Intestinal adaptation in response to Roux-en-Y Gastric Bypass Surgery

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To my family

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ABSTRACT

Background: Obesity is a condition with increasing prevalence that leads to morbidity, decreased quality of life and reduced life expectancy. The only effective evidence-based treatment is bariatric surgery. The most well documented procedure; Roux-en-Y Gastric Bypass (RYGB) results in a substantial and sustainable weight loss and an improved metabolic state. The mechanisms of action have not yet been fully elucidated but recent research has indicated that the proximal alimentary tract has a profound influence on central aspects of the body's metabolism such as appetite regulation and hepatic glucose production.

Aim: The general aim of this thesis was to explore alterations to the proximal small intestinal mucosa induced by RYGB, and thereby link functional aspects of the small intestine in the context of obesity and obesity related morbidity with the effects of RYGB surgery.

Method: Jejunal mucosal samples from patients were obtained during RYGB surgery and 6 months post-operatively via endoscopy. A proteomic analysis using 2-D gel electrophoresis and mass spectroscopy was then performed using a paired samples setting. The results from this exploratory proteomic analysis were then used as starting points for further in depth analysis of aspects of intestinal function such as barrier integrity, calcium uptake and lipid metabolism. For these studies additional human mucosal tissue samples were collected and analyzed with western blot, immunohistochemistry and in Ussing chambers. Also, previously collected bone densitometric data from a RCT comparing RYGB to vertical banded gastroplasty (VBG) were analyzed. Animal experiments using C57BL/6 mice and cell culture experiments using Caco-2 cell lines were performed as well.

Results: The proteomic analysis identified several proteins in the jejunal mucosa with markedly altered expression levels after RYGB surgery. Among these were cytokeratin (CK)8, Heat-shock protein (HSP) 90β and 3-hydroxy-3-methylglutaryl-CoA synthase 2 (HMGCS2) that were considered of particular interest.

CK8 has been reported to be of importance for intestinal mucosal barrier function. Further analysis with western blot revealed profound alterations in the expression levels of several proteins involved in tight junctions that are important in maintaining barrier integrity. Ussing chamber experiments linked an increase in Claudin-3 expression after RYGB to a decrease in intestinal permeability as reflected by reduced electrical resistance.

HSP90β has been reported to be a co-activator of vitamin-D in the small intestine. Additional western blot analysis suggested a decreased vitamin D receptor (VDR) activity in the small intestine after RYGB. VDR mediates active calcium uptake in the small intestine and analysis of DEXA data from patients that had undergone RYGB or VBG show that RYGB induced a weight loss independent decrease in bone mineral density.

Finally, analyses of human mucosal samples and animal experiments indicated that the production of ketone bodies in the proximal small intestinal mucosa could be induced by diet composition, and that this effect may be reversed by RYGB surgery indicating that lipid metabolism in the proximal small intestine is altered in obesity and in response to RYGB.

Conclusion: In our study, RYGB induces several changes to the proteome of the small intestinal mucosa indicating alterations in central aspects of small intestinal function such as barrier integrity, calcium absorption and lipid metabolism. These alterations could be of importance for linking the clinical effects of RYGB surgery to the largely unexplained pathophysiological mechanisms that link obesity with morbidity.

Keywords: obesity, bariatric surgery, proteomic analysis, intestinal permeability, calcium, bone mineral density, lipid metabolism

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SAMMANFATTNING PÅ SVENSKA

I dagens samhälle intar många människor mer energi med födan än de gör av med. Denna överskottsenergi lagras i form av fett och ökar kroppsvikten i förhållande till kroppslängden vilket leder till att det s.k. body–mass index (BMI) ökar. Fetma definieras som ett BMI över 30 och är kopplat till en kraftigt ökad risk för flera sjukdomar, t ex typ-2 diabetes, högt blodtryck, försämrad livskvalité och i förlängningen även överdödlighet. Forskning har visat att det är svårt att åstadkomma långsiktiga livsstilsförändringar som leder till varaktig viktnedgång. Sjukvården har haft begränsade möjligheter att erbjuda bevisat effektiva behandlingar mot fetma. Den enda behandling som hos flertalet patienter har visat sig fungera på sikt och reducera överdödligheten är Roux-en-Y Gastric Bypass (RYGB) operation. Denna typ av "övervikts-operation" innebär i korthet att man kopplar förbi större delen av magsäcken och tolvfingertarmen varefter galla och mat blandas först efter ca 120cm i tunntarmen. Man vet inte säkert varför operationen leder till viktnedgång och andra positiva effekter på hälsan. Det verkar som om operationen förändrar mekanismer som påverkar aptiten och därigenom delvis leder till ett förändrat ätbeteende.

Forskning på djurmodeller har påvisat hur tunntarmen kan känna av innehållet i födan och påverka andra organ som är viktiga för regleringen av kroppens ämnesomsättning t.ex. levern och hjärnan. Vår hypotes har varit att något eller några av dessa reglersystem påverkas på ett ogynnsamt sätt när man utvecklar fetma, och att detta kan återställas av RYGB operationen.

Vi har samlat in vävnadsprover från tunntarmens slemhinna hos patienter i samband med RYGB operation och sedan ånyo från samma patienter 6 månader efter operation. Vi har analyserat dessa prover för att hitta skillnader bl.a. i uttrycket av olika proteiner som kan vara delaktiga i effekterna av RYGB. Vi kan visa att flera olika proteiner ändras efter operation och en del av dessa proteinförändringar skulle kunna kopplas till viktiga funktioner i tarmen såsom upptag av kalk, barriärfunktion och fettnedbrytning, som i sin tur kan påverka signaleringsmekanismer för avkänning av näringsinnehållet i födan. Vi har även studerat dessa eventuella förändringar i en del av tarmens funktioner med andra experimentella metoder och ytterligare försök med friska frivilliga försökspersoner. Sammanfattningsvis talar våra resultat för att RYGB operationen påverkar flera viktiga funktioner i tunntarmen, som kan vara av betydelse för effekterna av operationen och som kan bidra med nya uppslag till de delvis okända mekanismerna som ligger bakom fetmans följdsjukdomar.

LIST OF PAPERS

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

I. **Erik Elias**, Anna Casselbrant, Emma Spak, Lars F ändriks, Ville Wallenius. Global proteomic analysis of proximal small intestinal mucosa before and after Roux-en-Y Gastric Bypass Surgery for obesity (Manuscript)

II. Anna Casselbrant, **Erik Elias**, Lars Fändriks, Ville Wallenius, Expression of tight-junction proteins in human proximal small intestinal mucosa before and after Roux-en-Y gastric bypass surgery Surgery for Obesity and Related Diseases, (2014); 11(1): 45-53

III. **E. Elias**, A. Casselbrant, M. Werling, K. Abegg, R. P. Vincent, J. Alaghband-Zadeh, T. Olbers, C. W. le Roux, L. Fändriks and V. Wallenius. Bone mineral density and expression of vitamin D receptor-dependent calcium uptake mechanisms in the proximal small intestine after bariatric surgery. British Journal of Surgery (2014); 101: 1566–1575

IV. **Erik Elias**, Anna Casselbrant, Lars Fändriks, Ville Wallenius. Altered lipid metabolism in the jejunum in obesity and after RYGB surgery. (Manuscript.)

CONTENT

ABBREVIATIONS

RYGB	Roux-en-Y Gastric Bypass
BMI	Body mass index
DEXA	Dual-energy X-ray absorptiometry
CNS	Central Nervous System
T2DM	Type 2 Diabetes Mellitus
HMGCS2	3-hydroxy-3-methylglutaryl-CoA synthase 2
VDR	Vitamin D receptor
CK8	Cytokeratin 8
HSP90 β	Heat shock protein 90 β
BHB	β - hydroxybutyrate

1 INTRODUCTION

1.1 Obesity, cause and consequence

1.1.1 In general

Throughout the course of evolution, energy has been a limited resource. Consequently, a common theme in living organisms has been to utilize available energy in the most efficient way. This is reflected in all the biological processes that constitute life.

Also, for many organisms, energy availability has been unpredictable and this has led to the evolution of mechanisms for storing energy, thus maximizing the use of available energy. Finally, since energy has historically been a limited resource necessary for sustaining life, organisms in general have an inherent drive to maximize energy intake. Many intricate behaviors and complex interactions between organisms of the same species and also between other forms of organisms reflect the evolutionary process that has aimed to maximize the benefit of a limited resource.

