Microbial regulation of Insulin-like peptide 5 and its implication on metabolism and bariatric surgery

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Cover illustration: Imagination

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ISBN 978-91-628-9838-0

Printed in Gothenburg, Sweden 2016
INEKO AB

To my family

ABSTRACT

The microbial community in our gastrointestinal tract, the gut microbiota, has great impact on our physiology. Particularly, the role for gut microbiota in host health and disease has been associated with modulation of gut hormones which are key players in the regulation of energy homeostasis. Recently, a new gut hormone, insulin-like peptide (INSL5) has been identified. In this thesis, we have studied the microbial regulation of INSL5 and its role on metabolism.
Bariatric surgery is the most effective treatment for obesity and obesity-related diseases such as type 2 diabetes. There is increasing evidence that supports a role for gut hormones and gut microbiota in mediating the beneficial effects of bariatric surgery. Thus, in this thesis, we also investigated whether INSL5 and the gut microbiota directly contributes to the metabolic improvements following the bariatric procedure called vertical sleeve gastrectomy (VSG).

In paper I, we found that *Insl5* expression is higher in the colon of germ-free mice (mice that lack a microbiota), compared with their conventionally-raised control animals. We demonstrated that the elevated *Insl5* expression in GF mice is a response to low energy levels, which could be restored by increasing the energy availability. In addition, we found that mice lacking INSL5 have slightly impaired hepatic glucose production during fasting. Thus we speculate that INSL5 might play a role in low energy conditions.

In paper II, we observed that circulating fasting INSL5 levels were increased in human individuals following VSG. The high INSL5 levels were declined upon a meal test, suggesting a postprandial response. To test whether INSL5 contributes to the beneficial effects mediated by VSG, we performed VSG surgeries on wild-type and *Insl5*-knockout mice. The metabolic improvements in both groups of mice were similar after VSG. Therefore, we conclude that INSL5 is not required for the beneficial effects observed after VSG.

In paper III, we characterized the longitudinal changes of the human gut microbiota after VSG, and we found that VSG strongly altered the microbiota composition. We showed that by transferring the VSG-altered gut microbiota from humans to mice, we also transferred the improvements in metabolic effects of VSG patients. We also showed that VSG surgery produced greater metabolic improvements in mice having a normal microbiota compared with germ-free mice. These results indicate that the gut microbiota is directly contributing to the beneficial effects mediated by VSG.

In conclusion, INSL5 is a microbially regulated gut hormone which promotes hepatic glucose production during low energy conditions. INSL5 is also a gut hormone which increases after fasting following sleeve gastrectomy in humans, but it appears not to contribute to the beneficial effects observed after sleeve gastrectomy in mice. However, the gut microbiota plays an important role for the metabolic improvements mediated by sleeve gastrectomy.

Keywords: gut microbiota, gut hormones, INSL5, Bariatric surgery

ISBN: 978-91-628-9838-0

SAMMANFATTNING PÅ SVENSKA

De bakterier som lever i vår mag-tarm kanal, tarmfloran, har stor inverkan på kroppens fysiologi. Tarmflorans påverkan på människans hälsa och sjukdom anses delvis ske genom modulering av tarmhormoner som i sin tur är viktiga aktörer inom reglering av kroppens energibalans. Insulin-like peptide 5 (INSL5) är ett nyligen identifierat tarmhormon. I den här doktorsavhandlingen har vi studerat hur tarmfloran reglerar nivåerna av INSL5 och dess roll inom metabolism.
Bariatrisk kirurgi (viktminskning kirurgi) är den effektivaste behandlingen mot fetma och fetma relaterade sjukdomar såsom typ 2 diabetes. Mer och mer forskning tyder på att tarmhormoner och tarmfloran är delaktiga i de hälsobringande effekterna av bariatrisk kirurgi. Därmed, har vi i den här doktorsavhandlingen också studerat ifall INSL5 och tarmfloran bidrar direkt till de hälsoförbättringarna som sker efter det bariatriska ingreppet vertical sleeve gastrectomy (VSG).

I delarbete I, upptäckte vi att gennivåerna av *Insl5* är högre i tjocktarmen i bakterie fria möss jämfört med kontroll mössen som har en normal bakterieflora. Vi visade att de förhöjda gennivåerna av *Insl5* i bakterie fria möss var en reaktion på låga energinivåer, som kunde återställas genom att öka energi tillgången i mössen. Dessutom upptäckte vi att möss som saknar INSL5 har något försämrad glukosproduktion från levern under fastetillstånd. Därför spekulerar vi att INSL5 möjligen har en roll i tillstånd med låg energi.

I delarbete II, observerade vi att serumnivåer av INSL5 efter fasta var förhöjda i människor som genomgått VSG. De höga INSL5 nivåerna sjönk efter intagandet av en standardiserad måltid. Vi testade ifall INSL5 bidrar till de hälsoförbättringar som orsakas av VSG genom att utföra VSG operationer på möss som saknar INSL5 och vanliga kontroll möss. VSG gav samma metabola förbättringar i båda mössen. Därför är vår slutsats att INSL5 inte krävs för att bidra till de hälsoförbättringar som sker efter VSG.

I delarbete III, studerade vi hur människors tarmflora förändrades med tiden efter VSG, och vi upptäckte att VSG kraftigt förändrade den bakteriella sammansättningen i tarmen. Vi visade att genom att överföra den VSG-förändrade tarmfloran från människor till möss så kunde vi också överföra de metabola förbättringarna från patienterna som genomgått VSG. Vi visade också att VSG operationer orsakade tydligare hälsoförbättringar på möss som har en normal bakterieflora jämfört med bakterie fria möss. Dessa resultat indikerar att tarmfloran har en direkt bidragande roll i de hälsobringande effekterna av VSG.

Vår slutsats är att INSL5 är ett tarmhormon som regleras av tarmfloran, och den stimulerar glukosproduktion från levern under låga energi förhållanden. INSL5 är också ett tarmhormon vars nivåer ökar efter VSG i människor, men den bidrar inte direkt till de hälsobringade effekterna som sker efter VSG i möss. Däremot spelar tarmfloran en betydande roll för de metabola förändringarna som sker efter VSG.

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I. Insulin-like peptide 5 is a microbially regulated peptide that promotes hepatic glucose production

Ying Shiuan Lee, Filipe De Vadder, Valentina Tremaroli, Anita Wichmann, Gilles Mithieux, Fredrik Bäckhed.

Mol Metab 2016; 5:263-270

II. Insulin-like peptide 5 is induced by sleeve gastrectomy in human serum but does not contribute to improved metabolism following sleeve gastrectomy in mice

Ying Shiuan Lee, Antonio Molinaro, Danila Capoccia, Carina Arvidsson, Rosie Perkins, Stefano Ginnani Corradini, Frida Leonetti, Gianfranco Silecchia, Fredrik Bäckhed.

Submitted

III. Mechanisms of sleeve gastrectomy and the role of the gut microbiota

Valentina Tremaroli, Antonio Molinaro, Lisa Olsson, Ying Shiuan Lee, Danila Capoccia, Louise Mannerås Holm, Robert Caesar, Carina Arvidsson, Jose Berger, Frida Leonetti, Stefano Ginnani Corradini, Gianfranco Silecchia, Randy Seeley, Fredrik Bäckhed.

Manuscript

CONTENT

ABBREVIATIONS

AGB	Adjustable gastric banding
BMI	Body mass index
CCK	Cholecystokinin
CNS	Central nervous system
CONV-D	Conventionalized
CONV-R	Conventionally-raised
EST	Expressed sequence tags
FXR	Farnesoid X receptor
G6Pase	Glucose 6-phosphatase
GI	Gastrointestinal
GIP	Glucose-dependent insulinotropic peptide
GF	Germ-free
GLP-1	Glucagon-like peptide-1
GLP-2	Glucagon-like peptide-2
GPR41	G-protein coupled receptor 41
GPR43	G-protein coupled receptor 43
HOMA	Homeostatic model assessment
INSL5	Insulin-like peptide 5
KO	Knockout
NTS	Nucleus tractus solitaris

PEPCK	Phosphoenolpyruvate carboxykinase
PYY	Peptide YY
qRT-PCR	Quantitative real-time polymerase chain reaction
RXFP4	Relaxin/insulin-like family peptide receptor-4
RYGB	Roux-en-Y gastric bypass
SCFA	Short chain fatty acid
SPF	Specific pathogen free
TGR5	Transmembrane G protein-coupled receptor 5
VBG	Vertical banded gastroplasty
VSG	Vertical sleeve gastrectomy
WT	Wild-type

1 INTRODUCTION

1.1 Gut microbiota

Our human body is home to roughly 100 trillion microbes, the majority of these microbes inhabit our gastrointestinal tract, called gut microbiota (1). We have evolutionary coevolved into a symbiotic relationship with the gut microbiota; we provide them with a nutrient-rich environment which sustains their continuous growth, while they perform several metabolic and biochemical functions like digest nutrients otherwise indigestible by ourselves, and provides protection against invading pathogens (2, 3). The gut microbiota composition in mammalians is clearly different from environmental communities residing in soils, sea-water, lakes and etc. (4).

The gut microbiota is composed by bacteria, archaea, yeasts, viruses and fungi (5, 6). However, the surge in gut microbiota research in the last decade has been focused on the bacterial species, although recently interest is growing on the non-bacterial components (7). But in the light of this thesis which focuses is on the bacterial population of the microbial community, will for simplicity´s sake be referred as the "gut microbiota". The microbiota populating our body is composed of at least as many bacteria as human cells (8). On earth, 100 different bacterial phyla have been detected, but only seven are found consistently in our gut; Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, Verrucomicrobia, Tenericutes, and Fusobacteria. Out of these, Firmicutes and Bacteroidetes represent together up to 90% of the bacterial population, while the remaining phyla make up the last 10% (9, 10). More than 1000 different species belonging to the previously mentioned phyla have been identified in healthy individuals (9). Although the composition of the microbiota varies tremendously between individuals, a common conserved "core" microbiota can be found in large cohorts of the human population (10).

We are colonized throughout our gastrointestinal tract, but the species and abundance residing in each section varies along the intestinal tract, this is partly due to nutrient availability, pH and oxygen gradient ranging from the stomach to the colon (11, 12), creating distinct environments which make different species thrive according to their preferred milieu. In humans, the bacterial density increases from the

upper to the lower part of the gastrointestinal tract, starting with the proximal intestine (duodenum) colonized by 10^3 bacteria/ml consisting of *Lactobacillus* and *Streptococcus*, continuing with the most distal part of the small intestine (ileum) with 10^6-10^8 bacteria/ml and consisting of *Enterobacteria*, *Enterococcus*, *Bacteroides*, *Clostridium*, *Lactobacillus* and *Veilonella*. Finally in the large intestine (colon), we end up with 10^{11} bacteria/ g content, with genera belonging to *Bacteroides*, *Eubacterium*, *Bifidobacterium*, *Ruminococcus*, *Peptostreptococcus*, *Propionbacterium*, *Clostridium*, *Lactobacillus*, *Escherichia* and *Streptococcus* (13, 14).

More importantly, the impact of gut microbiota on host physiology extends beyond the intestine. Either directly through microbial produced metabolites, such as bile acids and short chain fatty acids (SCFAs) (15), which are taken up from the intestinal lumen into our bloodstream, and act as signaling molecules on our cells (16), or indirectly by affecting the intestinal cells, which are in close proximity to the microbes, and alter their secretion of for example inflammation markers or hormones which in turn also leads to significant consequences for the whole body (17). Increasing evidence also suggest that the gut microbiota has an important impact on the gut-brain cross-talk, by not only local interaction with the enteric nervous system, but also with direct communication with the central nervous system (18). Moreover, various diseases have been associated with disturbances in the microbial composition (19), making it clear that the gut microbiota is both beneficial and potentially harmful for us. Since the gut microbiota can be manipulated through external factors such as diet (20-22), probiotics (23, 24), and antibiotics (25), studying the gut microbiota is a very interesting research topic for understanding and treating human diseases (26).

1.1.1 Studying the gut microbiota- a historical review

The study of gut microbiota is a rapidly growing research field that has received increasing attention the last decade-one might think that this is a relatively young research topic. However, already in 1885, the idea of using germ-free (GF) animals (animals that are completely sterile and free of microorganisms) and gnotobiotic animals (animals colonized with only one or several known strains of microorganisms) in nutritional studies was raised by Louise Pasteur. He made the following statement (27).

For several years during discussions with young scientists in my laboratory, I have spoken of an interest in feeding a young animal (rabbit, guinea pig, dog or chicken) from birth with pure nutritive products which have been artificially and totally deprived of the common microorganisms. Without affirming anything, I do not conceal the fact that if I had time, I would undertake such a study, with the preconceived idea that under these conditions life would have become impossible. If this work could be developed simply, one could then consider the study of digestion by the systematic addition to the pure food, of one or another single microorganisms or diverse microorganisms with well-defined relationships.

There are clearly several difficulties that will need to be overcome when rearing GF animals. But two main problems are: how and with what to feed the animal, and how to keep the GF animal isolated from its contaminating surroundings. Within 10 years after Pasteur´s first statement, Nuttal and Thierfelder delivered guinea pigs into a germ-free environment by caesarian section in 1895 (28). The guinea pigs survived for 13 days on a diet of diluted sterilized cow´s milk. The animals were examined post mortally and they showed enlarged caecums. Others followed Pasteur´s original concept. In 1898, Schottelius obtained the first GF chicken (29); however infection occurred in all their experiments. By 1912, Küster was able to rear two GF goats (30), which were fed with sterilized milk from the mother, to the age of 12 and 35 days respectively, before they became infected. Although the GF goats grew at the same rate as control goats reared on sterilized milk in a normal environment, Küster concluded that the importance of gut microbiota for the host cannot be determined until after the animals have been weaned.

In 1932, at the University of Lund, Sweden, Glimstedt resumed the study of GF guinea pigs in order to study the morphology and histology of lymphoid tissue (31). He made an effort on understanding the value of nutrition in rearing GF animals. They were fed sterilized milk together with solid food and vitamins which were sterile filtered, which resulted in the fact that GF guinea pigs now often survived up to 2 months. GF and control animals (animals reared in normal bacteria containing surroundings) fed on the same diet did not differ in viability, and growth was only slightly lower in GF animals compared with control animals. The GF animals showed greater appetite than the control animals, and they displayed large caecums and the consistency of their faeces was softer with higher water content. The GF animals

also exhibited immature lymphoid organs compared with the control animals.

During the 1940s, Reyniers and his co-workers at the Lobound Laboratory, University of Note Dame, US, attempted to rear higher organisms in GF conditions; they experimented with monkeys, dog, cats, rats, mice, guinea pigs and rabbits (32-34). He described the GF rearing system as a very complex program which requires a great deal of experience to design, construct and operate. It was also expensive. To run the system efficiently it required numerous specialized staff such as feeders, sterilization crews, maintenance machinist, bacteriologists, biochemists and pathologists etc. with their helpers and technicians (35-37) . The newborn pups were fed once every hour for the first 4 weeks of life which required 3 technicians to work by shifts. Reyniers used sufficient diet mixtures together with large amounts of pure vitamins and crude yeast and liver extract which resulted in good growth of the GF animals compared with the diet mixture alone (38). Reyniers and his colleagues succeeded to rear GF rats for a period of 5 months, and within this time, the GF rats gave birth to several litters which made Reyniers´ group the first one to successfully obtain a second generation of mammals in a GF environment, which circumvented the requirement for handfeeding the pups. They also succeeded in rearing GF guinea pigs for 8 months but their growth was poor, and most of the animals died of starvation after 2 months. Reyniers´ results point to the fact that the diet is of importance in rearing animals in an environment absent of microorganisms. As a matter of fact, a review of the literature from this period clearly shows that investigators who have taken care to supply large amounts of vitamins have been most successful (39).

During the same period, Gustafsson and his colleagues reestablished the GF work at Lund University. In 1948, he successfully reared GF rats (39), and by 1956 they achieved rearing of rats for consecutive generations (40).

In the 1950s, scientists had finally gained sufficient knowledge about nutritional requirements to establish and keep GF mice and rat colonies for generations to come. Altogether, it took 50 years to complete the germ-free experiment.

1.1.2 Gut microbiota and effects on host physiology

The gut microbiota is capable of many processes that are essential for several aspects of host biology. For example, they enable us to digest food particles that are otherwise indigestible and they produce vitamins (41), together with other physiological processes such as development and differentiation of the intestinal epithelium and immune system (42-44). They have also shown to be required for protection against invasion of pathogenic bacteria (3), development and maintenance of epithelial integrity and homeostasis (45), bone mass regulation (46), and host behavior (47, 48). In particular, the effect of gut microbiota on host metabolism (49, 50) has been the topic of intense research the last decade, for the purpose of this thesis, which will be discussed in more detail.

1.1.3 Gut microbiota and energy metabolism

Evolutionary, food has been scarce which have programmed animals to protect energy stores by promoting fat storage. Nowadays, diets have changed and energy-rich foods have become easily accessible; making obesity rather than undernutrition a concern for human health in many developed countries. Although, other factors than type of calorie and availability seem to be important. Recent studies performed during the past decade provide evidence which suggests that the gut microbiota might play a role in energy harvest, storage and expenditure.

Gut microbiota promotes energy harvest and storage

Since the 1950s, a number of animal species have been reared as GF, which have allowed scientists to investigate the role of microbiota in metabolism (51, 52). Two studies, using GF mice, conducted 10 years ago strongly supported the role of gut microbiota in obesity (49, 53). They showed that GF mice gain less weight compared with their conventionally-raised (CONV-R) control animals, despite that they ate more, because gut microbiota influenced energy harvest and storage and energy expenditure (49, 53). The microbiota increased energy storage by promoting *de novo* lipogenesis in the liver, and deposition of triglycerides into adipose tissue by suppressing the expression of angiopoietin-like protein 4 (a lipoprotein lipase inhibitor) (49). Furthermore, they demonstrated that introducing microbiota from CONV-R mice into GF mice resulted in increased body fat and insulin resistance within two weeks.

