Obesity - surgical treatment and molecular mechanisms

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ABSTRACT

Obesity is a condition of high prevalence and is associated with increased morbidity and mortality. Bariatric surgery is an effective treatment of obesity and reduces the risk for morbidity and mortality, but little is known of who would benefit the most from this treatment as well as of potential long-term side effects. Furthermore, there is a need for increased understanding of the molecular mechanisms in the adipose tissue and its association with obesity and morbidity.

The overall aim of this thesis was to increase our understanding on how obesity is associated with disease through molecular mechanisms, and to explore effects of bariatric surgery on different outcomes in different subgroups, as well as exploring potential side effects. Specific aims were to compare the effects of bariatric surgery on type 2 diabetes incidence and cardiovascular risk factors in subjects eligible and non-eligible for surgery according to today's eligibility criteria, to explore whether bariatric surgery is associated with increased incidence of alcohol use disorders, to explore the effects of bariatric surgery on overall cancer incidence, as well as specific cancers and groups of cancers, and to investigate the gene and protein expression of the *ITIH5* gene in different adipose tissue depots and its association with obesity.

Long-term effects of bariatric surgery have been assessed using the Swedish obese subjects (SOS) study, which is a prospective, controlled, intervention study. Outcomes have been evaluated using the SOS study follow-up examinations and questionnaires, as well as by cross-checking social security numbers with the Swedish Cancer Registry and the Swedish National Patient Register. The association between ITIH5 adipose tissue expression and obesity has been investigated in different study cohorts, using different methods for gene and protein expression.

Bariatric surgery was found to have a protective effect on type 2 diabetes incidence and cardiovascular risk factors in both eligible and non-eligible patients, indicating that eligibility criteria for bariatric surgery may need to be revised and not based primarily on body mass index. Bariatric surgery also reduces the risk for overall cancer incidence, and specifically female cancers. Meanwhile, bariatric surgery increased the risk for alcohol use disorders, especially in gastric bypass operated patients, and patients should be carefully followed-up in order to identify such potential side effects. The ITIH5 expression is increased in obesity, reduced after diet-induced weight loss, and is associated with measures of obesity and cardiovascular risk factors, suggesting that this gene is potentially involved in the molecular mechanisms linking obesity with morbidity.

Keywords: Obesity, bariatric surgery, vertical banded gastroplasty, gastric banding, roux-en-y gastric bypass, type 2 diabetes, cardiovascular risk factors, alcohol use disorders, cancer, ITIH5, adipose tissue

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SAMMANFATTNING PÅ SVENSKA

Fetma är en sjukdom vars förekomst ökar och som är associerat till ökad sjuklighet och förtida död. Fetmakirurgi är idag den mest effektiva behandlingen för viktnedgång och ger förbättrad hälsa och minskad risk för förtida död. Långtidseffekterna, positiva såväl som negativa, av fetmakirurgi är dock inte helt klarlagda. Det saknas också evidensbaserade indikationskriterier för kirurgi och det är oklart i vilka patientgrupper som fetmakirurgi gör störst nytta. En förhoppning är att kunna ersätta den kirurgiska behandlingen med medicinsk behandling varför vidare förståelse av fettvävens funktion vid fetma är viktig. Denna avhandling berör olika aspekter av fetma: positiva och negativa långtidseffekter av fetmakirurgi, vem som är mest lämpad för fetmakirurgi samt molekylära mekanismer i fettväven och dess koppling till fetma.

De tre första delarbetena i denna avhandling utgår från interventionsstudien ”Swedish obese subjects (SOS) study”, vars syfte är att undersöka långtidseffekter av kirurgiskt åstadkommen viktnedgång. Studien inkluderar 4047 individer med fetma, varav 2010 får kirurgisk behandling och 2037 får kontrollbehandling. Det primära effektmåttet för studien är förtida död och studiedeltagarna följs under 20 år. Data insamlas via kontinuerliga läkarundersökningar, enkäter och blodprovtagning, samt genom utdrag ur svenska hälso- och sjukvårdsregister samt befolkningsregister.