Figure 1. Chipmunk storing excess energy. Wikicommons media

In summary, three basic principles of energy management are shared among most living organisms:

1. Maximizing energy intake

2. Utilizing energy in the most efficient way

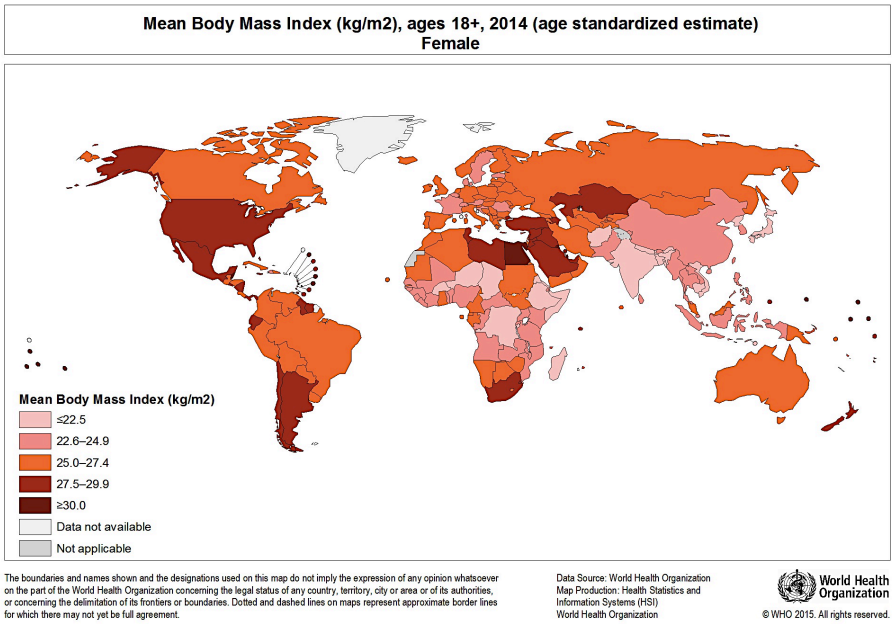
3. Storing excess energy

Figure 2. Ants cultivating aphids. Wikicommons media

1.1.2 In humans

The development of human civilization has created vastly different conditions for humans living today compared to the conditions when *Homo sapiens* evolved. For a large proportion of humanity, energy is no longer a limited resource and innovations have lead to reduced energy requirements for performing different tasks of daily living.

This has created an environment where many humans throughout their lives have access to an excess of energy. However this, in theory, beneficial environment where energy access is not restricted can in itself lead to increased morbidity and mortality. In *Homo sapiens* the main mechanism for storing energy is to synthesize triglycerides from lipids and store them within fat cells, adipocytes. Of the three principle nutrients available to mammals as an energy source: carbohydrates, protein and fat, fat is the most energy dense of the three and therefore provides the most efficient way to store excess energy for later use. In an environment that provides a continuous excess of energy this leads to an accumulation of stored energy, in the form of fat, which leads to increased body weight. **Body mass index** (BMI) is a commonly used measurement that describes the relationship between body height (which in the adult human is assumed to be constant) and body weight. It is calculated as BMI = weight (in kilograms)/ height * height (in meters).



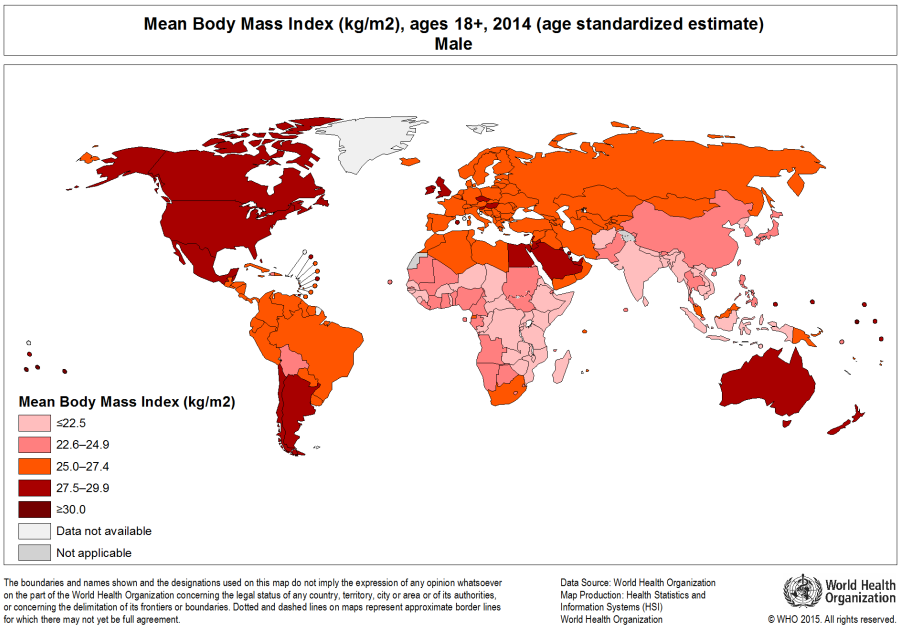


Figure 3. Average BMI in 2014. WHO

WHO has defined obesity has as a BMI of 30 or higher. It is a condition with increasing prevalence and is currently viewed as a major medical challenge as it is associated with increased morbidity and mortality and difficult to treat.

Obesity is associated with increased mortality and is an important risk factor for several medical conditions such as diabetes mellitus type 2, hypertension, and ischemic heart disease. As a consequence obesity reduces quality of life and life expectancy (1) (2).

It is currently not fully elucidated why the storage of excess energy causes morbidity. However, it has been shown that there is a link between obesity and increased inflammation (3). The causal link between inflammation and obesity is still unclear but the main theory suggests that macrophage infiltration in the adipose tissue results in release of pro-inflammatory substances such as cytokines (3). This inflammation has been suggested to result in decreased peripheral insulin sensitivity and decreased beta cell function, which are the hallmarks of Type 2 Diabetes Mellitus (T2DM). This systemic inflammation has also been associated with the development of arteriosclerosis and an increased risk of cancer. (4).

Obesity should, in theory, be easy to treat by adjusting energy intake according to energy requirements. Popular terms for these adjustments are "diet" i.e. to decrease energy intake and "exercise" i.e. increase energy expenditure. However, in practice, these life-style adjustments are difficult to maintain, often leading to weight-relapse. Pharmacological treatment options are currently limited as several substances have been withdrawn due to severe side affects and limited effects.

Intuitively, an appealing explanation as to why voluntary adjustment of energy intake is difficult to maintain is that the evolutionary process has generated mechanisms that serve to facilitate energy intake. Illustrative of this concept, several alterations in CNS appetite regulation have been described in the context of obesity. These alterations such as leptin resistance mostly act to reduce satiety and increase appetite (5).

In summary, as a result of a changed environment, obesity represents a major challenge to the healthcare industry. Obesity also represents a rather unique theoretical challenge as the causality is clearly defined and therefore therapeutic options should be readily available.

From a researchers perspective, several theoretical questions arise: Why does obesity lead to morbidity? Why is it resistant to treatment based on life-style changes? And why isn't everyone living in the same environment afflicted?

1.2 Bariatric surgery

Interestingly, surgical intervention is the only effective therapeutic option currently available in the treatment of obesity.

1.2.1 Brief history

Bariatric surgery is a form of gastro-intestinal surgery that has evolved over the last 50 years in order to offer a surgical treatment option for obesity (6). The initial operations were based on two principal mechanisms of action to achieve weight loss.

Restrictive procedures, which focused on restricting to amount of energy that a patient could consume. This is primarily accomplished by diminishing the volume of the stomach and thereby reducing its reservoir function. The most recent surgical procedure utilizing this mechanism is adjustable gastric banding, which is to some extent still used internationally. However, many patients experience side effects related to this procedure such as extensive vomiting and discomfort caused by excessive restriction.

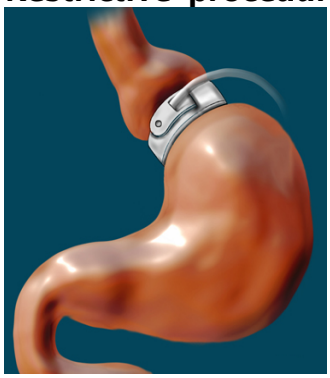
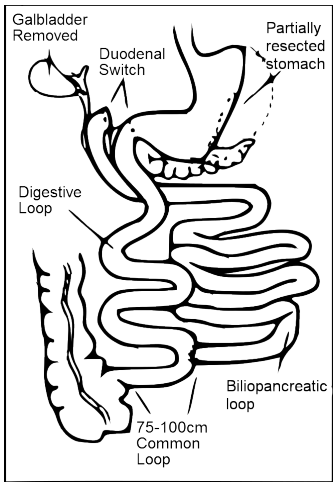


Figure 4. Image of adjustable gastric bandning. Wikicommons media

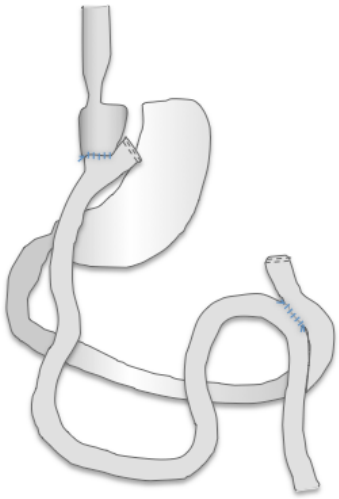


Also, adjustable gastric banding and similar procedures seem to have no or little effect on satiety and appetite. Actually, the combination of hunger and troublesome food intake is stressful and often makes the individual consume more energy dense food to compensate for the restriction.

