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Running title: Mechanistic review of gastric bypass and sleeve gastrectomy
Abstract

Obesity increases the likelihood of diseases like type 2 diabetes (T2D), heart disease and cancer, and is one of the most serious public health problems of this century. In contrast to ineffectual prevention strategies, lifestyle modifications and pharmacological therapies, bariatric surgery is a very effective treatment for morbid obesity, and also markedly improves associated comorbidities like T2D. However, weight-loss and resolution of T2D after bariatric surgery is heterogeneous and specific to type of bariatric procedure performed. Conventional mechanisms like intestinal malabsorption and gastric restriction do not fully explain this, and potent changes in appetite and the enteroinsular axis, as a result of anatomical reorganisation and altered hormonal, neuronal and nutrient signalling, are the portended cause. Uniquely these signalling changes appear to override vigorous homeostatic defences of stable body weight and compelling self-gratifying motivations to eat, and to reverse defects in beta cell function and insulin sensitivity. Here we review mechanisms of weight-loss and T2D resolution after Roux-en-Y gastric bypass and laparoscopic sleeve gastrectomy bariatric surgery, two markedly different procedures with robust clinical outcomes.

Keywords: Obesity, type 2 diabetes, bariatric surgery, roux-en-y gastric bypass, laparoscopic sleeve gastrectomy, mechanisms, ghrelin, PYY, GLP-1

Introduction

Obesity is one of the most rapidly increasing world-wide health-care challenges, confronting the medical and scientific community and governments. While the incidence and mortality rates for cancer and heart disease are stable or falling in the UK and USA (15, 140, 147, 176), the incidence of overweight (Body Mass Index (BMI) >25 kg/m²) and obesity (BMI >30 kg/m²) is increasing dramatically. Currently 61% of the adult UK population are overweight or obese (80), with 24.5% being obese (80) despite a target of less than 10% set out by the UK House of Commons Select committee in 1992 (73). In the USA more than 30% of adults are obese (144). This figure is estimated to increase to 50% by 2030 in the USA (211), and the UK is predicted to follow suit. Globally it is estimated that there will be 1.12 billion obese adults by 2030 (87). The prevalence in the hazardous extremes of obesity (morbid-obesity (BMI >40 kg/m²); super-obesity (BMI >50 kg/m²)) is increasing even faster (188). This problem is not unique to adulthood and in the USA 17% of children are obese (144), a further 30% will be by 2030 (211), and 75% will become obese adults (182).

Obesity carries significant morbidity and mortality (14, 55, 83, 220). It is a major risk factor for type 2 diabetes (T2D), hypertension, dyslipidaemia, atherosclerosis, heart failure, cancer, liver disease, obstructive sleep apnoea, infertility, degenerative joint disease, depression and dementia (21, 154, 214). Reduced life-expectancy is directly proportional to BMI (53) and, shockingly, obesity may have contributed to a reduction in overall life-expectancy in the USA (5).

Obesity arises when energy intake chronically outweighs energy expenditure (134). Adoption of Western life-style largely explains the dramatic global rise in obesity, by promoting over-consumption of energy-dense food and physical inactivity. Nonetheless obesity has a high heritability (142). Despite difficulty finding causative genes, those identified through rare monogenic obesity and genome-wide association studies implicate the brain (112, 209), indicating that obesity is primarily a neuro-behavioural disorder rather than a disorder of adipose tissue. However, sick fat or adiposopathy, the accumulation of mitochondrial dysfunction, endoplasmic reticulum stress and inflammation, is central to obesity-associated metabolic disease, leading to disturbed insulin and leptin signalling, with dysregulation of central appetite control (4, 47, 79, 142). Yet, apart from the rare Mendelian disorders like leptin deficiency, currently we have little explanation for why some people develop the hazardous extremes of obesity.
Here we review the mechanisms proposed to mediate the weight-loss and improvement in T2D after bariatric surgery, specifically roux-en-Y gastric bypass (RYGB) and laparoscopic sleeve gastrectomy (LSG) and relevant animal models. The topic of bariatric surgery as a whole, the control of appetite and energy homeostasis, and gut hormones are not covered in detail as these are well reviewed elsewhere (26, 52, 85, 134).
Failure of Medical and Success of Surgical Weight-loss

Medical management

Therapeutic lifestyle actions like dieting and exercise and weight-loss drugs deliver modest long-term weight-loss in obese individuals (221). For example in the prospective Swedish Obese Subjects Study, diet and conventional medical treatment, without use of drugs, resulted in a 1.6% increase in weight over a 10-year period (180). Highly intensive dieting and exercise programmes are capable of yielding significant weight-loss, however this is almost ubiquitously transient (74, 200). Pharmacological options are currently very limited. Absorption inhibitors, like Orlistat, typically only reduce weight by 3kg (149), and side-effects are common and unpleasant; appetite suppressants, like Sibutramine and Rimonabant, only by 4-5kg (149), but neither are currently available in the UK or USA, due to heterogeneous actions within the CNS and potentially harmful consequences. Drugs in development are some years away from implementation (149). Explanations for this failure include a robust homeostatic appetitive response to calorie deficit and compelling hedonistic and addictive drives to eat, which not only oppose weight-loss but also promote weight-regain after substantial weight-loss (134, 184, 193).

Surgical management

In contrast, bariatric surgery is highly efficacious and durable in yielding weight-loss. In a meta-analysis of 621 studies, encompassing 135,246 patients and four distinct procedures, mean total weight-loss was 38.5kg and mean % excess body weight-loss (%EWL) was 55.9% (27) (%EWL is calculated from the equation: (weight-loss in kg / excess-weight in kg) X 100; where excess-weight = total bodyweight – ideal bodyweight). Furthermore, complete resolution of T2D occurred in 78.1% of patients, verified by improved biochemical parameters (27). Likewise dyslipidaemia improves in 70%, hypertension in 61.7% and obstructive sleep apnoea in 85.7% (26), cancer incidence is significantly reduced, attributable to risk reduction in women but not men (108), and psycho-social well-being enhances (44, 72, 197). Despite surgical risk, bariatric surgery for morbid obesity is robustly associated with decreased overall mortality (3, 181).