Altered gut microbiota in obesity

There is also evidence showing that the gut microbiota residing in obese and lean individuals is different. A transfer of the intestinal content from obese mice (*ob/ob*) into GF mice resulted in greater increase of total body fat compared with the GF mice colonized with microbiota from lean donors (54). The 'obese microbiota' is suggested to have an increased capacity to harvest energy from the diet. The same researchers also demonstrated that both in mice and humans, obesity is associated with changes in the gut microbiota composition (55, 56).

Recent studies have identified that diet, especially fat, is a strong modulator of the microbiota (50, 57). For this reason, there is increasing evidence that the high fat intake rather than the obesity *per se* has a direct effect on the microbiota (58, 59). A later study, on discordant twins for obesity, where one twin is obese and the other is lean, showed that the phenotype can be transferred with the gut microbiota, where the change in total body fat is significantly greater in the mice that received fecal microbiota from the obese twin (60). In addition, several clinical studies have suggested a role of the gut microbiota in obesity (61). However, a causal relationship between microbiota and obesity in humans remains to be demonstrated.

Although, recent studies have established the microbiota as a contributing factor for obesity, we should consider that the major factor driving obesity is the diet through excess energy intake. Diet is also the primary factor that determines the microbiota composition, where studies in both animals and humans reveal that dietary changes rapidly alter the microbiota composition (21, 22). Increasing the understanding of the interactions between diet and microbiota will provide new strategies to potentially treat and/or prevent obesity in the future.

1.2 Fasting and feeding

Glucose is one of the main fuels for all animals. It is metabolized by our cells to produce adenosine triphosphate which is used as energy to power millions of biochemical processes that take place in the body (62). We obtain glucose from the food we eat, which is absorbed in the small intestine and travels to the liver through the hepatic portal vein. In the liver, glucose is converted and stored as glycogen, which can be

reconverted to glucose and released into the circulation when blood glucose levels are low (63). All cells in the body requires energy, and most can use other fuels such as lipids (64). However, neurons are almost exclusively dependent on glucose for their energy supply (65). Therefore, it is important for the body to maintain constant blood glucose levels for the nervous system to function properly.

During the day, blood glucose levels fluctuate according to the variation in food intake. After a meal, called post-absorptive or postprandial state, the blood glucose rise, and most of it is taken up by the cells in the body to make energy and the excess is converted and stored as glycogen by the liver (66, 67), which make the blood glucose level decrease.

Several hours after a meal, blood glucose level begins to drop, and the body enters an early-fasting state. Glycogen stored in the liver is now broken down into glucose (glycogenolysis) and released into the bloodstream (68). The liver also performs gluconeogenesis, which is endogenous production of glucose using substrates such as lactate, alanine, glycerol or amino acids (68-70). Together, these two processes in the liver maintain blood glucose level also during prolonged fasting.

After several days of starvation, the cells in the body shift from glucose to fatty acid utilization for fuel (64, 71-73). Free fatty acids are released from adipose tissue through lipolysis (74, 75). The liver utilizes free fatty acids to generate energy, resulting in increased acetyl-CoA formation, which is converted to ketone bodies (acetoacetate, D-β-hydroxybutyrate, and acetone), and released into the blood (76). Ketone bodies can be used as energy by the brain during times of starvation, which can provide a third of the energy requirements (77). The heart also uses ketone bodies as fuel during starvation (78).

The body needs to maintain the blood glucose level within a relatively narrow range, despite these daily fluctuations, and the mechanisms that maintain it within these limits are greatly controlled by two important hormones-insulin and glucagon. Insulin signals the fed state, whereas glucagon signals the fasting state (62). These two hormones and their role in glucose metabolism will be discussed in more detail in the next section.

1.2.1 The effects of fasting and feeding on circulating hormones

Hormones are signaling molecules produced by glands or cells that are released into the circulation to affect whole body physiology and behavior. Hormones enable communication between organs and tissues to regulate many physiological activities such as digestion, metabolism, stress, growth and reproduction (79-82). For the purpose of this thesis, the focus will be on the hormones involved in glucose metabolism, and how they regulate fuel utilization during fasting and feeding. The key regulatory hormones of glucose metabolism are insulin and glucagon.

Insulin

After a meal, the concentration of glucose and amino acids rises in the blood, which leads to the secretion of insulin from the β-cells of the pancreas (83-85). The most potent stimulus of insulin is blood glucose, but there are other factors that can stimulate insulin secretion. These stimuli include increased plasma levels of amino acids (86), hormones released from the gut following a meal (e.g. incretins such as GIP and GLP-1) (87); and parasympathetic stimulation *via* the vagus nerve (88, 89). Insulin has three ways to lower the blood glucose after meal ingestion: 1) it signals to the peripheral tissue, primarily muscle and adipose tissue to increase their glucose uptake (90); 2) accelerates the conversion of glucose to glycogen in the liver, to promote glycogen storage (91, 92); and 3) inhibits glucagon secretion from pancreatic α-cells, which signals to the liver to stop producing glucose through glycogenolysis and gluconeogenesis (93-95). Insulin also acts to stimulate fatty acid synthesis, triglyceride storage in the adipose tissue and protein synthesis in the liver and muscle tissue (90, 96). In essence, insulin provides an anabolic signal.

Glucagon

In contrast, the blood glucose level decrease below the normal range during fasting, which stimulates the release of glucagon from the α-cells in the pancreas (97). Glucagon is the major hormone stimulating hepatic glucose production which maintains the blood glucose level at a normal range during fasting. Glucose is produced by the liver via two

processes: glycogenolysis and gluconeogenesis. During the first hours of fasting, glucose is made available primarily through glycogenolysis. Over longer period of fasting, when the glycogen stores are depleted, glucose is produced by gluconeogenesis (98). The liver is the major organ performing endogenous glucose production; however at extreme starvation the kidney also significantly contributes to glucose production to maintain systemic blood glucose (99, 100). Both organs contain Phosphoenolpyruvate carboxykinase (PEPCK) and Glucose 6-phosphatase (G6Pase), two key enzymes involved in gluconeogenesis (101, 102). They are partially controlled by glucagon and markedly upregulated by fasting (103-106). In essence, glucagon provides a catabolic signal.

The regulation of glucose homeostasis is however not mediated by only two hormones; in fact it is a multi-hormonal system. Other hormones that can influence blood glucose level are: epinephrine, cortisol, somatostatin and growth hormone which are all blood glucose raising hormones (107).

1.2.2 Fasting and gut microbiota

The gut microbiota has been shown to provide beneficial effects during fasting periods. In 1968, Tennant *et al.* observed that mice containing microbiota survived significantly longer than GF mice when starved. Although body weight loss between the groups was similar, the GF animals died sooner (108). The difference in survival rate might instead be explained by alterations in the metabolic processes in GF animals, and that they have lower flexibility to switch between fuels. A more recent study revealed that the gut microbiota regulates ketone body metabolism during fasting. Fasted GF mice had reduced hepatic ketogenesis, which lead to unfavorable metabolic changes in the heart tissue (109), and may also indicate that they are less efficient in adapting to fasting.

However, fasting and energy deprivation shapes the gut microbial community which can affect host metabolism (110-112). A recent study reported that life-long calorie restriction in mice significantly changes the gut microbiota profile, consisting of an enrichment of bacteria species positively correlated with lifespan, and depletion of

species negatively correlated with lifespan (111). In addition, another study showed that the impaired growth phenotype of undernourished children could be transmitted by the microbiota to recipient animals (110). The gut microbiota also directly affects the local energy status in the gut. Microbes produce butyrate- a short chain fatty acid (SCFA), by fiber fermentation, which is used as fuel for the epithelial cells lining the colon (colonocytes) (113, 114). Thus the microbiota is affected by both nutrient composition and amount.

1.3 The gastrointestinal tract

The gastrointestinal (GI) system is the gateway for food to enter our body. Rather than being a passive player, it sends effective signals to influence feeding behavior and contributes to the maintenance of energy balance. It is responsible for the first interaction between the food we eat and our body, through digestion and absorption of the ingested nutrients (115). Simultaneously, functioning as a highly specialized sensory organ, composed of specialized cells called enteroendocrine cells, which are located throughout the GI tract. Enteroendocrine cells are able to sense and respond to specific nutrients, by releasing gut hormones, which control energy balance by their actions on peripheral target organs (116, 117). Signals from the gut hormones are important regulators of food intake, gut motility and glucose homeostasis. Gut hormones regulate glucose homeostasis by enhancing insulin secretion following food ingestion, and is thus called incretin hormones (79). Glucagon-like peptide (GLP-1) 1 is the most well studied incretin that in addition to maintain glucose homeostasis also suppress appetite (118).

More recently, there is a growing concept that the gut microbiota is also serving as an endocrine organ (16). The gut microbiota has metabolic capacity to produce and regulate multiple metabolites that reach the circulation and influence systems that are well beyond the GI tract (50, 119-121). One such class of metabolites is bile acids that can signal through either nuclear receptors such as FXR or G-protein coupled receptors such as TGR5, which influences whole body metabolism (122, 123). Thus, with this ability to impact the functions of distal organs and systems, in many aspects, the gut microbiota resembles an endocrine organ.

1.3.1 Gut hormones

Gut hormones are released by specialized epithelial cells called enteroendocrine cells which are distributed among other cell types in the GI tract. Enteroendocrine cells represent less than 1% of the total gut epithelial cell population, but the GI tract is still the largest endocrine organ in the body, as it produces over 20 hormones. Gut hormones are expressed by different genes and produced by a variety of enteroendocrine cells. Gut hormones produced by the enteroendocrine cells include: gastrin (G cells), ghrelin (A/X cells), cholecystokinin (CCK); (I cells), serotonin (enterochromaffin cells), glucose-dependent insulinotropic peptide (GIP) (K cells), GLP-1 and peptide YY (PYY) (L cells) (124-127). The peptides are accumulated in secretory granules and secreted upon stimulation, where they can act locally or on distant organs through the bloodstream (128-130). Food ingestion, digestion and absorption of nutrients such as fatty acids, amino acid, lipids and carbohydrate, regulate gut hormone release (131). Upon release, the gut hormones act locally, peripherally and centrally to regulate energy balance, including appetite, glucose metabolism and digestion (79, 118). Several hormones involved in regulation of energy homeostasis and their functions are listed in Table 1.

Table 1. Gastrointestinal hormones involved in food intake and/or glucose homeostasis

Gut hormone	**Site of release**	**Organ**	**Function**
Cholecystokinin	I-cells	Upper small intestine	• Stimulates gall bladder contraction • Stimulates bile and pancreatic secretions • Influences gut motility • Increases satiety
Gastrin	G-cells	Stomach and Duodenum	• Stimulates gastric acid production
Ghrelin	X/A-like cells	Stomach	• Increases appetite
Glucagon like peptide-1	L-cells	Distal small intestine and colon	• Stimulates insulin release • Inhibits gastric acid release and gastric emptying • Increases β-cell mass • Increases satiety
Glucose-dependent insulinotropic polypeptide	K-cells	Duodenum and jejunum	• Stimulates insulin secretion • Influences fatty acid metabolism • Increases β-cell proliferation
Peptide YY	L-cells	Distal small intestine and colon	• Inhibits gastric motility • Increases satiety

1.3.2 Gut hormones during fasting and after feeding

The gut secretes hormonal products which communicate with the brain to regulate appetite and energy expenditure. These endocrine signals are conveyed by vagal afferent nerves innervating the GI tract (132). Vagal afferent fibers respond to a large number of gut hormones (133), and mediate their actions through communication with the brainstem and hypothalamic areas. The gastrointestinal vagal fibers terminate mostly in the nucleus tractus solitaris (NTS) of the brainstem, thus establishing a direct communication between the enteroendocrine cells, through secreted hormones, with the central nervous system (134).

However, it is also possible for hormones to enter the circulation and pass through a certain part of the brain which has a leakier blood-brain barrier, to target the CNS directly (135).

During fasting, ghrelin is released from the stomach, which increases our appetite for food intake (136). During and after eating, the levels of hormones that promote appetite decrease. Instead there is an increase of hormones released from the enteroendodocrine cells in the duodenum and jejunum in response to food ingestion. These hormones include CCK, which increases satiety and stimulate gall bladder contraction and pancreatic enzyme release (137-139), as well as GIP which enhances insulin secretion (140, 141). Other hormones such as GLP-1 and PYY are released from more distal regions of the intestine, which actions stimulates insulin secretion and satiety, together with slowing gut motility (142-146).

1.3.3 Gut hormones and metabolic disorder

The role of gut hormones in obesity has been studied immensely. Some important gut hormones and their role in obesity will be briefly discussed in this section.

Ghrelin

Many studies conducted in healthy volunteers have confirmed that ghrelin treatment increases food intake (147). Furthermore, circulating ghrelin has been shown to be negatively correlated with BMI (148). Ghrelin is suggested to have a long term effect on the role of regulating body weight, since obese individuals displayed an altered ghrelin profile in response to fasting and eating. They do not demonstrate a peak of ghrelin after fasting and the level does not rapidly fall in food response (149-151). However, mice lacking ghrelin signaling appear to have normal body weight and normal food intake (152, 153), although mice treated with ghrelin demonstrated increased food intake (154).

PYY

Obese individuals have demonstrated lower fasting PYY levels (155), as well as a blunted PYY response after food intake (156). In addition, studies in mice showed that circulating PYY levels decreased in mice fed a high-fat diet (157). The *Pyy* knockout mice generated by Batterham and colleagues displayed increased food intake and fat tissue, which could be reversed by exogenous PYY treatment (157,

158). In line with this, one clinical study showed body weight reduction in obese individuals, with PYY administration (159). Also, increased postprandial PYY response has been reported to play a role in the beneficial effects following bariatric surgery (160, 161).

GLP-1

The development of GLP-1 receptor agonists has offered new ways to treat type 2 diabetes, which effectively reduce blood glucose levels and body weight while having low risk of hypoglycemia (162). In humans, GLP-1 has shown a dose dependent reduction of food intake in both obese and lean individuals (163). There are many studies providing evidence to support the role of GLP-1 in obesity, although several studies have shown that there is no difference in fasting GLP-1 level between obese and lean humans (164). Nonetheless, weight loss in obese adults and children is associated with reduced fasting GLP-1 (165, 166). GLP-1 secretion after food intake is considerably attenuated in obese individuals compared to lean controls (167, 168), however some studies showed a different post-prandial response (169). Furthermore, several studies have reported an increase in postprandial circulating levels of GLP-1 after bariatric surgery (170). The potential role of GLP-1 on the metabolic effects following bariatric surgery will be discussed in more detail later. In addition, studies on rodents demonstrated that the administration of GLP-1 significantly reduces the food intake (171). However, mice with complete disruption of GLP-1 receptor signaling eat normally and do not become obese with aging or after high-fat intake (172, 173). Similarly, as described above, the same phenomenon was observed for ghrelin. A mouse model lacking a specific hormone does not display any phenotype, while a pharmacological treatment with the same hormone results in an observable effect.

1.3.4 Insulin-like peptide 5

Insulin-like peptide 5 (INSL5) was first identified 1999 by screening the expressed sequence tags (EST) databases (174). Human and murine INSL5 are both polypeptides of 135 amino acids, with the classical protein structure of the insulin/relaxin superfamily, consisting of an A and B-chain connected with disulfide cross-links (175). A series of studies have detected the highest INSL5 expression in colonic tissue (174), more specifically in enteroendocrine cells (176-178), and some have also revealed the presence of INSL5 in a variety of human

tissues, including the brain (179). INSL5 has been reported to bind to the G protein coupled relaxin/insulin-like family peptide receptor-4 (Rxfp4) (179-181).

While, some members of the relaxin family have important roles in reproductive physiology and tissue remodeling, the function of INSL5 remains uncertain (182). However, a recent study showed that INSL5 is a hormone secreted by L-cells in the colon, which enhances food intake in mice (178). In agreement with being an appetite increasing hormone, circulating INSL5 was increased by fasting and calorie restriction, and declined with feeding. Another study reported a role of INSL5 in regulation of glucose homeostasis, where they observed reduced insulin secretion and β-cell mass in *Insl5*-/- mice (183). In addition, INSL5 has been shown to enhance glucose-stimulated insulin secretion both *in vivo* and *in vitro*, suggesting an incretin role of INSL5 (184).

1.3.5 Gut hormones and gut microbiota

As mentioned earlier, the gut microbiota is capable of producing metabolically active metabolites which functions much like hormones in our body (16). However, accumulating evidence shows that the gut microbiota can also influence host metabolism through their impact on gut hormones (16, 185, 186). Modulating the gut microbes with indigestible dietary fiber treatments has been associated with changes in gut hormone levels. Cani and colleagues have shown a connection between gut microbes and GLP-1 levels in several studies (187). In rats, oligofructose feeding (which shifted the bacterial composition) resulted in greater number of GLP-1 producing enteroendocrine cells in the colon. Obese mice treated with prebiotic carbohydrates had altered gut microbiota and increased levels of GLP-1 and GLP-2 (188).

The pathway of which the microbiota influences the gut hormone secretion is believed to be mediated through SCFAs actions on their receptors GPR41 and GPR43, which are expressed by the enteroendocrine cells (189, 190). SCFAs are produced by bacterial fermentation of carbohydrates from the diet. Thus, SCFAs (e.g., butyrate, propionate, acetate) function as an energy source as well as a signaling molecule, and their abundance is directly related to the bacterial species composition in the gut (191-193). A recent study performed by Wichmann *et al.,* showed that GF mice had increased circulating GLP-1 and GLP-1 producing enteroendocrine cells in the

GI tract, which was associated with slower gut transit (194). Furthermore, the gut microbiota may also stimulate GLP-1 secretion through modulation of bile acids which activate TGR5 receptors located on L-cells (195).