Mal-absorptive procedures instead focus on reducing energy uptake. These surgical procedures aim to reduce part of the physiological nutrient uptake by bypassing parts of the small intestine.

Figure 5. Image of duodenal switch. Wikicommons media

This induced a general malabsorption, which would then result in weight loss. Such procedures often have severe side effects related to overly extensive malabsorption or the presence of a ”closed end” segment of the small intestine promoting bacterial overgrowth. The most recent example of a mal-absorptive procedure is the biliopancreatic diversion with duodenal Switch. This procedure induces substantial weight loss, however it is also associated with significant side effects caused by excessive malabsorption and is therefore now only used very scarcely in some centers in super-obese patients (7).



1.2.2 Roux-en-Y Gastric Bypass

The most well-documented and effective treatment of obesity is Roux-en-Y gastric bypass surgery (RYGB) that originally evolved from similar procedures used for gastric resection. Bariatric surgery results in a substantial and sustainable weight loss and has been shown to reduce mortality and associated morbidity (8) (9) (10).

Figure 6. RYGB, image provided by V. Wallenius

Anatomical alterations to the alimentary tract induced by RYGB

The technique for the current gold standard RYGB operation was originally developed at Sahlgrenska Hospital in Gothenburg by Professor Hans Lönroth and colleagues (11). The standard RYGB operation is accomplished by: creating a small gastric pouch) and connecting it to the jejunum in an antecolic-antegastric Roux-en-Y construction.

As a consequence, RYGB induces several alterations to the proximal alimentary tract:

1. Diversion of ingested nutrients from the majority of the stomach, the duodenum and approx. 40-60 cm of the jejunum.

2. Diversion of bile, gastric and pancreatic juices from approx. 120 cm of the jejunum.

3. **Abolishing basic functions of the stomach such as digestion and the reservoir function.**

4. Rapid delivery of undigested nutrients to the roux-limb without passing the acid barrier of the stomach.

Effects of RYGB and mechanisms of action

Initially, it was believed that RYGB leads to weight loss by a combination of restriction and malabsorption (6). However this belief has not been supported by research (12). Both clinical and experimental studies suggest that RYGB surgery induces a profound alteration in body weight homeostasis (13). This is the fundamental difference between RYGB and conventional methods of achieving weight loss. The main concern with voluntary adjustment of energy intake is that it can be effective in the short term but it is not sustainable.

RYGB on the other hand seems to induce a permanent shift in the body weight that the patient can manage maintain. Illustrative of this, RYGB has been described to alter food preference and altered perception of satiety (14) (15) (16). Such fundamental alterations require an alternative mechanism of action other than the strictly mechanistic effects of the operation.

It has also been described that many metabolic improvements such as improved glucose homeostasis take place prior to weight loss. This phenomenon is often clinically apparent and requires adjusted diabetes medication during the primary post-operative care. Although some scientific data exist, this feature has not been as extensively documented as the effects on weight loss and other long-term effects. It is also unclear if the rapid improvement in glucose homeostasis and the long-term weight loss reflects two different presentations of the same mechanism or whether they are two principally different effects of RYGB.

It is currently not fully elucidated how RYGB leads to the observed clinical effects. Several mechanisms of action not relating to restriction or mal-absorption have been suggested.

Changed levels of hormones secreted by the gastrointestinal tract ("gut hormones") have been suggested to contribute to the clinical effects of RYGB (17). The hormones that have received the most attention are GLP-1, Grehlin and PYY. These hormones have been shown to affect insulin release and satiety, similar to the effects of RYGB (18). However, there has been mixed results from studies attempting to link GLP-1 and other "gut hormones" with effects of RYGB (19). Animal experiments have demonstrated that intact GLP-1 signaling is not required for the beneficial effects of RYGB (20).

Bile acids are secreted with bile from the liver into the duodenum where they solubilize ingested lipids and facilitate uptake. Recently it has been suggested that bile acids can act as hormones and via the factor Farnesoid x-receptor (FXR) exert metabolic regulation (21) (22). FXR has FGF19 as a gene target and FGF19 can influence bile acid secretion which implies a potential "entero-hepatic crosstalk" feedback mechanism were the small intestine and the liver can coordinate a systemic metabolic response (23). It has also been suggested that this concept interacts with another novel concept in metabolic regulation: the role of luminal microbiota (24).

The gut microbiota has in experimental animal studies been shown to have a profound influence on body weight and metabolic homeostasis (25) and experiments with germ-free mice have indicated that surgical effects can be mimicked with faecal transplantation (26). Gut microbiota can also, via bile acids, interact with the FXR receptor (24). However, human data is scarce and a causal link between gut microbiota and the suggested metabolic effects have not been identified.

Bile diversion and the loss of the acid barrier are consequences of RYGB, and this provides a reasonable explanation as to why these mechanisms could be affected by surgery.

Alterations to intestinal lipid metabolism and intestinal barrier integrity have also been suggested to contribute to the effects of RYGBP. These mechanisms are more thoroughly addressed in the next section and in paper II and IV.

In summary, RYGB represents a unique opportunity as it offers a clinically effective treatment to a major healthcare challenge. However, invasive procedures are always associated with a risk for complications and are expensive, especially in relation to the high prevalence of obesity. There are

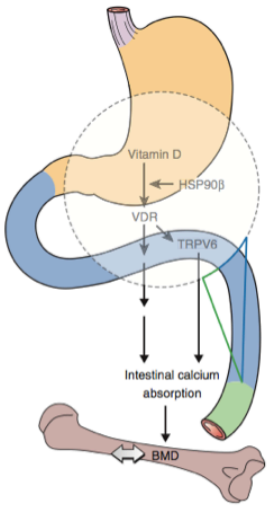
also potential operation-type specific side effects such as decreased absorption of essential micronutrients.

Identifying the molecular mechanisms that explain the observed clinical effects of RYGB can lead to novel insights regarding the pathogenesis of obesity related morbidity and offer new and less invasive treatment options.

1.3 Functional aspects of the small intestine in the context of obesity and bariatric surgery

1.3.1 Macro- and micro-nutrient absorption

The most obvious role of the small intestine, in the context of obesity, is being the primary organ for digesting and absorbing ingested nutrients. As mentioned previously, humans can utilize three principle nutrients for energy production: carbohydrates, fatty acids from lipids and amino acids from proteins. These are all primarily absorbed in the jejunum but through different mechanisms. Carbohydrates are transported into the epithelial cells with the aid of transporter proteins such as SLGT1. Fatty acids are solubilized in the intestinal lumen with the aid of bile acids and can then diffuse through the cell membrane. Amino acids are absorbed with the aid of specialized transporters. The small intestine also is the primary site for absorption of other essential substances. These include, among others, vitamins, iron and calcium. Absorption of these micro-nutrients is often achieved by the use of specialized transport mechanisms enabling the regulation of uptake. These specialized transport mechanisms are localized at different anatomical locations of the small intestine.



Calcium absorption

Taking calcium as an example, calcium is absorbed by two principally different pathways. Passive paracellular diffusion and an active transport mediated by the intestinal vitamin D receptor (VDR) (27). Passive diffusion occurs along the entire small intestine as well as in the colon. The active (facilitated) absorption occurs primarily in the proximal small intestine and requires adequate circulating levels of

Figure 7. Schematic image of some aspects of active intestinal calcium absorption.

activated Vitamin-D that together with co activators such as heat shock protein 90 beta (HSP90β) (28) activates the vitamin-D receptor that regulates the transcription of several proteins such as TRPV6 (29) that are believed to act as specific calcium transporters (30).

Macro- and micro-nutrient absorption, obesity and RYGB

Research has suggested that obesity could induce alterations of the basic mechanisms that regulate glucose uptake contributing to increasing energy absorption perhaps reflecting the evolutionary drive to maximize energy intake when it is available. As mentioned previously, inducing a general nutrient malabsorption was one of the two basic principles behind early examples of bariatric surgery. However, the resulting weight loss was hard to predict and severe side effects were common. There is currently no scientific data that indicate that decreased nutrient absorption could represent a mechanism of action for RYGB. There is, however, a concern that RYGB may induce more subtle changes to the absorption of essential micro-nutrients such as calcium, iron etc (31). Specifically, reduced active calcium absorption has been a concern as this could lead to decreased bone mineralization and eventually osteoporosis. Indeed, the anatomical alterations induced by RYGB i.e. the bypass of nutrient flow through the duodenum and the proximal part of the jejunum will omit calcium uptake in this part of the alimentary tract. If active calcium uptake in the Roux limb is also affected this would hypothetically influence overall calcium homeostasis.

