (471±4.3 g) was randomised for gastric bypass without vagal preservation (n=14) or gastric bypass with vagal preservation (n=14) or sham operation (n=11).

Hormone Assay

Animals from group 3 were fasted for 12 h from the beginning of the light cycle. At the onset of the dark cycle animals were offered 5 g of standard chow all of which was consumed within half an hour by the animals. Blood was then obtained by puncture of a sublingual vein under brief isoflurane anaesthesia from sham-operated controls, gastric bypass with and without vagal preservation (each n=6).

Blood was collected into EDTA-rinsed tubes and, immediately centrifuged at 3,000 rpm for 10 min at 4°C. The supernatant was stored at −80°C until further analysis. Concentrations of active GLP-1 and PYY were analysed using a rat endocrine lincoplex kit (RENDO-85 K, Labodia SA, Yens, Switzerland).

Measurement of Size of the Gastro-Jejunostomy

To exclude that the differences in body weight between bypass rats were due to different levels of restriction and subsequent differences in food intake, sizes of the gastro-jejunostomy were measured during necropsy in all gastric bypass rats of group 3.

CRP Analysis

Blood was obtained from all animals of group 3 by puncture of a sublingual vein under brief isoflurane anaesthesia. Blood was collected into EDTA-rinsed tubes and immediately centrifuged at 3,000 rpm for 10 min at 4°C. Plasma was stored at −80°C before analysis for C-reactive protein (Abbott, UK) to assess inflammation.

Faecal Analysis

To evaluate nutrient malabsorption, faeces were collected over 24 h on postoperative days 15 and 59 from all animals in group 3. Faeces were dried in an oven and weighed; calorie content was measured using a ballistic bomb calorimeter [20].

Statistics

All data were normally distributed and are expressed as mean ± SEM. Student's t test for independent samples and one-way ANOVA with repeated measures and post-hoc Bonferroni test for each time point were used to test for significant differences. $p<0.05$ was considered significant.

Results

Mortality

Overall surgical mortality was 13.4% (11/82). Gastric bypass-related mortality was 14.5% (8/55), while mortality after sham operation was 11.1% (3/27, $p=0.668$). There was no mortality difference between bypass rats with complete ligation and with preservation of the para-oesophageal bundle. All eight bypass rats showed signs of respiratory distress along with hypersalivation and dysphagia within the first two postoperative days after the operation and were euthanized immediately after onset of symptoms. Necropsy revealed that these symptoms originated at the level of the gastro-jejunostomy where food did not pass through and was retained in the oesophagus. Whether this was due to inflammatory swelling following anastomotic leakage or due to anastomotic constriction remains unclear. The three sham-operated rats died without prior noticeable symptoms. Necropsy revealed in two cases a small bowel ileus presumably due to a volvulus after inappropriate repositioning of the viscera into the abdominal cavity at the end of the operation. In one case, a leak at the site of the gastrotomy was found.

Energy Intake

In group 1, there was no difference in average daily energy intake between gastric bypass rats and sham-operated rats over a period of 60 days (sham, 97.4±2.5 kcal vs. bypass, 89.3±4.7 kcal, $p=0.3$). In contrast, gastric bypass rats of group 2 ate significantly less than the sham-operated rats (sham, 76.7±2.2 kcal vs. bypass, 52.5±4.8 kcal, $p<0.001$). In group 3, there was no difference in average energy intake between bypass rats without vagal preservation and sham-operated rats over a period of 75 days, while bypass rats with vagal preservation ate significantly less than sham-operated rats and rats without vagal preservation (sham, 118.7±3.9 kcal vs. bypass with vagal preservation, 84.4±3.3 kcal vs. bypass without vagal preservation, 102.8±7.5 kcal, $p<0.001$). The average daily energy intake is shown for all three groups in Fig. 1.

Body Weight

In all three groups gastric bypass rats had a significant lower body weight than sham-operated rats from day 5 after surgery throughout the rest of the observation period. After a short period of post-surgical weight loss, sham-operated rats of all three groups constantly gained weight for the rest of the study. In group 1, gastric bypass rats started to regain weight around postoperative day 25 and there was no difference between their body weight before surgery and after surgery at the end of the observation period (day 0, 457.0±7.4 g vs. day 60, 468.0±9.3 g, $p=0.36$). In group 2, gastric bypass animals lost about 20% of their preoperative weight by postoperative day 25 and their body weight then plateaued around 260 g (day 0, 330.8±5.8 g vs. day 60, 259.1±16.3 g, $p=0.001$). In group 3, there was no difference in body weight between bypass rats without vagal preservation and bypass rats with vagal preservation until postoperative day 40 (day 40: bypass with vagal preservation, 408.3±11.2 g vs. bypass without vagal preservation, 414.4±11.2 g, $p=0.70$). However, thereafter bypass rats without vagal preservation started to regain weight for the rest of the observation period, while bypass

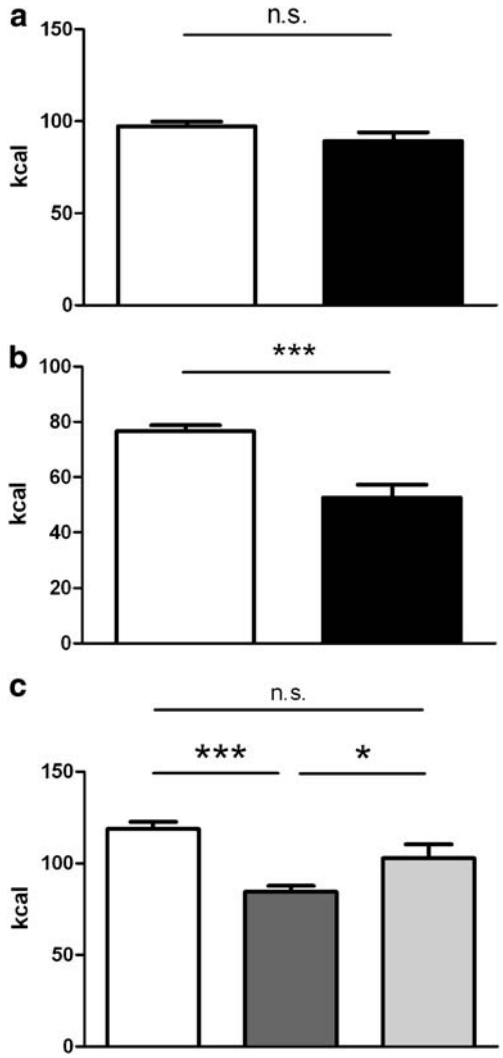


Fig. 1 **a** Average daily energy intake (group 1) over 60 days for sham-operated ad libitum fed rats (n=7, *white column*) and for gastric bypass rats (n=14, *black column*). Data are shown as mean values ± SEM. **b** Average daily energy intake (group 2) over 60 days for sham-operated ad libitum fed rats (n=8, *white column*) and for gastric bypass rats (n=8, *black column*). Data are shown as mean values ± SEM (***p<0.001). **c** Average daily energy intake (group 3) over 75 days for sham-operated ad libitum fed rats (n=10, *white column*) and for gastric bypass rats with vagal preservation (n=11, *dark grey*) or without vagal preservation (n=10, *light grey*). Data are shown as mean values ± SEM. Post-hoc differences between the three groups are indicated (***p<0.001 and *p<0.05)

rats with preserved vagal fibres maintained their low body weight (day 75: bypass with selective ligation, 365.8± 14.6 g vs. bypass with complete ligation, 468.0±9.3 g, p< 0.001). The development of body weight after surgery is shown for all groups in Fig. 2.

Postprandial Plasma Levels of PYY and Active GLP-1

One-way ANOVA revealed significant differences for levels of PYY and active GLP-1 in the gastric bypass groups in comparison to sham-operated controls (PYY: sham, 29.5±7.1 pg/ml vs. bypass with vagal preservation, 70.4±8.8 pg/ml vs. bypass without vagal preservation, 83.2± 14.3 pg/ml, p<0.01; GLP-1: sham, 85.8±2.1 pg/ml vs. bypass with vagal preservation, 146.9±23.7 pg/ml vs. bypass without vagal preservation, 155.4±24.1 pg/ml, p<0.05). However, post-hoc Bonferroni testing showed no significant

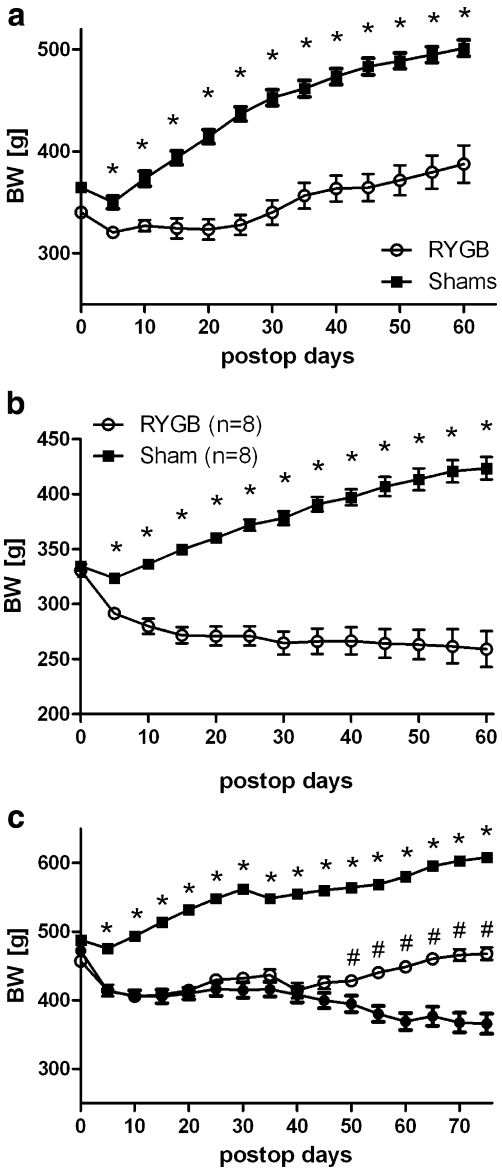


Fig. 2 **a** Body weight change in group 1 for the gastric bypass (*unfilled circle*; n=14) and sham-operated rats (*filled square*; n=7). Data are shown as mean values ± SEM (*p<0.05). **b** Body weight change in group 2 for the gastric bypass (*unfilled circle*; n=8) and sham-operated rats (*filled square*; n=8). Data are shown as mean values ± SEM (*p<0.05). **c** Body weight change in group 3 for the gastric bypass rats without vagal preservation (*unfilled circle*; n=10) and gastric bypass rats with vagal preservation (*filled circle*; n=11) and sham-operated rats (*filled square*; n=10). Data are shown as mean values ± SEM (*p<0.05 for sham vs. bypass; #p<0.05 for bypass without vagal preservation vs. bypass with vagal preservation)

difference for PYY and GLP-1 levels between gastric bypass rats with or without vagal preservation (Fig. 3).

Size of the Gastro-Jejunostomy

There was no gastrogastric fistula in any of the gastric bypass rats of group 3. The overall size of the gastro-jejunostomy in all gastric bypass rats was 15.4±0.4 mm. There was no difference in size of the anastomosis between rats in which the complete para-oesophageal bundle was ligated and rats in which the left gastric vessels were separated and selectively ligated (bypass with selective ligation, 15.2±0.4 mm vs. 15.6±0.7 mm, p=0.69).

CRP Analysis

C-reactive protein levels were below 2 mg/L in all animals of group 3 indicating that there was no postsurgical infection or inflammation 28 days after surgery.

Faecal Analysis

There was no increase in either fresh faecal mass (sham, 8.4±0.5 g vs. bypass with vagal preservation, 7.5±0.6 g vs. bypass without vagal preservation, 7.2±0.6 g, p=0.3) or faecal calorie content (sham, 3.56±0.04 kcal vs. bypass with vagal preservation, 3.43±0.05 kcal vs. bypass without vagal preservation, 3.65±0.06 kcal, p=0.24) in the gastric bypass animals compared to the sham-operated rats in group 3.