Surprisingly weight-loss and resolution of T2D is heterogeneous and specific to type of surgical procedure (Table 1). Conventional mechanisms like intestinal malabsorption and gastric restriction do not fully explain differences in weight-loss, and potent changes in appetite are the portended cause. Furthermore improved glycaemic control is in
part independent of weight-loss magnitude and caloric restriction. Thus energy and glucose homeostasis alter in an operation-specific fashion, which may be determined by intestinal factors.

**The Gut, Energy Homeostasis and Obesity**

Energy homeostasis is a complex biological process by which food intake and fuel expenditure are constantly adjusted to preserve stable long-term balance (134). This involves central assimilation of short-term as well as long-term hormonal, neural and nutrient signals concerning energy status, which emanate from the gastrointestinal tract, adipose tissue and hepatic stores. The net result is a level of appetite or satiety and of energy expenditure, which is integrated with cognitive, visual, olfactory and taste cues to provide volition for active food seeking and exertion.

Surgical reorganisation of gut anatomy causes changes in nutrient partitioning and absorption, and hence local stimulation, with resultant adaptation of short-term and possibly long-term hormonal, neuronal and nutrient signals. These signalling changes modify appetite, energy consumption and gut motility, and uniquely they appear to override vigorous homeostatic defences of stable body weight, and compelling self-gratifying motivations to eat. Unexpectedly through surgical modulation of gut anatomy, the previously unsolvable obesity pandemic may be solvable, and the previously irreversible natural history of T2D may be reversible. This makes study of gut function the vanguard for understanding obesity and T2D pathobiology and design of obesity and T2D drugs.

**RYGB and LSG as models for understanding weight-loss**

Weight-loss surgery is classified as restrictive, malabsorptive or hybrid, involving aspects of both. RYGB is a hybrid surgical procedure (Figure 1) endorsed as having the best balance of efficacy and risk by the American National Institute of Health (27). Bilio-pancreatic diversion (BPD), another hybrid procedure, is modestly more efficacious but less safe, whilst adjustable gastric banding (AGB), a purely restrictive procedure, is less efficacious but safer (Table 1). Hence RYGB is the commonest bariatric procedure in the USA and Canada (28), and the fastest growing procedure in Europe (28). The frequency of use of RYGB and the magnitude of appetite and gluco-regulatory benefits make it an excellent choice for mechanistic studies.

Laparoscopic sleeve gastrectomy (LSG) is a restrictive bariatric procedure, designed originally as a first-stage procedure to reduce weight in the super-obese (who have a high operative risk), which could be transformed into a hybrid procedure such as RYGB, following sufficient weight-loss (Figure 1). The medium-term efficacy of LSG is superior to laparoscopic adjustable gastric banding (LAGB) (1, 146, 168) and can rival RYGB, with EWL of 63-75% and
T2D resolution of 82-84% documented (1, 82, 84, 105, 207). However, as with other restrictive procedures (11, 31, 66, 175, 192), the long-term efficacy of LSG may be less favourable. Two recent studies demonstrate significant weight-regain after 5 years (17, 75), though this could be related to variation in surgical technique, the LSG procedure has evolved over time and on the whole sleeve diameter has been tightened. Nonetheless LSG efficacy remains superior when compared to other restrictive procedures, implying that mechanisms other than gastric restriction are key, and resultantly operative numbers are expanding. Therefore we will compare mechanisms of weight-loss and T2D resolution in RYGB and LSG, two leading bariatric operations for investigating obesity.

**Mechanisms of weight-loss after RYGB**

RYGB combines gastric restriction and intestinal bypass (Figure 1). It does not produce clinically significant malabsorption (121), but weight-loss magnitude is equivalent to procedures that do (26), and superior to purely restrictive procedures (26). By comparison with purely restrictive procedures RYGB can produce up to 50% greater EWL (143). Animal models of RYGB indicate that this additional weight-loss is not due sub-clinical intestinal malabsorption, which accounts for only 4%, but due to decreased food consumption (189). Post-operatively subjects experience a potent reduction in appetite, extending beyond the early postprandial period (24) during which gastric mechanoreceptors exert satiating effects. Despite energy deficit, meal and snack frequency reduces and predilections for sugary and fatty foods diminish (40, 69, 88). This is accompanied by rapid amelioration of T2D independently from weight reduction (170, 216), as evidenced by inferior gluco-regulatory effects following comparable weight-loss from dieting or restrictive surgery (103, 152).

It is increasingly clear that central energy and glucose homeostasis is extensively regulated by the intestine, the largest endocrine organ in the body (52, 129). Changes in gut hormones, like ghrelin, peptide YY (PYY) and glucagon-like peptide-1 (GLP-1), follow RYGB and generally support anorexia and euglycaemia.

**Ghrelin**

Ghrelin, uniquely, is the only orexigenic gut hormone. However, the regulation and action of ghrelin, in relationship with other energy signals, is incompletely understood (Box 1). After dieting and restrictive bariatric procedures fasting ghrelin levels increase in proportion to weight-loss (42, 70, 95, 135, 141, 185). Therefore a profound elevation in fasting ghrelin might be anticipated following RYGB, which produces proportionately more weight-loss. On the contrary fasting ghrelin levels change heterogeneously, and may decrease, not change or increase (160). These
heterogeneous changes in ghrelin following RYGB may be partially due to timing of ghrelin measurement after surgery and partially procedural.