1.3.2 Barrier function

The alimentary tract is continuously exposed to bacteria, either as contaminants from ingested nutrient or from the residing bacterial flora. Therefore, the inner mucosal layer facing the lumen must maintain barrier integrity to prevent pathogens from entering into the bloodstream and cause infections. The inside of the intestinal channel is coated with a mucus layer that particularly in the gastroduodenal area and in the large intestine is considerable and act as part of the mucosal barrier (32). In the jejunum, the mucus layer is very thin and permeable to allow the secretion/absorption processes involved in nutrient uptake. The barrier properties of the jejunal mucosa are therefore determined by the state of the surface epithelium. The luminal side of the jejunum consists mainly of cylinder-shaped enterocytes

that are linked together via proteincomplexes called tight junctions.

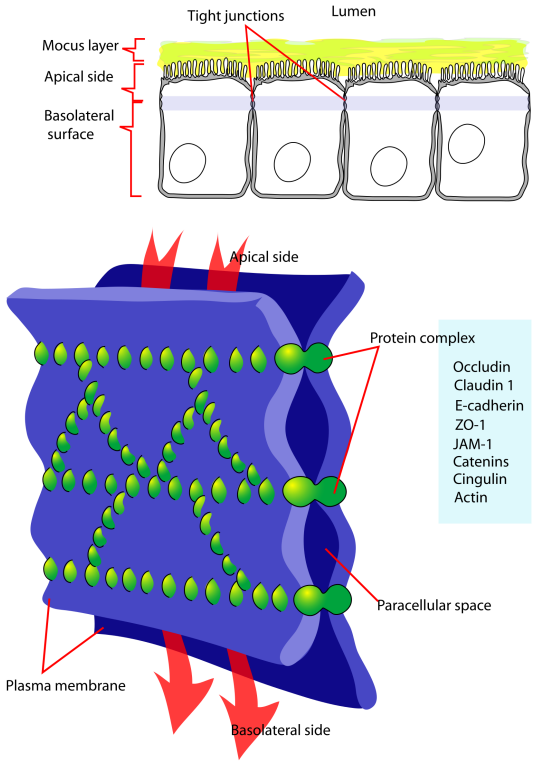


Figure 8. Tight junctions. Wikicommons media

Tight junctions influence paracellular permeability (33, 34). Tight junctions are composed of several proteins, among them are: transmembrane proteins claudin 1-5, occludin, and the scaffold protein zonnula occludens. The claudin family of proteins appear to have a central role in regulating paracellular intestinal permeability and this is accomplished by two separate mechanisms. Some claudins such as claudin-2 are pore-forming claudins and increase intestinal permeability whereas other claudins such as claudin-3 are sealing i.e. increase barrier integrity.

Changes in expression levels of claudins and other components of tight junctions have been shown to affect intestinal permeability (35).

Also, the anchoring of tight junctions to the epithelial cytoskeleton can affect intestinal permeability, and expression levels of components of the cell cytoskeleton such as cytokeratin 8 have been linked to alterations in intestinal permeability (36) (37).

Barrier function, obesity and RYGB

As mentioned previously, obesity has been described be associated with a state of chronic low-grade inflammation that has been suggested to be the causal link that leads to obesity-associated morbidity. It is however unclear how obesity in itself gives rise to the observed inflammation.

A recent theory links alterations in intestinal barrier integrity and the subsequent leakage of pro-inflammatory substances such as bacterial endotoxin or lipopolysaccharides (LPS) with obesity (33) (34). This provides an appealing theory of how obesity associated inflammation may be induced. Several publications have linked diet composition to increased intestinal permeability and increased circulating levels of bacterial endotoxins (LPS) that would result in an inflammatory response (38).

If obesity induced inflammation is derived from the alimentary tract this could provide an interesting mechanism as to why gastrointestinal surgery can reverse this inflammation and thereby affect aspects of obesity related morbidity.

It is unclear if and how RYGB could affect barrier integrity in the small intestine. In the light of the anatomical alterations induced by RYGB several factors could influence barrier integrity, such as reduced exposition of ingested nutrients to the gastric acid thus changing the intraluminal milieu and the diversion of bile and pancreatic juices from the roux limb.

As mentioned in the previous chapter, a changed intestinal microbiota has also been suggested to contribute to the observed clinical effects of RYGB. As the epithelium is the first structure where the microbiota and host interact, altered structural and functional barrier properties could have an influence on these interactions as well.

1.3.3 Intestinal lipid metabolism in response to ingested nutrients

Another novel concept, highlighting the role of the small intestine as a metabolic regulatory organ, is how intestinal lipid metabolism is affected by ingested nutrients. Intestinal lipid metabolism and metabolic signaling have been suggested to affect different aspects of metabolism such as hepatic glucose output and appetite regulation (39).

Several papers describe how the infusion of lipids in the proximal small intestine affects appetite (40) (41). This has been attributed to the intestinal synthesis of specific lipids belonging to the N-acylethanolamines (NAE) family such as oleoylethanolamide (OEA) (42) (43). These lipids have been described to reduce appetite and affect hepatic glucose output via vagal afferents (44). These effects require an intact signaling from peroxisome proliferator activated receptor alpha (PPARα) that regulates cellular pathways of lipid metabolism such as ketogenesis and beta-oxidation. Direct stimulation with an intraperitonial PPARα agonist has also been described to reduce food intake (45) (46).

Intestinal lipid metabolism, obesity and RYGB

The vast majority of research concerning intestinal lipid metabolism and metabolic signaling is experimental research using various animal models that attempt to describe the basic physiological concept. The results of these models indicate short-term beneficial effects of lipid infusion/consumption, such as increased insulin sensitivity. This leads to an interesting paradox as long-term overconsumption of dietary fat has been shown to induce obesity and obesity related morbidity. There are some publications that address this and suggest that long-term lipid consumption induces a pathological change in the basic intestinal lipid metabolism related mechanisms counteracting the initial beneficial effects (47).

The concept of altered or reversed pathological ” intestinal lipid metabolism and metabolic signaling” as a mechanism of action for RYGB is appealing from a research perspective for several reasons.

1. The intestinal lipid metabolism mechanisms described are anatomically localized in a region that is directly affected by RYGB.

2. Intestinal lipid metabolism has been described to influence many of the same aspects of metabolism as RYGB, i.e. satiety and glucose homeostasis.

3. It is believed that RYGB has a rapid onset effect on metabolic morbidity and this fits with the concept of post-operatively altered signaling mechanisms.

However, to our knowledge, no study to date has directly addressed the concept of altered intestinal lipid metabolism in the context of RYGB.

2 AIM

2.1 Overall aim and hypothesis

The overall aim of this thesis was to test the hypothesis that Roux-en-Y gastric bypass surgery induces alterations to the proximal small intestinal mucosa that contribute to the clinical effects of the operation.

2.2 Specific aims of the thesis

Aim 1: *The first aim* was to explore a potentially changed protein expression in the Roux-limb jejunal mucosa in response to RYGB surgery. (**I**)

A proteomic analysis identified several proteins in the jejunal mucosa with markedly altered expression levels in response to RYGB surgery. Among these, cytokeratin 8 (CK8), heat-shock protein 90 β (HSP90β) and HMGCS2 were considered of particular interest and were selected for more detailed analyses:

Aim 2: As CK8 is part of the junctional complexes between the epithelial cells, *the second aim* was to perform a broader investigation of tight junction proteins and paracellular permeability in the jejunum/Roux limb before and after RYGB. (**II**)

Aim 3: HSP90β has been described to affect vitamin D receptor activity (VDR) and *the third aim* was therefore to characterize potential alterations, induced by RYGB, to VDR-regulated proteins that have been described to affect intestinal calcium absorption. (**III**)

Aim 4: Altered expression levels of HMGCS2, the rate-limiting enzyme in ketogenesis, could reflect an effect of RYGB on intestinal lipid metabolism. *The fourth aim* of the present thesis project, therefore, was to characterize the effect that RYGB, dietary composition and obesity have on aspects of small intestinal ketogenesis. (**IV**)

3 PATIENTS AND METHODS

3.1 Patient cohorts and tissue sampling

3.1.1 Tissue sampling before, during RYGB surgery and 6-8 months after RYGB surgery

Papers **I-IV** all contain data from small intestinal tissue samples from human subjects collected in a similar way. These samples were obtained from a consecutive group of consenting patients undergoing first-time RYGB or a conversion from gastric banding to RYGB (Paper **I**, n=7), (Paper **II**, n=33), (Paper **III**, n=10), Paper **IV**, n= 15).