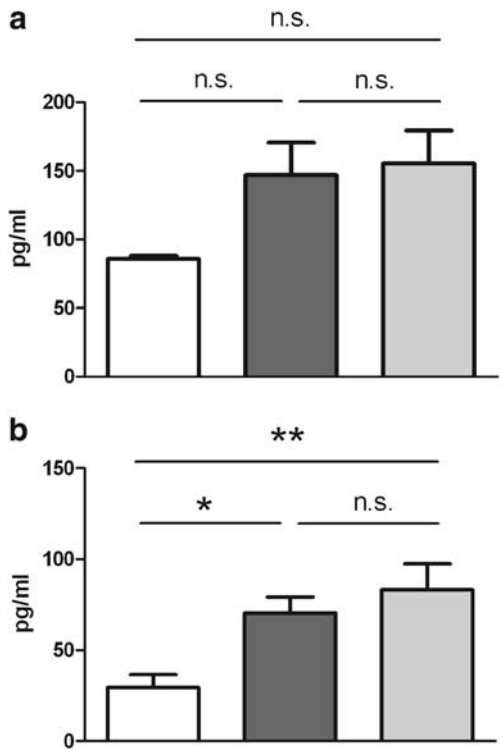


Fig. 3 Levels of active GLP-1 (**a**) and PYY (**b**) for sham-operated ad libitum fed rats (n=6, *white column*) and for gastric bypass rats with vagal preservation (n=6, *dark grey*) or without vagal preservation (n=6, *light grey*). Data are shown as mean values ± SEM. Post-hoc differences between the three groups are indicated (**p<0.01 and *p<0.05)

Discussion

Our data in the rat model for gastric bypass are consistent with previous findings that gastric bypass surgery can effectively induce food intake and body weight reduction [1, 21, 22]. In this randomised study, the weight loss and food intake outcome of gastric bypass surgery was dependent on whether vagal fibres were preserved or not during the formation of the gastric pouch. Rats in which the para-oesophageal bundle including the vagal fibres was completely ligated started to regain body weight up to preoperative levels and showed no difference in average daily energy intake compared to their sham-operated counterparts. In contrast, rats in which the para-oesophageal bundle including the vagal fibres was preserved and in which the left gastric vessels were selectively ligated, maintained the reduced body weight and ate significantly less than the sham-operated controls throughout the entire study period. Gastric bypass rats had higher postprandial GLP-1 and PYY levels compared to sham-operated controls, but there were no differences in GLP-1 and PYY levels between gastric bypass rats with our without preserved vagal fibres. Furthermore, differences in food intake and body weight were not related to the size of the gastro-jejunostomy in gastric bypass rats and there were no signs of malabsorption or inflammation after gastric bypass in any of the groups.

Our data confirm previous findings that gastric bypass in rats increases postprandial levels of peptide YY and glucagon-like peptide-1, which are satiation-inducing gut hormones and hence favour an anorectic state and facilitate body weight loss [5, 21]. Both hormones are thought to activate anorectic neurons in the hypothalamic arcuate nucleus which promote weight loss [23–26]. Gut hormones released from enteroendocrine cells in the distal ileum like GLP-1 and PYY can signal either through the circulation or via afferent vagal neurons [18].

In this study body weight and food intake after gastric bypass were related to whether the vagal fibres within the para-oesophageal bundle were preserved or not, while there were no differences in levels of GLP-1 and PYY between these two groups. This finding highlights the potential role of the vagal nerve for mediating the inhibitory effects of gut hormones such as PYY and GLP-1 on food intake and body weight after gastric bypass surgery in rats. Our findings are consistent with previous reports showing that the ablation

of the vagal–brainstem–hypothalamic pathway attenuates the inhibitory effects of PYY and GLP-1 on food intake [27]. Vagal preservation may thus be necessary for optimum weight loss after bariatric surgery [15–17].

In contrast to our observation, Wang and Liu [15] described greater weight loss after gastric bypass and total vagotomy in rats. The difference was only present at 20 days after surgery, but the difference in food intake and body weight between bypass rats with or without vagal dissection was lost thereafter. Another difference to our study is that Wang and Liu [15] used the bypass operation to prevent obesity in rats weighing 180–200 g while we performed surgery to cause weight loss in obese rats.

Weight loss after a gastric bypass operation might also be due to nutrient malabsorption or postoperative inflammation. However, we found no evidence for an increase in either faecal mass or faecal calorie content in the gastric bypass animals with or without vagal preservation. Moreover, we did not detect any evidence of increased inflammation in animals with or without vagal ligation post-surgery.

The size of the gastric pouch and the lengths of the different limbs used in this study have been proven to effectively induce weight loss [6]. An increasing body of evidence in humans indicates that up to certain limits the size of the gastric pouch and length of the different limbs is of less importance for the outcome of gastric bypass [28]. In support of this observation, we demonstrated that the level of restriction measured by the size of the gastro-jejunostomy has no impact on different levels of weight loss and food intake after gastric bypass in rats.

There are two major limitations of our study. Firstly, we cannot exclude the possibility that the ligation of the para-oesophageal bundle is functionally equivalent only to a partial dissection of the vagal nerve. In addition, we did not perform a secretin test or histological analysis to collect further informations on vagal function to confirm whether the complete ligation of the para-oesophageal bundle produced a total or partial vagotomy. Secondly, it remains unclear whether our results can be translated into humans. Most bariatric surgeons usually aim to preserve the anterior and posterior vagal trunk during formation of the gastric pouch, although there is a lack of supporting data indicating that this approach has beneficial effects. Recently, the dissection of the anterior vagal trunk during pouch formation has been reported to have no effect on clinical, functional and laboratory results of a gastric bypass operation [16].

In conclusion, our gastric bypass technique induces reliable weight loss in rats with an acceptable mortality. We propose that vagal nerve fibres should be preserved during gastric bypass in rats. Restriction at the gastro-jejunal anastomosis does not seem to be critical for the weight loss. Although the mechanisms have not yet been fully elucidated, vagal preservation may play an important role in inducing and maintaining weight loss after gastric bypass in humans and rats.

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Gastric Bypass Increases Energy Expenditure in Rats

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BACKGROUND & AIMS: Mechanisms underlying weight loss maintenance after gastric bypass are poorly understood. Our aim was to examine the effects of gastric bypass on energy expenditure in rats. **METHODS:** Thirty diet-induced obese male Wistar rats underwent either gastric bypass (n = 14), sham-operation ad libitum fed (n = 8), or sham-operation body weight-matched (n = 8). Energy expenditure was measured in an open circuit calorimetry system. **RESULTS:** Twenty-four-hour energy expenditure was increased after gastric bypass (4.50 ± 0.04 kcal/kg/h) compared with sham-operated, ad libitum fed (4.29 ± 0.08 kcal/kg/h) and sham-operated, body weight-matched controls (3.98 ± 0.10 kcal/kg/h, $P < .001$). Gastric bypass rats showed higher energy expenditure during the light phase than sham-operated control groups (sham-operated, ad libitum fed: 3.63 ± 0.04 kcal/kg/h vs sham-operated, body weight-matched: 3.42 ± 0.05 kcal/kg/h vs bypass: 4.12 ± 0.03 kcal/kg/h, $P < .001$). Diet-induced thermogenesis was elevated after gastric bypass compared with sham-operated, body weight-matched controls 3 hours after a test meal (0.41% ± 1.9% vs 10.5% ± 2.0%, respectively, $P < .05$). The small bowel of gastric bypass rats was 72.1% heavier because of hypertrophy compared with sham-operated, ad libitum fed rats ($P < .0001$). **CONCLUSIONS: Gastric bypass in rats prevented the decrease in energy expenditure after weight loss. Diet-induced thermogenesis was higher after gastric bypass compared with body weight-matched controls. Raised energy expenditure may be a mechanism explaining the physiologic basis of weight loss after gastric bypass.**

Keywords: Weight Loss; Diet-Induced Thermogenesis; Gut Hypertrophy.

The obesity epidemic is a major health concern that is associated with increased morbidity and mortality[1] as well as negative personal, social, and economic consequences.[2,3] Roux-en-Y gastric bypass (gastric bypass) is the most effective therapeutic option currently available for sustained weight loss with a proven mortality benefit.[4,5] Gastric bypass procedures are increasing rapidly,[6] but underlying mechanisms by which gastric bypass induces and sustains weight loss are poorly understood. Initially, it was speculated that weight loss after gastric bypass was due to mechanical restriction and malabsorption.[7] Experimental and clinical studies, however, have suggested that other mechanisms contribute to weight loss after gastric bypass.[8–13] The absence of a compensatory increase in appetite after gastric bypass-induced weight loss has been intriguing because nonsurgical intentional body weight loss is usually followed by body weight regain through increased appetite.[14]

A proposed mechanisms for reduced food intake after bypass surgery is the secretory stimulus to L-cells in the distal gut, resulting in increased levels of gastrointestinal satiation hormones such as peptide YY (PYY) and peptides of the enteroglucagon family.[9,11,15–17] These hormones stimulate anorectic pathways in the hypothalamus and brain stem leading to reduced food intake[18] and may also influence energy expenditure.[19]

Gastric bypass surgery has been successfully modeled in rat experiments. The body weight loss after gastric bypass in rats is not only due to decreased food intake because sham-operated, pair-fed controls weigh more than gastric bypass rats.[10,11,20–22] Possible explanations such as malabsorption and inflammation have been excluded,[10] thus the weight difference despite similar food intake raises the possibility of enhanced energy expenditure[10] as previously speculated.[23,24] We therefore tested the hypothesis that energy expenditure would be higher after bypass surgery.

Materials and Methods

Animals and Housing

Thirty adult diet-induced obese male Wistar rats weighing 480–500 g were used for energy expenditure experiments, and 16 adult male Wistar rats weighing 330–350 g were used for morphometric gut analysis. All animals were individually housed under artificial 12-hour/12-hour light-dark cycle and at a room temperature

Abbreviations used in this paper: ARC, arcuate nucleus; NPY, neuropeptide Y; POMC, pro-opiomelanocortin; PYY, peptide YY.

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of 21°C ± 2°C unless otherwise stated. Water and standard chow were available ad libitum. All experiments were performed under a license issued by the Home Office United Kingdom (PL70-6669) or were approved by the Veterinary Office of the Canton Zurich, Zurich, Switzerland.

Surgery

Surgery was performed according to an established protocol as described in the Supplementary Materials and Methods.[10] Figure 1 shows a schematic illustration of the pre- and postoperative anatomy.

Indirect Calorimetry

Measurements were conducted in an open circuit calorimetry system (AccuScan Inc, Columbus, OH) as described in the Supplementary Materials and Methods.[25]

Experimental Design

The 30 diet-induced obese rats used in the energy expenditure experiments were randomized to gastric bypass (n = 14) or sham operation (n = 16). After a recovery period of 7 days, sham-operated animals were randomly divided into 2 groups of 8 rats each: shams with no dietary manipulation (ad libitum fed, sham-operated rats weighing 488.8 ± 3.9 g) and food-restricted, sham-operated rats whose postoperative weight was matched to the weight of bypass animals (body weight-matched, sham-operated rats weighing 474.3 ± 4.2 g). Starting on day 7 after gastric bypass surgery, the body weight-matched, sham-operated rats received as much food daily as was necessary for them to maintain a similar body weight to the bypass rats. Based on experiences from previous studies, rats were given 10 g of standard chow in the beginning of food restriction. This amount of food was offered at dark onset and readjusted every third day depending on the body weight. Sixteen metabolic cages were used, and measurements were conducted in the following order on 3 consecutive days: bypass (n = 8) vs sham-operated, ad libitum fed (n = 8) (40 days after surgery) rats and bypass (n = 6) vs sham-operated, body weight-matched (n = 8) (75 days after surgery) rats. Diet-induced thermogenesis was measured in rats that were fasted for 12 hours from the beginning of the light cycle and received a 5-g meal at subsequent dark onset. Diet-induced thermogenesis was calculated as the cumulative increase in energy expenditure after a 5-g test meal compared with fasting values before the test meal (expressed as percentage of the energy content of the test meal: 17.6 kcal). Methods for fecal and blood analysis are described in the Supplementary Materials and Methods.

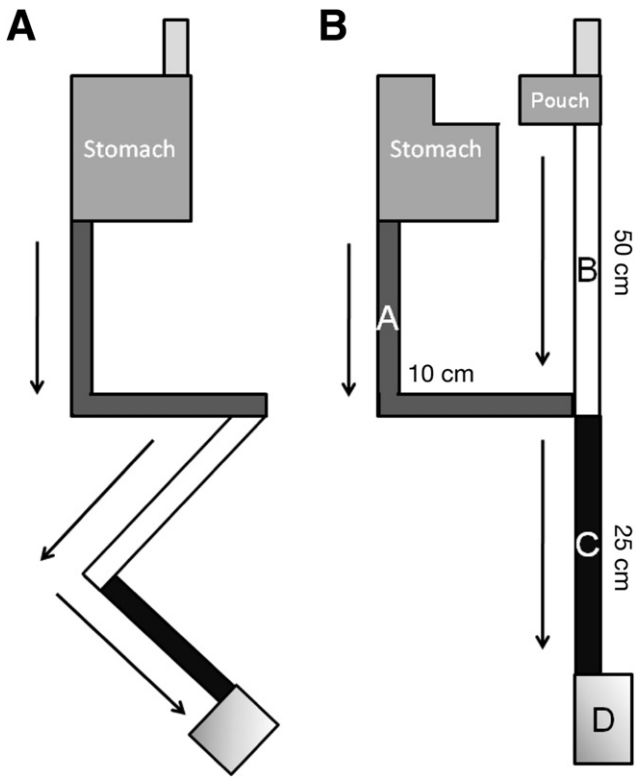


Figure 1. Diagrammatic representation of the gastrointestinal anatomy before (*A*) and after (*B*) the gastric bypass operation. Letter A within Figure part *B* represents biliopancreatic limb (~10 cm), B represents alimentary limb (~50 cm), C represents common channel (~25 cm), and D represents cecum.