There is mounting evidence for an immediate reduction in ghrelin following RYGB (20, 95, 131, 133, 191), and there is some indication that this change is intra-operative, most sizeably at division of the stomach (117). An explanation for this is transient vagal inhibition during surgery (117, 191), with heterogeneity resulting from inconsistent vagal manipulation (114). The vagus nerve plays an important role in ghrelin physiology, with afferent vagal tone being implicated in ghrelin functionality, and efferent vagal tone in ghrelin release (pharmacological and surgical inhibition of vagal neurotransmission in humans and animals abolishes both the stimulatory effect of peripheral ghrelin administration on eating behaviour, and the food deprivation-induced elevation of plasma ghrelin levels) (8, 23, 43, 109, 122, 217). Alternatively, acute caloric restriction has been proposed as contributory, as this modestly reduces serum ghrelin levels (41, 42, 111), but this mechanism is unlikely given the contrast between bariatric operations. Another explanation for a reduction in fasting ghrelin is division of the stomach to create a gastric pouch, given the importance of functionality of the gastric fundus for ghrelin secretion (57, 58), with variability resulting from differences in pouch configuration (157).

In the months after RYGB, fasting ghrelin levels appear to increase relative to immediate post-surgical levels (191). Weight-loss is an important determinant of this, and one study directly correlates increases in fasting ghrelin with reductions in BMI at 6 and 12 months after RYGB but not earlier (222). Nonetheless even at equivalent levels of weight-loss, fasting ghrelin levels are highly variable. Early studies of RYGB demonstrate this adroitly; some demonstrate an increase in fasting ghrelin levels in comparison to pre-surgical level in the context of significant weight-loss (61, 76, 206); others show lower or unchanged fasting ghrelin levels in the context of similar weight-loss (39, 51, 54, 57-59, 63, 84, 92, 95, 110, 114, 115, 117, 118, 131, 145, 164, 185, 198, 215), and therefore should be viewed as absolute or relative falls in ghrelin secretion respectively (in general these studies commenced ghrelin measurements beyond 6 months after surgery and thus fail to show an initial fall in fasting ghrelin). This heterogeneity could result from variations in compensatory ghrelin hyper-secretion and magnitude of weight-loss, in combination with the antagonistic effect of surgery. Heterogeneity could also be a consequence of intrinsic phenotypic differences like insulin resistance, which independently predicts fasting ghrelin level (124). Moreover, variations in methodology may be contributory; different ghrelin assays produce inconsistent results (161), sample
collection and storage is not standardised between studies, and some studies measure acyl (active) ghrelin whilst others measure total (acyl and des-acyl) ghrelin. Importantly in studies reporting increases in fasting ghrelin, weight-loss is reported as less profound in those with higher fasting ghrelin levels (222).

These findings suggest that circulating ghrelin levels are determined by a composite of potentially opposing surgical and weight-loss consequences, and also multiple acute effectors. Quantifying the effect of ghrelin or correlating changes in ghrelin with weight-changes will therefore require systematic analyses and modelling in large robustly matched cohorts. This should be achievable given the volume of bariatric procedures performed. Obtaining tissue for gene expression and using knock-out (KO) and knock-in (KI) animal models will further quantify the specific role of ghrelin. An increase in fasting ghrelin level in the months after surgery suggests that other, possibly more important, signalling changes are likely.

**PYY**

PYY3-36 is a potent anorectic hormone (Box 1). Dysregulation of PYY is implicated in obesity (12), and PYY secretion in response to feeding may be blunted in obese individuals (12). This is not replicated in all studies (165, 205), perhaps because of phenotypic variation. However, in obese individuals sensitivity to the anorectic effect of PYY3-36 is intact (52).

After RYGB meal-stimulated PYY increases significantly (92, 115, 130, 132), varying between 1.5-10 fold, depending on meal calorie content (19, 33, 95, 133, 164). This occurs as early as day 2 after RYGB, and before weight-loss occurs (110), indicating this is a direct consequence of surgery, and equivalent weight-loss from dieting (203) or LAGB (19) does not similarly alter PYY. The most likely cause for this difference is surgical re-routing of gut content.

One explanation is that after RYGB nutrients enter a limb of distal jejunum directly from a small stomach pouch (Figure 1), expediting delivery of nutrient-rich chyme to L-cells in the distal gut, and stimulating greater PYY secretion. Similar re-routing bariatric procedures replicate this PYY finding (86). Ileal transposition (IT) in rodents provides mechanistic support (Figure 1). By transferring a section of resected ileum into the proximal duodenum, early nutrient exposure achieves similar augmentation of PYY secretion and reduces food intake and weight (186, 187). Gastric restriction, malabsorption and foregut exclusion do not occur at IT indicating that the causal surgical manoeuvre here, and seemingly after RYGB, is connecting the distal gut to the stomach (36, 90, 91, 187).
Another explanation is gut adaptation with enteroendocrine cell hypertrophy or hyperplasia. In rodents RYGB increases bowel width, villus height, crypt depth and crypt proliferation, maximal in nutrient stimulated regions (194), and BPD (18), and IT (186) behave similarly. In humans, jejuno-ileal bypass has been shown to result in enteroendocrine cell hyperplasia (25). Interestingly some but not all studies show small time-dependent incremental increases in PYY level following RYGB, consistent with an adaptive response to prolonged stimulation (95). These gut adaptations may only sometimes manifest clinically, feasibly in good responders (133). Gut adaptation however is not rapid and does not explain the early dramatic RYGB effects.