Kriterierna för fetmakirurgi baseras idag huvudsakligen på BMI, vilket utesluter en stor del av patienterna med lindrigare fetma. I SOS-studien kan vi utvärdera effekten av kirurgi även hos de som inte uppfyller dagens kriterier. Vi undersökte effekten av kirurgi på typ 2-diabetes och riskfaktorer för hjärt- och kärlsjukdom och fann att, oavsett om dagens kriterier var uppfyllda eller inte, så hade kirurgi en skyddande effekt.

Fetmakirurgi, och framförallt gastrisk bypass, har i studier visat sig påverka upptag och nedbrytning av alkohol. I SOS-studien undersökte vi om risken att drabbas av alkoholrelaterad sjukdom skilde sig åt mellan de olika behandlingsgrupperna och fann att gastrisk bypass ökade risken för alkoholrelaterad sjukdom både jämfört med kontrollgruppen och med övriga operationstekniker.

Fetma är kopplat till cancersjukdom och tros vara en bidragande orsak till cancerutveckling. Tidigare resultat från SOS-studien har visat att fetmakirurgi har en skyddande effekt mot cancer hos kvinnor. I denna

avhandling ingår en fördjupad undersökning av kirurgins effekt på cancerinsjuknande, inklusive effekt på specifika cancrar eller grupper av cancrar. Vi fann att kirurgi minskade risken för all cancer, hudcancer inklusive malignt melanom och könsspecifika cancrar hos kvinnor såsom livmodercancer.

I denna avhandling undersöktes den fettcellsspecifika genen *ITIH5* med avseende på uttryck i fettväv och koppling till fetma samt riskfaktorer för hjärt- och kärlsjukdom. Vi fann att genen var högre uttryckt i underhudsfettväv än i bukhålans fettväv, uttrycket var högre hos individer med fetma jämfört med normalviktiga, och uttrycket minskade då individer med fetma minskade i vikt genom lågkaloridiet. Genuttrycket av *ITIH5* var också associerat till kroppsmått för fetma och riskfaktorer för hjärt- och kärlsjukdom. Resultaten talar för att ITIH5 kan vara en faktor som är involverad i sjukdomsutveckling vid fetma.

LIST OF PAPERS

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

I. **Evaluation of current eligibility criteria for bariatric surgery: diabetes prevention and risk factor changes in the Swedish obese subjects (SOS) study.** Sjöholm K, Anveden Å, Peltonen M, Jacobson P, Romeo S, Svensson PA, Sjöström L, Carlsson LM.
Diabetes care, 2013. 36(5): p. 1335-40.

II. **Alcohol consumption and alcohol problems after bariatric surgery in the Swedish obese subjects study.** Svensson PA, Anveden Å, Romeo S, Peltonen M, Ahlin S, Burza MA, Carlsson B, Jacobson P, Lindroos AK, Lönroth H, Maglio C, Näslund I, Sjöholm K, Wedel H, Söderpalm B, Sjöström L, Carlsson LM.
Obesity, 2013. 21(12): p. 2444-51.

III. **Cancer incidence up to 26 years after bariatric surgery – results from the Swedish obese subjects study.** Anveden Å, Peltonen M, Jacobson P, Andersson-Assarsson J, Taube M, Sjöholm K, Sjöström L, Svensson PA, Carlsson LM.
Manuscript.

IV. **ITIH-5 expression in human adipose tissue is increased in obesity.** Anveden Å, Sjöholm K, Jacobson P, Palsdottir V, Walley AJ, Froguel P, Al-Daghri N, McTernan PG, Mejhert N, Arner P, Sjöström L, Carlsson LM, Svensson PA.
Obesity, 2012. 20(4): p. 708-14.