Measurement of Body Composition

Adipose tissue mass was measured using a rodent computerized tomography scanner (Latheta, Aloka, Japan). Rats were anesthetized with isoflurane, and the area between vertebrae L1 and L5 was scanned using an x-ray source tube voltage of 50 kV, current of 1 mA, pitch size of 2 mm, and a speed of 4.5 seconds per image (roughly 25 images per rat). Aloka software (Zug, Switzerland) was used to estimate volumes of adipose tissue and nonadipose tissue using differences in x-ray density. Adipose tissue weights were computed using the density factor of 0.92 g/cm^3. Scanning was undertaken 70 days after surgery.

Gut Morphometry

For the study of gut morphometry, 16 male Wistar rats were randomized to gastric bypass (n = 8) or sham operation (n = 8). All rats were ad libitum fed throughout the complete observation period of 60 days. Rats were fasted for 24 hours before being killed to ensure the small bowel was free of chow residue. The entire small bowel from the duodenum to the ileocecal valve was collected. Total wet weight and length of the small bowel were measured in the sham-operated rats, whereas, in gastric bypass rats, the weight and length of the 3 limbs (alimentary, biliopancreatic, and common channel) were measured separately and then added. Sup-

plementary Materials and Methods describes gut tissue processing and analysis.

Statistical Analysis

All data were normally distributed and are expressed as mean ± standard error of mean. Student *t* test for independent samples and 1-way analysis of variance with repeated measures and post hoc Bonferroni test for each time point were used to test for significant differences. $P < .05$ was considered significant. For all analyses, data from the 2 gastric bypass groups were pooled because data did not differ between the 2 time points (day 40 and day 75 after surgery).

Results

Body Weight

Figure 2 shows the body weight changes for both groups. For the energy expenditure experiments (Figure 2*A*), body weight was significantly lower in gastric bypass rats compared with the sham-operated, ad libitum fed group from day 5 after surgery. On postoperative day 70, the difference in weight was almost 200 g (sham-operated, ad libitum fed: 603.2 ± 6.6 g vs bypass: 414.3 ± 13.8 g, $P < .0001$). After a short period of postsurgical weight loss, sham-operated, ad libitum fed rats fed constantly gained weight for the rest of the study. In contrast, gastric bypass animals lost 11.2% ± 1.4% of their preoperative weight by postoperative day 10; body weight then plateaued around 415 g.

Food restriction started 1 week after surgery for the body weight-matched, sham-operated (n = 8) rats. There was no significant difference in body weight between the gastric bypass group and the food-restricted body weight-matched rats on and after day 55 (sham-operated, body weight-matched: 412.2 ± 3.0 g vs bypass: 408.7 ± 9.4 g, $P = .78$).

There was no increase in either fresh fecal mass (sham-operated, ad libitum fed: 8.4 ± 0.5 g vs sham-operated, body weight-matched: 6.6 ± 0.6 g vs bypass: 7.3 ± 0.4 g, P = ns) or fecal calorie content (sham-operated, ad libitum fed: 3.56 ± 0.04 kcal/g vs sham-operated, body weight-matched: 3.51 ± 0.04 kcal/g vs bypass: 3.65 ± 0.04 kcal/g, P = ns) in the gastric bypass animals compared with the control groups. C-reactive protein levels were below the detection limit of the assay (<2 mg/L) in all animals, suggesting no postsurgical infection or inflammation 28 days after surgery.

In the gut morphometry experiments, body weight was significantly lower in gastric bypass rats compared with the sham-operated group from day 5 after surgery (Figure 2*B*); sham-operated rats gained weight for the rest of the study, whereas gastric bypass animals lost 15.4% ± 1.1% of their preoperative weight by postoperative day 10 and then plateaued around 260 g. The difference in body weight on day 60 was 164 g (sham-operated, ad libitum fed: 423.6 ± 10.2 g vs bypass: 259.1 ± 16.3 g, $P < .0001$).

Body Composition

Adipose tissue mass between vertebrae L1 and L5 in gastric bypass was lower than in sham-operated, ad libitum fed rats, but similar to body weight-matched shams (sham-operated, ad libitum fed: 27.6 ± 2.7 g vs sham-operated, body weight matched: 5.3 ± 0.9 g vs bypass: 11.6 ± 1.3 g, $P < .001$). Nonadipose tissue in gastric bypass was lower than in sham-operated, ad libitum fed rats but higher than in body weight-matched shams (sham-operated, ad libitum fed: 107.1 ± 2.9 g vs sham-operated, body weight matched: 71.0 ± 1.1 g vs bypass: 80.9 ± 2.4 g, $P < .001$).

Food Intake Outside Metabolic Cages

Food intake followed similar patterns as body weight. Figure 3*A* shows the average daily food intake for rats of the energy expenditure experiments (postoperative days 1–70). Daily food intake was consistently lower after gastric bypass (sham-operated, ad libitum fed: 34.0 ± 1.2 g vs bypass: 27.5 ± 0.8 g, $P < .0001$). Body weight-matched, sham-operated animals required significantly less food than gastric bypass animals to maintain the same level of body weight (sham-operated, body weight-matched: 16.2 ± 0.5 g vs bypass: 27.5 ± 0.8 g, $P < .0001$). Gastric bypass rats used for the analysis of gut morphometry also ate significantly less than their sham-

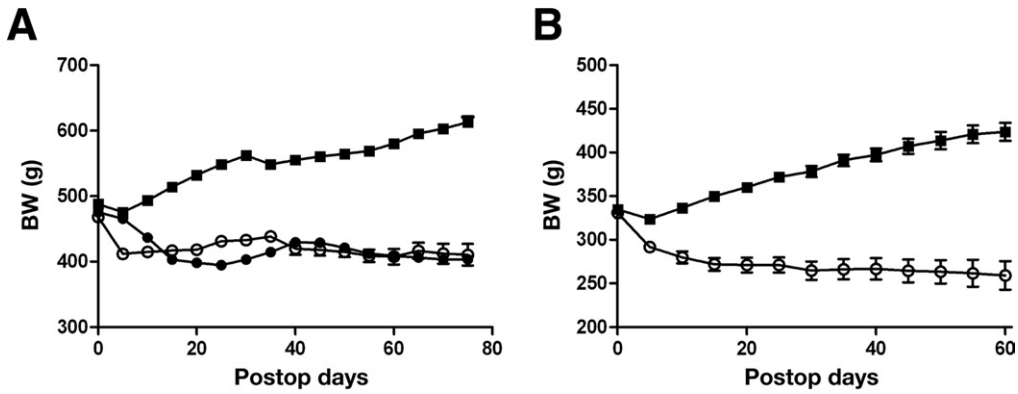


Figure 2. Body weight change for the gastric bypass (-○-) (n = 14) and sham-operated rats ad libitum fed (-■-) (n = 8) and sham-operated body weight matched (-●-) (n = 8) used for energy expenditure measurements (*A*) and for gastric bypass (-○-) (n = 8) and sham-operated rats ad libitum fed (-■-) (n = 8) used for gut morphometry analysis (*B*). Data are shown as mean values ± SEM.

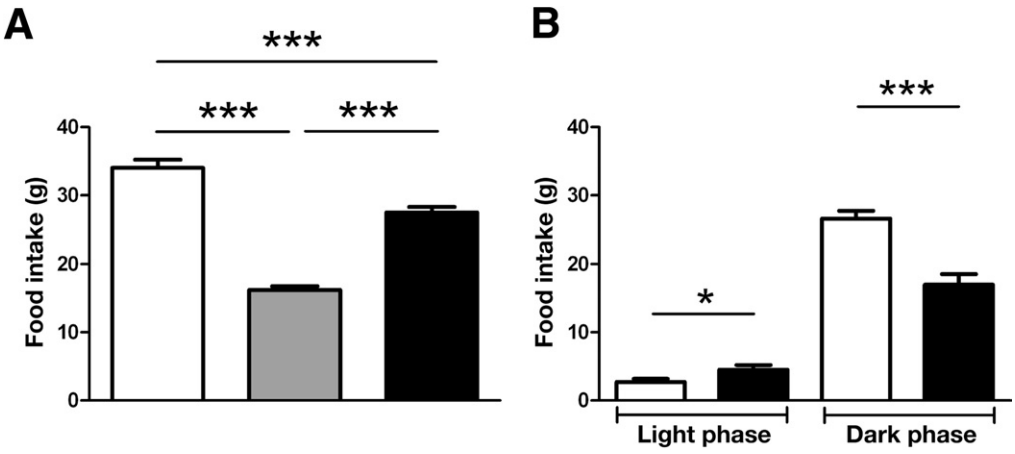


Figure 3. (*A*) Average daily food intake over 70 days for sham-operated ad libitum fed rats (n = 8, *open column*), for sham-operated body weight-matched rats (n = 8, *shaded column*), and for gastric bypass rats (n = 14, *solid column*). Data are shown as mean values ± SEM (***$P < .001$). (*B*) Average food intake during dark and light phase for sham-operated ad libitum fed (n = 8, *open columns*) and gastric bypass rats (n = 8, *solid columns*). Data are shown as mean values ± SEM (*$P < .05$, ***$P < .001$).

operated counterparts (sham-operated: 32.5 ± 0.4 g vs bypass: 26.0 ± 0.5 g, $P < .0001$).

Food Intake in Metabolic Cages

Meal patterns were different among the 3 groups in the energy expenditure experiment. In the dark phase, gastric bypass and sham-operated, ad libitum fed rats ate more than in the light phase. Dark phase food intake in gastric bypass rats was lower than in sham-operated, ad libitum fed rats (sham-operated, ad libitum fed: 26.6 ± 1.1 g vs bypass: 17.0 ± 1.5 g, $P < .0001$), whereas they ate more during the light phase (sham-operated, ad libitum fed: 2.7 ± 0.5 g vs bypass: 4.5 ± 0.7 g, $P < .05$, Figure 3*B*). Sham-operated, body weight-matched rats consumed all their food during the first half of the dark phase and are therefore not represented in Figure 3*B*.

Energy Expenditure

Twenty-four hour energy expenditure was increased after gastric bypass compared with sham-operated, ad libitum fed rats and sham-operated, body weight-matched controls (sham-operated, ad libitum fed: 4.29 ± 0.08 kcal/kg/h vs sham-operated, body weight matched: 3.98 ± 0.10 kcal/kg/h vs bypass: 4.50 ± 0.04 kcal/kg/h, $P < .001$). Sham-operated, body weight-matched rats had lower total energy expenditure than sham-operated, ad libitum fed rats ($P < .05$). When analyzing the 2 phases of the light/dark cycle separately, it was obvious that, during the light phase, when overall activity is typically low, energy expenditure in gastric bypass rats was significantly higher than in sham-operated, ad libitum fed animals and body weight-matched shams (sham-operated, ad libitum fed: 3.63 ± 0.04 kcal/kg/h vs sham-operated, body weight-matched: 3.42 ± 0.05 kcal/kg/h vs bypass: 4.12 ± 0.03 kcal/kg/h, $P < .001$). In the dark phase, when overall activity is typically higher, there was no difference in energy expenditure between gastric bypass and sham-operated, ad libitum fed rats, but energy expenditure in bypass rats was higher than in body weight-matched, sham-operated rats (sham-operated, ad libitum fed: 4.81 ± 0.06 kcal/kg/h vs sham-operated, body weight matched: 4.46 ± 0.15 kcal/kg/h vs bypass: 4.81 ± 0.04 kcal/kg/h, $P < .01$). Figure 4*A* shows average 24-hour light phase and dark phase energy expenditure for all groups.

Respiratory Quotient

Respiratory quotient was examined during 12 hours of fasting and for the subsequent 6 hours after offering a fixed test meal of 5 g. Results are shown in Figure 4*B*. During fasting, gastric bypass rats had a lower respiratory quotient than sham-operated, ad libitum fed rats, but there was no difference in sham-operated, body weight-matched rats. The pattern was similar for the 0- to 3-hour observation period after the test meal for gastric bypass; sham-operated, ad libitum fed; and sham-operated, body weight-matched rats (sham-operated, ad libitum fed: 0.89 ± 0.01 vs sham-operated, body weight matched: 0.78 ± 0.01 vs bypass: 0.77 ± 0.01, $P < .001$) and the 3- to 6-hour observation period after the test meal (sham-operated, ad libitum fed: 0.95 ± 0.01 vs sham-operated, body weight matched 0.73 ± 0.01 vs bypass: 0.74 ± 0.01, $P < .001$). Respiratory quotient between gastric bypass and sham-operated body weight-matched rats was not different during fasting or the 6 hours after the test meal.