After RYGB visual analogue scales (VAS) consistently demonstrate an increase in satiety and a decrease in hunger, in association with levels of PYY known to modify appetite (115) (VAS is a measurement instrument used to quantify a subjective measure, for example appetite, in an objective format by asking a respondent to specify their level of agreement with a statement by indicating a position along a continuous line between two end-points, for example Q: ‘How hungry do you feel?’, A: ‘Not hungry at all’ to ‘As hungry as I have ever felt’). While this might imply causality, most studies do not correlate changes in PYY with VAS (92, 94, 130, 203). One explanation is inadequate statistical power as VAS scores lack sensitivity (19). Alternatively PYY, in isolation, may not fully explain appetitive changes after RYGB.

Support for the involvement of PYY in RYGB mediated weight-loss is provided by good and poor surgical responder studies. Inferior weight-loss after RYGB is associated with significantly lower stimulated PYY as well as GLP-1 levels (110, 133). However, many studies do not directly correlate changes in PYY and weight-loss (19), but suffer from methodological problems including small numbers, use of weight surrogates for adipose mass, and the capricious nature of isolated gut hormone measurements. A recent animal model of gastro-intestinal bypass provides direct evidence that PYY plays a key mechanistic role in surgical weight-loss. Weight-loss in diet-induced obese (DIO) mice is greater after bypass surgery than after sham surgery, however in obese PYY KO mice weight-loss is equivalent after bypass surgery and sham surgery (where weight-loss results from caloric restriction and surgical stress but not anatomical modulation) (34). Nonetheless, a comparison of RYGB and IT in rats showed greater reduction in food intake and weight following RYGB despite similar PYY enhancement (35), suggesting other important contributory factors. Thus PYY may play a principal role, in synergy with other gut hormones uniquely affected by RYGB, including
reciprocal changes in ghrelin. Measuring changes in hormone ratios instead of isolated gut hormone levels might establish correlation with weight outcomes.

**Incretins**

Incretins are hormones secreted from the gastrointestinal tract, with potent insulinotropic activity, and further gluco-regulatory and energy homeostasis controlling functions, and are the key mediators of the enteroinsular axis (Box 1). T2D is characterised by defective insulin secretion and sensitivity, but the incretin-effect is also impaired, mainly as a result of blunted GLP-1 secretion and glucose-dependent insulinotropic peptide (GIP) resistance (10, 20, 99). The immediate weight-loss independent T2D resolution after RYGB suggests that surgery modifies the enteroinsular axis.

The changes in systemic GLP-1 levels after RYGB are well documented. Meal or glucose stimulated GLP-1 responses increase early (110), durably and by 5-10 fold in magnitude from pre-surgical levels, in non-diabetics and diabetics alike (37, 93, 115, 130, 132, 136, 204). GLP-1 levels do not change similarly following weight-loss by dieting (103) or LAGB (19, 93, 95, 164, 178). After RYGB fasting levels of GLP-1 do not appreciably alter (19, 37, 95, 101, 103, 130, 166). Thus, as with PYY, the rapid delivery of nutrient-rich chyme to the hindgut is the proposed primary stimulus, and in support of this GLP-1 secretion also increases after IT (186, 187).

In contrast with PYY, incremental increases in stimulated GLP-1 levels over time are not observed (95, 99, 110), suggesting that gut adaptation does not augment GLP-1 secretion. Nevertheless the rare late complication of RYGB, hyperinsulinaemic hypoglycaemia, could be a consequence of either enteroendocrine cell or islet cell hyper-function, as nesidioblastosis (abnormal islet cell histology accompanying this hyperinsulinaemia) is not a ubiquitous finding (195).

Altered gut motility is another possible mechanism. Accelerated gastric emptying and intestinal transit have been demonstrated following RYGB, with GLP-1 level magnitude correlating with gastric emptying time (130). Other studies report delayed gastric emptying to solids (77, 137) and faster emptying to liquids (77) following RYGB, but the interpretation of these findings is complicated because GLP-1 itself slows gut transit. Notably however changes in PYY level do not correlate with gastric emptying time (130), casting doubt on this mechanism.
After RYGB there is an early weight-independent increase in glucose stimulated oxyntomodulin (OXM) level, another important incretin originating in L-cells (Box 1), which strongly correlates with GLP-1 and PYY suggesting concordant L-cell secretion (102). To date there has been no assessment of whether this effect persists.

Meal-stimulated GIP levels may increase transiently after RYGB (101, 103), and then normalise or fall (37, 67, 93, 100), but results of studies of stimulated and fasting GIP levels are inconsistent. GIP is predominantly secreted in the bypassed duodenum (10), making a sizeable role in weight-loss and diabetes resolution doubtful in theory. In practice however, GIP levels do appear to paradoxically fluctuate after RYGB, necessitating further clarification.

In association with increases in incretin levels after RYGB, patients with T2D show improved control, with reduced glycaemic excursion after oral glucose, and return of the incretin effect on insulin secretion and of the first-phase insulin response to the level of non-diabetic obese controls (100, 101). Weight-loss does not cause these glucose-regulatory changes following dieting or LAGB (20), and GLP-1 antagonist administration in rats following RYGB inhibits GLP-1 secretion and reverses improvements in glucose tolerance (89). Moreover, lower fasting glucose and insulin levels and increased insulin sensitivity also follow RYGB (132, 156, 158, 166, 170, 216), and notionally GLP-1 contributes to this through inhibition of gastric emptying, restoration of insulin sensitivity and inhibition of glucagon secretion (10). The exact role of these other basic incretin functions will require more detailed study, as highlighted by reports of failure to suppress, or discordant increases in blood glucagon levels after RYGB, in the face of antagonistic increases in GLP-1 (65, 103). Nonetheless, dramatic weight-loss is also a key determinant of fasting improvements (99), due to a reduction in adiposity, inflammation, glucotoxicity, lipotoxicity, and altered circulating adipokines and cytokines.