CONTENT

ABBREVIATIONS

WHO	World Health Organization
BMI	Body Mass Index (kg/m^2)
T2DM	Type 2 Diabetes Mellitus
VBG	Vertical Banded Gastroplasty
GB	Gastric Banding
GBP	Roux-en-y Gastric Bypass
NIH	National Institute of Health (U.S.A.)
The SOS study	The Swedish Obese Subjects study
TNF-α	Tumor Necrosis Factor - α
ITIH5	Inter (α) globulin Inhibitor Heavy chain -5
FPG	Fasting Plasma Glucose
OGTT	Oral Glucose Tolerance Test
AUD	Alcohol Use Disorders
DSM	Diagnostic and Statistical Manual of Mental Disorders
SCID	Structured Clinical Interview for DSM disorders
AUDIT	Alcohol Use Disorders Identification Test
ICD	International Classification of Disease
VLCD	Very Low Calorie Diet
PCR	Polymerase Chain Reaction
C_T	Cycle of Threshold

ELISA	Enzyme-linked Immunosorbent Assay
HR	Hazard Ratio
CI	Confidence Interval
NNT	Number Needed to Treat
ABV	Alcohol By Volume

1 INTRODUCTION

Obesity and overweight has risen to a global epidemic, causing more deaths in the world than does starvation, and today it is estimated that 13 % of the global adult population have obesity [1]. The obesity epidemic needs to be tackled from different angles, both in the prevention of a further increase in prevalence, and in the treatment of obesity already present. Both for prevention and treatment of obesity, there is a need for increased knowledge of the physiological mechanisms of obesity, and especially pathophysiological mechanisms linking obesity with morbidity and mortality.

Since humans started to inhabit this planet, enormous changes in the way we live have taken place. In western society today, the supply of food seems to be never-ending, and stocking up the fridge is just a click away using our smartphones. There is even a phrase for moving about since it is becoming so rare – "physical activity", because today being physically inactive is the standard. The evolvement of our convenient lifestyle, where periods scarce of food supplies are essentially non-existent, has been of short duration compared to the preceding time period when generations have lived with regular periods of starvation. It is easy to be attracted by the thrifty genes hypothesis [2] saying that evolution has selected gene variants that enhance survival throughout longer starvation periods, i.e. facilitate energy storage in our bodies, and thereby cause increased susceptibility for becoming obese. Nevertheless, obesity is not a new phenomenon, as is illustrated by the Venus figurines produced around 23 000 years ago [3], and the thrifty genes hypothesis has not stood uncriticized [4]. Regardless of obesity being or not being a new phenomenon in human history, the obesity epidemic is a concrete threat to global health, and we have limited experience on how to deal with it.

This thesis will focus on obesity intervention, which today is best achieved with gastrointestinal surgical procedures, collectively called bariatric surgery. In addition, it will highlight specific molecular mechanisms in the adipose tissue of the obese state.

2 BACKGROUND

2.1 Obesity – definition, epidemiology and causes

Obesity, classified as a disease by the World health organization (WHO), is defined as a condition of excess fat accumulation in the adipose tissue. Body Mass Index (BMI), originally proposed by Quetelet in 1832, has become the most commonly used index to quantify obesity. Quetelet tried to characterize a human population by anthropometry and could not fit a Gaussian curve of normal distribution to body weight alone, but for the ratio of body weight and squared height, i.e. the weight was proportional to the squared height [5]. BMI correlates well with the amount of adipose tissue, at least in large populations, and is therefore still the standard proximate of obesity and excess adipose tissue [6]. The most important reasons however to use BMI is that it is a strong predictor of overall mortality [7] and an easily obtainable measurement. The classification of obesity using BMI categories is shown in Table 1 [8].

Table 1 Classification of obesity using BMI ranges, including examples of length and approximate weights for the different classes.

BMI (kg/m^2)	Classification	Example height (m)	Example weight (kg)
< 25	Normal weight	1,70	< 72
25 – 30	Overweight	1,70	72 – 86.5
30 – 35	Obesity class I	1,70	86.5 – 101
35 – 40	Obesity class II	1,70	101 – 115.5
≥ 40	Obesity class III	1,70	≥ 115.5

The prevalence of obesity is increasing globally and today 600 million people, or 13 % of the global population, are classified as having obesity [1]. In Sweden, the prevalence among adults above 20 years of age is 18.9 % in men and 19.8 % in women [9], with a remarkable increase in prevalence during the last 30 years.