Body Temperature

Body temperature as measured during the light and dark phase is shown in Figure 4*C*. Body temperature in gastric bypass rats was lower than in sham-operated, ad libitum fed rats but higher compared with body weight-matched, sham-operated rats during the light phase (sham-operated, ad libitum fed: 36.8°C ± 0.02°C vs sham-operated, body weight matched: 36.3°C ± 0.06°C vs bypass: 36.5°C ± 0.03°C, $P < .001$). During the dark phase, average body temperature in gastric bypass rats was lower than in sham-operated, ad libitum fed rats but no different compared with body weight-matched, sham-operated rats (sham-operated, ad libitum

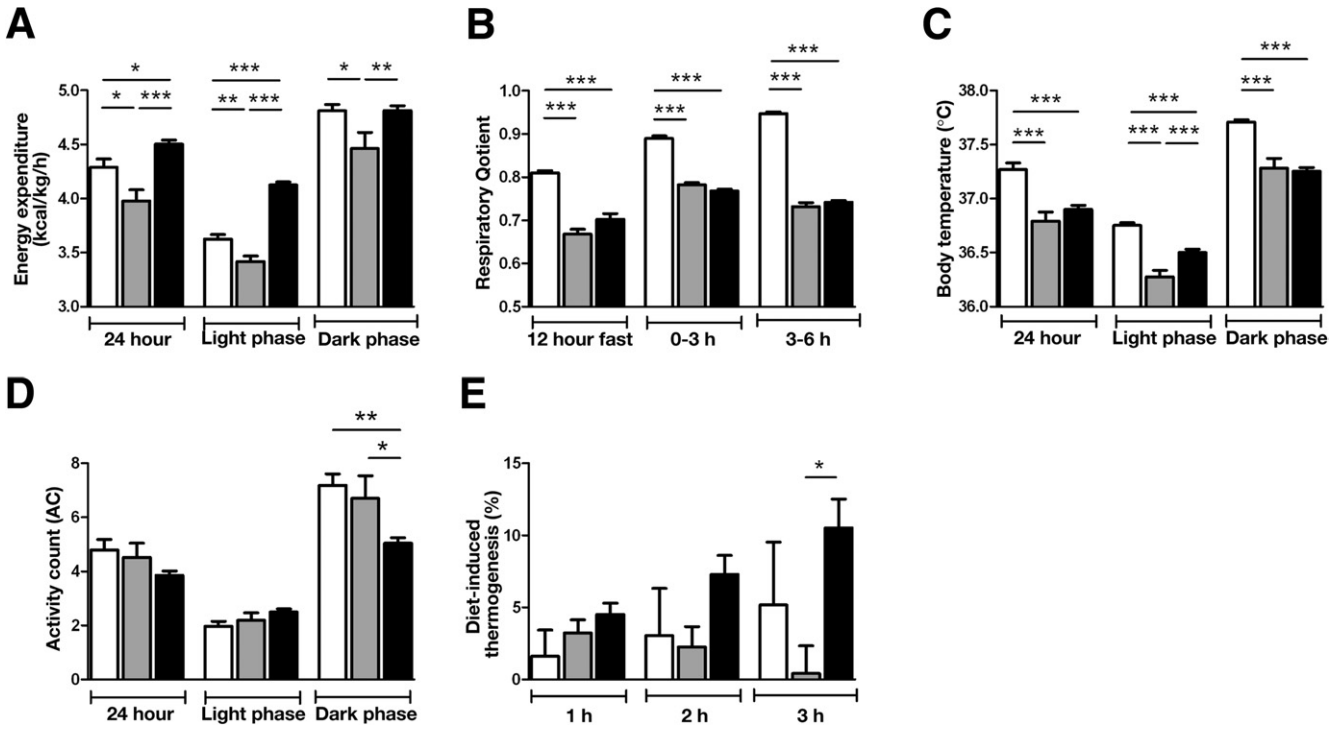


Figure 4. Differences in maintenance energy expenditure (*A*), respiratory quotients (*B*), average body temperature (*C*), activity (*D*), and diet-induced thermogenesis (*E*) for sham-operated ad libitum fed (n = 8, *open columns*), for sham-operated body weight matched (n = 8, *shaded columns*), and for gastric bypass rats (n = 14, *solid columns*). Whereas data for energy expenditure, body temperature, and activity are shown during 24 hours, the light and dark phase, respiratory quotients are shown during 12-hour fasting and within the first 6 hours after a 5-g test meal. Data for diet-induced thermogenesis are expressed as a percentage of the energy content of a 5-g test meal and shown at 1 hour, 2 hours, and 3 hours after refeeding with the test meal after a 12-hour fasting period. All data are shown as mean values ± SEM (*$P < .05$, **$P < .01$, ***$P < .001$).

fed: 37.7°C ± 0.02°C vs sham-operated, body weight matched: 37.3°C ± 0.09°C vs bypass: 37.3°C ± 0.03°C, $P < .001$).

Physical Activity

A dissociation between total energy expenditure and body temperature was observed and, thus, physical activity was analyzed (Figure 4*D*). No difference in activity over 24 hours or during the light phase was seen among all 3 groups. During the dark phase, however, gastric bypass rats were less active than sham-operated, ad libitum fed rats and sham-operated, body weight-matched rats (sham-operated, ad libitum fed: 7.19 ± 0.4 activity counts vs sham-operated body weight matched: 6.70 ± 0.8 activity counts vs bypass: 5.04 ± 0.2 activity counts, $P < .001$).

Diet-Induced Thermogenesis

Diet-induced thermogenesis was measured over 3 hours after a 5-g standard test meal after a 12-hour fast. The sham-operated, ad libitum fed and the sham-operated body weight-matched groups consumed all 5 g within 20 minutes; the gastric bypass animals required 30 minutes. Figure 4*E* shows the diet-induced thermogenesis for all groups for the first 3 hours after the test meal. Three hours after the 5-g test meal, gastric bypass rats had a significantly greater diet-induced thermogenesis than the body weight-matched controls, but bypass was not different from the sham-operated, ad libitum fed rats (sham-operated, ad libitum fed: 5.2% ± 4.4% vs sham-operated, body weight-matched: 0.41% ± 1.9% vs bypass: 10.5% ± 2.0%, $P < .05$).

Gut Morphometry

Differences in gut morphometry are summarized in Figure 5. There was no difference in total length of the complete small bowel between sham-operated and gastric bypass rats (sham-operated, ad libitum fed: 108.6 ± 1.7 cm vs bypass: 110 ± 2.2 cm, $P = .8$). In contrast, the wet weight of the small bowel was 72.1% higher after gastric bypass than after sham operations (sham-operated, ad libitum fed: 12.2 ± 0.6 g vs bypass: 21.0 ± 1.2 g, $P < .001$). Average weight of the alimentary limb was 10.6 ± 0.8 g, of the biliopancreatic limb 2.7 ± 0.2 g, and of the common channel 7.8 ± 0.6 g. Muscle thickness (sham-operated, ad libitum fed: 95.0 ± 8.7 μm vs bypass: 247.9 ± 32.5 μm, $P < .001$), mucosal height (sham-operated, ad libitum fed: 530.8 ± 19.1 μm vs bypass: 969 ± 58.2 μm, $P < .001$), villus height (sham-operated, ad libitum fed: 390.4 ± 21.7 μm vs bypass: 673.6 ± 63.8 μm, $P < .001$), and crypt depth (sham-operated, ad libitum fed: 140.4 ± 8.0 μm vs bypass: 295.4 ± 20.6 μm, $P < .001$) were

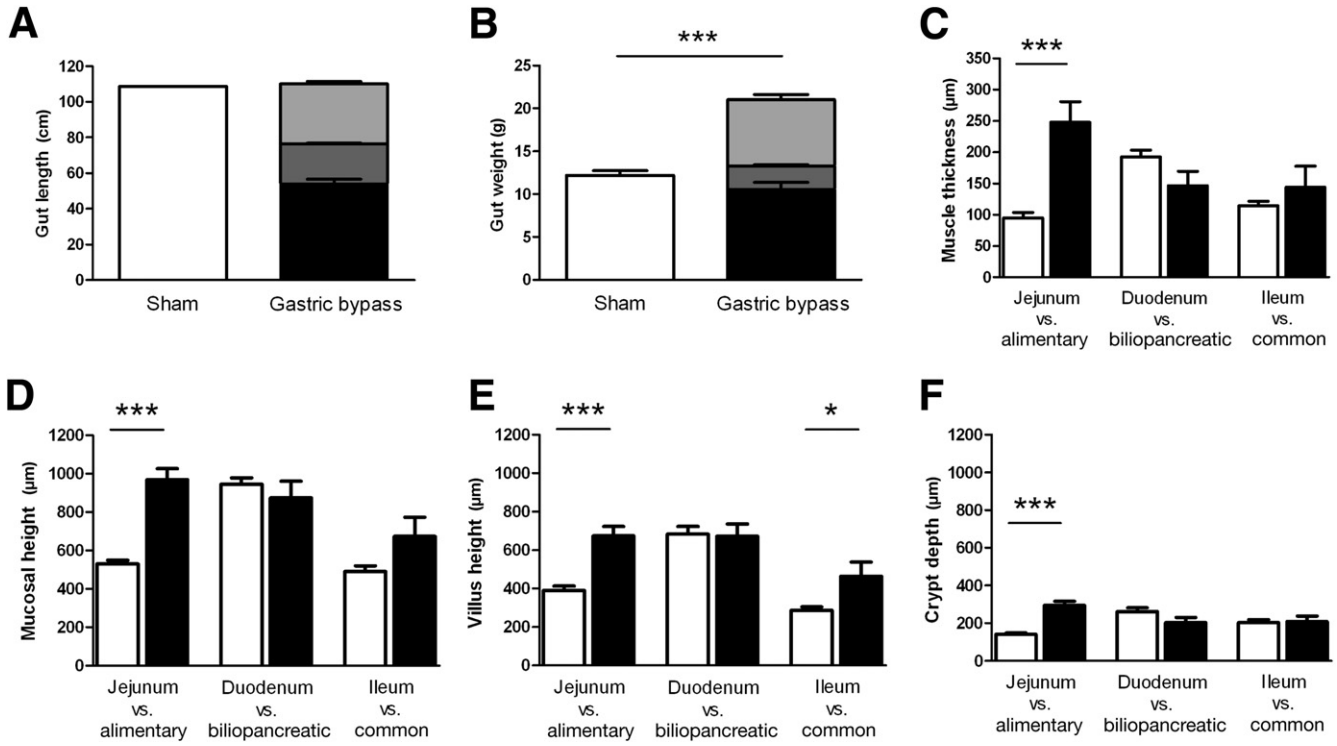


Figure 5. Length (*A*) and weight (*B*) of the entire small bowel and differences in gut morphometry in rats 60 days after gastric bypass (n = 8) and sham operation (n = 8). Differences in muscle thickness (*C*), mucosal height (*D*), villus height (*E*), and crypt depth (*F*) are shown for the alimentary limb, the biliopancreatic limb, and the common channel after gastric bypass in comparison with the corresponding parts of jejunum, duodenum, and ileum after sham operation, respectively. Data are shown as mean values ± SEM (***$P < .001$, *$P < .05$).

significantly increased in the alimentary limb after gastric bypass in comparison with the corresponding section of the jejunum of the sham-operated controls. Gastric bypass rats had a significantly greater villus height of the common channel than sham-operated animals (sham-operated, ad libitum fed: 287.1 ± 18.1 µm vs bypass: 464.6 ± 73.9 µm, $P < .05$). There was a trend toward an increase in mucosal height (sham-operated, ad libitum fed: 490.4 ± 29.6 µm vs bypass: 673.8 ± 99.7 µm, $P = .09$) and muscle thickness (sham-operated, ad libitum fed: 490.4 ± 29.6 µm vs bypass: 673.8 ± 99.8 µm, $P = .09$) in the common channel.

Discussion

Our data in the rat gastric bypass model are consistent with previous findings that gastric bypass surgery is effective to reduce body weight and especially to maintain body weight loss.[4,9,10,12,16] We confirmed that body weight loss after gastric bypass was associated with a significant loss of fat mass and to a lesser degree of nonadipose body mass.[26,27] Food intake was reduced in gastric bypass rats, which may be partly explained by hormonally mediated mechanisms.[9,16,28] Importantly, the lower food intake after gastric bypass compared with sham-operated, ad libitum fed rats only partly explains body weight loss because the sham-operated, body weight-matched group required on average 40% less food than the bypass group to maintain the same level of body weight. Consequently, reduced calorie consumption is important but not the sole cause of weight loss after gastric bypass. We found no increased fecal mass, fecal calorie content, or inflammation in the gastric bypass animals; therefore, nutrient malabsorption or inflammation are unlikely to play a major role in this weight loss.[10]

We demonstrate a higher total energy expenditure in rats after gastric bypass compared with ad libitum fed and body weight-matched sham groups, which is in accordance with some but not all previous reports of energy expenditure in humans.[29–31] Our differences in energy expenditure were mainly due to changes during the light phase when physical activity is typically low. Gastric bypass surgery did not only prevent the expected decrease in energy expenditure subsequent to body weight loss but actually increased 24-hour and in particular light phase energy expenditure in comparison with the control groups.