Postprandial GLP-1 responses following RYGB reach the supra-physiological levels required to reduce energy intake and weight in humans (10). OXM administration to animals and humans also causes weight-loss by reducing food intake in combination with increasing energy expenditure (119, 218, 219), and early indicators suggest a rise in meal stimulated OXM after RYGB. However, long-acting therapeutic incretins (e.g. Exenatide and Liraglutide) for T2D produce only modest weight-reduction (1.5-3kg) (10), nothing like the magnitude of RYGB. Whilst not directly comparable, it is likely that incretins play a supportive or synergistic but not dominant role in RYGB weight-loss.
Of note, gut hormones receptors are widely conserved throughout the gut and ANS, and gut hormones may act locally near their site of release to produce some of their effects (Box 1). For this reason measuring systemic gut hormone levels may not accurately reflect the contribution of local gut hormone activity on appetite, weight-loss, beta-cell function and insulin sensitivity. Thus, measuring levels of gut hormones in the portal circulation would be of great interest, and might resolve some of the discrepancies between systemic gut hormone levels and outcome.

**Cholecystokinin, Amylin, others**

Other gastrointestinal hormones including cholecystokinin, amylin, and pancreatic polypeptide have no clearly defined role in weight-loss and T2D resolution after RYGB to date (143, 159, 160).

**Leptin and insulin sensitivity.**

The appetite changes and weight reduction after RYGB occur in the face of a dramatic antagonistic homeostatic response to negative energy balance. Leptin, the archetypical satiety factor, is a long-term energy signal secreted in proportion to adipose tissue mass (9). It controls feeding behaviour, substrate partitioning and substrate metabolism through negative feedback at central homeostatic centres (113). When body-weight reduces leptin levels fall, and central pathways increase hunger and food intake and decrease energy expenditure as a result. When lean individuals gain weight the converse occurs. This mechanism fails in the obese due to central leptin resistance (196). Equivalent central insulin resistance similarly deregulates energy homeostasis, and in combination with peripheral insulin resistance deregulates glucose homeostasis (45, 169). Indeed insulin and leptin resistance share pathobiological mechanisms in the brain (174, 196, 223). Acute caloric intake is also a major determinant of leptin level, and fasting causes a larger reduction in leptin than that would be predicted after a short-term loss of adipose tissue mass alone (64). Leptin is also produced in the stomach, and may act locally via leptin receptors activation on vagal afferents, or through systemic release, to mediate this effect (162).

After RYGB leptin levels fall (51, 63, 92), and in this context a marked reduction in satiety and increase in caloric intake would be expected. Significantly, this homeostatic response does not manifest. Furthermore in some studies reductions in leptin level do not directly correlate with weight-loss or fat mass-loss, suggesting an independent cause (19, 94). Moreover, greater reductions in leptin level have been suggested after RYGB, than after LAGB, despite equivalent weight-loss (19), whilst in obese rats leptin levels fall as early as one week after duodenal exclusion.
surgery, prior to significant weight changes (148). This may be because BMI is a deficient surrogate for adipose mass
or because leptin levels also reflect energy usage irrespective of adipose tissue mass (64). RYGB reduces weight more
rapidly than LAGB, and proportionately negative energy balance is greater despite quantitatively similar weight-loss.
Also early after surgery subjects are in negative energy balance, potentially lowering leptin levels before weight-loss.
In support, weight-stable women adjusted for weight-loss and total adipose mass have similar leptin levels after
RYGB and LAGB (96). Alternatively, leptin sensitivity could improve after RYGB (19), thereby explaining the absence
of appetitive behavioural and weight-conserving responses.

Weight-loss independent reductions in fasting glucose, fasting insulin, and insulin resistance occur early after RYGB
(123, 156, 216). Isolated foregut exclusion in bariatric models also improves glucose homeostasis (167), and
intestinal gluconeogenesis is a recently proposed mechanism for this beneficial foregut effect. In murine models,
intestinal gluconeogenesis increases glucose fluxes in the portal vein, which are signalled to the hypothalamus via
neural circuits, and result in decreased food consumption and improved insulin sensitivity (127, 128, 151, 210). In
addition, RYGB in mouse models increases intestinal gluconeogenesis with associated beneficial effects on food
intake and fasting glucose homeostasis, whilst gastric banding does not. These effects are abolished in glucose
transporter 2 (GLUT-2) KO mice with portal vein denervation (199). It is not clear what happens to leptin sensitivity
in these models.

**Other signalling pathways**

Parasympathetic nervous signals via vagal afferents provide discrete information regarding nutrients in the gut and
portal circulation, and food storage in the liver and adipose tissue (113, 174). Vagal efferents control motility and
nutrient partitioning in the intestine, and nutrient utilisation for energy or storage. Furthermore the ANS may affect
long-term energy homeostasis, through primitive memory mechanisms like synaptic plasticity (134). New
approaches in animals are delineating a mechanistic role for the autonomic nervous system in RYGB outcomes;
variable vagus-sparing during surgery decreases post-operative food intake independently from gut hormone
changes (29).

Short-term nutrient fluctuations also affect nervous signalling (38, 134, 174), and anatomical modification after RYGB
may alter nutrient absorption and intestinal nutrient metabolism. These nutrients act directly on hypothalamic
neurones to affect feeding behaviour (104). Furthermore, free-fatty acid metabolites like oleoylethanolamide and long-chain fatty acyl CoA, produced in the duodenum in response to lipid ingestion, signal through vagal neurocircuits, to control feeding behaviour (173) and hepatic insulin sensitivity (210). If these regulatory mechanisms are stimulated by undiluted bile acids or nutrient reflux into the excluded duodenum, or have downstream lipid-sensors, then they could play an important role in RYGB efficacy (195).