1.4 Bariatric surgery

Over the past decade, obesity has become a major health concern all around the world (196, 197). Obesity has now reached pandemic levels in the United States, as well as other countries (198). In the United States, two thirds of the population meets the criteria for overweight, and over one third of the population is obese (199). There is growing evidence that support bariatric surgery as the most effective treatment option for severe obesity and its related diseases (200). As a result, the number of bariatric procedures has increased significantly during the past few decades (201, 202).

Bariatric surgery has been proven to be much more effective in treating obesity than the traditional non-surgical treatments such as diet and exercise (200). In contrast, dieting has been associated with only a modest long-term weight loss for most patients (203-206). In a study, less than 20% of the individuals succeeded to achieve and maintain a weight loss of 10% over 1 year (206). In contrast, bariatric surgery produces weight loss that ranges between 50% - 75% of excess body weight (207), which is maintained longer than can be achieved by changes in lifestyle (208). Some studies indicate that the weight loss can be sustained for up to 16 years after surgery (209), whereas lifestyle and pharmacological interventions often results in weight regain after 6 to 24 months (203, 210). Therefore, bariatric surgery is currently considered the most effective treatment against obesity.

1.4.1 Bariatric surgery types

Bariatric surgery is suggested to promote decreased energy intake in two ways, that is by restriction and malabsorption (211). The restriction method limits the amount of food to be consumed. Procedures such as adjustable gastric banding (AGB) and vertical sleeve gastrectomy (VSG) are examples of the restriction type of surgery. In combination with food restriction, energy intake can be limited by bypassing segments of the small intestine, which results in caloric malabsorption, an example of this type is Roux-en-Y gastric bypass (RYGB) (211). A global overview of bariatric surgery in 2013,

revealed that the most commonly performed procedure in the world was RYGB, 45%; followed by VSG, 37%; and AGB, 10% (212). In addition, VSG is currently the most popular procedure in the USA/Canada and in the Asia/Pacific regions, whereas RYGB is more frequently performed in Europe and Latin/South America. While, vertical banded gastroplasty (VBG) was the predominant procedure during the 1980s, it has now rapidly fallen in popularity due to insufficient long-term maintenance of weight loss, and long-term complications (213). A short description of some procedures follows below.

Vertical banded gastroplasty (VBG)

A partitioning of the stomach is created with staples and a rubber band, resulting in the formation of a small pouch in the upper part of the stomach. The passage of the food is restricted by the small pouch before reaching the stomach (213).

Adjustable gastric banding (AGB)

A synthetic adjustable gastric band is placed below where the esophagus is attached to the stomach, creating a stomach pouch. However, the stomach remains intact. The band can be inflated and deflated to adjust the degree of constriction around the stomach, in order to limit the amount of food the patient can eat, as well as maintaining satiety by slowing gastric emptying (211).

Vertical sleeve gastrectomy (VSG)

In the VSG procedure, 80% of the greater curvature of the stomach is removed, creating a tight funnel for the food to pass through the esophagus to the duodenum (211).

Roux-en-Y gastric bypass (RYGB)

RYGB procedure consists of a restrictive and a malabsorptive component. It involves creating a small stomach pouch which is connected to a limb of distal intestine (jejunum), bypassing the proximal intestine (duodenum). This procedure results in bypassing the potential absorption of nutrients in the duodenum and proximal jejunum. It also reduces the time in which bile and pancreatic enzymes can mix with the food (211).

A recent meta-analysis comparing studies of RYGB, VSG and AGB demonstrated similar weight loss of approximately 60% of excess body weight, was produced after RYGB and VSG, that was superior to the weight loss induced by AGB (214). AGB also had the highest number of complaints from patients (20%) who failed to achieve sufficient weight loss. In addition, resolution rate of comorbidities after surgery, such as type-2 diabetes, was lowest in AGB (207). However, AGB seems to be a safer procedure, with frequent but less severe complications (214). The more recent VSG procedure achieves similar reduction in HbA1c and also rates (24% of patients) of type 2 diabetes resolution have been reported for RYGB (38% of patients) after 3 years (215).

1.4.2 Bariatric surgery and metabolic improvements beyond weight loss

A significant cause of death in obese patients is cardiovascular disease (216). Obese patients have dyslipidemia, a plasma lipid profile which is associated with increased risk of cardiovascular disease (217). Bariatric surgery has other powerful effects than only to reduce body weight; it also produces improvements on other metabolic parameters such as glucose tolerance and plasma lipids. One study reported that at least 70% of the patients, which have been subjected to different types of bariatric procedures, displayed an improved plasma lipid profile post-surgery (207). Specially, improvements have been reported in humans after RYGB (218), AGB (219) and VSG (220). However, RYGB has been uniquely reported to lower the total cholesterol in comparison to the other two surgery types (221, 222). Since RYGB appear to induce weight loss superior to AGB, but comparable to VSG, the key question is whether the improved lipid profile occur independent of weight loss.

Diabetes is also a comorbidity frequently related to obesity (223). Typically, patients with abnormal glucose tolerance have higher insulin levels after glucose administration, which is a compensation for reduced insulin sensitivity. Prediabetes and type 2 diabetes represent different degrees of insufficiency in glucose-induced insulin response. As the disease progresses, β-cell mass is reduced, and eventually the β-cells fail to secrete insulin (224). Some bariatric surgeries result in drastic improvements of glucose homeostasis. Improved glucose homeostasis can be a result of improved insulin secretion and sensitivity, which can be achieved by weight loss. This is also the case

after bariatric surgery, but the effects occur sooner than expected. Fasting glucose has been reported to reduce 1 week after RYGB (225, 226) and VSG (227, 228) procedure, which was before any substantial weight loss could be observed. The rapid effects on glucose regulation support the evidence of the improvements being independently of weight loss produced by the procedures (229, 230). In contrast to RYGB and VSG, improvements in glucose homeostasis after AGB are entirely dependent on weight loss (231). In addition, both RYGB and VSG had greater increase in insulin sensitivity than that observed in AGB patients (232).

1.4.3 Bariatric surgery and potential mechanisms

Bariatric surgery is currently the most effective treatment for sustained weight loss and reduction of obesity-related comorbidities. The underlying mechanisms mediating the beneficial effects of bariatric surgery are not yet fully understood. Although it is generally thought that restriction and malabsorption are the main mechanisms mediating weight loss in bariatric surgery, metabolic improvement occur before substantial weight loss takes place, indicating that other mechanisms are also involved. Recent research has proposed that bariatric procedures achieve their physiological effects through the so-called BRAVE mechanisms (Bile flow alteration, Reduction of gastric size, Anatomical gut rearrangement and altered flow of nutrients, Vagal manipulation and Enteric gut hormone modulation) (233). Some of these mechanisms will be discussed in this section.

Food intake and preference

A basic principle of weight loss is reducing energy intake. A simple explanation to the successful weight loss following bariatric procedures is that the reduced stomach volume is physically limiting the food intake. As a matter of fact, many reports show that patients who undergo bariatric procedures decrease their food intake and eat smaller meals after surgery (234-236). However, the mechanisms important for the reduction in food intake appear to be more complex than reduced stomach size.

Bariatric patients have reported a change of food preference, selecting different foods and losing interest of other foods after surgery. Bariatric procedures, in both rodents and humans, decreased the preference for high-fat intake (237-241). Together, reduced intake of

sweet foods (242, 243), with reduced sweet taste stimuli have also been reported (244). One explanation for altered food choices after bariatric procedures can be due to the presence of adverse symptoms after the consumption of certain kinds of foods, which then drives patients to avoid these foods. These adverse symptoms occur when the nutrients reach the small intestine too quickly, which causes nausea, abdominal pain, diarrhea, palpitations, and flushing (dumping syndrome) (245, 246). Several studies have also suggested that both bariatric patients and rodents may experience decreased food reward in general, but also to certain type of foods (247-249). In addition, bariatric patients also report decreased hunger and increased satiety (250-252).

Interestingly, increased postprandial release of gut hormones including, GLP-1 and PYY, have been associated with bariatric surgery (160, 161, 253, 254). These hormones promote satiety, which can in part explain the increased feeling of satiety after surgery.

Glucose homeostasis

Besides weight loss, rapid improvement on glucose homeostasis is produced by some bariatric surgeries. This phenomenon has been hypothesized to be an effect of increased postprandial GLP-1 release (170, 255). In agreement, a series of studies have reported a correlation between the increased incretin effect and improvements in glucose metabolism after VSG and RYGB (256-258)

Another interesting hypothesis stems from the observation of increased circulating bile acids after VSG and RYGB (259). Bile acids not only facilitate the absorption of fat from the intestine, they also enter the circulation and act on nuclear transcription factors (FXR) that regulate genes involved in glucose homeostasis in the liver (123, 260). Bile acids can also activate the receptor TGR5 present in the gut, which has been linked to GLP-1 secretion (195). Bariatric surgeries performed on mice lacking the TGR5 receptor (261) and FXR receptor (262), demonstrated that the beneficial effects of surgery require an intact bile acid signaling. Thus, the increased circulating bile acids in both VSG and RYGB can be important mediators of the metabolic improvements in these procedures (263, 264).

Recent studies have also indicated that the gut microbiota may mediate some of the beneficial effects of bariatric surgery. Both short-term and

long-term changes to the microbiota composition have been observed after RYGB in humans (265-267), as well as after VSG and RYGB in mice (262, 268). Recent studies showed that GF mice which received the surgically altered microbiota gained less fat, which suggests that these microbial changes are capable of modulating host metabolism (267, 268). Interestingly, gut microbiota has also been reported to regulate the bile acid pool (269). Future studies will elucidate the interaction between bile acids and gut microbes and their role in bariatric surgery.

2 AIM

The overall aim of my thesis was to address how the gut microbiota modulates the expression of INSL5 and to investigate whether it was regulated by bariatric surgery, and its potential involvement in mediating the beneficial effects of the procedure. My third aim was to investigate whether the microbiota contributed to the beneficial effects of bariatric surgery.

3 RESULTS

3.1 Paper I

3.1.1 *Insl5* expression in the colon is regulated by microbiota and energy availability

Insl5 in the colon was found to be one of the most significantly regulated genes in a micro-array based screen (270). We confirmed this finding by quantitative real-time polymerase chain reaction (qRT-PCR) in two separate mouse strains. We showed that *Insl5* expression was significantly upregulated in colon of GF Swiss Webster and C57Bl/6 mice compared with their CONV-R counterparts (Paper I Fig 1A and E). We also showed that reducing the bacterial load in the mice with antibiotic treatment significantly increased the *Insl5* expression in the colon (Paper I Fig 1B).

Cells in the colon use primarily SCFAs (microbial metabolites produced as end-products of fermentation activities) as energy substrate (271). Thus, in GF mice the absence of microbiota leads to significantly reduced SCFAs levels (194), and as a result energy-deprived colonocytes (113). The lack of energy in GF colonocytes has been previously reported to increase *Gcg* (the gene coding for GLP-1) (194). The increased *Gcg* expression and GLP-1 levels in the GF mice resulted in slower intestinal transit. We hypothesize that this is a physiological adaptation which allows more nutrient and energy to be absorbed in an energy deficit state and that the elevated *Insl5* expression in GF colon is caused by reduced energy levels.

Indeed, we found that increasing the energy availability with energy-rich diets or colonization in the GF mice restored the *Insl5* expression to normal levels (Paper I Fig 1D, and F). Our data suggest that INSL5 may play a role when energy status is low, which is in agreement with an earlier study reporting that plasma INSL5 is induced by fasting, and reduced by feeding (178).

3.1.2 The physiological role of INSL5

By performing immunohistochemistry, we confirmed that INSL5 is an L-cell hormone which is specifically expressed in the colon (178). Physiologic functions commonly described for L-cell hormones are

appetite regulation and gut transit (79, 118). To investigate whether INSL5 is involved in similar processes we compared wild-type and *Insl5*-/- mice, but did not obtain evidence for a role of INSL5 in regulation of food intake and gut transit (Paper I Fig S2D and E). These results contradict an earlier study by Grosse et al. reporting that INSL5 is a hormone that increases appetite. However, Grosse et al. studied the acute effect of an INSL5 injection, while we used a whole-body *Insl5* knockout mouse model. It is not uncommon to observe a significant effect of an exogenous peptide administration, while knocking out the same peptide in a mouse fails to demonstrate any phenotype (272). This has been previously described for ghrelin and GLP-1 (152, 154, 171, 172). A lack of effect in knockout mice can be due to redundant systems that can compensate for the loss of function and other adaptive responses. Another possibility is that while lacking an appetite hormone may not have an effect on feeding behavior, the increase of the same hormone might exhibit a pronounced effect (172). Thus, it will be interesting to investigate the role of INSL5 on appetite in transgenic mice with *Insl5* overexpression. Interestingly, previous studies have reported increased food intake in GF mice (49), which we here report have increased *Insl5* expression. However, the role of INSL5 in GF mice remains unclear.

3.1.3 INSL5 and hepatic glucose production

We found that *Insl5*-/- mice had impaired intraperitoneal glucose tolerance (Paper 1 Fig 3C), which is in agreement with a recent report by Burnicka-Turek et al (183). However, we did not observe any difference in oral glucose tolerance between the two genotypes (Paper I Fig 3A). A possible explanation for the different responses of the two glucose tolerance tests is the fact that oral but not intraperitoneal administered glucose activates the parasympathetic gut-brain axis which promotes increased glycogen storage and inhibits glycogenolysis (273-276). Interestingly, *Insl5*-/- shows lower glycogen levels after 6 hours fast (Paper I Fig 3J), indicating an altered glycogenolysis, which can contribute to the increased glucose level observed after an intraperitoneal glucose injection.

Surprisingly, we next found that *Insl5*-/- mice have improved insulin tolerance (Paper I Fig 3E), which usually is not accompanied with an impaired glucose tolerance. The blood glucose profile after insulin administration, suggested that the insulin sensitivity is similar between *Insl5*-/- and WT mice, it is rather the counter-regulatory responses (i.e.,

release of hormones such as glucagon and catecholamines) that are delayed or impaired in the *Insl5*-deficient mice. An alteration in counter-regulatory responses may affect the hepatic glucose production during conditions of low energy. Indeed, we found that *Insl5*-/- mice displayed a slightly reduced hepatic glucose production following a pyruvate tolerance test (Paper I Fig 3F), together with reduced liver G6Pase levels and activity (Paper I Fig 3G and H) after 12 fasting, which suggest reduced gluconeogenesis in the mice. We speculate that the reduced glycogen availability observed after 6 hours fast, together with the reduced gluconeogenesis could potentially explain the delayed ability to counterbalance the reduction in blood glucose during an insulin tolerance and further implies that INSL5 may be important to mediate response to energy deprived conditions (e.g., increasing glucose production).

The INSL5 receptor Rxfp4 has been detected in various tissues, including liver (176) and myenteric neurons (178), therefore INSL5 might act on liver directly or indirectly through a gut-brain-liver axis to stimulate hepatic glucose production. INSL5 might also mediate its effect indirectly through modulation of counter-regulatory hormones such as glucagon and catecholamines.

Taken together, our data suggests that INSL5 might play a role in hepatic glucose production during fasting or low blood glucose conditions, although its effect is mild. We speculate that we might observe more pronounced effects of INSL5 on glucose maintenance in mice exposed to extreme starvation or long-term calorie restriction.

3.2 Paper II

3.2.1 INSL5 levels are increased following sleeve gastrectomy in humans

To investigate whether INSL5 is regulated in obesity we analyzed serum levels in obese and lean individuals, but did not observe significant differences. In contrast, we found that fasting circulating INSL5 levels were increased 6 months following VSG. We also observed that INSL5 levels were reduced upon a test meal in VSG patients (Paper II Fig 1). Attenuated postprandial gut hormone responses have previously been associated with obesity (149, 167), and increased following bariatric surgery (160, 255, 258). INSL5 is an

orexigenic hormone which is increased by fasting and calorie restriction and declined with feeding in mice (178), and we have reported that *Insl5* expression in colon is upregulated by reduced energy availability (277). Thus we speculate that the increased fasting INSL5 levels following VSG surgery is due to low energy status in the body and in particular in the colon due to reduced stomach size and overnight fast. Ingestion of a meal increases the energy availability in the body and may thus reduce the INSL5 levels.

It is currently unknown if the increased INSL5 levels can be attributed to the physiological changes in the gut anatomy produced by the VSG *per se*, or indirectly by the significant weight loss caused by calorie restriction and a 'fasting' condition following VSG. In addition, the fact that we did not observe any difference in serum INSL5 between obese and lean individuals suggest that the increased INSL5 levels after VSG is not a result of a lower body weight *per se*, but rather a negative energy balance caused by reduced energy intake compared with previous eating behavior which is the case in the VSG patients but not the lean individuals.

Ghrelin is an orexigenic hormone, similar to INSL5; it rises with prolonged fasting and decreases after a meal. Therefore, it has been shown to increase after weight loss via calorie restriction (278). Interestingly, anorexia nervosa patients demonstrated increased fasting ghrelin levels which decrease upon an oral glucose tolerance (279). In contrast, clinical studies on RYGB and VSG have reported a significant decrease in ghrelin levels, which might be due to reduced contact between the ghrelin producing cells and ingested nutrients (280, 281). However, beneficial effects of VSG surgery in rodents proved to be ghrelin independent (282).

Future studies may reveal whether calorie restriction or anorexia induce INSL5 in humans. In addition, it will be interesting to study whether circulating INSL5 in healthy lean individuals demonstrate a postprandial response.