Higher energy expenditure after gastric bypass was associated with lower respiratory quotients, suggesting that fat rather than carbohydrates was burnt to sustain higher energy expenditure. However, food-restricted, body weight-matched controls showed similar respiratory quotient levels to the gastric bypass group, suggesting that body weight loss rather than a specific effect by the gastric bypass procedure was an important determinant for the observed decrease in respiratory quotient.

Because higher levels of total energy expenditure usually result either from greater heat generation or increased physical activity,[32] some of our findings remain unexplained. First, bypass rats were not more physically active than the control groups. The bypass rats showed no difference in spontaneous activity during the light phase to indicate reduced sleep time, but we have not formally evaluated sleep patterns. In fact, at least during the dark phase, when spontaneous activity is usually high, physical activity was lower in the bypass rats than in the sham-operated controls. Because gastric bypass induces an increase in postprandial levels of PYY and glucagon-like peptide-1 (GLP-1),[15] which reduce food intake, the reduced dark phase physical activity may possibly indicate reduced appetite and hence less foraging or food-seeking behavior. The second unexpected finding was the lower body temperature in gastric bypass rats compared with ad libitum fed, sham-operated controls. This was observed throughout the light-dark cycle. However, during the light phase, the body temperature of the gastric bypass rats was higher than in the body weight-matched controls, despite no difference in physical activity. It must be emphasized that, during the light phase, gastric bypass rats continued to consume some food, whereas the body weight-matched, sham-operated rats consumed all food during the first half of the dark cycle. Thus, differences in light phase body temperature might be related to food intake and subsequently diet-induced thermogenesis.[33,34]

After a 5-g test meal, gastric bypass rats had greater diet-induced thermogenesis than body weight-matched controls, but no difference was observed between gastric bypass rats and the ad libitum fed, sham-operated group. Our data suggest that gastric bypass induces profound changes in food intake, energy expenditure, and the mechanisms by which the body controls energy expenditure. Because gastric bypass significantly rearranges the gastrointestinal anatomy, we suggest that gastrointestinal and central neuroendocrine signaling contribute to increased energy expenditure.[32] Neurons in the hypothalamic arcuate nucleus (ARC) coexpress neuropeptide Y (NPY) and agouti-related peptide, which stimulate food intake and weight gain.[35] Another population of ARC neurons coexpress pro-opiomelanocortin (POMC) and cocaine-and-amphetamine-regulated transcript, which both promote weight loss.[36] The balance between NPY and POMC is critical for the maintenance of body weight.[35–37] Gastric bypass increases postprandial levels of PYY and GLP-1,[9,10] which are satiating-inducing gut hormones and hence favor an anorectic state and facilitate body weight loss through modulation of the hypothalamus and brain stem,[38,39] also being involved in the control of energy expenditure.[18] In fact, PYY has been shown to activate anorectic POMC expressing neurons in the ARC[40] and to inhibit NPY neurons,[41] suggesting a potential to increase energy expenditure.

Gastrointestinal effects of GLP-1 and PYY can be resolved by ablation of vagus/brain stem/hypothalamus pathways,[38] indicating a role for the vagus in mediating effects on food intake and potentially energy expenditure. However, it was beyond the scope of this study to assess the potential role of vagal or visceral neural afferent information to the central nervous system.

GLP-1 increases endogenous amylin levels.[42] Amylin may be another potential candidate decreasing food intake and increasing energy expenditure.[25] Of note, the reduced food intake after amylin is independent of GLP-1 and vice versa (Lutz TA, unpublished data). Nonetheless, chronic amylin administration reduces food intake,[43] and it prevents the decrease in energy expenditure that would typically result from lower food intake and body weight loss[44,45] (Lutz TA, unpublished data).

The increase in total energy expenditure might also represent a higher energy requirement after bypass surgery. We also demonstrated significant morphometric changes of the small intestine after gastric bypass surgery.[11,15] The observed increase in muscle thickness and mucosal mass after gastric bypass resulted in a 72% increase of the total small bowel weight. The gut is metabolically very active and the mean in vitro rates of oxygen consumption in gastrointestinal tissues in rats have been reported to be 15%–22% of total oxygen consumption.[46,47] Thus, gut hypertrophy may at least in part explain the higher maintenance energy requirement that contributes to body weight loss.

Postoperative inflammation secondary to infection can lead to a higher energy demands, but we found no evidence of an inflammatory response in our study. Other mechanisms that should be considered but may be less likely include decreased leptin after gastric bypass. Usually high leptin and not low leptin contributes to increased energy expenditure.[48] Although low leptin levels may explain the lower body temperature in bypass rats than in ad libitum fed controls, it does not explain the observed difference in body temperature between bypass and body weight-matched rats.

This study does not explain why average body temperature was reduced while total energy expenditure was higher after gastric bypass. One possible explanation is that more heat was dissipated to the immediate environment of the rats especially because gastric bypass rats had significantly less body fat and hence less thermal isolation. We did not assess cutaneous vasodilation to further explore potential mechanisms. Another explanation may be an up-regulated activity of brown adipose tissue, but our measuring system did not allow the separate assessment of brown adipose tissue and tail temperature.

In summary, not only did gastric bypass surgery prevent the expected decrease in energy expenditure subsequent to body weight loss in this diet-induced obese rat model, but 24-hour and in particular light phase energy expenditure were higher than in sham controls. Diet-

induced thermogenesis was also higher after gastric bypass surgery compared with body weight-matched controls. Increased energy expenditure may offer an additional explanation why gastric bypass surgery is superior to dieting for successfully maintaining long-term body weight loss.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at doi: 10.1053/j.gastro.2009.11.012.

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Conflicts of interest

The authors disclose no conflicts.

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Supplementary Materials and Methods

Surgery

At the beginning of the gastric bypass procedure, the stomach was transected close to the gastro-oesophageal junction to create a small gastric pouch. Subsequently, the pouch was anastomosed to a loop of jejunum 10 cm distal to the pylorus in an end-to-side fashion. A 7-mm side-to-side small bowel anastomosis was performed between the biliopancreatic and the alimentary limbs to create a common channel of 25 cm; similar to the technique in humans, the omega loop of small bowel was then divided. The sham operation consisted of a laparotomy, a 7-mm gastrotomy on the anterior wall of the stomach with subsequent closure and a 7-mm jejunotomy with subsequent closure. Preoperatively, gentamicin, 8 mg/kg, and carprofen, 5 mg/kg, were administered intraperitoneally as prophylaxis for postoperative infection and pain relief.

To assess changes of body temperature, intra-abdominal temperature sensors were used during indirect calorimetry. Under brief isoflurane anesthesia, a temperature transmitter was implanted intraperitoneally (VM-FA disc; DataScience ART4.0 telemetry system; DataScience, St. Paul, MN). Animals were given at least 1 week to recover before measurements in metabolic cages were started.

Indirect Calorimetry

Rats were individually housed in Plexiglas airtight metabolic cages (41 × 41 × 31 cm) on a layer of wood shavings under the same light and temperature conditions as described above. Water and standard powder chow (GLP3433; Provimi Kliba Ag, Kaiseraugst, Switzerland) were available ad libitum, unless otherwise stated. Food intake and water intake were measured continuously. Physical activity was monitored by a 3-dimensional array of infrared light beams and sensors. Thus, the activity data provided represent a relative measure of locomotor activity of the rats. The activity data do not relate to an absolute measurement of distance moved or to a spatial position. Measurements were conducted in an open circuit calorimetry system (AccuScan Inc, Columbus, OH).[1] Energy expenditure was calculated for each 2-minute sample according to Weir[2] using the following equation: total energy expenditure (kcal/h) = 3.9 × $V(O_2)$L/h + 1.1 × $V(CO_2)$L/h. The respiratory quotient was defined as the quotient of CO_2 production and O_2 consumption.

Gut Morphometry

For analysis of gut morphometry, 2-cm segments of the alimentary, biliopancreatic limb, and common channel from bypass operated rats and corresponding segments of jejunum, duodenum, and ileum of sham-operated rats were opened on the mesenteric border and fixed overnight at 4°C in Zamboni's fixative (2% paraformaldehyde, 15% picric acid, pH 7.4). Transverse segments from each segment were incubated in 20% sucrose in phosphate-buffered saline overnight at 4°C and then embedded in optimum cutting temperature compound. Sections of intestine (12 μm) were cut on a cryostat, thaw mounted onto slides coated with poly-D-lysine, and stored at −20°C until use. Sections were then processed for H&E staining. Sections were washed 3 times at 10-minute intervals in phosphate-buffered saline containing 0.1% Triton X-100 and then rinsed in distilled water. Sections were immersed in Ehrlich's Alum Hematoxylin for 4 minutes and then rinsed in distilled water. Sections were then dipped 2 or 3 times in 0.5% acid alcohol and rinsed in distilled water. Sections were soaked in Scott's Blueing for 30 seconds before being rinsed in distilled water for 30 seconds. Next, the sections were dipped once in Eosin Y acid, washed stain, and again rinsed in distilled water. Slides were then coverslipped with bicarbonate-buffered glycerol, and sections were examined for morphometric analysis. Muscle thickness (circular + longitudinal muscle), mucosal height (villus height + crypt depth), villus height and crypt depth were measured in well-orientated sections under a Zeiss Axioplan (Zeiss, Jena, Switzerland) microscope fitted with an eyepiece graticule by an observer blinded to the group. Three measurements per tissue were taken, and an average was obtained.

Fecal Analysis

To evaluate nutrient absorption, feces were collected over 24 hours on postoperative days 15 and 59 from all animals. Feces were dried in an oven and weighed; calorie content was measured using a ballistic bomb calorimeter.[3]

Blood Analysis

Blood was obtained by puncture of a sublingual vein under brief isoflufrane anesthesia. Blood was collected into EDTA-rinsed tubes and immediately centrifuged at 3000 rpm for 10 minutes at 4°C. Plasma was stored at −80°C before analysis. Measurements of C-reactive protein (Abbott, Maidenhead, UK) were made to assess inflammation.

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SURGERY FOR OBESITY AND RELATED DISEASES

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Original article

Sodium and water handling after gastric bypass surgery in a rat model

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Abstract

Background: To investigate the influence of gastric bypass on renal sodium and water handling at a university hospital. The relationship between sodium and water absorption along the gastrointestinal tract and their renal excretion is poorly understood. Beneficial effects on blood pressure have been seen after bariatric surgery before significant weight loss has occurred.

Methods: Male Wistar rats (348 ± 19 g) underwent either gastric bypass (n = 14) or sham operation (n = 7) and were given a low-sodium diet with deionized water ad libitum. Before and after surgery, the rats received an oral sodium load (1.5 mmol/kg) as hyperosmolar saline (616 mM), and were then placed in individual metabolic cages so the urine volume, sodium content, and water intake for 8 hours could be recorded. The urine sodium concentration was also measured.

Results: The rats that had undergone gastric bypass had a significantly lower body weight than the sham-operated controls throughout the follow-up period (346 ± 21 g versus 501.3 ± 8.0 g at day 60; $P = .0004$). An oral sodium load after gastric bypass led to an increase in water intake (.07 ± .01 mL/g versus .03 ± .01 mL/g; $P = .023$), urine output (.03 ± .01 mL/g versus .02 ± .002 mL/g; $P = .027$), and sodium excretion (65.99 ± 10.7 mol versus 31.71 ± 8.7 mol; $P = .020$). No change was seen in water intake, urine output, or sodium excretion after sham surgery.

Conclusion: Urine output, water intake, and sodium excretion are all increased after gastric bypass surgery in rats given an oral sodium load compared with sham-operated controls. More rapid excretion, and less retention, of a dietary sodium load could be a part of the mechanism underlying the beneficial effect of bariatric surgery on blood pressure. (Surg Obes Relat Dis 2010;xx:xxx.) © 2010 Published by Elsevier Inc. on behalf of American Society for Metabolic and Bariatric Surgery.

Keywords: Gastrointestinal tract; Hypertension; Sodium; Water

Gastric bypass surgery is currently the most effective treatment of morbid obesity, and its beneficial effects on obesity-related co-morbidities, such as diabetes and hypertension, are now well documented [1]. In the Swedish Obese Subjects study, the systolic blood pressure decreased by approximately 11 mm Hg and the diastolic blood pressure by approximately 7 mm Hg in the first 6 months after bariatric surgery [2,3]. Several other studies have reported similar findings [1,4–6]. The improvement in blood pressure seen after bypass surgery was initially thought to be a medium-term effect related to weight loss, with the first documented reductions seen at 8 weeks postoperatively. However, Ahmed et al. [7] observed significant reductions in systolic (9 mm Hg) and diastolic (7 mm Hg) blood pressure as early as 1 week after gastric bypass surgery, before any significant change in weight. Furthermore, this beneficial effect was maintained for ≥1 year after surgery,

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and the postoperative use of antihypertensive drugs was reduced by one third [7].