After RYGB patients report profoundly reduced preference for sweet and fat tastes, unexplained by common taste effectors (193). Rodents following IT develop conditioned taste aversion (186) and following RYGB respond more positively to low-sucrose foods, with subsequent reduction in pursuit of calorie rich foods. This implies that gustatory pathways change after RYGB, and also that this may alter food reward value (179).

Different gut micro-floral phyla are associated with lean, obese and post-bariatric phenotypes (30, 116, 201). Although the cause of this is uncertain, obese-gut-derived micro-flora can generate obesity in germ-free mice (202). Potential mechanisms include increased lipopolysaccharide production (177), altered energy harvesting (202) and hormonal modulation (32). Conversely, dietary composition can change gut micro-flora, and diet is significantly altered after surgery (208). It remains to be seen whether changes in micro-flora play a contributory role in either obesity pathogenesis or in weight-loss after bariatric surgery, or whether changes in micro-flora result directly from differing dietary contents (or a combination of both).

**Learning from RYGB failures**

Despite excellent efficacy, failure rates after RYGB are significant; durable weight-loss is not achieved in 20% (26, 120), T2D improves without resolving in 15% (26), and more recent studies highlight recurrence of T2D after post-surgical resolution (48). Ascertaining how putative signalling changes alter in RYGB failures could clarify which are the necessary effectors for weight-loss and T2D resolution.

Retrospective epidemiological studies associate a number of pre-surgical phenotypes and post-surgical behavioural factors with poor outcomes for weight-loss and T2D resolution. Pre-operative associations for inferior weight-loss include T2D and male gender, and post-operative associations include poorer follow-up attendance, lower levels of physical activity, un-married status, poor self-esteem and abnormal eating behaviour (120, 126). Pre-operative associations for lack of T2D resolution include higher BMI, longer T2D duration, more severe T2D, larger number of
T2D medications and insulin use, and post-operative associations include weight-loss failure and weight-regain (48, 170, 171). Causation is not established but a number of basic mechanistic inferences are possible; RYGB outcomes are complex, and failure most likely results from overlapping but heterogeneous causes; pre-surgical phenotypes influence outcome, implying a core biological underpinning to failure mechanisms; post-operative behaviours influence outcome, so the potential exists for behavioural override of RYGB mechanisms; and post-surgical weight-loss outcomes and T2D resolution are intrinsically linked but not directly correlative, meaning either that causal mechanisms are shared or improvements in one drive improvements in the other (or both), but that non-shared pathways also exist.

In T2D resolution pre-surgical phenotype is essential and poor outcomes associate with features consistent with beta-cell malfunction (48, 170, 171). Thus signalling changes in isolation are not sufficient to resolve T2D but residual beta-cell function appears necessary.

In humans, putative biological mechanisms for weight-loss failure have been investigated in good and poor human responders, as discussed previously. Good responders to RYGB achieve significantly higher nutrient stimulated PYY and GLP-1 levels than poor responders (110, 133), but a direct correlation between change in weight and change in PYY and GLP-1 is not seen. One explanation is deficient powering due to small numbers and inadequate phenotyping; poor responder groups combine inadequate weight-loss and weight-regain, with their potentially different root causes. However BMI itself is also an important predictor of gut hormone levels, and poor PYY and GLP-1 response may purely reflect a higher current BMI. In this context, a causal role for gut hormones in RYGB failure remains unsubstantiated.

Studies in rodents endorse a biological basis. In a mechanistic study, DIO rats after RYGB, when compared with sham-operated ad-libitum fed rats and with sham-operated rats pair-fed to match the RYGB arm, failed to achieve durable weight-loss at a similar frequency and in a similar pattern to human equivalents (68). At re-introduction of solid diet, caloric intake was greater in RYGB-failures than in successes. Furthermore weight-loss in RYGB-failures was inferior to successes, but equivalent to the pair-fed cohort, and superior to the ad-libitum group. Thus RYGB-successes lost more weight than pair-fed counterparts despite equivalent caloric intake, and RYGB-failures lost comparable weight to pair-fed counterparts despite greater caloric intake. Seemingly, in this model, RYGB-successes and failures either expend more energy (are less able to conserve energy), or utilise less ingested calories than sham
controls. Thus the fundamental difference between failure and success may be retaining (or regaining) the capacity to compensate caloric deficit and weight-loss by overeating.

In the same study, plasma PYY and hypothalamic PYY expression levels in RYGB-failures were significantly lower than in the successes (68), but as the rats were culled in the fed state but in a non-standardised manner, the timing between last feed and PYY measurement is potentially highly variable between the two groups and causation is unreliable. Also energy expenditure was greater after RYGB (successes and failures) than sham-operated (pair- and non-pair fed), a finding replicated in separate studies and in other animal models (189).

The study of RYGB failure mechanisms may yet prove highly informative, but given the greater levels of complexity in humans, it may be prudent to investigate failures in rodent models first, then replicate findings in much larger prospectively investigated human cohorts, employing sub-group analyses in extreme good and poor responders.

**Mechanisms of weight-loss after LSG**

The importance of gut hormones in mediating weight-loss and resolution of T2D after RYGB is clear. This suggests that other efficacious bariatric procedures may behave similarly, and after LSG, changes in gut hormones occur and favour anorexia and weight-loss.