3.2.2 *Insl5* deficiency in mice does not affect VSG-mediated beneficial effects

To investigate whether the elevated levels of fasting INSL5 observed after VSG in humans contributed to beneficial outcomes of the surgery we performed VSG in wild-type and *Insl5*-deficient mice. We

observed that VSG produced similar reduction in body weight, body fat and food intake in *Insl5* WT and KO mice (Paper II Fig 2A-C), as well as similar improvements in oral glucose tolerance (Paper II Fig 3A,B). The relative insulin release (expressed as a percentage of basal insulin) increased to a similar extent in WT and KO mice (Paper II Fig 3D). Overall, our findings indicate that in mice INSL5 is not essential for mediating the beneficial effects produced by VSG, but we cannot exclude that it may confer the beneficial effects in humans. Similarly, recent studies reported that bariatric surgery produced similar metabolic improvements between mice genetically disrupted for the GLP-1 receptor and WT mice (283, 284). This suggests that although increased GLP-1 has been reported following RYGB and VSG (255, 258), GLP-1 activity does not appear to contribute to the improved metabolic features following bariatric surgery.

To investigate whether circulating INSL5 levels also were increased in mice following VSG, we attempted to measure circulating INSL5 in serum obtained from WT mice using ELISA. However, we failed to achieve any conclusive results, due to nonspecific signal produced by the ELISA; this issue has been previously reported (178). Thus, we measured *Insl5* expression in the colon on RNA level instead. Interestingly, we did not observe any gene expressional changes on *Insl5* (data not shown) in WT-VSG compared with WT-sham mice. We speculate that it might be due to the fact that we harvested the tissues from the mice 12 weeks post-surgery, which is a time when the mice have already recovered from the acute weight loss produced by VSG initially, and instead they have reached a state where they are steadily gaining weight which is surpassing their starting weight pre-surgery.

The elevated INSL5 in humans might just be a physiological adaptation to the altered physiology following bariatric surgery, rather than contributing to the improved metabolism. However, in general, a dietary change post-surgery also contributes to the beneficial effects of bariatric surgery. Furthermore, altered food preference after bariatric surgery has been observed in both rodent and clinical studies (241, 247, 285). A difference between the human cohort and our rodent study is that the human subjects were to choose freely what to eat post-surgery, whereas the WT and KO mice were restricted to the high fat diet we provided them with. Interestingly, INSL5 has been suggested to be involved in food preference, where mice lacking *Rxfp4* (the putative receptor for INSL5) demonstrated reduced preference for

high-fat food (178). Thus it would be interesting to speculate whether we would observe a different metabolic improvement of the mice lacking INSL5 if they were allowed to choose what type of food to eat.

In summary, we have found that VSG induces fasting circulating INSL5 levels in humans following the procedure; however by performing VSG on *Insl5*-deficienet mice we conclude that INSL5 seems not to contribute to the beneficial effects mediated by VSG.

3.3 Paper III

3.3.1 Sleeve gastrectomy alters the composition of the human gut microbiota

Our previous observations that the microbiota is altered after RYGB (267) led us to test the hypothesis that the altered microbiota can contribute to the beneficial effects of bariatric surgery. To this end we had to switch to VSG as this methodology is more easily performed in mice and can be adapted to GF mice. First, we confirmed that VSG strongly affects the microbiota using a longitudinal approach. Six months after surgery we observed an increased abundance in lactic acid bacteria, Actinobacteria and Proteobacteria, together with decreased abundance of several clostridia species (Paper III Fig 1A). 18 months after surgery we observed further increased abundance of Proteobacteria and in lactic acid bacteria, and decreased abundance of several *Prevotella* species (Paper III Fig 1B). Reduced abundance of clostridia has also been shown in a small cohort of VSG patients (three individuals followed up to 6 months after surgery) (286). In addition, the increased abundance of Proteobacteria and lactic acid bacteria has also been reported in two recent mouse studies (261, 262). We also observed a similar microbiota profile in our conventionally-raised VSG mice (Paper III Fig S1). Taken together, our findings, in combination with previous studies, suggest that there is a conserved gut microbial composition shift following VSG. The microbial profile after VSG consists of an enrichment of Proteobacteria and lactic acid bacteria and a depletion of clostridia and *Prevotella*. Clostridia and *Prevotella* are microbial species that are able to metabolize complex dietary fiber and produce SCFAs (287).

Interestingly, the shifts in microbiota composition could be due to changes in dietary regimes, because reduced abundance of clostridia

has also been associated with a low calorie diet which consists of high protein/low carbohydrate intake (288, 289). Also, the decrease in *Prevotella* could be due to reduced consumption of complex dietary fiber (290), which is supported by the fact that VSG patients initially consume liquid food postoperatively. However, similar microbiota compositional shifts (increase in Proteobacteria and lactic acid bacteria, along with a decrease in clostridia) have been observed in individuals taking proton pump inhibitor drugs (291, 292), indicating that the increase in intestinal pH may serve as the main driver of changes in gut microbiota after VSG surgery.

3.3.2 Surgically-altered gut microbiota may directly contribute to the beneficial effects of sleeve gastrectomy on glucose metabolism

We showed that by transferring the VSG-altered gut microbiota from humans to GF recipient mice, we also transferred the improved glucoregulatory phenotype of VSG patients (Paper III Fig 2). These results indicate that the surgically-altered gut microbiota might directly contribute to the beneficial effects on glucose metabolism induced by VSG. Next, we showed that VSG surgery induced greater improvement on weight loss, glucose and insulin metabolism in CONV-R mice compared with GF mice, indicating that the gut microbiota is required for mediating the beneficial effects following VSG (Paper III Fig 3). We showed consistently reduced fasting glucose and HOMA index, together with increased relative insulin release during a glucose tolerance test in CONV-R VSG, but not GF VSG mice in comparison to sham-surgery control animals. In addition, the improvements in glucose and insulin levels in VSG mice could be transferred by microbiota transplantation to VSG-microbiota recipient mice (Paper III Fig 5). Our results indicate that the gut microbiota is involved in mediating the improved glucose metabolism following VSG.

Bariatric surgery mediated beneficial metabolic effects have been shown to require the bile acids receptor TGR5 (261), as well as the bile acids receptor FXR (262). As the gut microbiota play an important role in the regulation of bile acid metabolism (269), we will continue to investigate whether the beneficial effects of VSG might be mediated by the gut microbiota´s modulation of bile acid composition and the actions of bile acids on their receptors.

4 METHODOLOGICAL CONSIDERATIONS

Animal models are indispensable tools in biomedical research, as they have enabled us to understand underlying mechanisms to human diseases. Here, I will briefly discuss some considerations that I had in mind regarding the animal models that have been used in this thesis. For specific descriptions of the methods included in the thesis, please refer to the material and methods sections of Paper I, Paper II and Paper III.

4.1 The utilization of two different mouse strains-C57Bl/6 vs. Swiss Webster

In paper I we used two different mouse strains, C57Bl/6J and Swiss Webster mice. Interestingly, we observed that *Insl5* expression was 80-fold higher in GF compared with CONV-R mice on Swiss Webster background, whereas it only was 7-fold higher in GF compared to CONV-R mice on C57Bl/6J background. Thus, I thought it would be interesting to discuss the differences between the two mouse models.

The C57Bl/6 is the most widely used inbred strain. More than 90% of all world´s publications based on mouse studies were referenced to C57Bl/6 (293). It is very well-characterized and the first mouse genome to be fully sequenced in 2005. The genome is fairly stable and homogenous which allows for reproducible experiments (294). C57BL/6 mice are commonly used for generating transgenic mouse models to serve as physiological and pathological models for *in vivo* experiments as well as for elucidating signaling mechanisms. C57Bl/6 mice breed well, are long-lived, have a low susceptibility to tumors and have fairly low body weights (male weighs approx. 25 grams) at 10 weeks of age. Other typical characteristics of this mouse strain include high susceptibility to diet-induced obesity and development of insulin resistance (295). Thus, C57Bl/6 is a popular model for studying metabolic disorders.

The Swiss Webster mouse model is an outbred strain. In comparison to inbred strains, outbred strains are more genetically heterogeneous. Most commercial suppliers set up breeding strategies which aim to maintain the maximum heterozygosity in the population (296). Genetic traits of being an outbred strain include long life span, high disease

resistance, large and frequent litters, and large size (approx. 40 grams at 10 weeks of age). Swiss Webster mice are preferably used in research where the genotype is not important. For that reason it has been widely used as an all-purpose stock and for drug safety testing. Also, female Swiss Webster mice have excellent nurturing abilities, and thus they are often used as recipient mothers to nurture pups that have been delivered by caesarian section from other strains (297).

Interestingly, we have recently observed that C57Bl/6 and Swiss Webster mice harbor slightly different microbiota profiles (Kovatcheva-Datchary, unpublished data, 2016). Moreover, the same strain of mice also harbors unique microbial communities in different animal facilities associated with different metabolic outcomes (298). The different microbial species present in the two models or perhaps other intrinsic host factors might cause distinct responses to an environment absent of microbes, and thus also different levels of *Insl5*.

4.2 The germ-free mouse model

The GF mouse model is widely used for studying the role of microbiota in mammalian physiology (52, 299). GF animals are born in a sterile environment, which is supplied by filtered sterile air, as well as sterile autoclaved food and water (300). The use of the GF mouse model has enabled us to answer questions regarding how a complete absence of microbes will affect different aspects of host physiology. Rederivation of transgenic mice into the GF environment has also allowed us to study whether actions of specific genes are microbiota dependent. The fast growing development of new sequencing platforms has enabled us to study the microbiota composition in various conditions. Although, many recent clinical studies report an altered microbiota composition in relation to different diseases, they have often only been association studies and have not established whether the microbiota is a cause or consequence of a disease. However, colonization of GF mice with microbiota from hosts with different phenotypes or with specific bacterial strains has enabled us to study the causal link between the microbes and the disease conditions.

To study GF mice we use their CONV-R counterparts as controls. CONV-R mice have a normal microbiota and are kept SPF (specific pathogen free), and they are fed the same autoclaved diet as the GF mice. There are some important physiological differences between GF

and CONV-R mice: GF mice are leaner, have improved glucose tolerance and an immature immune system compared with their CONV-R counterparts. In addition, the GF and CONV-R mice have been bred in separate colonies for several generations, which increase the risk of genetic drift between the two populations. Taken these factors into account, an observed difference in phenotype might be due to not only the microbiota but also other intrinsic factors. Therefore, while designing experiments we also include CONV-R mice treated with antibiotics that reduce a large amount of the microbiota to mimic the GF condition, to confirm our findings in the GF mice. We also colonize GF mice with cecum microbiota obtained from CONV-R mice to render conventionalized (CONV-D) control animals.

4.3 Pros and cons of using knockout mice

The ability to selectively delete specific target genes in mice, generating knockout mice, has provided a powerful tool to understand the underlying mechanisms to diseases. This technique can also be used to generate mouse models that express specific disease-associated proteins by replacing the normal gene with one containing a specific mutation. Production of knockout mice has revolutionized biomedical research, and it is still a powerful tool for modeling human diseases in mice. However, there are some potential limitations.

Although, many genes linked to diseases in humans have a homolog in mice and approximately 70% of the gene-coding sequences are shared between humans and mice, there are many DNA sequences and variations that are not shared (301). This could potentially limit the mouse as a disease model, because a deletion of the gene may not produce an observable change in the mouse, or even produce different characteristics compared with humans with the same deletion. In addition, unexpected compensatory effects or activation of redundant mechanisms can occur when the gene of interest is missing and potentially mask the actual contribution of the gene in normal physiology. In principle, knocking out a gene, the absence of the gene is being studied and not the effects of the gene directly. Therefore, for some genes which convey their effects when they are increased, removal of the same genes may not necessarily produce an observable effect.

Nonetheless, there are many important advantages to using knockout mice in research: 1) deletion of a gene is usually a very precise and

"clean" approach; because 2) the effects of a gene product can be studied without the potential side-effects and/or off-target effects of a pharmacological intervention; 3) there is no uncertainties regarding the degree of activation/ blockade of a specific gene product, in contrast to pharmacological treatments; and 4) deletion of a gene generates a chronic ablation which facilitates long-term studies without the need to invest in long-term pharmacological interventions.

4.4 Bariatric surgery in mice and rodents

Despite the large degree of similarities between humans and mice in anatomy, physiology and genetics, there are some differences worth mentioning in interpreting and extrapolating findings from rodent studies to the human condition after bariatric surgery. In rodent experiments, after surgery the animals encounter a situation that would never be the case for humans: they are restricted to the same high-fat diet that induced their obesity in the first place. Despite this, dramatic weight loss occurs in rodents after RYGB and VSG (240, 302). Even though the animals are not given the opportunity to choose different or healthier foods, the procedures result in significant weight loss and improvements of metabolic parameters. Therefore, it seems that altered food preference is a side effect of RYGB and VSG surgeries rather than the primary factor for mediating the beneficial effects.

In contrast to humans, rodents keep growing throughout life (303), which together with high fat diet, results in animals regaining their weight after the initial weight loss a few weeks postoperatively. However, the animals that underwent bariatric surgery will remain a lower body weight compared to their sham controls, during the course of the study.

An altered food choice after bariatric surgery can possibly be a reaction to food intolerance, which are aversive symptoms following the consumption of certain types of foods (245, 246). Thus, bariatric patients are driven to avoid those foods. Vomiting is one of the most common food intolerances (304). However, animal studies of food intolerance are few, because of the difficulties of assessing those symptoms in rodents, due to the fact that they cannot vomit (305). In addition, a reduction in sweet taste detection and a loss of cravings for sweets have been reported for humans (243). However, assessing these changes in rodents is less conclusive. Although, this has been studied in rodents, the procedures used (e.g., the licking rate test and two-

bottle choice test) do not distinguish between detection and liking of the sweet stimuli (247, 306).

Finally, considering the increasing number of reports that support the role of microbiota and bile acids in mediating the metabolic improvements following bariatric surgery, we need to address the fact that there are considerable differences in bile acid and microbiota profiles between humans and mice (307, 308). This may potentially differentiate the response to bariatric surgery between the two species.

5 CONCLUSION

The conclusions from this thesis are:

- INSL5 is regulated by the gut microbiota and energy availability.
- INSL5 is an L-cell hormone that could play a role in promoting hepatic glucose production during energy deprived conditions.
- Circulating fasting INSL5 is increased following VSG in human individuals, but INSL5 is not required for the beneficial effects observed after VSG in mice.
- VSG alters the composition of the gut microbiota.
- VSG-altered gut microbiota plays a direct role and is required for the improvements in glucose metabolism following VSG.

ACKNOWLEDGEMENT

This was all about the journey and not the destination, but without you I would not have arrived at all. Therefore, I would like to thank:

Fredrik Bäckhed, my supervisor. You have been an amazing mentor to me. You are brilliant in the way you think and in the way you do things which inspires me to become a better scientist. You guided and supported me through some tough times, thank you for believing in me, my abilities and my data, in times when I did not. I feel grateful to have a supervisor like you, who can be so organized, sharp and focused, but also so relaxed and funny about things at the same time. Our meetings have been so much fun; I never know what we will end up discussing☺

Valentina Tremaroli, my co-supervisor. You are one of the smartest and most kindhearted people I know; I look up to yo you as a person and as a scientist. Thank you for teaching me labwork and patiently mentoring me through it all, you were always there when I needed you.

Jan Borén, thank you for being my co-supervisor.

Bäckhed lab, I couldn´t have wished for better colleagues. I feel very fortunate to work with people that are as friendly and inspiring as you guys. Everyone is always ready to help, no problem is too big or too small. There is always someone to turn to whenever I face a problem or when I just need to talk. Thank you everyone!

Lisbeth, for so smoothly and efficiently dealing with all the administrative stuff. I feel so lucky to have had your help during the past years; there are very few things that you do not know or cannot fix.

Coauthors of my papers, **Filipe**, for all your help with the glycogen and G6Pase measurements, without your help we wouldn't have any story to tell, **Anita**, your work on GLP-1 in GF mice lead my project into the right direction; your samples have been invaluable. **Antonio**, first of all, thank you for getting the human bariatric samples,

secondly, it was awesome to work with you, I like the way you think that we should not think, and hopefully we will find more “cats” at EBM in the future. **Carina**, the best hobby surgeon, thank you for all the surgeries you have done for my project, me and you are a great VSG team; remember if nothing works out I am going into the shaving business. **Lisa**, for analyzing the metagenome data, thank you for spending all those hours in front of the smelly hood. **Rosie**, thank you for all your help with the papers, you were great coaching me through it all. I am so grateful that you were there when we faced all the deadlines. In fact I don´t think I could have made it without you.

All the members of **Wlab**, for making it such a great environment to work in. During the past years, Wlab has become my second home away from home, and I am happy that I am sharing this place with you. And I feel so spoiled to have you **Sven**, **Magnus**, **Merja** and **Ulrika** around. Thank you for making sure everything runs smoothly in the lab, and thank you for all the great talks.

My fellow PhD students, **Aditi, Ismena, Marta, Matias, Tony, Yangzhen,** for being there to share my ups and downs. And for all the crazy laughs we had about silly things. You are all unique in your own way, and I admire you all for it, and together we make an awesome team.

People in my writing room, **Anwar, Elin, Malin, Ismena, Marta, Sama, Tony, Yangzhen,** for making me feel so comfortable and welcomed in the writing room. For all the discussions we have had between heaven and earth. I may be biased, but I think we have the best writing room in Wlab.

Apotekarna, **Anna, Elisabet, Jennifer, Karima, My, och Trang, Le** för att ni fick mig att komma tillbaka till Apotekarprogrammet. Att få lära känna er var nog det bästa med hela utbildningen, ni är bland de goaste och snällaste personerna jag känner.

”Utflyktsgruppen”, **Jenny och Karin**, för alla roliga utflykter vi har gjort tillsammans. Tack för att ni påminner mig om att det finns en

värld utanför jobbet också. Och det finns så mycket kvar att upptäcka ihop.

Mobina, för att du är en otrolig bra vän och för att du alltid är så glad. Tack för att du alltid får mig på bra humör.