After informed consent was obtained, a full thickness sample from the jejunum was excised per-operatively and the mucosa was dissected using biopsy forceps before it was snap frozen for further analysis (Paper **I-IV**) or kept on ice and mounted for Ussing-chamber analysis (Paper **II**). 6-8 months post-operatively an upper GI-endoscopy was performed and mucosal biopsies from the Roux-limb were retrieved and also snap frozen for further analysis enabling a paired samples analysis (see figure 9).

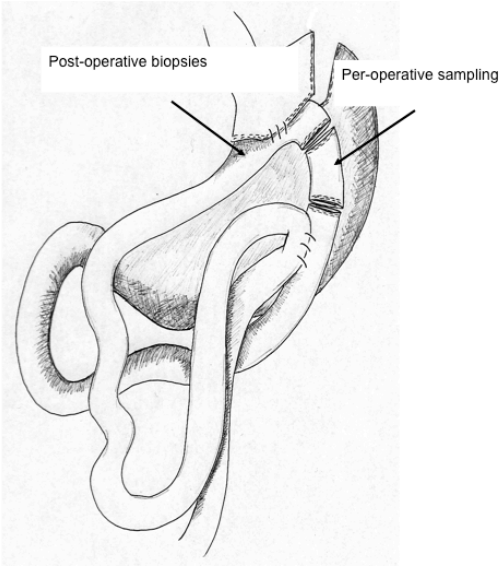


Figure 9. Image of tissue sampling.

Paper IV also contains data from a subgroup of patients within the original cohort that consented to an additional upper-GI endoscopy 4 weeks before surgery. This endoscopy was performed using an extra long instrument making it possible to retrieve mucosal biopsies distally to ligament of Treitz (n=12). The biopsies were retrieved before patients started the pre-operative

Very Low Calorie Diet (VLCD) making it possible to analyse the impact of this diet on protein expression in the jejunal mucosa.

3.1.2 Blood samples

Paper III contains data from an additional cohort of patients

This cohort study comprised a hormone analysis of bone resorption markers after RYGB. Consecutive patients with a body mass index (BMI) over 35 kg/m2 were recruited. Fasting samples taken before and 18 months after RYGB surgery were used for the analysis of bone resorption markers.

3.1.3 The human randomized controlled laparoscopic obesity surgery trial

The human laparoscopic obesity surgery trial was a randomized controlled trial comparing RYGB (n=37) with VBG (n=45), which commenced in 1999. The primary endpoint was weight loss (48). DXA data was collected at baseline (n=28 and n=25, respectively), one year (n=28 and n=25, respectively and six years post-operatively (n=17 and n=14, respectively) (49)

3.1.4 Experimental study on diet composition and protein expression

Paper **IV** contains data from jejunal tissue samples collected by upper GI-endoscopy in a study with healthy volunteers designed to evaluate the impact of diet composition on protein expression. In short, healthy volunteers were recruited to study the effects of diet composition on protein expression in the jejunal mucosa. Inclusion criteria included age between 18-65 years, BMI between 18-30 kg/m2 and no medication or other medical condition. Exclusion criteria included history of substance abuse, pregnancy or known allergy.

15 subjects were included and instructed to alter their diet for 2 weeks so that 60% of the energy would come from either lipids or carbohydrates. After the initial 2 weeks the subjects went through push endoscopy and biopsies were retrieved approximately 50cm distal to ligament of Treitz. After this the subjects had a “wash-out period” of at least 11 days during which they were instructed to eat as they normally would. After the “wash-out period” the subjects then altered their diet so that the subjects that had consumed a lipid-dominated diet would switch to carbohydrates and vice versa. After 2 weeks on this diet an additional endoscopy was performed in a similar way.

Mucosal tissue specimens were snap-frozen in liquid nitrogen and kept frozen (-70°C) for later analysis of protein expression.

3.1.5 Animal experiments

Finally, in paper **IV,** tissue and blood samples from C57BL/6 mice fed a high fat diet or normal chow diet were collected and analyzed.

3.2 Methods

3.2.1 Proteomic analysis (Paper I)

Jejunal tissue samples from patients sampled during surgery and 6-8 months post-operatively were used for a paired samples proteomic analysis (n=7).

The basic principle for the proteomics analysis was 1) sample preparation, protein solubilisation and protein labeling 2) separation using 2d-gel electrophoresis 3) computer assisted spot analysis and picking of selected spots 4) peptide sequencing of selected spots with tandem mass spectroscopy and 5) analysis of obtained peptide sequences using a protein database.

3.2.2 Western blot (Paper I-IV)

Western blotting was used for validation of results from the proteomics analysis as well as detecting novel protein regulations. Commercially available antibodies were used and GAPDH was used as a loading control.

3.2.3 Ussing chamber (Paper II)

Ussing chamber experiments were carried out to evaluate the significance of observed protein changes in the context of barrier integrity. The pulse-Ussing method was used as previously described (50). In short, the basic principle of the pulse-Ussing method is that electric variables measured during certain experimental conditions reflect physiological properties in the epithelial layer. In our setting, intestinal mucosa was mounted as a wall separating two chambers containing oxygenated Krebs solution. Each chamber was supplied with an electrode that was used to measure the potential difference across the mucosa and also to emit standardized current pulses.

The measured potential difference was used as a measurement of sample viability and intact epithelium. A brief current was then applied and the epithelial layer was charged. The epithelial resistance was calculated based

on the rate of epithelial discharge and this value represents paracellular permeability (50).

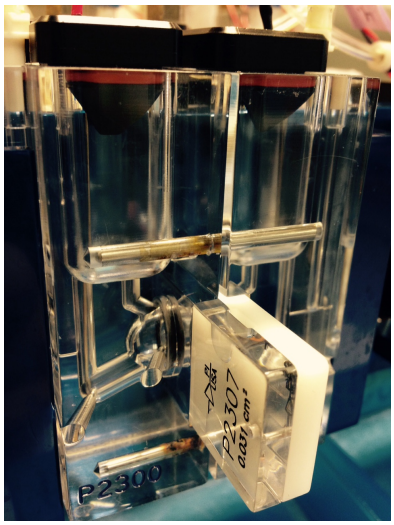


Figure 10. Mounting tissue specimens in ussing chamber. Image by Erik Elias

3.2.4 Other

DEXA (Paper III)

Dual-energy X-ray absorptiometry was used to asses bone mineral density in subjects before and after surgery.

Ketone body analysis (Paper IV)

Ketone body concentration was determined using a commercially available kit. It utilizes a ph-sensitive reaction induced by the enzyme 3-hydroxybutyrate dehydrogenase. The equilibrium between β-hydroxy butyrate (BHB) and acetoacetate (AcAc) is shifted and accompanied by a change in the NADH concentration measured by absorbance at 340 nm. The NADH concentration is proportional to the concentration of either AcAc or BOH depending on what buffer is used.

Statistical analysis

In general, due to limited sample size and unknown distribution of measured variables, non-parametric tests were used to analyze statistical significance in data from human subjects. Data generated from mice and cell cultures were analyzed with parametric tests.

Ethics

The study procedures were performed in accordance with the Declaration of Helsinki and approved by the Regional Ethical Review Board in Gothenburg. All animal procedures involved humane care and use of animals and were approved by the animal ethical committee of University of Gothenburg.

4 SUMMARY OF RESULTS AND INTERPRETATION

4.1 Paper I

4.1.1 Results

The aim of this study was to identify changes to the proteome of the jejunal mucosa induced by RYGB surgery.

Jejunal mucosa samples were collected from seven patients at laparoscopic RYGB surgery and six months post-operatively by endoscopy. A global protein expression analyses was performed by 2-D gel electrophoresis and mass spectrometry (MS)

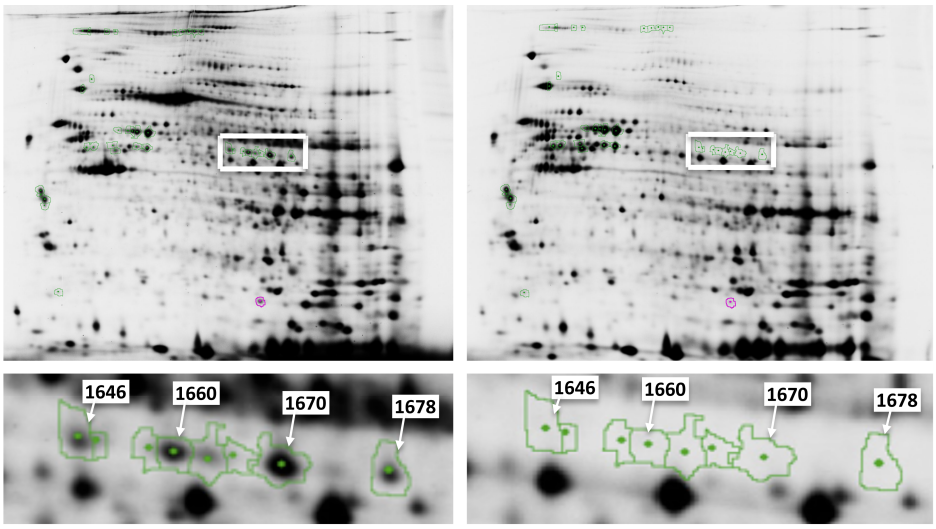


Figure 11. Image of 2D gels and spots

As evident of the image above there were, as expected, a several of regulated protein spots present. We decided to use relatively conservative criteria to determine which spots that would be selected for further analysis. Computer assisted analysis of the 2-D gels indicated 27 spots in eight clusters that were significantly regulated in a similar manner in all included patients.