Ghrelin consistently decreases following LSG (46, 84, 98, 107, 153, 212), and in contrast does not change or increases after other restrictive procedures (49, 57, 59, 71, 117, 141, 172). A prospective comparison shows ghrelin reduction at 1 and 12 months following LSG, but increased ghrelin proportionate to weight-loss following LAGB (107). This fall is particularly significant because the superior weight-loss following LSG compared to LAGB would be expected to promote increased ghrelin levels (107). A head-to-head comparison of LSG and RYGB shows a fall in fasting ghrelin level after LSG and an unchanged fasting ghrelin level after RYGB despite equivalent weight-loss (84). Moreover falls in ghrelin following LSG are durable to five years after surgery (17). A single study in rats supports a reduction in ghrelin after sleeve gastrectomy, and an increase in ghrelin after gastric banding (212). However, in this study hypothalamic GHS-R1a protein levels increase after sleeve gastrectomy, but do not change after gastric banding, suggesting increased ghrelin sensitivity following LSG. This may explain the comparable weight changes in both the surgical groups, and could challenge the proposed role of ghrelin in LSG weight-loss, if ghrelin sensitivity increases as ghrelin levels fall.
Removal of ghrelin secreting tissue is one proposed mechanism for reduction in ghrelin after LSG. Gastric resection and removal of ghrelin secreting tissue is the major difference between LSG and other restrictive procedures that do not lower ghrelin levels. In support, a comparable reduction in ghrelin is observed following total gastrectomy (6). The importance of complete fundus resection at LSG is highlighted in a single case of high post-operative ghrelin and complete weight-regain, where at reoperation a remnant of herniated fundus was found (106). Furthermore, in studies combining complete gastric fundus removal at LSG with IT or BPD, a fall in ghrelin is demonstrable (46, 97, 98), and this fall does not occur after isolated IT and after BPD without complete fundus removal (2, 57, 62). Theoretically residual fundus tissue could adapt and hyper-secrete ghrelin (106). An auxiliary role for vagal interruption in ghrelin down-regulation is anatomically unlikely given the caudal pathway of both anterior or posterior vagal trunks and the vertical not horizontal stomach transection.

Unexpectedly, changes in anorectic distal intestinal hormones follow LSG, in the absence of intestinal manipulation. Increases in meal stimulated PYY levels occur at one week after LSG (153), are sustained at 12 months (84, 203), and can be equivalent to those after RYGB (84, 153, 203). Corollary changes in meal stimulated GLP-1 are observed, but are less marked than after RYGB (84, 153).

Hindgut exposure through faster gastric emptying, which expedites sequential nutrient delivery to the duodenum, jejunum and ileum, does not provide a convincing mechanism for these PYY and GLP-1 changes. Studies examining gastric emptying after LSG are discordant, and LSG with antrum removal increases emptying to solids (125), whilst LSG with antrum preservation has no effect (13). Interestingly, gastric antrum preservation is one hypothesis for outcome variability following LSG (60). Alternatively, duodenal nutrient sensing is sufficient stimulus for PYY secretion (155), and less-well digested chyme delivering greater stimulus for PYY secretion is one possible cause.

A direct role for LSG in leptin regulation needs more investigation. One animal study shows significantly lower plasma leptin levels after sleeve gastrectomy compared to pair-fed sham operated rats (183). Corresponding changes in hypothalamic leptin and melanocortin-4 receptor expression and sensitivity to intra-peritoneal leptin were not observed (183), suggesting no reduction in leptin resistance.

In obese patients with T2D, LSG causes early and significant improvements in glucose homeostasis, prior to weight-loss (207). Acute caloric restriction causes some, but not a comparable improvement in obese controls with T2D.
undergoing laparoscopic cholecystectomy (163), and thus is not the primary mechanism. Furthermore foregut exclusion plays no mechanistic role in LSG. Observed gut hormone changes after LSG support glycaemic improvement, but whether these alone or in conjunction with acute caloric restriction are sufficient for early T2D resolution is unclear. Of these changes augmented GLP-1 response is likely to have the most substantive insulinogenic and gluco-regulatory effects, and increases in post-prandial insulin secretion accompany LSG (153). Ghrelin also exerts several diabetogenic effects (22, 23, 190), so a reduction, coupled with an increase in PYY may improve glucose homeostasis (16). Unquestionably, the enormity of weight-loss maintains and strengthens long-term T2D resolution.

Gastric restriction is a key additional weight-loss mechanism after LSG. The importance of gastric restriction is highlighted by significant weight-loss after other restrictive procedures like LAGB, which yield lesser gut hormone deviations (27). In head-to-head comparison LSG produces about 10-15% greater EWL than LAGB (146, 212). LSG reduces gastric volume to 10% of its pre-surgical volume (150). This can be predicted to restrict food intake and activate stretch mechanoreceptors earlier, to terminate eating (satiation). In addition the potential of the removed gastric fundus to form a pseudo-pouch and permit larger food volumes is lost. Currently there is no evidence that reducing bougie size, the major operative determinant of sleeve diameter, improves weight-loss (56, 82). Nonetheless sleeve diameter also varies by proximity of staple-line application and mechanical tissue stretch around the bougie (150), and late sleeve dilatation may be more common when larger bougie diameters are employed (213), one proposed mechanism for weight-regain following LSG.

**Perspectives and significance**

**Significance**

RYGB and LSG robustly reduce weight and improve T2D, whilst anatomical modifications at RYGB and LSG alter gut hormones in a procedure specific fashion. By implication gut hormone changes are a fundamental difference between these and other less efficacious bariatric procedures. After RYGB, augmentations of meal-stimulated PYY and GLP-1 are robust, whilst a general reduction of fasting ghrelin (or failure in the setting of weight-loss to maximally increase) is likely. After LSG, augmentations of meal-stimulated PYY and GLP-1 and reduction of fasting ghrelin level are apparent, but will require further replication in larger cohorts. Whether gut hormone changes are sufficient in isolation to explain the dramatic clinical outcomes that accompany these procedures remains uncertain.
The role of other signalling changes, including neuronal, nutrient and micro-floral modification, within this complex regulatory system should not be underestimated.