Kristina, för att du har varit min vän sedan vi var små. Tack för att du är en fantastisk vän, och för att du alltid har varit den starkare av oss två. Jag skulle också vilja tacka dig, **Markus, Sigrid** och **Hjalmar**, för att jag får vara en i familjen.

Pappa, for your positive and encouraging ways. Thank you for pushing me out of my comfort zone when you knew I could handle it, but at the same time being so supportive when I was feeling weak. There is never a dull moment spent with you.

Pengeh, för att du är min syster, så olika men ändå så lika, tack för att du ibland får ta hand om mig även om du är min lillasyster. För allt vi har delat hittills och för allt som komma skall. Om jag mot all förmodan skulle vinna på lotto eller om allt skiter sig så är du den första jag kommer dela allt med.

Mamma, för att du har varit allt för mig. Det finns ingen lika bra som du att ha vid sin sida när det är kris. Jag beundrar dig för din energi och styrka, det finns inget du inte klarar av. Du har lärt mig att livet både är för kort och för långt för att hålla på med saker man ogillar, men om man har ett mål så ska man kämpa för att nå det. Men allt brukar ändå ordna sig till slut.

REFERENCES

1. Ley RE, Peterson DA, Gordon JI. Ecological and evolutionary forces shaping microbial diversity in the human intestine. Cell 2006;124:837-848.
2. Gill SR, Pop M, Deboy RT, Eckburg PB, Turnbaugh PJ, Samuel BS, Gordon JI, Relman DA, Fraser-Liggett CM, Nelson KE. Metagenomic analysis of the human distal gut microbiome. Science 2006;312:1355-1359.
3. Smith K, McCoy KD, Macpherson AJ. Use of axenic animals in studying the adaptation of mammals to their commensal intestinal microbiota. Semin Immunol 2007;19:59-69.
4. Ley RE, Lozupone CA, Hamady M, Knight R, Gordon JI. Worlds within worlds: evolution of the vertebrate gut microbiota. Nat Rev Microbiol 2008;6:776-788.
5. Dave M, Higgins PD, Middha S, Rioux KP. The human gut microbiome: current knowledge, challenges, and future directions. Transl Res 2012;160:246-257.
6. Reyes A, Haynes M, Hanson N, Angly FE, Heath AC, Rohwer F, Gordon JI. Viruses in the faecal microbiota of monozygotic twins and their mothers. Nature 2010;466:334-338.
7. Scarpellini E, Ianiro G, Attili F, Bassanelli C, De Santis A, Gasbarrini A. The human gut microbiota and virome: Potential therapeutic implications. Dig Liver Dis 2015;47:1007-1012.
8. Sender R, Fuchs S, Milo R. Are We Really Vastly Outnumbered? Revisiting the Ratio of Bacterial to Host Cells in Humans. Cell 2016;164:337-340.
9. Structure, function and diversity of the healthy human microbiome. Nature 2012;486:207–214.
10. Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, Nielsen T, Pons N, Levenez F, Yamada T, Mende DR, Li J, Xu J, Li S, Li D, Cao J, Wang B, Liang H, Zheng H, Xie Y, Tap J, Lepage P, Bertalan M, Batto JM, Hansen T, Le Paslier D, Linneberg A, Nielsen HB, Pelletier E, Renault P, Sicheritz-Ponten T, Turner K, Zhu H, Yu C, Li S, Jian M, Zhou Y, Li Y, Zhang X, Li S, Qin N, Yang H, Wang J, Brunak S, Dore J, Guarner F, Kristiansen K, Pedersen O, Parkhill J, Weissenbach J, Meta HITC, Bork P, Ehrlich SD, Wang J. A human gut microbial gene catalogue established by metagenomic sequencing. Nature 2010;464:59-65.
11. Albenberg L, Esipova TV, Judge CP, Bittinger K, Chen J, Laughlin A, Grunberg S, Baldassano RN, Lewis JD, Li H, Thom SR, Bushman FD, Vinogradov SA, Wu GD. Correlation between intraluminal oxygen gradient and radial partitioning of intestinal microbiota. Gastroenterology 2014;147:1055-1063 e1058.

12. Kohl KD, Stengel A, Samuni-Blank M, Dearing MD. Effects of anatomy and diet on gastrointestinal pH in rodents. J Exp Zool A Ecol Genet Physiol 2013;319:225-229.
13. Ridlon JM, Kang DJ, Hylemon PB. Bile salt biotransformations by human intestinal bacteria. J Lipid Res 2006;47:241-259.
14. Sekirov I, Russell SL, Antunes LC, Finlay BB. Gut microbiota in health and disease. Physiol Rev 2010;90:859-904.
15. Tremaroli V, Backhed F. Functional interactions between the gut microbiota and host metabolism. Nature 2012;489:242-249.
16. Clarke G, Stilling RM, Kennedy PJ, Stanton C, Cryan JF, Dinan TG. Minireview: Gut microbiota: the neglected endocrine organ. Mol Endocrinol 2014;28:1221-1238.
17. Sommer F, Backhed F. The gut microbiota--masters of host development and physiology. Nat Rev Microbiol 2013;11:227-238.
18. Carabotti M, Scirocco A, Maselli MA, Severi C. The gut-brain axis: interactions between enteric microbiota, central and enteric nervous systems. Ann Gastroenterol 2015;28:203-209.
19. Carding S, Verbeke K, Vipond DT, Corfe BM, Owen LJ. Dysbiosis of the gut microbiota in disease. Microb Ecol Health Dis 2015;26:26191.
20. Albenberg LG, Wu GD. Diet and the intestinal microbiome: associations, functions, and implications for health and disease. Gastroenterology 2014;146:1564-1572.
21. Wu GD, Chen J, Hoffmann C, Bittinger K, Chen YY, Keilbaugh SA, Bewtra M, Knights D, Walters WA, Knight R, Sinha R, Gilroy E, Gupta K, Baldassano R, Nessel L, Li H, Bushman FD, Lewis JD. Linking long-term dietary patterns with gut microbial enterotypes. Science 2011;334:105-108.
22. David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, Ling AV, Devlin AS, Varma Y, Fischbach MA, Biddinger SB, Dutton RJ, Turnbaugh PJ. Diet rapidly and reproducibly alters the human gut microbiome. Nature 2014;505:559-563.
23. Degirolamo C, Rainaldi S, Bovenga F, Murzilli S, Moschetta A. Microbiota modification with probiotics induces hepatic bile acid synthesis via downregulation of the Fxr-Fgf15 axis in mice. Cell Rep 2014;7:12-18.
24. Petschow B, Dore J, Hibberd P, Dinan T, Reid G, Blaser M, Cani PD, Degnan FH, Foster J, Gibson G, Hutton J, Klaenhammer TR, Ley R, Nieuwdorp M, Pot B, Relman D, Serazin A, Sanders ME. Probiotics, prebiotics, and the host microbiome: the science of translation. Ann N Y Acad Sci 2013;1306:1-17.
25. Cox LM, Blaser MJ. Antibiotics in early life and obesity. Nat Rev Endocrinol 2015;11:182-190.

26. Holmes E, Li JV, Marchesi JR, Nicholson JK. Gut microbiota composition and activity in relation to host metabolic phenotype and disease risk. Cell Metab 2012;16:559-564.
27. Wostmann BS. The germfree animal in nutritional studies. Annu Rev Nutr 1981;1:257-279.
28. Nuttal GHF, Thierfelder H. Thierosches Leben ohne Bacterien im Verdauungskanal. Z Physiol Chem 1895-1896;21:109-121.
29. Schottelius M. Die Bedeutung der Darmbacterein für die Ernährung. Arch Hyg 1902;42:48-70.
30. Kuster E. Die Gewinnung Haltung und Aufzucht keimfreier Tiere und ihre Bedeutung für die Erforschung natürliches Lebensvorgänge. Arb Kaiserlich Gesundh Amtes 1915;48:1-79.
31. Glimstedt G. Bakterienfreie Meerschwinchen, Aufzucht, Lebensfähigkeit und Wachstum, nebst untersuchungen über das lymphatische Gewebe. Acata Pathol Microbiol Scand Suppl 1936;30:1-295.
32. Reyniers JA, Trexler PC, Ervin RF. Rearing germfree albino rats. In: Reyniers JA, ed. *Lobound Rep. No. 1*. Notre Dame: Ind: Univ Notre Dame; 1946: 1-84.
33. Reyniers JA, Trexler PC, Ervin RF, Wagner M, Luckey TD, Gordon HA. Rearing germfree chickens. In: Reyniers JA, ed. *Lobound Rep. No. 2*. Notre Dame: Ind: Univ Notre Dame; 1946: 1-115.
34. Reyniers JA. Rearing of a Caesarian-born M. Rhesus monkey under sterile conditions. A preliminary report. J Bact 1942;43:778.
35. Reyniers JA. The use of germ-free guinea pigs in bacterilogy. 1. Preliminary report concerned especially with technique. Proc Indiana Acad Sci 1932-1933;42:35-37.
36. Reyniers JA. The germ-free technique and its application to rearing animals free from contamination. Micrurgical and germ-free methods. 1943:114-143.
37. Reyniers JA. Introduction to the general problem of isolation and elimination of contamination. Micrurgical and germ-free methods. 1943:95-113.
38. Reyniers JA. Germ-free life applied to nutrition studies. Lobound Rep. . 1946;1:87-120.
39. Gustafsson BE. Germfree rearing of rats, general technique. Acta Pathol Microbiol Scand 1948;73:1-130.
40. Gustafsson BE. Lightweight stainless steel systems for rearing germ-free animals. Ann NY Acad Sci 1959;78:17-28.
41. Nicholson JK, Holmes E, Kinross J, Burcelin R, Gibson G, Jia W, Pettersson S. Host-gut microbiota metabolic interactions. Science 2012;336:1262-1267.
42. Eberl G, Lochner M. The development of intestinal lymphoid tissues at the interface of self and microbiota. Mucosal Immunol 2009;2:478-485.

43. Mazmanian SK, Liu CH, Tzianabos AO, Kasper DL. An immunomodulatory molecule of symbiotic bacteria directs maturation of the host immune system. Cell 2005;122:107-118.
44. Arrieta MC, Finlay BB. The commensal microbiota drives immune homeostasis. Front Immunol 2012;3:33.
45. Sanos SL, Bui VL, Mortha A, Oberle K, Heners C, Johner C, Diefenbach A. RORgammat and commensal microflora are required for the differentiation of mucosal interleukin 22-producing NKp46+ cells. Nat Immunol 2009;10:83-91.
46. Sjogren K, Engdahl C, Henning P, Lerner UH, Tremaroli V, Lagerquist MK, Backhed F, Ohlsson C. The gut microbiota regulates bone mass in mice. J Bone Miner Res 2012;27:1357-1367.
47. Neufeld KM, Kang N, Bienenstock J, Foster JA. Reduced anxiety-like behavior and central neurochemical change in germ-free mice. Neurogastroenterol Motil 2011;23:255-264, e119.
48. Diaz Heijtz R, Wang S, Anuar F, Qian Y, Bjorkholm B, Samuelsson A, Hibberd ML, Forssberg H, Pettersson S. Normal gut microbiota modulates brain development and behavior. Proc Natl Acad Sci U S A 2011;108:3047-3052.
49. Backhed F, Ding H, Wang T, Hooper LV, Koh GY, Nagy A, Semenkovich CF, Gordon JI. The gut microbiota as an environmental factor that regulates fat storage. Proc Natl Acad Sci U S A 2004;101:15718-15723.
50. Kovatcheva-Datchary P, Arora T. Nutrition, the gut microbiome and the metabolic syndrome. Best Pract Res Clin Gastroenterol 2013;27:59-72.
51. Pollard M. Germfree Animals and Biological Research. Science 1964;145:247-251.
52. Glimstedt G. The germfree animal as a research tool. Ann N Y Acad Sci 1959;78:281-284.
53. Backhed F, Manchester JK, Semenkovich CF, Gordon JI. Mechanisms underlying the resistance to diet-induced obesity in germ-free mice. Proc Natl Acad Sci U S A 2007;104:979-984.
54. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature 2006;444:1027-1031.
55. Ley RE, Backhed F, Turnbaugh P, Lozupone CA, Knight RD, Gordon JI. Obesity alters gut microbial ecology. Proc Natl Acad Sci U S A 2005;102:11070-11075.
56. Ley RE, Turnbaugh PJ, Klein S, Gordon JI. Microbial ecology: human gut microbes associated with obesity. Nature 2006;444:1022-1023.
57. Brown K, DeCoffe D, Molcan E, Gibson DL. Diet-induced dysbiosis of the intestinal microbiota and the effects on immunity and disease. Nutrients 2012;4:1095-1119.

58. Claesson MJ, Jeffery IB, Conde S, Power SE, O'Connor EM, Cusack S, Harris HM, Coakley M, Lakshminarayanan B, O'Sullivan O, Fitzgerald GF, Deane J, O'Connor M, Harnedy N, O'Connor K, O'Mahony D, van Sinderen D, Wallace M, Brennan L, Stanton C, Marchesi JR, Fitzgerald AP, Shanahan F, Hill C, Ross RP, O'Toole PW. Gut microbiota composition correlates with diet and health in the elderly. Nature 2012;488:178-184.
59. Hildebrandt MA, Hoffmann C, Sherrill-Mix SA, Keilbaugh SA, Hamady M, Chen YY, Knight R, Ahima RS, Bushman F, Wu GD. High-fat diet determines the composition of the murine gut microbiome independently of obesity. Gastroenterology 2009;137:1716-1724 e1711-1712.
60. Ridaura VK, Faith JJ, Rey FE, Cheng J, Duncan AE, Kau AL, Griffin NW, Lombard V, Henrissat B, Bain JR, Muehlbauer MJ, Ilkayeva O, Semenkovich CF, Funai K, Hayashi DK, Lyle BJ, Martini MC, Ursell LK, Clemente JC, Van Treuren W, Walters WA, Knight R, Newgard CB, Heath AC, Gordon JI. Gut microbiota from twins discordant for obesity modulate metabolism in mice. Science 2013;341:1241214.
61. Graham C, Mullen A, Whelan K. Obesity and the gastrointestinal microbiota: a review of associations and mechanisms. Nutr Rev 2015;73:376-385.
62. Berg JM. *Biochemistry*. 7 ed: W.H. Freeman Co Ltd; 2011.
63. MacDonald IA, Webber J. Feeding, fasting and starvation: factors affecting fuel utilization. Proc Nutr Soc 1995;54:267-274.
64. Jensen MD, Ekberg K, Landau BR. Lipid metabolism during fasting. Am J Physiol Endocrinol Metab 2001;281:E789-793.
65. Mergenthaler P, Lindauer U, Dienel GA, Meisel A. Sugar for the brain: the role of glucose in physiological and pathological brain function. Trends Neurosci 2013;36:587-597.
66. Holness MJ, French TJ, Sugden MC. Hepatic glycogen synthesis on carbohydrate re-feeding after starvation. A regulatory role for pyruvate dehydrogenase in liver and extrahepatic tissues. Biochem J 1986;235:441-445.
67. Huang MT, Veech RL. Role of the direct and indirect pathways for glycogen synthesis in rat liver in the postprandial state. J Clin Invest 1988;81:872-878.
68. Rui L. Energy metabolism in the liver. Compr Physiol 2014;4:177-197.
69. Marliss E, Aoki TT, Felig P, Pozefsky T, Cahill GF, Jr. Hormones and substrates in the regulation of gluconeogenesis in fasting man. Adv Enzyme Regul 1970;8:3-11.
70. Chiasson JL, Atkinson RL, Cherrington AD, Keller U, Sinclair-Smith BC, Lacy WW, Liljenquist JE. Effects of fasting on

gluconeogenesis from alanine in nondiabetic man. Diabetes 1979;28:56-60.
71. Browning JD, Baxter J, Satapati S, Burgess SC. The effect of short-term fasting on liver and skeletal muscle lipid, glucose, and energy metabolism in healthy women and men. J Lipid Res 2012;53:577-586.
72. Cahill GF, Jr. Starvation in man. N Engl J Med 1970;282:668-675.
73. McGarry JD, Foster DW. Regulation of hepatic fatty acid oxidation and ketone body production. Annu Rev Biochem 1980;49:395-420.
74. Landau BR, Wahren J, Previs SF, Ekberg K, Chandramouli V, Brunengraber H. Glycerol production and utilization in humans: sites and quantitation. Am J Physiol 1996;271:E1110-1117.
75. Klein S, Holland OB, Wolfe RR. Importance of blood glucose concentration in regulating lipolysis during fasting in humans. Am J Physiol 1990;258:E32-39.
76. Balasse EO, Fery F. Ketone body production and disposal: effects of fasting, diabetes, and exercise. Diabetes Metab Rev 1989;5:247-270.
77. Laffel L. Ketone bodies: a review of physiology, pathophysiology and application of monitoring to diabetes. Diabetes Metab Res Rev 1999;15:412-426.
78. Kodde IF, van der Stok J, Smolenski RT, de Jong JW. Metabolic and genetic regulation of cardiac energy substrate preference. Comp Biochem Physiol A Mol Integr Physiol 2007;146:26-39.
79. Drucker DJ. The role of gut hormones in glucose homeostasis. J Clin Invest 2007;117:24-32.
80. Meier U, Gressner AM. Endocrine regulation of energy metabolism: review of pathobiochemical and clinical chemical aspects of leptin, ghrelin, adiponectin, and resistin. Clin Chem 2004;50:1511-1525.
81. Ranabir S, Reetu K. Stress and hormones. Indian J Endocrinol Metab 2011;15:18-22.
82. Hull KL, Harvey S. Growth hormone and reproduction: a review of endocrine and autocrine/paracrine interactions. Int J Endocrinol 2014;2014:234014.
83. Schuit FC, Huypens P, Heimberg H, Pipeleers DG. Glucose sensing in pancreatic beta-cells: a model for the study of other glucose-regulated cells in gut, pancreas, and hypothalamus. Diabetes 2001;50:1-11.
84. Del Prato S, Marchetti P, Bonadonna RC. Phasic insulin release and metabolic regulation in type 2 diabetes. Diabetes 2002;51 Suppl 1:S109-116.
85. Strubbe JH, Steffens AB. Rapid insulin release after ingestion of a meal in the unanesthetized rat. Am J Physiol 1975;229:1019-1022.
86. Floyd JC, Jr., Fajans SS, Conn JW, Knopf RF, Rull J. Stimulation of insulin secretion by amino acids. J Clin Invest 1966;45:1487-1502.