These spots that matched our selection criteria were excised and analyzed

with MS/MS to identify peptide sequences present in the spot.

Peptide sequences were then run against the MASCOT search database to identify corresponding proteins. Proteins were assigned a score based on the probability that they were present in the spot.

Among the proteins that received the highest scores were cytokeratin (CK) 8, HMGCS2 and HSP90β.

Using protein preparations remaining from the proteomic analysis, western blotting was performed in order to validate the expression changes in the identified proteins selecting the top scoring protein present in each of the eight clusters of spots.

For example, one cluster of spots contained two top scoring proteins; HMGCS2 and CSK. Western blot verified a regulation of HMGCS2 but not of CSK.

4.1.2 Interpretation

When interpreting and discussing the potential implications of these findings there are several methodological considerations that need to be addressed.

The first concerns the characteristics of the patients that were sampled. These patients were consecutively RYGB operated patients that met the criteria for RYGB surgery (i.e. BMI more than 40 or BMI more than 35 *and* comorbidity) at the Sahlgrenska University Hospital surgical clinic and thus represented a diverse cohort of patients. Male and female patients were included, some had metabolic conditions related to obesity and some had previously undergone a restrictive procedure and were converted to a RYGB, usually due to weight regain or vomiting due to the previous restrictive procedure.

The diversity of this patient cohort together with the relatively small sample size (n=7) limits the potential to link specific protein regulations to patient characteristics such as gender or metabolic morbidity. However, one could argue that this study design increases variance and decreases the risk of over-interpretation of results that might have been attributable to a certain subgroup of patients. In other words, results that are present despite the small sample size and relatively large variance in this small patient sample should also be valid for a larger population. In larger clinical materials there are no indications that certain patient characteristics influence the outcome of

surgery, thus we hypothesized that any detectable change to the proteome that was relevant to the outcomes of surgery should be present in all our included patients.

A limitation in our study was that the per- and postoperative samples were not retrieved exactly in the same way; this is relevant to all Papers I-IV. Peroperative tissue samples where dissected (with biopsy forceps) to extract the mucosa in order to mimic the way the endoscopic samples were retrieved. There is however, still a possibility that this approach results in systematic error with unequal amounts of submucosa tissue in the samples. These raises the possibility that the observed protein regulations could in part reflect the difference in sampling and are not directly an effect of the surgery itself.

However, there are arguments against a sampling bias. **First**, the reported proteins were according to the present literature predominantly expressed in the mucosa, our immunohistochemical analysis of one the proteins confirm this (Paper IV). **Second**, the reported proteins that were localized in the mucosa either increased or decreased systematically indicating that there were not simply unequal amounts of mucosa present in the samples. **Finally**, additional experiments in another experimental animal model reproduce the results from the initial study (Paper II-IV).

The proteomic analysis utilized 2D-gel electrophoresis that has currently been replaced with peptide labeling. The major disadvantage with the methodology used is that the MS/MS identifies many proteins present in a regulated spot but not which of these proteins that reflect the change in the spot intensity. Additional western blotting or another similar method is required to confirm that a specific protein is regulated. This is demonstrated in the example of HMGCS2 and CSK that were identified in the same spot, whereas only HMGCS2 expression was changed when analyzed by western blot.

In summary, Paper I presents the results of exploratory research using a proteomic analysis in a novel setting. The data presented are descriptive in nature and cannot be directly linked to the effects of RYGB surgery. However, they can serve as a starting point for additional targeted studies.

4.2 Paper II

4.2.1 Results

This study used the previous finding of an increased expression of Cytokeratin 8 (CK8) in the jejunal mucosa after RYGB as a basis for an in-depth analysis of tight junction protein expression after RYGB. First, the significant upregulation of CK8 was validated in a larger group of patients sampled in the same way as in paper **I**. Vinculin was also analyzed in the same material and consistent with the results from the previous study there was no significant regulation of vinculin after RYGB in this material.

Next, we performed a western blot analysis of several of the tight junction proteins such as the various claudins, occludin and found changed expression levels of several of these proteins after RYGB.

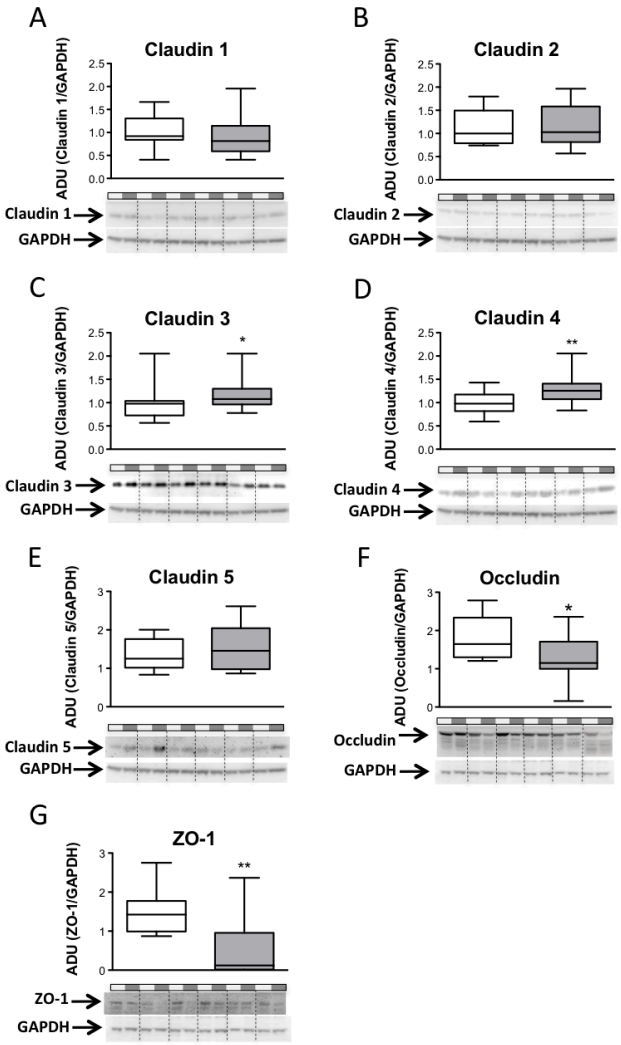


Figure 12. Image of western blot anaylsis

To evaluate the functional relevance of these observed protein regulations we performed an Ussing chamber analysis on per-operatively obtained jejunal tissue samples.

Some of these tissue samples were also used for western blot analysis. This enabled a correlation analysis where intestinal permeability measured *ex vivo* in the Ussing chamber was correlated with the expression levels of the tight junctional proteins.

The only statistically significant correlation between protein expression levels and intestinal permeability assessed with the Ussing chamber technique was the levels of claudin-3.

4.2.2 Interpretation

The results indicate that the jejunal mucosa in the part of the small intestine that constitutes the Roux limb responds to RYGB surgery with a remodeling of the epithelial architecture and significantly changes the expression pattern of many proteins that are central to regulating properties of tight junctions and paracellular permeability. The functional relevance of the changed protein levels is unclear but we can see a consistent pattern where ”sealing” proteins are increased and ”pore forming” proteins are decreased. We can also show that one of the proteins that increases after RYGB was the ”sealing” protein claudin 3 that is correlated with increased epithelial resistance (decreased permeability). Taken together, these results may suggest that RYGB decreases intestinal permeability in the Roux-limb. Alternatively, it could also indicate that the patients preoperatively had increased permeability and that this is normalized after RYGB.

Methodological considerations regarding this paper concern the method of tissue sampling that has been discussed in the preceding section. Although we had an increased sampled size compared to Paper I, the patient material was diverse. We reasoned in a similar way as in Paper I, i.e. that relevant protein regulations would be found in all the patients.