Obesity arises after a longer period of positive energy balance, when energy intake exceeds energy output and the excess energy is stored in the adipose tissue. The weight regulation of the human body is influenced by multiple factors, acting independently or together, including genetic predisposition,

somatic or psychiatric disease, endocrine changes, medication, other exogenous factors, and life style factors. The latter is the most commonly given explanation for the obesity epidemic, including decreased physical activity and high calorie intake, but evidence is scarce and several other contributing factors in society have been suggested [10]. These include diverse factors such as increased mean age of mothers at first birth, increased prescription of antidepressants and other psychiatric medication, increased time spent awake, and decreased prevalence of smoking [10].

Regardless of the mechanisms responsible for a positive energy balance, the result is the same: an increased storage of triglycerides in adipocytes of the adipose tissue. However, the resulting obesity may be of different characters, as the storage of excess energy can occur in different adipose tissue depots – e.g. the subcutaneous adipose tissue or the visceral adipose tissue. Men are more prone to increase their visceral adipose tissue depot surrounding the organs inside the abdomen, whereas women are more prone to increase their subcutaneous adipose tissue depot [11-13]. The adipose tissue is also considered to have different properties in men and women [11, 14]. After menopause however, women shift their storage to the visceral depot with a more “male” pattern of obesity [12].

2.2 Consequences of obesity

Although some individuals with obesity may be relatively healthy, on a population level, obesity is associated with an increased risk of premature death [7, 15, 16]. In white individuals aged between 20 and 30 years, with a BMI exceeding 45, the years of life lost due to obesity has been estimated to be 13 in men and 8 in women [17]. This increased likelihood of premature death is most probably due to the association between obesity and several serious medical conditions [18, 19].

Obesity is closely associated with insulin resistance and type 2 diabetes mellitus (T2DM) [18-20], but the association seems to diminish with age [20]. T2DM is a condition characterized by hyperglycemia due to a relative insulin deficiency and insulin resistance disabling the uptake of glucose in tissues [21]. Long term hyperglycemia leads to severe medical complications, such as cardiovascular disease, kidney disease, and neurological disease, making T2DM a disease to be taken seriously. Obesity is also associated with hypertension and dyslipidemia [22], which increases the risk for cardiovascular disease, and obesity is also an independent risk factor for cardiovascular disease, including coronary heart disease [23, 24].

Furthermore, increasing BMI is associated with an increased risk for cancer [25-28] and it is estimated that approximately 3.6 % of all new cancer cases are attributed to obesity [29]. Cancer is one of the leading causes of morbidity and mortality in the world [30] and a very common disease, affecting approximately one third of the Swedish population during their lifetime [31]. Cancer includes all types of malignant disease, where abnormal cells proliferate and expand uncontrollably, forming a fast growing tumor or a fast growing population of abnormal cells [32]. The development of cancer is a multistep, complex process, starting with mutations of cells caused by carcinogenic agents, such as toxic substances, viruses or ultraviolet light. In preventing cancer development, the immune system plays an important role, and chronic inflammation is one factor promoting cancer [32]. Among the most common cancers in Sweden are prostate, breast, colon, lung and malignant melanoma [31].

Apart from increasing the risk of these serious medical conditions, obesity has implications on well-being and is associated with reduced health-related quality of life [33]. Psychiatric mood and anxiety disorders have also been associated with obesity [34], as well as conditions of the musculoskeletal system [35]. Hence, obesity is a substantial threat to an individual's health and well-being.

2.3 Treatment of obesity

Examples of successful obesity treatments in a specific individual or group of individuals are easily found. However, to find obesity treatments with good results in an entire obese population is more difficult. It is not easy to predict if a certain method will lead to weight loss and increased health in a specific individual with obesity. It is also important to remember that the goal of obesity treatment may differ between individuals, and between care giver and patient.