87. Holst JJ, Gromada J. Role of incretin hormones in the regulation of insulin secretion in diabetic and nondiabetic humans. Am J Physiol Endocrinol Metab 2004;287:E199-206.
88. D'Alessio DA, Kieffer TJ, Taborsky GJ, Jr., Havel PJ. Activation of the parasympathetic nervous system is necessary for normal meal-induced insulin secretion in rhesus macaques. J Clin Endocrinol Metab 2001;86:1253-1259.
89. Kiba T. Relationships between the autonomic nervous system and the pancreas including regulation of regeneration and apoptosis: recent developments. Pancreas 2004;29:e51-58.
90. Dimitriadis G, Mitrou P, Lambadiari V, Maratou E, Raptis SA. Insulin effects in muscle and adipose tissue. Diabetes Res Clin Pract 2011;93 Suppl 1:S52-59.
91. Fleig WE, Enderle D, Steudter S, Nother-Fleig G, Ditschuneit H. Regulation of basal and insulin-stimulated glycogen synthesis in cultured hepatocytes. Inverse relationship to glycogen content. J Biol Chem 1987;262:1155-1160.
92. Roden M, Perseghin G, Petersen KF, Hwang JH, Cline GW, Gerow K, Rothman DL, Shulman GI. The roles of insulin and glucagon in the regulation of hepatic glycogen synthesis and turnover in humans. J Clin Invest 1996;97:642-648.
93. Claus TH, Pilkis SJ. Regulation by insulin of gluconeogenesis in isolated rat hepatocytes. Biochim Biophys Acta 1976;421:246-262.
94. Marks JS, Botelho LH. Synergistic inhibition of glucagon-induced effects on hepatic glucose metabolism in the presence of insulin and a cAMP antagonist. J Biol Chem 1986;261:15895-15899.
95. Ito K, Maruyama H, Hirose H, Kido K, Koyama K, Kataoka K, Saruta T. Exogenous insulin dose-dependently suppresses glucopenia-induced glucagon secretion from perfused rat pancreas. Metabolism 1995;44:358-362.
96. Lukens FD. Insulin and Protein Metabolism. Diabetes 1964;13:451-461.
97. Marliss EB, Aoki TT, Unger RH, Soeldner JS, Cahill GF, Jr. Glucagon levels and metabolic effects in fasting man. J Clin Invest 1970;49:2256-2270.
98. Wahren J, Ekberg K. Splanchnic regulation of glucose production. Annu Rev Nutr 2007;27:329-345.
99. Ekberg K, Landau BR, Wajngot A, Chandramouli V, Efendic S, Brunengraber H, Wahren J. Contributions by kidney and liver to glucose production in the postabsorptive state and after 60 h of fasting. Diabetes 1999;48:292-298.
100. Gerich JE, Meyer C, Woerle HJ, Stumvoll M. Renal gluconeogenesis: its importance in human glucose homeostasis. Diabetes Care 2001;24:382-391.

101. Granner D, Pilkis S. The genes of hepatic glucose metabolism. J Biol Chem 1990;265:10173-10176.
102. Lawrence GM, Jepson MA, Trayer IP, Walker DG. The compartmentation of glycolytic and gluconeogenic enzymes in rat kidney and liver and its significance to renal and hepatic metabolism. Histochem J 1986;18:45-53.
103. Band G, Jones CT. Activation by glucagon of glucose 6-phosphatase activity in the liver of the foetal guinea pig. Biochem Soc Trans 1980;8:586-587.
104. Striffler JS, Garfield SA, Cardell EL, Cardell RR. Effects of glucagon on hepatic microsomal glucose-6-phosphatase in vivo. Diabete Metab 1984;10:91-97.
105. Beale E, Andreone T, Koch S, Granner M, Granner D. Insulin and glucagon regulate cytosolic phosphoenolpyruvate carboxykinase (GTP) mRNA in rat liver. Diabetes 1984;33:328-332.
106. Postic C, Dentin R, Girard J. Role of the liver in the control of carbohydrate and lipid homeostasis. Diabetes Metab 2004;30:398-408.
107. Lager I. The insulin-antagonistic effect of the counterregulatory hormones. J Intern Med Suppl 1991;735:41-47.
108. Tennant B, Malm OJ, Horowitz RE, Levenson SM. Response of germfree, conventional, conventionalized and E. coli monocontaminated mice to starvation. J Nutr 1968;94:151-160.
109. Crawford PA, Crowley JR, Sambandam N, Muegge BD, Costello EK, Hamady M, Knight R, Gordon JI. Regulation of myocardial ketone body metabolism by the gut microbiota during nutrient deprivation. Proc Natl Acad Sci U S A 2009;106:11276-11281.
110. Blanton LV, Charbonneau MR, Salih T, Barratt MJ, Venkatesh S, Ilkaveya O, Subramanian S, Manary MJ, Trehan I, Jorgensen JM, Fan YM, Henrissat B, Leyn SA, Rodionov DA, Osterman AL, Maleta KM, Newgard CB, Ashorn P, Dewey KG, Gordon JI. Gut bacteria that prevent growth impairments transmitted by microbiota from malnourished children. Science 2016;351.
111. Zhang C, Li S, Yang L, Huang P, Li W, Wang S, Zhao G, Zhang M, Pang X, Yan Z, Liu Y, Zhao L. Structural modulation of gut microbiota in life-long calorie-restricted mice. Nat Commun 2013;4:2163.
112. Kohl KD, Amaya J, Passement CA, Dearing MD, McCue MD. Unique and shared responses of the gut microbiota to prolonged fasting: a comparative study across five classes of vertebrate hosts. FEMS Microbiol Ecol 2014;90:883-894.
113. Donohoe DR, Garge N, Zhang X, Sun W, O'Connell TM, Bunger MK, Bultman SJ. The microbiome and butyrate regulate energy metabolism and autophagy in the Mammalian colon. Cell Metab 2011;13:517-526.

114. Donohoe DR, Wali A, Brylawski BP, Bultman SJ. Microbial regulation of glucose metabolism and cell-cycle progression in mammalian colonocytes. PLoS ONE 2012;7:e46589.
115. Duca FA, Lam TK. Gut microbiota, nutrient sensing and energy balance. Diabetes Obes Metab 2014;16 Suppl 1:68-76.
116. Sternini C, Anselmi L, Rozengurt E. Enteroendocrine cells: a site of 'taste' in gastrointestinal chemosensing. Curr Opin Endocrinol Diabetes Obes 2008;15:73-78.
117. Helander HF, Fandriks L. The enteroendocrine "letter cells" - time for a new nomenclature? Scand J Gastroenterol 2012;47:3-12.
118. Cummings DE, Overduin J. Gastrointestinal regulation of food intake. J Clin Invest 2007;117:13-23.
119. Evans JM, Morris LS, Marchesi JR. The gut microbiome: the role of a virtual organ in the endocrinology of the host. J Endocrinol 2013;218:R37-47.
120. Ichimura A, Hirasawa A, Hara T, Tsujimoto G. Free fatty acid receptors act as nutrient sensors to regulate energy homeostasis. Prostaglandins Other Lipid Mediat 2009;89:82-88.
121. Clarke G, Grenham S, Scully P, Fitzgerald P, Moloney RD, Shanahan F, Dinan TG, Cryan JF. The microbiome-gut-brain axis during early life regulates the hippocampal serotonergic system in a sex-dependent manner. Mol Psychiatry 2013;18:666-673.
122. Thomas C, Auwerx J, Schoonjans K. Bile acids and the membrane bile acid receptor TGR5--connecting nutrition and metabolism. Thyroid 2008;18:167-174.
123. Lefebvre P, Cariou B, Lien F, Kuipers F, Staels B. Role of bile acids and bile acid receptors in metabolic regulation. Physiol Rev 2009;89:147-191.
124. Rehfeld JF. The new biology of gastrointestinal hormones. Physiol Rev 1998;78:1087-1108.
125. Dockray G. Gut endocrine secretions and their relevance to satiety. Curr Opin Pharmacol 2004;4:557-560.
126. Dockray GJ, Varro A, Dimaline R. Gastric endocrine cells: gene expression, processing, and targeting of active products. Physiol Rev 1996;76:767-798.
127. Strader AD, Woods SC. Gastrointestinal hormones and food intake. Gastroenterology 2005;128:175-191.
128. Buchan AM. Nutrient Tasting and Signaling Mechanisms in the Gut III. Endocrine cell recognition of luminal nutrients. Am J Physiol 1999;277:G1103-1107.
129. Dockray GJ. Luminal sensing in the gut: an overview. J Physiol Pharmacol 2003;54 Suppl 4:9-17.
130. Hofer D, Asan E, Drenckhahn D. Chemosensory Perception in the Gut. News Physiol Sci 1999;14:18-23.

131. Diakogiannaki E, Gribble FM, Reimann F. Nutrient detection by incretin hormone secreting cells. Physiol Behav 2012;106:387-393.
132. Berthoud HR. Vagal and hormonal gut-brain communication: from satiation to satisfaction. Neurogastroenterol Motil 2008;20 Suppl 1:64-72.
133. Powley TL, Spaulding RA, Haglof SA. Vagal afferent innervation of the proximal gastrointestinal tract mucosa: chemoreceptor and mechanoreceptor architecture. J Comp Neurol 2011;519:644-660.
134. Dockray GJ. Gastrointestinal hormones and the dialogue between gut and brain. J Physiol 2014;592:2927-2941.
135. Banks WA. The blood-brain barrier: connecting the gut and the brain. Regul Pept 2008;149:11-14.
136. Druce MR, Wren AM, Park AJ, Milton JE, Patterson M, Frost G, Ghatei MA, Small C, Bloom SR. Ghrelin increases food intake in obese as well as lean subjects. Int J Obes (Lond) 2005;29:1130-1136.
137. Gibbs J, Young RC, Smith GP. Cholecystokinin elicits satiety in rats with open gastric fistulas. Nature 1973;245:323-325.
138. Byrnes DJ, Borody T, Daskalopoulos G, Boyle M, Benn I. Cholecystokinin and gallbladder contraction: effect of CCK infusion. Peptides 1981;2 Suppl 2:259-262.
139. Konturek SJ, Konturek JW, Lamers CB, Tasler J, Bilski J. Role of secretin and CCK in the stimulation of pancreatic secretion in conscious dogs. Effects of atropine and somatostatin. Int J Pancreatol 1987;2:223-235.
140. Dupre J, Ross SA, Watson D, Brown JC. Stimulation of insulin secretion by gastric inhibitory polypeptide in man. J Clin Endocrinol Metab 1973;37:826-828.
141. Pederson RA, Schubert HE, Brown JC. Gastric inhibitory polypeptide. Its physiologic release and insulinotropic action in the dog. Diabetes 1975;24:1050-1056.
142. Kreymann B, Williams G, Ghatei MA, Bloom SR. Glucagon-like peptide-1 7-36: a physiological incretin in man. Lancet 1987;2:1300-1304.
143. Mojsov S, Weir GC, Habener JF. Insulinotropin: glucagon-like peptide I (7-37) co-encoded in the glucagon gene is a potent stimulator of insulin release in the perfused rat pancreas. J Clin Invest 1987;79:616-619.
144. Hellstrom PM, Naslund E, Edholm T, Schmidt PT, Kristensen J, Theodorsson E, Holst JJ, Efendic S. GLP-1 suppresses gastrointestinal motility and inhibits the migrating motor complex in healthy subjects and patients with irritable bowel syndrome. Neurogastroenterol Motil 2008;20:649-659.
145. Flint A, Raben A, Astrup A, Holst JJ. Glucagon-like peptide 1 promotes satiety and suppresses energy intake in humans. J Clin Invest 1998;101:515-520.

146. Karra E, Chandarana K, Batterham RL. The role of peptide YY in appetite regulation and obesity. J Physiol 2009;587:19-25.
147. Wren AM, Seal LJ, Cohen MA, Brynes AE, Frost GS, Murphy KG, Dhillo WS, Ghatei MA, Bloom SR. Ghrelin enhances appetite and increases food intake in humans. J Clin Endocrinol Metab 2001;86:5992.
148. Feigerlova E, Diene G, Conte-Auriol F, Molinas C, Gennero I, Salles JP, Arnaud C, Tauber M. Hyperghrelinemia precedes obesity in Prader-Willi syndrome. J Clin Endocrinol Metab 2008;93:2800-2805.
149. Tschop M, Weyer C, Tataranni PA, Devanarayan V, Ravussin E, Heiman ML. Circulating ghrelin levels are decreased in human obesity. Diabetes 2001;50:707-709.
150. Shiiya T, Nakazato M, Mizuta M, Date Y, Mondal MS, Tanaka M, Nozoe S, Hosoda H, Kangawa K, Matsukura S. Plasma ghrelin levels in lean and obese humans and the effect of glucose on ghrelin secretion. J Clin Endocrinol Metab 2002;87:240-244.
151. Erdmann J, Lippl F, Wagenpfeil S, Schusdziarra V. Differential association of basal and postprandial plasma ghrelin with leptin, insulin, and type 2 diabetes. Diabetes 2005;54:1371-1378.
152. Sun Y, Ahmed S, Smith RG. Deletion of ghrelin impairs neither growth nor appetite. Mol Cell Biol 2003;23:7973-7981.
153. Sun Y, Butte NF, Garcia JM, Smith RG. Characterization of adult ghrelin and ghrelin receptor knockout mice under positive and negative energy balance. Endocrinology 2008;149:843-850.
154. Muller TD, Muller A, Yi CX, Habegger KM, Meyer CW, Gaylinn BD, Finan B, Heppner K, Trivedi C, Bielohuby M, Abplanalp W, Meyer F, Piechowski CL, Pratzka J, Stemmer K, Holland J, Hembree J, Bhardwaj N, Raver C, Ottaway N, Krishna R, Sah R, Sallee FR, Woods SC, Perez-Tilve D, Bidlingmaier M, Thorner MO, Krude H, Smiley D, DiMarchi R, Hofmann S, Pfluger PT, Kleinau G, Biebermann H, Tschop MH. The orphan receptor Gpr83 regulates systemic energy metabolism via ghrelin-dependent and ghrelin-independent mechanisms. Nat Commun 2013;4:1968.
155. Pfluger PT, Kampe J, Castaneda TR, Vahl T, D'Alessio DA, Kruthaupt T, Benoit SC, Cuntz U, Rochlitz HJ, Moehlig M, Pfeiffer AF, Koebnick C, Weickert MO, Otto B, Spranger J, Tschop MH. Effect of human body weight changes on circulating levels of peptide YY and peptide YY3-36. J Clin Endocrinol Metab 2007;92:583-588.
156. Zwirska-Korczala K, Konturek SJ, Sodowski M, Wylezol M, Kuka D, Sowa P, Adamczyk-Sowa M, Kukla M, Berdowska A, Rehfeld JF, Bielanski W, Brzozowski T. Basal and postprandial plasma levels of PYY, ghrelin, cholecystokinin, gastrin and insulin in women with moderate and morbid obesity and metabolic syndrome. J Physiol Pharmacol 2007;58 Suppl 1:13-35.