A major shortcoming is that we were not able to directly assess changes in intestinal permeability *in vivo* in the patient cohort.

Unfortunately we could not compare the paired samples using the Ussing chamber, because of technical limitations regarding reproducibility of the exact values of the electric parameters used. Comparison over time of repeated samples would have produced unreliable results. We also considered *in viv*o measurements of intestinal permeability using radioactively labeled carbohydrates. However, as RYGB induces several alterations in the gastrointestinal tract that could affect transit time we felt that such results could be difficult to interpret. We also attempted to measure levels of bacterial endotoxemia before and after RYGB but due to technical difficulties limiting reproducibility of endotoxin measurements the results were inconclusive.

In summary, the results presented in this paper show that RYGB affects several components of the tight junction complex that regulates barrier integrity in the intestinal mucosa. This should motivate additional research focused on small intestinal barrier integrity in the context of obesity and RYGB.

4.3 Paper III

4.3.1 Results

The aim of this study was to study the effect of RYGB on proteins mediating active vitamin-D induced calcium absorption and evaluate if it was possible to detect procedure dependant effects on bone mineral density (BMD).

As HSP90β, which affects vitamin-D receptor (VDR) activity, was decreased after RYGB, we hypothesized that this could result in a decrease of VDR activity. Consequently, we first confirmed the decreased levels of HSP90β in additional jejunal tissue samples before and after RYGPB. We also measured expression levels of the calcium channel protein TRPV6, which is transcriptionally regulated by VDR, in jejunal mucosa before and after RYGB. Expression levels of TRPV6 could reflect alterations to VDR activity. TRPV6 significantly decreased after RYGB and changes to expression levels of TRPV6 and HSP90β correlated significantly with each other. Finally, we measured expression levels of VDR, which increased after RYGBP.

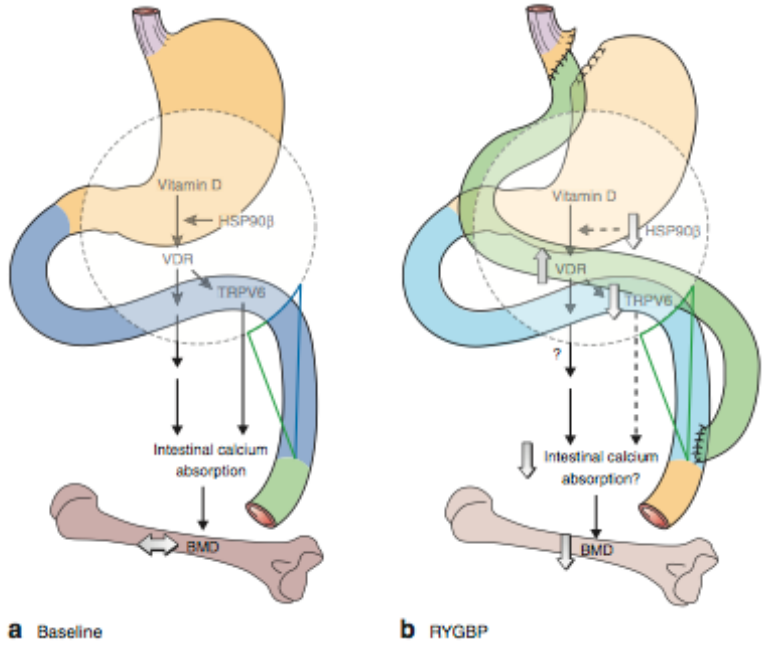


Figure 13. Schematic image on alterations to calcium uptake after RYGB

We then obtained data from a RCT comparing the clinical outcomes of the restrictive procedure VBG with RYGB. We performed a retrospective cohort analysis of these patients DEXA measurements at baseline, one and six years after surgery. These data indicated a weightloss-independent specific effect of RYGB on patient BMD (Paper III).

4.3.2 Interpretation

This paper combines results from two separate studies. One was designed to examine changes in the expression levels of proteins that have been described to affect active vitamin-D induced intestinal calcium absorption. The basis for this study was the observation that HSP90β decreases in Roux-limb mucosa after RYGB (Paper I). HSP90β has been reported to influence VDR activity and we therefore measured levels of a calcium channel protein downstream of VDR (TRPV6) and could show that levels of this protein were also decreased.

The results of this study suggest that RYGB may reduce active vitamin-D induced calcium absorption even in the part of the proximal small intestine that is still exposed to micro-nutrients in the diet. Whether this suggested reduction of active calcium absorption may be of clinical relevance is unclear. It may however represent a mechanism that could explain the reduction of bone mineral density (BMD) that we saw in the patients that had undergone RYGB surgery.

One complicating factor when studying BMD after bariatric surgery is that the weight loss induced by surgery in itself can affect BMD, probably by decreasing pressure load on weight-bearing parts of the skeleton. We attempted to address this by re-assessing specific BMD measurements, both regarding weight-bearing and non-weight bearing parts, in patients that had undergone two different types of bariatric surgery, VBG which is purely restrictive in nature and RYGB that is not primarily restrictive. Originally, these data had been collected previously in a randomized clinical trial comparing the clinical outcomes of VBG and RYGB surgery (48).

Since the groups had similar weight loss after one year these data enabled us to **1:** Compare the two procedures with each other with regards to BMD and **2:** Do a subset analysis of the data selecting skull BMD as a non-weight-bearing part of skeleton that would not be affected by the weight-loss per se.

Our analysis of DEXA data indicated an procedure dependant decrease in BMD after RYGB.

4.4 Paper IV

4.4.1 Results

The aim of paper IV was to further characterize changes to mucosal lipid metabolism in the Roux-limb after RYGB. Based on the finding that HMGCS2 was decreased after RYGB, we hypothesized that the obese patients had a changed intestinal lipid metabolism.

First we confirmed that HMGCS2 was reduced after RYGB in a larger cohort of patients sampled in the same way. We also analyzed levels of the keteolytic enzyme SCOT in the same group of patients as an indicator of whether or not levels of ketone bodies were changed. SCOT decreased after RYGB indicating reduction in intestinal ketogenesis and intestinal ketolysis.

We also analyzed the levels of HMGCS2 in patients sampled at three occasions: before the preoperative Very-Low Calorie Diet (VLCD), at the time of surgery and 6-8 months post-operatively. HMGCS2 has been reported to be transcriptionally regulated by PPARα and therefor we analyzed changes in levels of PPARα as well. Our results indicated that preoperative diet alone increased levels of PPARα and showed a trend towards a decrease in HMGCS2 expression. As our results indicated that diet might affect protein expression levels we designed an experimental study in which study subjects were instructed to alter their diet as to provide energy from mainly lipids or carbohydrates. Two weeks with an altered diet was sufficient in this cohort to affect protein expression in the small intestinal mucosa as HMGCS2 increased and PPARα decreased in response to lipid-dominated diet compared to the carbohydrate diet. To further analyze HMGCS2 expression in the context of obesity we performed animal experiments in which animals were fed a high-fat energy dense "western diet" or normal chow. Animals were sacrificed and intestinal and liver protein expression levels of HMGCS2 and SCOT were analyzed. Interestingly, HMGCS2 was increased in the small intestine but not in the liver after high-fat diet. Also the increase in HMGCS2 was most pronounced in the jejunum, corresponding to the Roux-limb after RYGB surgery in humans.

Finally, we analyzed ketone bodies in portal vein blood and peripheral venous blood from mice fed either high-fat diet or normal chow to determine if the enzyme was functionally active. Mice fed high-fat diet had significantly higher levels of ketone bodies in the portal vein blood compared to peripheral venous blood, whereas the lean normal chow-fed controls had higher ketone levels in peripheral compared to portal vein blood, suggesting that an active and predominantly intestinal ketogenesis is induced by high-fat diet, as compared to normal chow-fed animals.

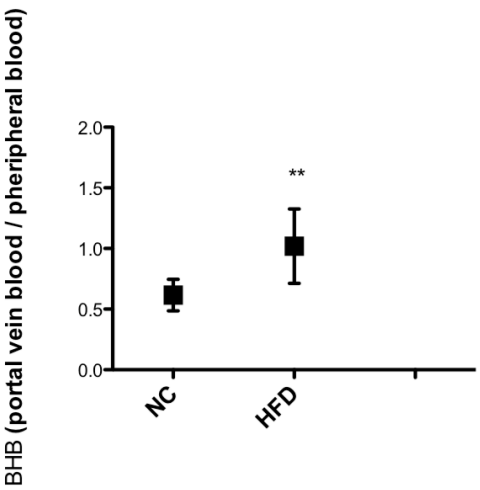


Figure 14. Levels of β – hydroxy butyrate (BHB) in portal blood of mice, NC =Normal chow, HFD= High fat diet

4.4.2 Interpretation

The results from this paper indicate that lipid dominated diet induces an increased HMGCS2 expression in the small intestine and that this is reversed after RYGB. This finding was confirmed in both animal and human experiments. Furthermore, our animal experiments indicate that the enzyme is functionally active thus producing ketonebodies in response to ingested lipids.