The common goal – to lose weight – is achieved by a negative energy balance, where energy intake is less than energy output. The first line of treatment is making life style changes regarding meal habits and physical activity. A large German study examined the effects of a 52 week intensive life style and diet intervention in patients with obesity and found weight reductions of 15.2 kg in women and 19.4 kg in men with beneficial effects on co-morbidities up to 3 years after the start of intervention [36]. A recent review and meta-analysis found a mean weight loss of 10.8 kg up to one year with life style intervention [37]. Maintaining weight loss is however difficult, and the combination of dietary and physical activity programs have been

shown to result in a modest minimizing effect of weight regain up to 24 months [37]. The large LOOK AHEAD study investigated long term effects of lifestyle intervention in patients with overweight or obesity and T2DM up to 11.5 years, and found significant difference in weight loss and other risk factors but not for the primary outcome cardiovascular events [38, 39]. The Diabetes prevention program study and the Finnish diabetes prevention study, both including patients with at least overweight and impaired glucose tolerance, found that lifestyle intervention resulted in modest weight loss and a reduced risk for T2DM during up to 10 years follow-up [40, 41].

Throughout the years, many pharmacological strategies have been tested, but several have had to be withdrawn due to unacceptable side effects [42]. Today, Orlistat is the only anti-obesity drug available in Sweden. Orlistat inhibits the breakdown of lipids in the gastro-intestinal tract leading to decreased lipid uptake but also to unpleasant side effects when large amounts of fat are consumed. It results in modest weight loss, but has positive effects on diabetes and cardiovascular risk factors [43]. Earlier this year, Liraglutide was approved by the European medical association for the treatment of obesity. This is an appetite-regulating drug, leading to increased satiety and reduced food intake, that has promising results on weight loss and glucose homeostasis in clinical trials [42].

2.4 Bariatric surgery

Bariatric surgery is the only treatment of obesity today proven to lead to sustainable weight loss [44]. The history of bariatric surgery starts in the 1950's, when the first operation aimed at curing obesity was performed by Dr Henriksson who removed the major part of the patients' small intestine, resulting in malabsorptive weight loss [45]. This operation was followed by several different procedures bypassing the small intestine to achieve malabsorption – jejuno-ileal bypasses [46]. The procedures resulted in weight loss and improvements in co-morbidities, but they were associated with serious complications. In the 1960's Dr Mason developed a new technique in order to avoid the side effects of the jejuno-ileal bypass [47]. This procedure resulted in a reduced stomach size and a mild malabsorption and was the precedent of the roux-en-y gastric bypass (GBP) commonly used today. Today, several other techniques have been developed and although GBP is still the most commonly used globally, the technique called sleeve gastrectomy is rapidly gaining popularity and is the most frequently used technique in the USA [48]. In the sleeve gastrectomy procedure a tube is

formed alongside the lesser curvature of the ventricle removing the fundus and the greater curvcature part of the ventricle [49].

2.4.1 Surgical techniques used in this thesis

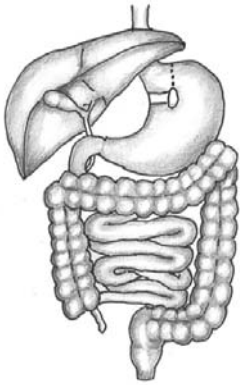

Figure 1 Vertical banded gastroplasty (VBG). Illustration by Per-Arne Svensson.

Vertical banded gastroplasty (VBG) is a restrictive procedure where a small vertical pouch is created along the smaller curvature of the ventricle, and its exit is stabilized by a non-elastic band (Figure 1). This is the most common procedure in the cohort of this thesis, but it is rarely performed today.