Hypertension is associated with central adiposity and insulin resistance [8–10], but the pathophysiologic mechanism remains unclear. Several hypotheses are plausible, including insulin resistance [11,12], aldosterone and so-called aldosterone-releasing factors [13,14], and hyperleptinemia [15,16], leading to sodium retention, increased blood volume, and, finally, elevated blood pressure. Alternatively, it has been suggested that increased aldosterone levels might be secondary to increased intra-abdominal pressure [17,18]. A proposed mechanism is that the increased intra-abdominal pressure raises the diaphragm, which increases pleural pressure, decreasing venous return to the heart. An increased intra-abdominal pressure would also increase the inferior vena cava pressure, resulting in increased renal venous pressure and decrease renal perfusion. Both mechanisms would activate the renin-angiotensin-aldosterone system, leading to increased renal sodium and water retention [17,18]. However, a reduction of the visceral fat mass and decrease in sympathetic nerve activity and/or sodium retention do not occur immediately after gastric bypass surgery, and they do not explain the early reductions in blood pressure reported by Ahmed et al. [7]. Therefore, we hypothesized that renal sodium and water handling might be altered by bypass surgery and that this might contribute to the early improvement in blood pressure control that occurs.

Thus, our aim in the present study was to evaluate the water intake, urine output, and renal sodium excretion in rats before and shortly after gastric bypass surgery in response to an acute oral sodium challenge.

Methods

Animals

A total of 21 male Wistar rats (body weight 348 ± 19 g) were randomized to undergo either gastric bypass (n = 14) or sham surgery (n = 7). The study was performed under U.K. Home Office license (PL 70-5569), and all rats were kept in identical environmental conditions (temperature 24°C, humidity 60%, light cycle 7:00–7:00) with normal chow (RM1 diet, Special Diet Services, Essex, UK) and tap water ad libitum, unless otherwise stated. The body weight was measured daily.

Metabolic cage experiments

The urine output, water intake, and sodium excretion were measured at 3 different points. First, before surgery and after an oral sodium load; second, after surgery, but without an oral sodium load (baseline measurements); third, after surgery and after an oral sodium load. Before each experiment, the rats were maintained on a low-sodium diet and given deionized water ad libitum for 1 week to establish a stable urinary excretion rate for sodium and to enhance the endogenous mechanisms for sodium retention [19]. The low-sodium diet was identical to normal chow, except for its sodium content (D02051701, Research Diets, New Brunswick, NJ; sodium content 102.6 ppm). For the measurements after an oral sodium load, sodium (1.5 mmol Na/kg body weight) was given intragastrically by oral gavage for 10 seconds as a hyperosmolar NaCl solution (616 mM) at the beginning of the light phase (7:00 AM). The rats were then placed in individual metabolic cages for urine collection and to record the water intake for 8 hours. For the baseline measurements, the rats were placed in metabolic cages without having received the oral sodium load. In all experiments, urine was collected in preweighed plastic tubes. Water was given in preweighed plastic bottles that were reweighed at the end of the experiment. The cages were cleaned and rinsed with deionized water after each experiment.

Surgery

Surgery was performed according to an established and standardized protocol using isoflurane inhalation anesthesia [20]. All operations were performed by 1 surgeon (M.B.). The rats were fasted overnight but had access to tap water ad libitum. During surgery, the stomach was transected close to the gastroesophageal junction, which was subsequently anastomosed to a loop of jejunum 7 cm distal to the ligament of Treitz in an end-to-side fashion. A 7-mm side-to-side small bowel anastomosis was performed between the biliopancreatic and the alimentary limbs to create a common channel of 25 cm, and the omega loop of small bowel was then divided. Figure 1 shows a diagrammatic representation of the gastric bypass rodent model. The sham operation consisted of laparotomy, a 7-mm gastrotomy on the anterior wall of the stomach with subsequent closure, and a 7-mm jejunotomy with subsequent closure. Preoperatively, gentamycin 8 mg/kg and carprofen .01 mL were administered intraperitoneally as prophylaxis for postoperative pain and infection. F1

Measurement of urinary sodium

The urine sodium concentration was measured with integrated chip technology using the Architect ci16200 (Abbott Diagnostics, Abbott Park, IL). It obtains millivolt readings and converts them to assay-specific analyte conversion units. The measurement of the integrated chip technology reference solution and integrated chip technology samples were used to calculate the assay results.

Statistical analysis

The data are presented as the mean ± standard error of the mean. The data were compared using the 2-tailed, paired Student *t* test (GraphPad Prism, GraphPad Software, La Jolla, CA). $P < .05$ was considered significant.

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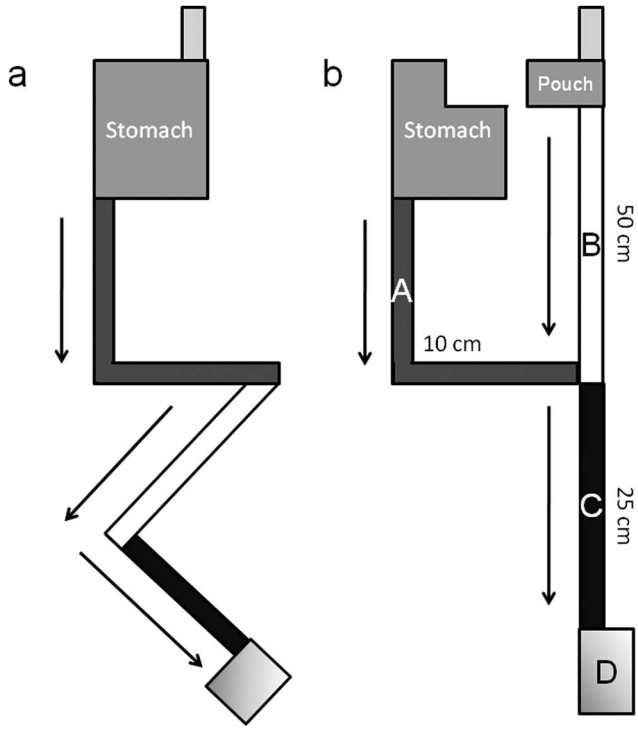


Fig. 1. Diagrammatic representation of gastrointestinal anatomy (A) before and (B) after gastric bypass operation. A, biliopancreatic limb (~10 cm); B, alimentary limb (~50 cm); C, common channel (~25 cm); and D, cecum.

Results

Weight loss

The body weight was significantly lower in the gastric bypass rats than in the sham-operated group from day 5 after surgery (sham 349.9 ± 6.1 g versus bypass 313.6 ± 6.4 g; $P < .01$). The sham-operated rats gained weight for the rest of the study. The difference in body weight on day 60 was 165 g (sham 501 ± 8 g versus bypass 346 ± 21 g; $P < .001$). Figure 2 shows the percentage of initial body weight for all bypass (n = 14) and sham-operated (n = 7) rats. F2

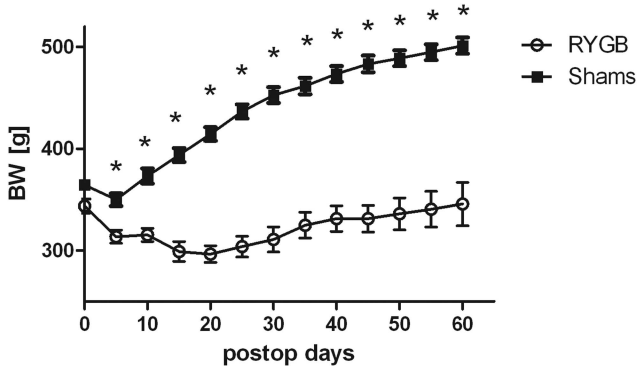


Fig. 2. Body weight (BW) change in group 1 for gastric bypass (circles; n = 14) and sham-operated rats (black squares; n = 7). Data shown as mean ± standard error of mean. $^{*}P < .05$. RYGB = Roux-en-Y gastric bypass.

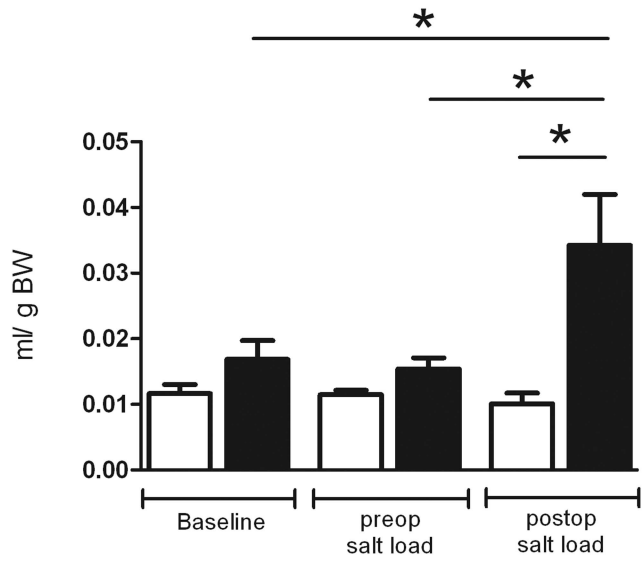


Fig. 3. Urine production of bypass rats (black columns; n = 14) and sham-operated rats (white columns; n = 7) after oral sodium loading (1.5 mmol Na/kg body weight of 616 mM NaCl solution) preoperatively and on postoperative day 30. Baseline urine output measured without oral sodium loading. Data shown as mean ± standard error of mean. $^{*}P < .05$ considered significant. AQ4

Urine output (volume)

In the gastric bypass rats, sodium loading after surgery led to a greater increase in urine output compared with the urine output after the same sodium load before surgery (preoperatively .015 ± .002 mL/g body weight versus postoperatively .034 ± .007 mL/g body weight; $P = .03$). No change was seen in the urine output in the sham-operated group after the sodium load compared with their preoperative response after sodium loading (preoperatively .011 ± .001 mL/g body weight versus postoperatively .010 ± .002 mL/g body weight; $P = .44$). The gastric bypass rats produced significantly more urine than the sham-operated rats after sodium loading (sham .010 ± .002 versus bypass .034 ± .007 mL/g body weight; $P = .038$). No difference was seen in the baseline urine production between the 2 groups after surgery (sham .011 ± .001 mL/g body weight versus bypass .015 ± .002 mL/g body weight; $P = .12$). Figure 3 summarizes the data for urine output. F3

Water intake

The data for water intake are summarized in Fig. 4. The gastric bypass rats consumed significantly more water after the sodium load compared with before surgery (preoperatively .033 ± .006 mL/g body weight versus postoperatively .065 ± .012 mL/g body weight; $P = .02$). No changes were F4

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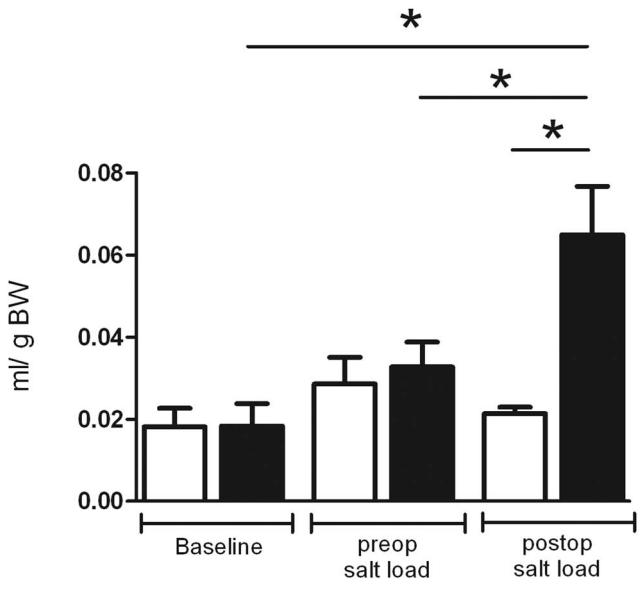


Fig. 4. Water intake of bypass rats (black columns; n = 14) and sham-operated rats (white columns; n = 7) after oral sodium loading (1.5 mmol Na/kg body weight of 616 mM NaCl solution) preoperatively and on postoperative day 30. Baseline water intake measured without oral sodium loading. Data shown as mean ± standard error of mean. $^{*}P < .05$ considered significant.

observed for water intake before and after sham surgery after the sodium load (preoperatively .029 ± .006 mL/g body weight versus postoperatively .021 ± .002 mL/g body weight; $P = .3$). The bypass rats also drank significantly more water than did the sham-operated rats (sham .021 ± .002 mL/g body weight versus bypass .065 ± .012 mL/g body weight; $P = .02$) after the sodium load. No difference was seen in the baseline water intake between the 2 groups (sham .029 ± .006 mL/g body weight versus bypass .033 ± .006 mL/g body weight; $P = .68$).