157. Batterham RL, Heffron H, Kapoor S, Chivers JE, Chandarana K, Herzog H, Le Roux CW, Thomas EL, Bell JD, Withers DJ. Critical role for peptide YY in protein-mediated satiation and body-weight regulation. Cell Metab 2006;4:223-233.
158. Batterham RL, Bloom SR. The gut hormone peptide YY regulates appetite. Ann N Y Acad Sci 2003;994:162-168.
159. Gantz I, Erondu N, Mallick M, Musser B, Krishna R, Tanaka WK, Snyder K, Stevens C, Stroh MA, Zhu H, Wagner JA, Macneil DJ, Heymsfield SB, Amatruda JM. Efficacy and safety of intranasal peptide YY3-36 for weight reduction in obese adults. J Clin Endocrinol Metab 2007;92:1754-1757.
160. Korner J, Bessler M, Cirilo LJ, Conwell IM, Daud A, Restuccia NL, Wardlaw SL. Effects of Roux-en-Y gastric bypass surgery on fasting and postprandial concentrations of plasma ghrelin, peptide YY, and insulin. J Clin Endocrinol Metab 2005;90:359-365.
161. Karamanakos SN, Vagenas K, Kalfarentzos F, Alexandrides TK. Weight loss, appetite suppression, and changes in fasting and postprandial ghrelin and peptide-YY levels after Roux-en-Y gastric bypass and sleeve gastrectomy: a prospective, double blind study. Ann Surg 2008;247:401-407.
162. Trujillo JM, Nuffer W, Ellis SL. GLP-1 receptor agonists: a review of head-to-head clinical studies. Ther Adv Endocrinol Metab 2015;6:19-28.
163. Verdich C, Flint A, Gutzwiller JP, Naslund E, Beglinger C, Hellstrom PM, Long SJ, Morgan LM, Holst JJ, Astrup A. A meta-analysis of the effect of glucagon-like peptide-1 (7-36) amide on ad libitum energy intake in humans. J Clin Endocrinol Metab 2001;86:4382-4389.
164. Wadden D, Cahill F, Amini P, Randell E, Vasdev S, Yi Y, Church J, Sun G. Circulating glucagon-like peptide-1 increases in response to short-term overfeeding in men. Nutr Metab (Lond) 2013;10:33.
165. Adam TC, Jocken J, Westerterp-Plantenga MS. Decreased glucagon-like peptide 1 release after weight loss in overweight/obese subjects. Obes Res 2005;13:710-716.
166. Reinehr T, de Sousa G, Roth CL. Fasting glucagon-like peptide-1 and its relation to insulin in obese children before and after weight loss. J Pediatr Gastroenterol Nutr 2007;44:608-612.
167. Vilsboll T, Krarup T, Deacon CF, Madsbad S, Holst JJ. Reduced postprandial concentrations of intact biologically active glucagon-like peptide 1 in type 2 diabetic patients. Diabetes 2001;50:609-613.
168. Ranganath LR, Beety JM, Morgan LM, Wright JW, Howland R, Marks V. Attenuated GLP-1 secretion in obesity: cause or consequence? Gut 1996;38:916-919.
169. Fukase N, Igarashi M, Takahashi H, Manaka H, Yamatani K, Daimon M, Tominaga M, Sasaki H. Hypersecretion of truncated

glucagon-like peptide-1 and gastric inhibitory polypeptide in obese patients. Diabet Med 1993;10:44-49.
170. Cummings DE, Overduin J, Shannon MH, Foster-Schubert KE, Conference ABSC. Hormonal mechanisms of weight loss and diabetes resolution after bariatric surgery. Surg Obes Relat Dis 2005;1:358-368.
171. Turton MD, O'Shea D, Gunn I, Beak SA, Edwards CM, Meeran K, Choi SJ, Taylor GM, Heath MM, Lambert PD, Wilding JP, Smith DM, Ghatei MA, Herbert J, Bloom SR. A role for glucagon-like peptide-1 in the central regulation of feeding. Nature 1996;379:69-72.
172. Scrocchi LA, Brown TJ, MaClusky N, Brubaker PL, Auerbach AB, Joyner AL, Drucker DJ. Glucose intolerance but normal satiety in mice with a null mutation in the glucagon-like peptide 1 receptor gene. Nat Med 1996;2:1254-1258.
173. Scrocchi LA, Drucker DJ. Effects of aging and a high fat diet on body weight and glucose tolerance in glucagon-like peptide-1 receptor -/- mice. Endocrinology 1998;139:3127-3132.
174. Conklin D, Lofton-Day CE, Haldeman BA, Ching A, Whitmore TE, Lok S, Jaspers S. Identification of INSL5, a new member of the insulin superfamily. Genomics 1999;60:50-56.
175. Akhter Hossain M, Bathgate RA, Kong CK, Shabanpoor F, Zhang S, Haugaard-Jonsson LM, Rosengren KJ, Tregear GW, Wade JD. Synthesis, conformation, and activity of human insulin-like peptide 5 (INSL5). Chembiochem 2008;9:1816-1822.
176. Mashima H, Ohno H, Yamada Y, Sakai T, Ohnishi H. INSL5 may be a unique marker of colorectal endocrine cells and neuroendocrine tumors. Biochem Biophys Res Commun 2013;432:586-592.
177. Thanasupawat T, Hammje K, Adham I, Ghia JE, Del Bigio MR, Krcek J, Hoang-Vu C, Klonisch T, Hombach-Klonisch S. INSL5 is a novel marker for human enteroendocrine cells of the large intestine and neuroendocrine tumours. Oncol Rep 2013;29:149-154.
178. Grosse J, Heffron H, Burling K, Akhter Hossain M, Habib AM, Rogers GJ, Richards P, Larder R, Rimmington D, Adriaenssens AA, Parton L, Powell J, Binda M, Colledge WH, Doran J, Toyoda Y, Wade JD, Aparicio S, Carlton MB, Coll AP, Reimann F, O'Rahilly S, Gribble FM. Insulin-like peptide 5 is an orexigenic gastrointestinal hormone. Proc Natl Acad Sci U S A 2014;111:11133-11138.
179. Liu C, Kuei C, Sutton S, Chen J, Bonaventure P, Wu J, Nepomuceno D, Kamme F, Tran DT, Zhu J, Wilkinson T, Bathgate R, Eriste E, Sillard R, Lovenberg TW. INSL5 is a high affinity specific agonist for GPCR142 (GPR100). J Biol Chem 2005;280:292-300.
180. Sutton SW, Bonaventure P, Kuei C, Nepomuceno D, Wu J, Zhu J, Lovenberg TW, Liu C. G-protein-coupled receptor (GPCR)-142 does not contribute to relaxin-3 binding in the mouse brain: further

support that relaxin-3 is the physiological ligand for GPCR135. Neuroendocrinology 2005;82:139-150.
181. Belgi A, Hossain MA, Shabanpoor F, Chan L, Zhang S, Bathgate RA, Tregear GW, Wade JD. Structure and function relationship of murine insulin-like peptide 5 (INSL5): free C-terminus is essential for RXFP4 receptor binding and activation. Biochemistry 2011;50:8352-8361.
182. Bathgate RA, Halls ML, van der Westhuizen ET, Callander GE, Kocan M, Summers RJ. Relaxin family peptides and their receptors. Physiol Rev 2013;93:405-480.
183. Burnicka-Turek O, Mohamed BA, Shirneshan K, Thanasupawat T, Hombach-Klonisch S, Klonisch T, Adham IM. INSL5-deficient mice display an alteration in glucose homeostasis and an impaired fertility. Endocrinology 2012;153:4655-4665.
184. Luo X, Li T, Zhu Y, Dai Y, Zhao J, Guo ZY, Wang MW. The insulinotrophic effect of insulin-like peptide 5 in vitro and in vivo. Biochem J 2015;466:467-473.
185. Vrieze A, Holleman F, Zoetendal EG, de Vos WM, Hoekstra JB, Nieuwdorp M. The environment within: how gut microbiota may influence metabolism and body composition. Diabetologia 2010;53:606-613.
186. Uribe A, Alam M, Johansson O, Midtvedt T, Theodorsson E. Microflora modulates endocrine cells in the gastrointestinal mucosa of the rat. Gastroenterology 1994;107:1259-1269.
187. Cani PD, Hoste S, Guiot Y, Delzenne NM. Dietary non-digestible carbohydrates promote L-cell differentiation in the proximal colon of rats. Br J Nutr 2007;98:32-37.
188. Cani PD, Possemiers S, Van de Wiele T, Guiot Y, Everard A, Rottier O, Geurts L, Naslain D, Neyrinck A, Lambert DM, Muccioli GG, Delzenne NM. Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. Gut 2009;58:1091-1103.
189. Samuel BS, Shaito A, Motoike T, Rey FE, Backhed F, Manchester JK, Hammer RE, Williams SC, Crowley J, Yanagisawa M, Gordon JI. Effects of the gut microbiota on host adiposity are modulated by the short-chain fatty-acid binding G protein-coupled receptor, Gpr41. Proc Natl Acad Sci U S A 2008;105:16767-16772.
190. Brown AJ, Goldsworthy SM, Barnes AA, Eilert MM, Tcheang L, Daniels D, Muir AI, Wigglesworth MJ, Kinghorn I, Fraser NJ, Pike NB, Strum JC, Steplewski KM, Murdock PR, Holder JC, Marshall FH, Szekeres PG, Wilson S, Ignar DM, Foord SM, Wise A, Dowell SJ. The Orphan G protein-coupled receptors GPR41 and GPR43 are activated by propionate and other short chain carboxylic acids. J Biol Chem 2003;278:11312-11319.

191. Hoverstad T, Midtvedt T. Short-chain fatty acids in germfree mice and rats. J Nutr 1986;116:1772-1776.
192. Bergman EN. Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. Physiol Rev 1990;70:567-590.
193. den Besten G, van Eunen K, Groen AK, Venema K, Reijngoud DJ, Bakker BM. The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. J Lipid Res 2013;54:2325-2340.
194. Wichmann A, Allahyar A, Greiner TU, Plovier H, Lunden GO, Larsson T, Drucker DJ, Delzenne NM, Cani PD, Backhed F. Microbial modulation of energy availability in the colon regulates intestinal transit. Cell Host Microbe 2013;14:582-590.
195. Thomas C, Gioiello A, Noriega L, Strehle A, Oury J, Rizzo G, Macchiarulo A, Yamamoto H, Mataki C, Pruzanski M, Pellicciari R, Auwerx J, Schoonjans K. TGR5-mediated bile acid sensing controls glucose homeostasis. Cell Metab 2009;10:167-177.
196. Prentice AM. The emerging epidemic of obesity in developing countries. Int J Epidemiol 2006;35:93-99.
197. World Health Organization. Obesity: preventing and managing the global epidemic: WHO Obesity Technical Report Series 894, World Health Organization, Geneva, Switzerland 2000.
198. OECD. OECD Obesity update 2014. www.oecd.org/health/obesity-update.htm. 2014.
199. Flegal KM, Carroll MD, Ogden CL, Curtin LR. Prevalence and trends in obesity among US adults, 1999-2008. JAMA 2010;303:235-241.
200. Gloy VL, Briel M, Bhatt DL, Kashyap SR, Schauer PR, Mingrone G, Bucher HC, Nordmann AJ. Bariatric surgery versus non-surgical treatment for obesity: a systematic review and meta-analysis of randomised controlled trials. BMJ 2013;347:f5934.
201. Zhao Y, Encinosa W. Bariatric Surgery Utilization and Outcomes in 1998 and 2004: Statistical Brief #23. *Healthcare Cost and Utilization Project (HCUP) Statistical Briefs*. Rockville (MD); 2006.
202. Buchwald H, Williams SE. Bariatric surgery worldwide 2003. Obes Surg 2004;14:1157-1164.
203. Wadden TA, Butryn ML, Byrne KJ. Efficacy of lifestyle modification for long-term weight control. Obes Res 2004;12 Suppl:151S-162S.
204. Weiss EC, Galuska DA, Kettel Khan L, Gillespie C, Serdula MK. Weight regain in U.S. adults who experienced substantial weight loss, 1999-2002. Am J Prev Med 2007;33:34-40.
205. Safer DJ. Diet, behavior modification, and exercise: a review of obesity treatments from a long-term perspective. South Med J 1991;84:1470-1474.

206. Kraschnewski JL, Boan J, Esposito J, Sherwood NE, Lehman EB, Kephart DK, Sciamanna CN. Long-term weight loss maintenance in the United States. Int J Obes (Lond) 2010;34:1644-1654.
207. Buchwald H, Avidor Y, Braunwald E, Jensen MD, Pories W, Fahrbach K, Schoelles K. Bariatric surgery: a systematic review and meta-analysis. JAMA 2004;292:1724-1737.
208. Sjostrom L, Lindroos AK, Peltonen M, Torgerson J, Bouchard C, Carlsson B, Dahlgren S, Larsson B, Narbro K, Sjostrom CD, Sullivan M, Wedel H, Swedish Obese Subjects Study Scientific G. Lifestyle, diabetes, and cardiovascular risk factors 10 years after bariatric surgery. N Engl J Med 2004;351:2683-2693.
209. Christou NV, Sampalis JS, Liberman M, Look D, Auger S, McLean AP, MacLean LD. Surgery decreases long-term mortality, morbidity, and health care use in morbidly obese patients. Ann Surg 2004;240:416-423; discussion 423-414.
210. Yanovski SZ, Yanovski JA. Long-term drug treatment for obesity: a systematic and clinical review. JAMA 2014;311:74-86.
211. Ward M, Prachand V. Surgical treatment of obesity. Gastrointest Endosc 2009;70:985-990.
212. Angrisani L, Santonicola A, Iovino P, Formisano G, Buchwald H, Scopinaro N. Bariatric Surgery Worldwide 2013. Obes Surg 2015;25:1822-1832.
213. Elder KA, Wolfe BM. Bariatric surgery: a review of procedures and outcomes. Gastroenterology 2007;132:2253-2271.
214. Franco JV, Ruiz PA, Palermo M, Gagner M. A review of studies comparing three laparoscopic procedures in bariatric surgery: sleeve gastrectomy, Roux-en-Y gastric bypass and adjustable gastric banding. Obes Surg 2011;21:1458-1468.
215. Schauer PR, Bhatt DL, Kirwan JP, Wolski K, Brethauer SA, Navaneethan SD, Aminian A, Pothier CE, Kim ES, Nissen SE, Kashyap SR, Investigators S. Bariatric surgery versus intensive medical therapy for diabetes--3-year outcomes. N Engl J Med 2014;370:2002-2013.
216. Rimm EB, Stampfer MJ, Giovannucci E, Ascherio A, Spiegelman D, Colditz GA, Willett WC. Body size and fat distribution as predictors of coronary heart disease among middle-aged and older US men. Am J Epidemiol 1995;141:1117-1127.
217. Howard BV, Ruotolo G, Robbins DC. Obesity and dyslipidemia. Endocrinol Metab Clin North Am 2003;32:855-867.
218. Nguyen NT, Varela E, Sabio A, Tran CL, Stamos M, Wilson SE. Resolution of hyperlipidemia after laparoscopic Roux-en-Y gastric bypass. J Am Coll Surg 2006;203:24-29.
219. Dixon JB, O'Brien PE. Health outcomes of severely obese type 2 diabetic subjects 1 year after laparoscopic adjustable gastric banding. Diabetes Care 2002;25:358-363.

220. Zlabek JA, Grimm MS, Larson CJ, Mathiason MA, Lambert PJ, Kothari SN. The effect of laparoscopic gastric bypass surgery on dyslipidemia in severely obese patients. Surg Obes Relat Dis 2005;1:537-542.
221. Woodard GA, Peraza J, Bravo S, Toplosky L, Hernandez-Boussard T, Morton JM. One year improvements in cardiovascular risk factors: a comparative trial of laparoscopic Roux-en-Y gastric bypass vs. adjustable gastric banding. Obes Surg 2010;20:578-582.
222. Benaiges D, Goday A, Ramon JM, Hernandez E, Pera M, Cano JF, Obemar G. Laparoscopic sleeve gastrectomy and laparoscopic gastric bypass are equally effective for reduction of cardiovascular risk in severely obese patients at one year of follow-up. Surg Obes Relat Dis 2011;7:575-580.
223. Guh DP, Zhang W, Bansback N, Amarsi Z, Birmingham CL, Anis AH. The incidence of co-morbidities related to obesity and overweight: a systematic review and meta-analysis. BMC Public Health 2009;9:88.
224. Mahler RJ, Adler ML. Clinical review 102: Type 2 diabetes mellitus: update on diagnosis, pathophysiology, and treatment. J Clin Endocrinol Metab 1999;84:1165-1171.
225. Wickremesekera K, Miller G, Naotunne TD, Knowles G, Stubbs RS. Loss of insulin resistance after Roux-en-Y gastric bypass surgery: a time course study. Obes Surg 2005;15:474-481.
226. Rubino F, Gagner M, Gentileschi P, Kini S, Fukuyama S, Feng J, Diamond E. The early effect of the Roux-en-Y gastric bypass on hormones involved in body weight regulation and glucose metabolism. Ann Surg 2004;240:236-242.
227. Rizzello M, Abbatini F, Casella G, Alessandri G, Fantini A, Leonetti F, Basso N. Early postoperative insulin-resistance changes after sleeve gastrectomy. Obes Surg 2010;20:50-55.
228. Basso N, Capoccia D, Rizzello M, Abbatini F, Mariani P, Maglio C, Coccia F, Borgonuovo G, De Luca ML, Asprino R, Alessandri G, Casella G, Leonetti F. First-phase insulin secretion, insulin sensitivity, ghrelin, GLP-1, and PYY changes 72 h after sleeve gastrectomy in obese diabetic patients: the gastric hypothesis. Surg Endosc 2011;25:3540-3550.
229. Rubino F, R'Bibo S L, del Genio F, Mazumdar M, McGraw TE. Metabolic surgery: the role of the gastrointestinal tract in diabetes mellitus. Nat Rev Endocrinol 2010;6:102-109.
230. Pories WJ, Swanson MS, MacDonald KG, Long SB, Morris PG, Brown BM, Barakat HA, deRamon RA, Israel G, Dolezal JM, et al. Who would have thought it? An operation proves to be the most effective therapy for adult-onset diabetes mellitus. Ann Surg 1995;222:339-350; discussion 350-332.

231. Korner J, Inabnet W, Conwell IM, Taveras C, Daud A, Olivero-Rivera L, Restuccia NL, Bessler M. Differential effects of gastric bypass and banding on circulating gut hormone and leptin levels. Obesity (Silver Spring) 2006;14:1553-1561.
232. Abbatini F, Rizzello M, Casella G, Alessandri G, Capoccia D, Leonetti F, Basso N. Long-term effects of laparoscopic sleeve gastrectomy, gastric bypass, and adjustable gastric banding on type 2 diabetes. Surg Endosc 2010;24:1005-1010.
233. Ashrafian H, Bueter M, Ahmed K, Suliman A, Bloom SR, Darzi A, Athanasiou T. Metabolic surgery: an evolution through bariatric animal models. Obes Rev 2010;11:907-920.
234. Dias MC, Ribeiro AG, Scabim VM, Faintuch J, Zilberstein B, Gama-Rodrigues JJ. Dietary intake of female bariatric patients after anti-obesity gastroplasty. Clinics (Sao Paulo) 2006;61:93-98.
235. Warde-Kamar J, Rogers M, Flancbaum L, Laferrere B. Calorie intake and meal patterns up to 4 years after Roux-en-Y gastric bypass surgery. Obes Surg 2004;14:1070-1079.
236. Naslund I, Jarnmark I, Andersson H. Dietary intake before and after gastric bypass and gastroplasty for morbid obesity in women. Int J Obes 1988;12:503-513.
237. Trostler N, Mann A, Zilberbush N, Charuzi II, Avinoach E. Nutrient Intake following Vertical Banded Gastroplasty or Gastric Bypass. Obes Surg 1995;5:403-410.
238. Brolin RE, Robertson LB, Kenler HA, Cody RP. Weight loss and dietary intake after vertical banded gastroplasty and Roux-en-Y gastric bypass. Ann Surg 1994;220:782-790.
239. Thomas JR, Marcus E. High and low fat food selection with reported frequency intolerance following Roux-en-Y gastric bypass. Obes Surg 2008;18:282-287.
240. Zheng H, Shin AC, Lenard NR, Townsend RL, Patterson LM, Sigalet DL, Berthoud HR. Meal patterns, satiety, and food choice in a rat model of Roux-en-Y gastric bypass surgery. Am J Physiol Regul Integr Comp Physiol 2009;297:R1273-1282.
241. Wilson-Perez HE, Chambers AP, Sandoval DA, Stefater MA, Woods SC, Benoit SC, Seeley RJ. The effect of vertical sleeve gastrectomy on food choice in rats. Int J Obes (Lond) 2013;37:288-295.
242. Ernst B, Thurnheer M, Wilms B, Schultes B. Differential changes in dietary habits after gastric bypass versus gastric banding operations. Obes Surg 2009;19:274-280.
243. Lindroos AK, Lissner L, Sjostrom L. Weight change in relation to intake of sugar and sweet foods before and after weight reducing gastric surgery. Int J Obes Relat Metab Disord 1996;20:634-643.
244. Tichansky DS, Boughter JD, Jr., Madan AK. Taste change after laparoscopic Roux-en-Y gastric bypass and laparoscopic adjustable gastric banding. Surg Obes Relat Dis 2006;2:440-444.