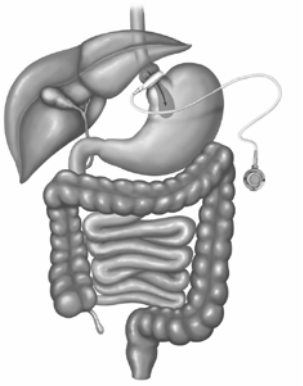

Figure 2 Gastric banding (GB). © Ethicon, Johnson & Johnson.

Gastric banding (GB) is also a restrictive technique, where an adjustable or a non-adjustable band is implanted right below the ventricular cardia, creating

a small pouch and narrowing the passage down to the ventricular fundus (Figure 2). It is a reversible technique and is becoming less frequent today [48].

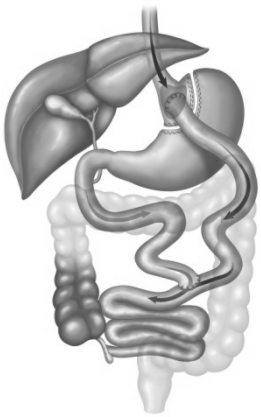

Figure 3 Roux-en-y gastric bypass. © Ethicon, Johnson & Johnson.

Today, Roux-en-y gastric bypass (GBP) is the most common surgical technique in Sweden [48], and is a development of the technique described by Mason in 1967 [47]. It is a combined restrictive and malabsorptive technique. In this procedure a pouch of 10-25 ml is formed from the ventricle alongside the lesser curvature, just below cardia. The jejunum is cut approximately 50-60 cm from the duodeno-jejunal junction and its proximal part is brought up to this gastric pouch. The main ventricle is stapled away and becomes, together with the duodenum and upper part of jejunum, by-passed. The distal end of this limb is then connected to the jejunum 100-150 cm down, allowing for gastric juice, biliary fluids and pancreatic enzymes to mix with ingested food in the distal part of the jejunum (Figure 3).

2.4.2 Effects of bariatric surgery

Bariatric surgery leads to substantial and sustainable weight loss, most of which occur during the first year following the intervention [44]. It also prevents T2DM [50, 51], cardiovascular events [52, 53], cancer in women [54-56] and premature death [57, 58]. In patients with obesity and T2DM, bariatric surgery often leads to remission of T2DM and a reduced risk for its complications [53, 59-65] both when compared with an intensive medical therapy group [61-65], and conventional obesity treatment [59, 60]. Several other positive effects of bariatric surgery have been reported, such as

improved health-related quality of life [66], improved fertility and pregnancy outcomes [67], and improvements of musculoskeletal disease [68].

Today, bariatric surgery is mostly performed laparoscopically, which means less invasive surgery and minimized traumatic injury to the body. Still, these surgical procedures are major operations on the gastrointestinal tract and there is a risk for short term as well as long term surgical complications with risk of reoperations [69]. Other long term side effects have been reported, such as nutritional deficiencies [70], increased sensitivity to alcohol [71, 72] and even an increased risk for suicide [73].

An increased sensitivity to alcohol may be a risk factor for developing alcohol use disorders (AUD), which is a serious psychiatric condition. Alcohol, i.e. ethanol, is both water and fat soluble and distributes in all body tissues after consumption, crossing the blood-brain barrier and affecting multiple neurotransmitter systems [74]. This makes alcohol a very potent substance in the human body. At low doses it has anxiolytic, rewarding and socially facilitating effects, but at increased doses it impairs cognitive and psychomotor function. Alcohol is causally associated to 60 different diseases, and is contributing to premature death through its association with injury, cancers and cardiovascular disease [74]. Multiple factors can contribute to the development of harmful use of alcohol or AUD, such as cultural settings, inheritance, and presence of mood or neuropsychiatric disorders.