Sodium excretion

Postoperative sodium loading led to a greater increase in cumulative sodium excretion in the gastric bypass rats compared with their preoperative response (preoperative 31.7 ± 8.7 μmol versus postoperative 65.9 ± 10.7 μmol; $P = .02$). No changes in sodium excretion were observed before or after sham surgery after the oral sodium load (preoperative 40.9 ± 16.0 μmol versus postoperative 36.2 ± 10.7 μmol; $P = .81$). The gastric bypass rats had greater sodium excretion than their sham-operated counterparts after sodium loading (sham 36.2 ± 10.7 μmol versus bypass 80.9 ± 14.4 μmol; $P = .03$). No significant difference was found between the baseline sodium excretion between the gastric bypass rats and the sham-operated rats (sham 40.9 ± 16.0 μmol versus bypass 31.7 ± 8.7 μmol; $P = .59$). The data are summarized in Fig. 5.

Discussion

Both central and peripheral abnormalities account for the development and maintenance of high arterial pressure in the presence of obesity [21]. Visceral obesity is considered an important risk factor for hypertension and cardiovascular disease [22]. It is linked to hyperinsulinemia, hyperleptinemia, and increased levels of aldosterone and so-called aldosterone-releasing factors, all of which lead to activation of the sympathetic and renin-angiotensin-aldosterone systems [23–25]. In addition, increased aldosterone levels might also result from increased intra-abdominal pressure activating the renin-angiotensin-aldosterone system and leading to increased sodium and water reabsorption [17].

The beneficial effect of gastric bypass surgery on arterial hypertension has been well documented [26,27]. The reduction of visceral fat mass and the subsequent decrease in sympathetic activation and sodium retention is not immediate and does not explain the early reduction in blood pressure observed after gastric bypass described by Ahmed et al. [7]. Thus, we reasoned that other mechanisms might be involved in the early resolution of hypertension after gastric bypass and that alteration of renal sodium and water handling could be 1 of them.

We have demonstrated a significant increase in urine output, water intake, and sodium excretion after gastric bypass surgery compared with preoperatively. The sham-

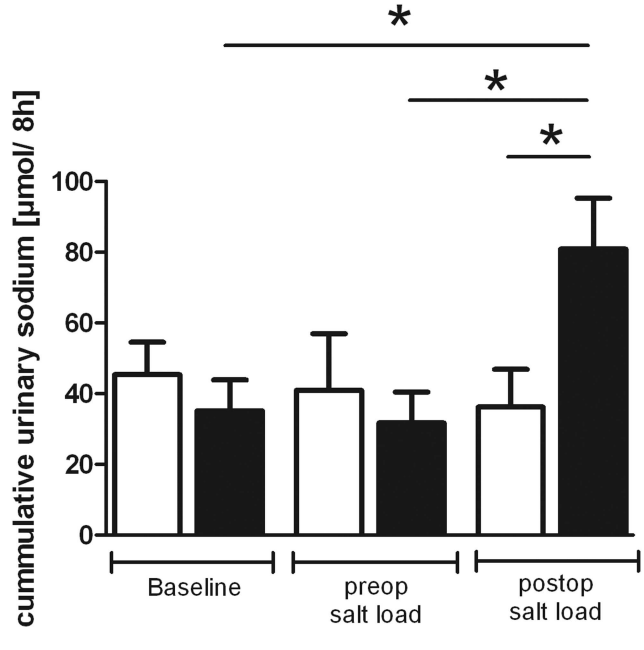


Fig. 5. Cumulative sodium excretion of bypass rats (black columns; n = 14) and sham-operated rats (white columns; n = 7) after oral sodium loading (1.5 mmol Na/kg body weight of 616 mM NaCl solution) preoperatively and on postoperative day 30. Baseline sodium excretion measured without oral sodium loading. Data shown as mean ± standard error of mean. $^{*}P < .05$ considered significant.

operated rats showed no such changes in water intake, urine production, or sodium excretion after surgery.

In many groups of patients with hypertension, the sodium balance is disturbed [28], attributed to impaired renal sodium excretion. However, only a few studies have focused on the possible role of the gastrointestinal tract in the control of the sodium balance and, thus, systemic blood pressure. The concept that dietary intake and composition can affect renal function is perhaps self-evident, but a detailed characterization of this relationship is still lacking. Several physiologic mechanisms are involved in controlling the sodium balance, in particular, the hormones aldosterone, angiotensin II [29], and atrial natriuretic peptide [30]; however, some evidence has supported involvement of the gastrointestinal tract. Analogous to the "incretin effect," characterized by an exaggerated plasma insulin response to an oral glucose load compared with the same amount of glucose given intravenously, the oral ingestion of sodium chloride has a greater natriuretic effect than when the same amount has been given intravenously to subjects consuming a low-sodium diet [31]. This effect has been shown to be independent of changes in aldosterone and atrial natriuretic peptide [31]. In the case of insulin release, the important incretin gut hormone has been shown to be glucagon-like peptide-1, which has since been developed into a successful treatment for type 2 diabetes [32]. Although the mechanism for the analogous effect on sodium excretion and, potentially, blood pressure control, has yet to be identified, the glucagon-like peptide-1 response after gastric bypass remains a candidate, because it is a known natriuretic [33,34].

Animal studies have provided some evidence that the gastrointestinal tract can exert a direct influence on renal function. Morgan et al. [35] observed that salt-sensitive Harlan Sprague-Dawley rats with transplanted kidneys from salt-resistant Harlan Sprague-Dawley rats developed significant salt-induced hypertension, suggesting that extrarenal factors also contribute to hypertension in this model of hypertension [35]. These findings were not accounted for by any changes in established hormones known to control renal sodium excretion, including aldosterone, renin [29], angiotensin II [29], or atrial natriuretic peptide [30]. Hence, the presence of an intestinal natriuretic factor for renal sodium excretion was proposed [35].

Our data have suggested that gastric bypass induces profound changes in sodium and water handling. Because gastric bypass significantly rearranges the gastrointestinal anatomy, we suggest that gastrointestinal and central neuroendocrine signaling contribute to the increased sodium and water excretion [36]. Potential mediators between the gut and the kidney include both peptide YY [37] and glucagon-like peptide-1, which have been shown to have diuretic and natriuretic properties [38]. Thus, it is reasonable to speculate that glucagon-like peptide-1 and peptide YY could mediate a link between the gastrointestinal tract and kidney in terms of sodium and water excretion [33,38,39].

However, our study could not distinguish between a direct effect of hypertonic saline to stimulate thirst with an increase in water intake after gastric bypass and an indirect effect of increased renal sodium excretion to stimulate thirst and offset salt and water loss. Also, a nonhypertensive rat strain was used, and the blood pressure was not measured to determine whether the observed increase in sodium excretion led to any change in the blood pressure.

Conclusion

Gastric bypass surgery in humans and in the rat have provide us with a valuable model in which to explore the role of the gastrointestinal tract in sodium and water homeostasis, other electrolytes, and perhaps also in salt-sensitive hypertension. Gastric bypass resulted in a greater urine output, water intake, and sodium excretion in salt-restricted rats after an oral sodium load. This observation could provide insight into the mechanism of the early improvement in arterial hypertension seen in patients after gastric bypass surgery.

Disclosures

The authors have no commercial associations that might be a conflict of interest in relation to this article.

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Conference on 'Malnutrition matters'

Sir David Cuthbertson Medal Lecture
Bariatric surgery as a model to study appetite control

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The obesity epidemic and its associated morbidity and mortality have led to major research efforts to identify mechanisms that regulate appetite. Gut hormones have recently been found to be an important element in appetite regulation as a result of the signals from the periphery to the brain. Candidate hormones include ghrelin, peptide YY, glucagon-like peptide-1 and gastric inhibitory polypeptide, all of which are currently being investigated as potential obesity treatments. Bariatric surgery is currently the most effective therapy for substantial and sustained weight loss. Understanding how levels of gut hormones are modulated by such procedures has greatly contributed to the comprehension of the underlying mechanisms of appetite and obesity. The present paper is a review of how appetite and levels of gastrointestinal hormones are altered after bariatric surgery. Basic principles of common bariatric procedures and potential mechanisms for appetite regulation by gut hormones are also addressed.

Bariatric surgery: Appetite control model: Gut hormones: Obesity

Obesity is a major health problem that is associated with increased morbidity and mortality[1]. Its personal, social and economic consequences can be devastating[1–3]. Substantial research efforts are being directed towards the development of successful weight-loss therapies. Consequently, the understanding of neuroendocrine regulation of food intake and weight gain, especially in relation to the role of gut hormones, has substantially increased over recent years, but new therapies are still awaited[4–6]. Current anti-obesity drugs are moderately effective at achieving weight loss, but considerable adverse effects can occur. Presently, the only effective treatment with a proven mortality benefit is bariatric surgery[7,8]. The mechanisms underlying the effectiveness of these surgical techniques are not completely understood but alterations in circulating gut hormone levels have been shown to be an important factor[9–11]. The gut–brain axis refers in part to gut hormones communicating information from the gastrointestinal tract to the appetite centres within the central nervous system. Changes in these hormones following bariatric surgery may partly explain the mechanism by which surgery reduces appetite and sustains weight loss.

Bariatric surgery

Bariatric surgery, also known as weight-loss surgery, refers to the various surgical procedures performed to treat obesity by modification of the gastrointestinal tract in order to reduce nutrient intake and/or absorption. Procedures for surgical removal of body fat such as liposuction or abdominoplasty are not considered bariatric surgical procedures. Patients who have a BMI ≥ 35 kg/m^2 with an obesity-related comorbidity or patients with a BMI ≥ 40 kg/m^2 who have instituted an adequate exercise and diet programme (with or without adjunctive drug therapy) that has failed meet the National Institute of Clinical Excellence criteria for bariatric surgery[12]. Surgical procedures can be grouped in two main categories: restrictive procedures, e.g. gastric banding (Fig. 1); bypass procedures, e.g. Roux-en-Y gastric bypass (Fig. 2). Restrictive surgery works by reducing the volume of the stomach and physically preventing excessive consumption of food[13]. However, the most common form of bariatric surgery worldwide is Roux-en-Y gastric bypass surgery[14,15]. Here, a small stomach pouch is created with a stapler

Abbreviations: ARC, arcuate nucleus; GIP, gastric inhibitory polypeptide; GLP-1, glucagon-like peptide-1; PYY, peptide YY.

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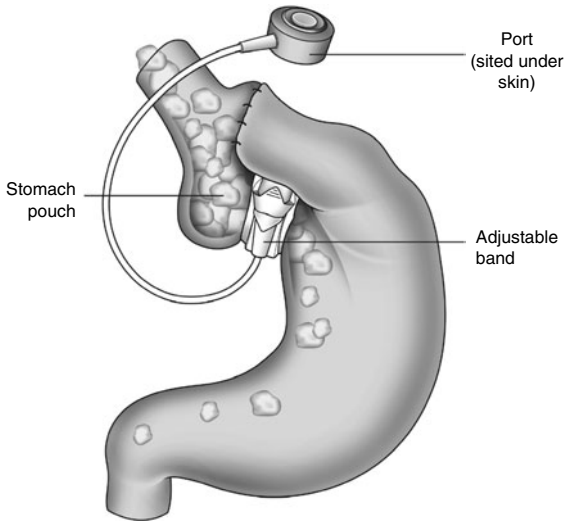


Fig. 1. Gastric banding, a restrictive procedure performed to treat obesity.

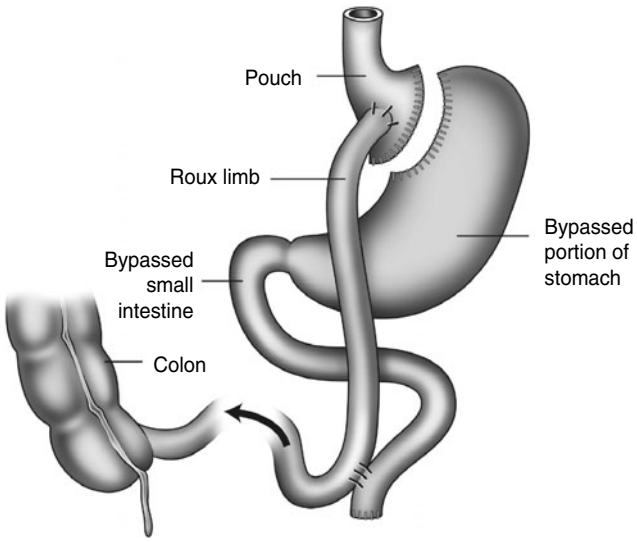


Fig. 2. Roux-en-Y gastric bypass, a bypass procedure performed to treat obesity.

device and connected to the distal small intestine. The upper part of the small intestine is then re-attached in a 'Y'-shaped configuration (Fig. 2). In general, the bypass procedures lead to more weight loss than the restrictive procedures[8]. Typically, gastric banding results in a weight loss of approximately 20%, whilst the Roux-en-Y gastric bypass results in approximately 30% weight loss[16]. Weight loss after bypass-type procedures has been shown to be a result of energy intake rather than malabsorption[17]. Several recent studies have reported a dramatic improvement in obesity-related comorbidities and a decrease in mortality after bariatric surgery[8,18,19]. Adverse effects after gastric bypass include dumping syndrome in about 20% of patients, leaks at the surgical anastomosis (12%), incisional hernia (7%), infections (6%), deep-vein thrombosis (1–3%)[20], pulmonary embolism (2%)[21] and pneumonia (4%)[22]. To reduce the incidence of complications, patients should be cared for in high-volume centres with clinicians experienced in bariatric surgery[23].