245. Abell TL, Minocha A. Gastrointestinal complications of bariatric surgery: diagnosis and therapy. Am J Med Sci 2006;331:214-218.
246. Tack J, Arts J, Caenepeel P, De Wulf D, Bisschops R. Pathophysiology, diagnosis and management of postoperative dumping syndrome. Nat Rev Gastroenterol Hepatol 2009;6:583-590.
247. Shin AC, Zheng H, Pistell PJ, Berthoud HR. Roux-en-Y gastric bypass surgery changes food reward in rats. Int J Obes (Lond) 2011;35:642-651.
248. Tichansky DS, Glatt AR, Madan AK, Harper J, Tokita K, Boughter JD. Decrease in sweet taste in rats after gastric bypass surgery. Surg Endosc 2011;25:1176-1181.
249. Ochner CN, Kwok Y, Conceicao E, Pantazatos SP, Puma LM, Carnell S, Teixeira J, Hirsch J, Geliebter A. Selective reduction in neural responses to high calorie foods following gastric bypass surgery. Ann Surg 2011;253:502-507.
250. Schultes B, Ernst B, Wilms B, Thurnheer M, Hallschmid M. Hedonic hunger is increased in severely obese patients and is reduced after gastric bypass surgery. Am J Clin Nutr 2010;92:277-283.
251. Delin CR, Watts JM, Saebel JL, Anderson PG. Eating behavior and the experience of hunger following gastric bypass surgery for morbid obesity. Obes Surg 1997;7:405-413.
252. Himpens J, Dapri G, Cadiere GB. A prospective randomized study between laparoscopic gastric banding and laparoscopic isolated sleeve gastrectomy: results after 1 and 3 years. Obes Surg 2006;16:1450-1456.
253. le Roux CW, Aylwin SJ, Batterham RL, Borg CM, Coyle F, Prasad V, Shurey S, Ghatei MA, Patel AG, Bloom SR. Gut hormone profiles following bariatric surgery favor an anorectic state, facilitate weight loss, and improve metabolic parameters. Ann Surg 2006;243:108-114.
254. Peterli R, Wolnerhanssen B, Peters T, Devaux N, Kern B, Christoffel-Courtin C, Drewe J, von Flue M, Beglinger C. Improvement in glucose metabolism after bariatric surgery: comparison of laparoscopic Roux-en-Y gastric bypass and laparoscopic sleeve gastrectomy: a prospective randomized trial. Ann Surg 2009;250:234-241.
255. Clements RH, Gonzalez QH, Long CI, Wittert G, Laws HL. Hormonal changes after Roux-en Y gastric bypass for morbid obesity and the control of type-II diabetes mellitus. Am Surg 2004;70:1-4; discussion 4-5.
256. Korner J, Bessler M, Inabnet W, Taveras C, Holst JJ. Exaggerated glucagon-like peptide-1 and blunted glucose-dependent insulinotropic peptide secretion are associated with Roux-en-Y gastric bypass but not adjustable gastric banding. Surg Obes Relat Dis 2007;3:597-601.

257. Korner J, Inabnet W, Febres G, Conwell IM, McMahon DJ, Salas R, Taveras C, Schrope B, Bessler M. Prospective study of gut hormone and metabolic changes after adjustable gastric banding and Roux-en-Y gastric bypass. Int J Obes (Lond) 2009;33:786-795.
258. Valderas JP, Irribarra V, Rubio L, Boza C, Escalona M, Liberona Y, Matamala A, Maiz A. Effects of sleeve gastrectomy and medical treatment for obesity on glucagon-like peptide 1 levels and glucose homeostasis in non-diabetic subjects. Obes Surg 2011;21:902-909.
259. Nakatani H, Kasama K, Oshiro T, Watanabe M, Hirose H, Itoh H. Serum bile acid along with plasma incretins and serum high-molecular weight adiponectin levels are increased after bariatric surgery. Metabolism 2009;58:1400-1407.
260. Hylemon PB, Zhou H, Pandak WM, Ren S, Gil G, Dent P. Bile acids as regulatory molecules. J Lipid Res 2009;50:1509-1520.
261. McGavigan AK, Garibay D, Henseler ZM, Chen J, Bettaieb A, Haj FG, Ley RE, Chouinard ML, Cummings BP. TGR5 contributes to glucoregulatory improvements after vertical sleeve gastrectomy in mice. Gut 2015.
262. Ryan KK, Tremaroli V, Clemmensen C, Kovatcheva-Datchary P, Myronovych A, Karns R, Wilson-Perez HE, Sandoval DA, Kohli R, Backhed F, Seeley RJ. FXR is a molecular target for the effects of vertical sleeve gastrectomy. Nature 2014;509:183-188.
263. Patti ME, Houten SM, Bianco AC, Bernier R, Larsen PR, Holst JJ, Badman MK, Maratos-Flier E, Mun EC, Pihlajamaki J, Auwerx J, Goldfine AB. Serum bile acids are higher in humans with prior gastric bypass: potential contribution to improved glucose and lipid metabolism. Obesity (Silver Spring) 2009;17:1671-1677.
264. Pournaras DJ, Glicksman C, Vincent RP, Kuganolipava S, Alaghband-Zadeh J, Mahon D, Bekker JH, Ghatei MA, Bloom SR, Walters JR, Welbourn R, le Roux CW. The role of bile after Roux-en-Y gastric bypass in promoting weight loss and improving glycaemic control. Endocrinology 2012;153:3613-3619.
265. Graessler J, Qin Y, Zhong H, Zhang J, Licinio J, Wong ML, Xu A, Chavakis T, Bornstein AB, Ehrhart-Bornstein M, Lamounier-Zepter V, Lohmann T, Wolf T, Bornstein SR. Metagenomic sequencing of the human gut microbiome before and after bariatric surgery in obese patients with type 2 diabetes: correlation with inflammatory and metabolic parameters. Pharmacogenomics J 2013;13:514-522.
266. Zhang H, DiBaise JK, Zuccolo A, Kudrna D, Braidotti M, Yu Y, Parameswaran P, Crowell MD, Wing R, Rittmann BE, Krajmalnik-Brown R. Human gut microbiota in obesity and after gastric bypass. Proc Natl Acad Sci U S A 2009;106:2365-2370.
267. Tremaroli V, Karlsson F, Werling M, Stahlman M, Kovatcheva-Datchary P, Olbers T, Fandriks L, le Roux CW, Nielsen J, Backhed F. Roux-en-Y Gastric Bypass and Vertical Banded Gastroplasty

Induce Long-Term Changes on the Human Gut Microbiome Contributing to Fat Mass Regulation. Cell Metab 2015;22:228-238.
268. Liou AP, Paziuk M, Luevano JM, Jr., Machineni S, Turnbaugh PJ, Kaplan LM. Conserved shifts in the gut microbiota due to gastric bypass reduce host weight and adiposity. Sci Transl Med 2013;5:178ra141.
269. Sayin SI, Wahlstrom A, Felin J, Jantti S, Marschall HU, Bamberg K, Angelin B, Hyotylainen T, Oresic M, Backhed F. Gut microbiota regulates bile acid metabolism by reducing the levels of tauro-beta-muricholic acid, a naturally occurring FXR antagonist. Cell Metab 2013;17:225-235.
270. Larsson E, Tremaroli V, Lee YS, Koren O, Nookaew I, Fricker A, Nielsen J, Ley RE, Backhed F. Analysis of gut microbial regulation of host gene expression along the length of the gut and regulation of gut microbial ecology through MyD88. Gut 2012;61:1124-1131.
271. Roediger WE. Utilization of nutrients by isolated epithelial cells of the rat colon. Gastroenterology 1982;83:424-429.
272. Nelson RJ. The use of genetic "knockout" mice in behavioral endocrinology research. Horm Behav 1997;31:188-196.
273. Stumpel F, Jungermann K. Sensing by intrahepatic muscarinic nerves of a portal-arterial glucose concentration gradient as a signal for insulin-dependent glucose uptake in the perfused rat liver. FEBS Lett 1997;406:119-122.
274. Myers SR, Biggers DW, Neal DW, Cherrington AD. Intraportal glucose delivery enhances the effects of hepatic glucose load on net hepatic glucose uptake in vivo. J Clin Invest 1991;88:158-167.
275. Moore MC, Pagliassotti MJ, Wasserman DH, Goldstein R, Asher J, Neal DW, Cherrington AD. Hepatic denervation alters the transition from the fed to the food-deprived state in conscious dogs. J Nutr 1993;123:1739-1746.
276. Moore MC, Satake S, Baranowski B, Hsieh PS, Neal DW, Cherrington AD. Effect of hepatic denervation on peripheral insulin sensitivity in conscious dogs. Am J Physiol Endocrinol Metab 2002;282:E286-296.
277. Lee YS, De Vadder F, Wichmann A, Tremaroli V, Mithieux G, Bäckhed F. Insulin-like peptide 5 is a microbially regulated peptide that promotes hepatic glucose production. Molecular Metabolism 2016;In press.
278. Cummings DE, Weigle DS, Frayo RS, Breen PA, Ma MK, Dellinger EP, Purnell JQ. Plasma ghrelin levels after diet-induced weight loss or gastric bypass surgery. N Engl J Med 2002;346:1623-1630.
279. Nakai Y, Hosoda H, Nin K, Ooya C, Hayashi H, Akamizu T, Kangawa K. Plasma levels of active form of ghrelin during oral glucose tolerance test in patients with anorexia nervosa. Eur J Endocrinol 2003;149:R1-3.

280. Fruhbeck G, Diez-Caballero A, Gil MJ, Montero I, Gomez-Ambrosi J, Salvador J, Cienfuegos JA. The decrease in plasma ghrelin concentrations following bariatric surgery depends on the functional integrity of the fundus. Obes Surg 2004;14:606-612.
281. Fruhbeck G, Rotellar F, Hernandez-Lizoain JL, Gil MJ, Gomez-Ambrosi J, Salvador J, Cienfuegos JA. Fasting plasma ghrelin concentrations 6 months after gastric bypass are not determined by weight loss or changes in insulinemia. Obes Surg 2004;14:1208-1215.
282. Chambers AP, Kirchner H, Wilson-Perez HE, Willency JA, Hale JE, Gaylinn BD, Thorner MO, Pfluger PT, Gutierrez JA, Tschop MH, Sandoval DA, Seeley RJ. The effects of vertical sleeve gastrectomy in rodents are ghrelin independent. Gastroenterology 2013;144:50-52 e55.
283. Mokadem M, Zechner JF, Margolskee RF, Drucker DJ, Aguirre V. Effects of Roux-en-Y gastric bypass on energy and glucose homeostasis are preserved in two mouse models of functional glucagon-like peptide-1 deficiency. Mol Metab 2014;3:191-201.
284. Wilson-Perez HE, Chambers AP, Ryan KK, Li B, Sandoval DA, Stoffers D, Drucker DJ, Perez-Tilve D, Seeley RJ. Vertical sleeve gastrectomy is effective in two genetic mouse models of glucagon-like Peptide 1 receptor deficiency. Diabetes 2013;62:2380-2385.
285. Pepino MY, Bradley D, Eagon JC, Sullivan S, Abumrad NA, Klein S. Changes in taste perception and eating behavior after bariatric surgery-induced weight loss in women. Obesity (Silver Spring) 2014;22:E13-20.
286. Damms-Machado A, Mitra S, Schollenberger AE, Kramer KM, Meile T, Konigsrainer A, Huson DH, Bischoff SC. Effects of surgical and dietary weight loss therapy for obesity on gut microbiota composition and nutrient absorption. Biomed Res Int 2015;2015:806248.
287. Graf D, Di Cagno R, Fak F, Flint HJ, Nyman M, Saarela M, Watzl B. Contribution of diet to the composition of the human gut microbiota. Microb Ecol Health Dis 2015;26:26164.
288. Cotillard A, Kennedy SP, Kong LC, Prifti E, Pons N, Le Chatelier E, Almeida M, Quinquis B, Levenez F, Galleron N, Gougis S, Rizkalla S, Batto JM, Renault P, consortium ANRM, Dore J, Zucker JD, Clement K, Ehrlich SD. Dietary intervention impact on gut microbial gene richness. Nature 2013;500:585-588.
289. Duncan SH, Lobley GE, Holtrop G, Ince J, Johnstone AM, Louis P, Flint HJ. Human colonic microbiota associated with diet, obesity and weight loss. Int J Obes (Lond) 2008;32:1720-1724.
290. De Filippo C, Cavalieri D, Di Paola M, Ramazzotti M, Poullet JB, Massart S, Collini S, Pieraccini G, Lionetti P. Impact of diet in shaping gut microbiota revealed by a comparative study in children

from Europe and rural Africa. Proc Natl Acad Sci U S A 2010;107:14691-14696.
291. Imhann F, Bonder MJ, Vich Vila A, Fu J, Mujagic Z, Vork L, Tigchelaar EF, Jankipersadsing SA, Cenit MC, Harmsen HJ, Dijkstra G, Franke L, Xavier RJ, Jonkers D, Wijmenga C, Weersma RK, Zhernakova A. Proton pump inhibitors affect the gut microbiome. Gut 2016;65:740-748.
292. Jackson MA, Goodrich JK, Maxan ME, Freedberg DE, Abrams JA, Poole AC, Sutter JL, Welter D, Ley RE, Bell JT, Spector TD, Steves CJ. Proton pump inhibitors alter the composition of the gut microbiota. Gut 2016;65:749-756.
293. Johnson M. Laboratory Mice and Rats. MATER METHODS 2012;2:113 http://dxdoiorg/1013070/mmen2113 2015.
294. Charles River. C57/Bl6 research model. http://www.criver.com/files/pdfs/rms/c57bl6/rm_rm_d_c57bl6n_mouse.aspx.
295. The Jackson Laboratory. C57Bl/6 strain. Mouse strain data sheet. https://www.jax.org/strain/000664.
296. The Jackson Laboratory. Outbred stocks. http://www.informatics.jax.org/silver/chapters/3-2.shtml.
297. Taconic. Swiss webster outbred. http://www.taconic.com/mouse-model/swiss-webster.
298. Ussar S, Griffin NW, Bezy O, Fujisaka S, Vienberg S, Softic S, Deng L, Bry L, Gordon JI, Kahn CR. Interactions between Gut Microbiota, Host Genetics and Diet Modulate the Predisposition to Obesity and Metabolic Syndrome. Cell Metab 2015;22:516-530.
299. Grover M, Kashyap PC. Germ-free mice as a model to study effect of gut microbiota on host physiology. Neurogastroenterol Motil 2014;26:745-748.
300. Arvidsson C, Hallen A, Backhed F. Generating and Analyzing Germ-Free Mice. Curr Protoc Mouse Biol 2012;2:307-316.
301. NIH. New comprehensive view of the mouse genome finds many similarities and striking differences with human genome. https://www.nih.gov/news-events/news-releases/new-comprehensive-view-mouse-genome-finds-many-similarities-striking-differences-human-genome.
302. Stefater MA, Perez-Tilve D, Chambers AP, Wilson-Perez HE, Sandoval DA, Berger J, Toure M, Tschop M, Woods SC, Seeley RJ. Sleeve gastrectomy induces loss of weight and fat mass in obese rats, but does not affect leptin sensitivity. Gastroenterology 2010;138:2426-2436, 2436 e2421-2423.
303. Rosenbaum M, Knight R, Leibel RL. The gut microbiota in human energy homeostasis and obesity. Trends Endocrinol Metab 2015;26:493-501.

304. Broadbent R. Endoscopically Assisted Gastric Stomal Dilation for Reflux and Vomiting after Gastric Banding. Obes Surg 1994;4:47-50.
305. Horn CC, Kimball BA, Wang H, Kaus J, Dienel S, Nagy A, Gathright GR, Yates BJ, Andrews PL. Why can't rodents vomit? A comparative behavioral, anatomical, and physiological study. PLoS One 2013;8:e60537.
306. Hajnal A, Kovacs P, Ahmed T, Meirelles K, Lynch CJ, Cooney RN. Gastric bypass surgery alters behavioral and neural taste functions for sweet taste in obese rats. Am J Physiol Gastrointest Liver Physiol 2010;299:G967-979.
307. Setchell KD, Rodrigues CM, Clerici C, Solinas A, Morelli A, Gartung C, Boyer J. Bile acid concentrations in human and rat liver tissue and in hepatocyte nuclei. Gastroenterology 1997;112:226-235.
308. Nguyen TL, Vieira-Silva S, Liston A, Raes J. How informative is the mouse for human gut microbiota research? Dis Model Mech 2015;8:1-16.