2.4.3 Eligibility criteria

In 1992, the National Institute of Health (NIH) stated criteria to select patients for bariatric surgery that are still used today [75]: BMI of at least 40 kg/m^2 or a BMI between 35 and 40 kg/m^2 in the presence of obesity-related co-morbidities such as T2DM or sleep apnea. However, these criteria, with BMI as the primary criterion, are based on expert consensus rather than clear evidence, influenced by the association between increasing BMI and premature death [7, 15, 16]. Evidence on which patients that benefit the most from bariatric surgery is not clear. Several studies have documented the effects of bariatric surgery in patients with lower degree of obesity, i.e. class I obesity with a BMI between 30 and 35, most of them including only patients with T2DM, and similar positive effects, as for the higher BMI categories, have been reported [76-79]. In the large Swedish obese subjects (SOS) study, no difference in treatment effect between different BMI categories has been found with regards to T2DM, cardiovascular events, cancer or mortality [50, 52, 54, 57]. Hence, today's eligibility criteria may not select the patients that benefit the most from bariatric surgery.

2.5 Adipose tissue

Exploring the adipose tissue and its characteristics is an important part of clarifying how obesity, i.e. excess adipose tissue, is associated with morbidity. The adipose tissue mainly consists of adipocytes, supported by the extracellular matrix (Figure 4). Other cells of the adipose tissue include endothelial cells, fibroblasts and immune cells [80]. Important functions of the adipocytes are storage of excess energy in the form of triglycerides, and releasing this energy in the form of free fatty acids in periods of starvation. The adipose tissue also offers thermal insulation and a protection against mechanical injuries [80].

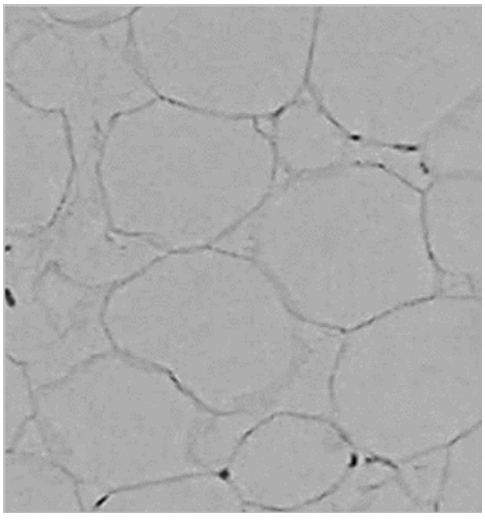

Figure 4 Histological photograph of the adipose tissue showing the adipocytes and their large single lipid droplet dominating. Photo by Jenny Hoffmann.

2.5.1 The adipose tissue in obesity

Today, the adipose tissue is considered to be an active endocrine tissue communicating with surrounding tissues and organs [80]. The communicating signaling proteins secreted by the adipocytes are termed adipokines. Adipokines influence body metabolism, blood homeostasis, and inflammation, and today more than 600 adipokines have been presented [81]. Obesity alters the expression of adipokines, and these changes are thought to play a role in the development of obesity-related co-morbidities. The most famous adipokine is leptin which was first discovered in 1994 [82]. It is almost exclusively produced in the adipose tissue and mainly exerts its

effects centrally in the hypothalamus of the brain, suppressing hunger and appetite and increasing energy expenditure [83]. The secretion of leptin, and levels of circulating leptin, is positively associated with increasing obesity, and communicates to the brain how much adipose tissue there is in the body. However, in obesity, leptin resistance may develop, with high circulating levels but limited action of leptin [84]. Another well-known adipokine is Adiponectin, which is inversely associated with obesity and increases insulin sensitivity [81].

Apart from secreting these adipokines, the adipose tissue displays an increased inflammation in obesity and this contributes to the low-grade chronic inflammation seen in obesity [85]. Macrophages in the adipose tissue have been shown to be increased in obesity and are the main source of adipose tissue derived pro-inflammatory TNF-α [86, 87]. Other immunological cells are also present in the adipose tissue, contributing to the increased inflammation, and the adipose tissue shows features similar to that of an immunological organ [88].

Another feature of the adipose tissue in obesity is the increased presence of fibrosis, especially in the subcutaneous depot [89]. This fibrosis is hypothesized to influence the impaired function of adipocytes in obesity and to contribute to the dysfunction of adipose tissue, including both increase and modification of the extracellular matrix components [90].