**Perspectives**

Many outstanding questions require further investigation. Perhaps the most important of these is how and where these signalling changes have their affect. Calorie restriction is a potent stimulus for increased appetite and following most restrictive bariatric procedures subjects consume more frequent small volume energy dense meals (24). After LSG and RYGB this adaptive response is not observed (193). The role of gut hormones and other signalling changes that accompany RYGB and LSG might be to negate homeostatic centres that increase meal frequency during calorie deficit, and positively alter food reward centres, thus preventing obesogenic eating behaviours. Animal and human studies demonstrate this adroitly. In rats, combining chronic food restriction on top of LSG increases meal frequency (183). This implies that appetite and not physical restriction prevents adaptive increases in meal frequency, and that the ability to overeat remains, but is reset to defend a lower energy threshold. In humans following RYGB and LSG, brain regions relevant to eating behaviour imaged by positron emission tomography CT show decreased dopamine (DA D2) receptor availability, reflecting an increase in dopamine levels (50). This links well known gut hormone influences on dopaminergic neurotransmission to functional alterations in reward centres following bariatric surgery.
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Box 1 – Gut hormone regulation

- **Ghrelin**: is a 28 amino-acid peptide synthesised primarily in the gastric fundus, but also throughout the intestine and in the hypothalamus. It is activated by gastric O-acyl transferase mediated post-translational esterification (85, 139). The control of ghrelin release is not fully understood, however circulating ghrelin levels increase during fasting and decrease rapidly in response to feeding. This implies a role in meal initiation (40), and ghrelin increases food intake in humans and rodents, but other roles in long-term energy balance, memory and immunity are likely (81, 85, 138). Ghrelin’s actions are mediated through the growth hormone secretagogue receptor (GHS-R1a), located in the brain and autonomic nervous system (ANS), the gut, adipose tissue, heart and lungs (78). Effects on appetite occur principally via direct GHS-R1a activation of neuropeptide Y (NPY) and agouti-related protein producing neurones in the hypothalamic arcuate nucleus (7), though GHS-R1a activation in non-homeostatic brain regions suggests more a more complicated role in appetite regulation (7, 85).

- **Peptide YY (PYY)**: is a 36 amino-acid peptide, synthesised in enteroendocrine L-cells located principally in the distal jejunum, ileum and colon, and released into the circulation in response to nutrient stimulus (7, 85). The ubiquitously expressed enzyme dipeptidyl peptidase IV (DPP-IV) is thought to cleave the N-terminus from PYY1-36 to form the major circulating peptide PYY3-36 (85). PYY3-36 is a potent satiety factor, reducing food intake in humans and animals, primarily acting at the high-affinity Y2-receptor (Y2R). Y2Rs are located throughout the central nervous system, nodose ganglia and vagal afferents (85). Appetite is controlled primarily by direct Y2R mediated inhibition of NPY and reciprocal activation of pro-opiomelanocortin producing neurones in the hypothalamic arcuate nucleus, but also via brainstem, vagal and higher behavioural cortical signals mediated via the Y2R (7).

- **The Incretins**: are a group of functionally related gut-derived peptides, including glucagon-like peptide-1 (GLP-1), glucose-dependent insulinoetrophic peptide (GIP) and oxyntomodulin (OXM). GLP-1 and OXM are formed from a common precursor, pre-proglucagon, primarily in the jejuno-ileal L-cells, whilst GIP is synthesised, via pro-GIP, primarily in duodenal K-cells (99). In response to direct nutrient stimulus following a meal these incretins are released into the circulation, and act to amplify normal pancreatic insulin secretion in response to glucose – the incretin effect. Thus a ten-fold greater insulin release occurs after oral glucose compared to an equivalent dose of intravenous glucose. GLP-1 and OXM share the widely expressed GLP-1 receptor, with ligand specific actions (7). GLP-1 is the more potent incretin, and has other key functions including inhibiting gastric emptying, blunting glucagon secretion, restoring insulin sensitivity, inducing β-cell proliferation and promoting satiety, whilst OXM is also a potent satiety signal (10). GIP acts at the distinct GIP receptor, and also promotes energy storage and bone formation (10). In contrast to PYY, GLP-1 and GIP are rapidly inactivated by DPP-IV (10).
Figure 1 – Illustrations of bariatric procedures; red markers indicate surgical manipulations, green arrows indicate nutrient flow. The entire small bowel is not included pictorially for clarity reasons, and to demonstrate this in our diagrams the linear contour of the distal small bowel is interrupted.

**RYGB:** Roux-en-Y gastric bypass, a small gastric pouch is created by division of the stomach at (A). The jejunum is divided 30-75cm from the ligament of Treitz, and the distal end is anastomosed to the gastric pouch (B), creating the roux limb. The incongruent proximal end is re-anastomosed to the alimentary limb 75-150cm from the gastrojejunostomy (C). Thus nutrients bypass the entire duodenum and part of the jejunum, by entering the distal jejunum directly from the gastric pouch and contact the distal bowel (where enteroendocrine L-cell density is greater) earlier.

**LSG:** Laparoscopic sleeve gastrectomy, a bougie is inserted into the stomach to calibrate the size of gastric sleeve. The stomach is transected from the greater curve (A) in a cephalad direction, in parallel to the sleeve, until the angle of His is reached. Thus the complete gastric fundus and body are removed, with variable degrees of antrum removal, whilst the entire small bowel is left intact.

**IT:** Ileal transposition, the small bowel is divided at (A) and a section of ileum is removed and re-sited in the proximal jejunum. As a result nutrient flow contacts this re-sited section of ileum (where enteroendocrine L-cell density is greater) earlier.
Table 1 – efficacy and mortality data after RYGB, BPD and AGB; adapted from Buchwald 2004 (26).

<table>
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<tr>
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<th>Roux-en-Y Gastric Bypass (RYGB)</th>
<th>Bilio-pancreatic Diversion (BPD)</th>
<th>Adjustable Gastric Banding (AGB)</th>
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<td>%EWL</td>
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