Appetite regulation via the gut–brain axis

The hypothalamus contains part of the central melanocortin system and plays a critical role in the regulation of food intake. It has a number of nuclei, including the arcuate nucleus (ARC), paraventricular nucleus, ventromedial nucleus and the dorsomedial nucleus, all of which are interconnected by circuits that regulate energy homeostasis[24]. The ARC receives and acts on circulating appetite signals including the modulated release of several key amino acid neurotransmitters[25,26]. The neurons in the medial ARC co-express neuropeptide Y and agouti-related peptide, which stimulate food intake and weight gain by increasing appetite[26]. By contrast, the neurons in the lateral ARC co-express pro-opiomelanocortin (also known as corticotrophin–lipotropin) and cocaine-and-amphetamine-regulated transcript, which both promote weight loss by decreasing appetite[25]. Both the ARC and the brainstem are ideally positioned to interact with circulating humoral factors and to receive signals from the periphery[26]. Thus, gut hormones may act directly in the brain after being released into the circulation and entering through the circumventricular organs. Neuropeptide Y can suppress appetite and is a selective ligand for the Y4 receptor subtype, which is expressed at the area postrema and the other appetite-regulating areas of the melanocortin pathway[27,28]. The balance between the activities of neuropeptide Y–pro-opiomelanocortin neuronal circuits is critical for the maintenance of body weight[25,26,29]. After food is ingested sensory input to the central nervous system is forwarded by vagal and somatosensory afferent fibres in the gastrointestinal tract that all end in the nucleus tractus solitarius within the brainstem. Reciprocal pathways between the hypothalamus and brainstem pass on information about energy stores and recent food intake, influencing the perception of satiety[26]. These brain centres can respond independently to peripheral signals when communication with higher brain centres is surgically interrupted[30]. Peripheral feedback to the hypothalamus is complex. Many circulating signals, including gut hormones, can have direct access to the ARC[29]. These neuronal interactions through central melanocortin pathways therefore reveal the critical role this system has in the regulation of hunger, satiety and energy expenditure[31]. However, the homeostatic melanocortin system may protect against weight loss more robustly than it does against weight gain[32]. In case of changes in body adiposity, the brain triggers physiological mechanisms that resist weight change through compensatory changes in appetite and metabolic rate[33,34].

Gut hormones

Ghrelin

Ghrelin is a twenty-eight-amino acid gut peptide derived predominantly from the stomach and pituitary gland[35]. So

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far, it is the only gut hormone with an orexigenic action. It acts via the growth hormone secretagogue receptor to increase food intake in rodents[36] and also stimulate food intake in human subjects[24]. Clinical studies have thus concentrated on its use as an orexigenic agent in conditions characterized by anorexia and cachexia[37–39]. Circulating ghrelin levels peak in the fasting state and fall after a meal[40]. Energy intake seems to be the primary regulator of plasma ghrelin levels[41]. Ghrelin stimulates appetite and food intake also in obese individuals[42]. Ghrelin levels are lower in weight-stable obese individuals and rise after diet-induced weight loss[43]. The postprandial decrease in plasma ghrelin is absent or attenuated in the obese, which suggests that ghrelin might be involved in the pathophysiology of obesity[44,45].

Glucagon-like peptide-1

Glucagon-like peptide-1 (GLP-1) is a neuropeptide hormone produced by post-translational processing of the preproglucagon gene in the central nervous system and the gastrointestinal tract[46]. Preproglucagon is secreted in the gastrointestinal tract by the endocrine L-cells that also secrete peptide YY (PYY)[46]. The GLP-1 receptor belongs to the G-protein-coupled receptors[47]. These receptors have been identified in neurons of the nucleus tractus solitarius, extending to regions of the hypothalamus that are important for the regulation of food intake[48]. Peripheral as well as central GLP-1 administration activates neurons in the ARC, the hypothalamic paraventricular nucleus, the nucleus tractus solitarius and the area postrema, inducing increased satiety and decreased hunger[47,49]. Usually, GLP-1 is released after energy intake, but differences have been observed between normal-weight and obese individuals[50–52]. GLP-1 is a potent incretin. It also suppresses gastric acid secretion and delays gastric emptying[53,54]. These effects can be resolved by vagotomy, indicating an important role of the vagus nerve in mediating the anorectic effects of GLP-1[49]. Peripheral GLP-1 infusions have been found to cause a dose-dependent reduction in food intake, while administration of exenatide (an agonist of the GLP-1 receptor) markedly reduces food intake[55,56]. Central actions of GLP-1 might also lead to increased energy expenditure by raising body temperature[57,58]. GLP-1 has been shown to promote lipolysis[59,60], although some studies have suggested a role in lipogenesis[60]. Glycaemic control in patients with type 2 diabetes mellitus improves after 3 weeks of treatment with subcutaneous GLP-1[61], while the agonist exenatide improves HbA1c in the long term[62]. Furthermore, GLP-1 has been shown to up regulate the expression of pancreatic β-cell genes, promoting β-cell proliferation and inhibiting apoptosis[63]. Exenatide enhances insulin secretion and suppresses glucagon release[64]. In phase III clinical trials exenatide has been found to reduce body weight by 3–4 kg, although not all patients respond equally[64,65]. Exenatide is not currently approved as an obesity treatment but has been approved for the treatment of type 2 diabetes mellitus. However, nausea is a common adverse effect of this treatment and this effect may relate to reduced gastric emptying or direct effects of the central nervous system[65].

Peptide YY

As a thirty-six-amino acid peptide PYY is a member of the pancreatic polypeptide family[66]. It is found throughout the human small intestine, with highest levels in the colon and rectum[67]. PYY is released after a meal from the endocrine L-cells of the gastrointestinal tract, where it is co-stored with GLP-1[67,68]. PYY is secreted in proportion to the amount of energy ingested and is independent of gastric distension[67]. PYY inhibits gastric, pancreatic and intestinal secretion as well as gastrointestinal motility[69,70]. The major form of circulating PYY is the N-terminally truncated PYY3–36, which has high affinity for the Y2 receptor and a lesser affinity for Y1 and Y5 receptors[71]. Although initially controversial, peripheral administration of PYY3–36 at physiological doses has now been accepted to reduce food intake in rodents, primates and human subjects in the short term[72–75]. PYY-knock-out mice are characterized by dysregulation of energy homeostasis[76]. PYY3–36 activates anorectic pro-opiomelanocortin-expressing neurons in the ARC and direct intra-ARC administration of PYY3–36 reduces food intake in rats[77]. Furthermore, it inhibits neuropeptide Y neurons, which might also contribute to its anorectic effects[78]. These effects of PYY3–36 can be blocked by the administration of a specific Y2 antagonist. In addition, PYY3–36 does not reduce appetite in Y2-knock-out mice[77,79]. Similar to GLP-1, ablation of the vagus–brainstem–hypothalamus pathway leads to a moderation of the anorectic effects, indicating a role of the vagus nerve in the neuronal messaging of PYY[49]. Obese individuals are sensitive to the effects of PYY, as peripheral PYY administration in the obese reduces food intake to the same extent as in normal-weight individuals[80], but circulating postprandial PYY levels are lower in the obese[80]. Exogenous administration of PYY3–36 has attracted considerable interest as a possible therapeutic strategy[81]. Long-term augmentation of dietary protein induces an increase in plasma PYY levels in mice, leading to less food intake and reduced adiposity[82]. PYY3–36 administration in human subjects to levels within the physiological range reduces food intake without causing nausea[77,80], whereas higher pharmacological doses can result in nausea[73]. Sensations of hunger, satiety and nausea might all be points along the same physiological spectrum[83], and nausea is associated with all high-dose satiety-inducing gastrointestinal hormones, including cholecystokinin[83], oxyntomodulin[63] and GLP-1[84]. Elevated fasting levels of PYY have also been observed in several gastrointestinal diseases associated with appetite loss, including inflammatory bowel disease, steatorrhoea as a result of small intestinal mucosal atrophy and chronic destructive pancreatitis[85]. Furthermore, in healthy elderly individuals high cholecystokinin and PYY levels are associated with delayed gastric emptying and reduced gallbladder contractility[86]. These high cholecystokinin and PYY levels facilitate long-lasting satiety and hunger suppression after meals and can lead to restriction of energy intake and malnutrition in the elderly[86].

Gastric inhibitory polypeptide

Gastric inhibitory polypeptide (GIP) is a forty-two-amino acid incretin peptide, which is released from endocrine

K-cells in the duodenum and proximal jejunum within minutes after food ingestion[87]. The main stimulus for GIP secretion is the presence of glucose and fat[88]. GIP promotes energy storage by direct actions on adipose tissue. The peptides exert several anabolic adipocyte actions[88,89] as well as lipolytic effects. GIP-receptor-knock-out mice have lower adipocyte mass and display a resistance to diet-induced obesity[90]. GIP on its own has no acute impact on food intake[87], but acts in concert with GLP-1 to control food intake and energy absorption. Similar to GLP-1, GIP increases glucose-dependent insulin secretion, β-cell proliferation and resistance to apoptosis[91]. GIP levels have been found to be elevated in obese individuals[87].

Gut hormones and appetite after bariatric surgery

Changes in appetite are evident within days of bariatric surgery[10]. Postprandial levels of gastrointestinal hormones that induce satiety, such as GLP-1 and PYY, are elevated after gastric bypass surgery[92], but not after gastric banding[93]. It has been shown that hunger is reduced and satiety is elevated if gastric bands are optimally inflated[13]. These changes in appetite appear independent of any gut hormone alterations[93]. Administration of octreotide, which would inhibit gut hormone responses, does not affect food intake after gastric banding[93]. Thus, non-hormonal mechanisms have been suggested[93]. In contrast, studies have demonstrated that postprandial PYY and GLP-1 levels start rising as early as 2 d after gastric bypass and can remain elevated for many months after surgery[10,11]. In patients with only 20% weight loss after gastric-bypass operations the postprandial PYY and GLP-1 responses are attenuated compared with patients with 40% post-operative weight loss[10]. Moreover, inhibition of the satiety gastrointestinal hormone response with octreotide after gastric bypass increases appetite and food intake[10]. The proposed mechanism behind these findings is that bariatric surgery gives a secretory stimulus to the distal L-cells, resulting in an increased level of gastrointestinal hormones such as PYY and the enteroglucagon family of peptides[93]. As a result, patients have long-term decreased appetite after gastric bypass. The combined effect of exogenous elevation of PYY and GLP-1 reduces food intake more than predicted by individual hormone infusions alone[94]. This combination of gastrointestinal hormone responses might, therefore, contribute to the successful weight loss and its maintenance after bariatric surgery.

On the other hand, changes in ghrelin levels after bariatric surgery are controversial. Ghrelin levels have been reported to be markedly suppressed after gastric bypass, while diet-induced weight loss is associated with increased levels of plasma ghrelin[43]. It was suggested that reduced ghrelin contributes to the weight loss after gastric bypass[43]. Other authors have published conflicting results[95–99]. Thus, the role of ghrelin after gastric bypass remains unclear. Ghrelin secretion might in fact be modified by other gastrointestinal hormones, the levels of which change in response to the altered gastrointestinal anatomy. However, since obesity is associated with lower levels of ghrelin, it seems unlikely that reducing the level of ghrelin would, by itself, induce weight loss[100].

Long-term follow-up data on the changes in gastrointestinal hormones after bariatric surgery are still awaited. Surgery modulates a number of the gut hormones and probably allows them to act in concert in such a way as to affect appetite optimally. Understanding the contribution each hormone makes to appetite control within the setting of gastric-bypass surgery may be the stepping stone to future anti-obesity treatments.

Conclusions

Gastrointestinal hormones have attracted a remarkable amount of research interest in recent years because of their physiological effects on energy balance and appetite effects. Gastric bypass surgery is associated with elevated satiety and satiety-inducing gut hormones. Blocking these hormones reverses the satiety effects. Although surgery has been shown to be beneficial for the time being, it carries a risk for complications for patients. Bariatric surgery may thus be used as a model to understand physiological weight loss. This knowledge may help to guide future surgical and non-surgical weight-loss treatments.

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