Intestinal ketogenesis coupled with an increase in HMGCS2 has been previously described (51) (52). Interestingly this was observed among suckling rats and mice, and was decreased after weaning. Again, the functional implications in this context are unclear but it suggests a mechanism activated during infancy that is "re-activated" in response to diet composition.

Finally, the observation that PPARα decreases in response to a lipid dominated diet and increases in response to RYGB is interesting in the light of previous reports concerning "intestinal lipid metabolism" mechanisms. As mentioned, the PPAR α transcription factor has been shown to be essential in mediating the effects of "intestinal lipid metabolism" signaling. Among the natural activators of PPARα are lipids and in the short term lipid consumption is reported to increase levels of PPARα. However, if consumption of a lipid dominated diet over an extended period of time (two weeks in our experimental study) suppresses PPARα expression this provide an explanation to the discrepancy that short term lipid consumption is beneficial to glucose homeostasis but long-term lipid consumption clinically leads to obesity and morbidity. In fact, preliminary data from our most recent experiments suggest that ketone bodies may directly suppress PPARα expression (unpublished data).

In summary, this paper provides descriptive data that suggest a diet-induced shift in lipid metabolism in proximal small intestine that could be linked to dysfunctional intestinal lipid metabolism induced by obesity. This is reversed after RYGB and could therefore represent a potential novel mechanism of action for RYGB.

5 CONCLUSIONS

1. The results from our proteomic analysis indicate that RYGB surgery consistently alters the mucosal proteome in the part of jejunum that constitutes the Roux-limb.

2. The expression pattern of several proteins present in the intercellular tight junctions of jejunal epithelium is altered after RYGB surgery. The changes suggest a decreased intestinal permeability in the Roux-limb after RYGB surgery.

3. Proteins involved in active vitamin-D dependent calcium absorption is altered by RYGB surgery, suggesting a decrease in calcium absorption after RYGBP.

4. RYGB induces a weight independent reduction of BMD. This, taken together with the previous conclusion, could indicate that RYGB surgery decreases active intestinal calcium absorption in the small intestine.

5. High fat diet seems to induce intestinal ketogenesis in the small intestine and this is diminished after RYGB surgery. High fat diet and RYGB surgery affects expression levels of intestinal PPARα. These findings could suggest that high fat diet induces an altered intestinal lipid metabolism with altered metabolic signaling and that this is reversed in the Roux-limb after RYGB surgery.

6 GENERAL DISCUSSION

This section discusses some general features shared among the presented papers.

First, the main findings in all papers were based on samples from an unselected cohort of patients that were eligible for RYGB surgery at the Sahlgrenska University Hospital surgical clinic. The rationale for this was that RYGB seems to have remarkably similar clinical effects regardless of patient characteristics. This suggests that RYGB surgery achieves its effect by a common mechanism of action that therefore should be present in an unselected cohort of patients.

In an exploratory setting with an elaborate, costly and time-consuming technique like proteomics it was, for practical reasons, only possible to examine samples from a limited number of patients. A certain heterogeneity could aid in "filtering" the data and to only allow for detection of robust changes that would, at best, be relevant for the whole group of patients.

There are however several limitations to this approach. One is that the natural biological variance (due to genetic diversity) and the limited sample sizes tend to induce type 2 errors, i.e. failure to detect a real difference. Our approach to this was to use paired samples and thereby eliminate the biological variance. This has been proven useful to obtain statistically significant results in heterogeneous groups. However, this is obviously a major limitation when attempting to compare groups of patients with different clinical characteristics.

Second, the data presented in these papers are mainly descriptive by nature. Working with human subjects limits the possibility for interventional procedures necessary to establish causality. However, one could argue that the main intervention is the RYGB surgery where the clinical effects are well established but the effects on the molecular levels are largely unknown.

Third, we have tried to address some of the limitations when working with human subjects by complementing the human data with experimental models such as animal models and cell cultures i.e. a *translational research* approach.

This concept utilizes a combination of experimental models and human studies to link experimental data to clinical relevance. The initial data can e g be experimental findings *or* observations from a patient cohort.

As an example of complementary experiments presented in **paper IV**, we wanted to determine if the decreased expression of HMGCS2 after RYGB could represent a "normalization" of HMGCS2 expression i.e. that this protein was abnormally elevated in the obese patients *or* if the decreased protein expression represented a novel change to proteome induced by RYGB.

In order to determine this, we examined jejunal tissue samples from mice fed normal chow or high fat diet and found that high fat diet increased HMGCS2 expression. This suggests that obese humans have an increased expression of HMGCS2 in the jejunal mucosa, possibly due to consumption of high-fat diet, and that this is "normalized" after RYGB as a consequence of the operation. Using jejunal tissue samples from mice, we also examined the distribution of HMGCS2 in the small intestinal tract and HMGCS2 expression in the liver in response to high fat diet, which would be difficult to analyze in human subjects.

We have also performed other complementary experiments such as inhibiting a specific protein in Caco-2 cell cultures and evaluating the impact on expression levels of other proteins. One example relates to the alterations in active calcium uptake after RYGB described in **paper III.** We performed a series of basic cell line experiments using intestinal Caco-2 cell lines and a known HSP90β inhibitor, Geldanamycin. As an example of the type of novel data these experiments generated, we found an effect on the expression of the pore-forming and calcium uptake-facilitating tight junction protein claudin-2 in these experiments (unpublished data). However, we could not verify this regulation *in vivo* in our human jejunal mucosa samples. This illustrates the need to verify experimental data in a human setting.

Another example is our experiments using the Ussing chamber. As reported in **paper II** we linked expression levels of claudin -3 to *ex vivo* measurements of intestinal permeability in human mucosal samples. In my experience, setting up novel experimental models such as the Ussing chamber is time-consuming and requires commitment, however, once these models are established they can be used to test a hypothesis generated from descriptive human data as well as generate novel hypotheses that can be studied in human subjects.

One of our planned future projects is based on observations made during work with **paper II** and **IV.** We observed that intervention with intestinal ketogenesis induced alterations to barrier integrity studied in Ussing chamber experiments (unpublished data). This suggests a novel link between a diet-induced intestinal ketogenesis and a diet-induced decrease in intestinal mucosal barrier integrity in the obese adult.

Previous observations report that intestinal ketogenesis is present in suckling neonate rats *and* that infants have an increased intestinal permeability (compared with healthy adults). If intestinal ketogenesis *and* increased intestinal permeability is present in the infant as well as in the obese adult, this could hypothetically suggest an evolutionary mechanism that could explain why the adult *Homo sapiens* might react with increased intestinal permeability in response to ingestion of high fat diet.

If high fat diet induces intestinal ketogenesis and this affects intestinal permeability, a shared mechanism between infant and adult could be similarities between the main diet of suckling infants i.e. breast milk and the "western diet" i.e. a diet with high lipid content and high caloric content that is believed to contribute to the increasing prevalence of obesity.

Increased intestinal permeability induced by high fat feeding and intestinal ketogenesis could be of value in the suckling infant to allow large macromolecules, such as immunoglobulins, to pass over the mucosal barrier. In obese adults, the same dormant function might be reactivated by high fat diet and allow the passage of bacterial endotoxins contributing to obesity associated inflammation.

These examples illustrate that an advantage with *translational research* is the ability to integrate clinical data and experimental methods and thereby addressing different facets of a research hypothesis within a planned project.

However, the greatest advantage, in my opinion, is the possibility to react to unexpected or puzzling results and in other experimental models or humans perform investigations that can assist in differing between irrelevant results and novel findings.

7 CONCLUDING REMARKS

In several ways, RYGB surgery offers a unique opportunity to address one the major challenges to the healthcare industry and the scientific community.

However, research concerning RYGB surgery has usually been *either* strictly clinical studies measuring clinically relevant endpoints such as mortality or glucose homeostasis *or* more or less complex animal models presenting potential effects of various interventions in a strictly controlled environment.

Clinical studies have provided evidence for the efficiency of surgery but do not provide information on how these effects are achieved. Experimental animal models on the other hand can provide robust results effectively determining causality and provide in depth analysis of interactions on a molecular level. However, they rarely attempt to correlate these experimental findings to human subjects.

In this thesis I have attempted to address this by analyzing the effects of RYGB in human patients on a molecular level thereby linking the clinical intervention with alterations in molecular mechanisms. This approach has generated novel data that, although mainly descriptive by nature, links RYGB surgery to previously reported experimental data and could provide a contribution to the fundamental question regarding RYGB surgery; It works, but how?

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