2.5.2 Inter- α (globulin) inhibitor H5 (ITIH5)

As there seems to be numerous amounts of unknown factors produced by the adipose tissue, our research group has actively searched for adipocyte-specific genes using whole genome searches [91-94]. In these searches, the gene inter-α (globulin) inhibitor H5 (*ITIH5*) was identified as highly expressed in adipocytes. *ITIH5* was first described in 2004 and was abundantly expressed in human placenta [95]. It had a gene sequence with high homology to the mouse gene but not to the other members of the ITIH family, although they all contain a signal peptide at the N-terminus [95]. The ITIH gene family of heavy chain peptides are part of the inter-α-trypsin inhibitor (IαI) family which are protein complexes composed of bikunin and one or two heavy chains. This complex functions as a protease inhibitor, and the heavy chains are also able to bind hyaluronic acid [96].

Little is known about the *ITIH5* gene, or its encoded protein, but as it contains a signal peptide sequence it is likely to encode a secreted protein which may have signaling properties acting in an endocrine/paracrine

manner. A few studies have shown an altered expression in tumor disease [97, 98].

3 AIM

3.1 General aim

The aim of this thesis was to increase our understanding on how obesity is associated with disease through molecular mechanisms, and to explore effects of bariatric surgery on different outcomes in different subgroups, as well as exploring potential side effects.

3.2 Specific aims

Paper I

The aim of this paper was to compare the effects of bariatric surgery on T2DM incidence and cardiovascular risk factors in subjects eligible and non-eligible for surgery according to today's eligibility criteria.

Paper II

The aim of this paper was to explore whether bariatric surgery is associated with increased incidence of alcohol use disorders.

Paper III

In this paper, the aim was to explore the effects of bariatric surgery on overall cancer incidence, as well as specific cancers and groups of cancers.

Paper IV

The aim of this paper was to investigate the gene and protein expression of the *ITIH5* gene in different adipose tissue depots and its association with obesity.

4 METHODS

4.1 Study populations

4.1.1 The Swedish Obese Subjects (SOS) study

The SOS study is an ongoing non randomized, prospective, controlled intervention study with the aim of studying long-term effects of surgically induced weight loss. The study started in 1987 and recruited patients until 2001. It includes 2010 surgically treated individuals, and a matched control group of 2037 individuals receiving conventional care for obesity. The different surgical techniques used were VBG (Figure 1, N=1369), GB (Figure 2, N=366) and GBP (Figure 3, N=265). All study participants were aged between 37 and 60 years, had a BMI exceeding 34 in men and 38 in women, and the exclusion criteria were few and aimed at obtaining operable subjects. The predefined primary endpoint was mortality and secondary endpoints type 2 diabetes, myocardial infarction and stroke. The participants are followed for 20 years, and the mean follow-up time today is approximately 18 years.

4.1.2 The Sib Pair study

The Sib Pair study is a cross-sectional study designed to investigate genetics in obesity. It includes all members of 154 families where the BMI difference between two siblings exceeded 10 units. In this thesis, a subpopulation of this study was used including 90 BMI-discordant, sex concordant sibling pairs, 156 women and 24 men. In each sibling pair, one sibling was classified as lean and the other as obese. Abdominal subcutaneous adipose tissue biopsies, blood samples, and anthropometric measurements were obtained from all subjects.

4.1.3 Very low calorie diet (VLCD) studies

Two studies of VLCD treatment are included in this thesis. The larger VLCD study (hereinafter “VLCD study I”) includes 24 individuals with obesity, 18 men and 6 women, who were non smokers and not on regular medication. The participants were treated with a very low calorie diet of 450 kcal per day during 16 weeks, followed by a two-week period of gradual reintroduction of regular food. Blood samples, anthropometrical measurements, and subcutaneous adipose tissue biopsies were obtained before week 0, and at week 8, 16 and 18. This study was used to measure *ITIH5* gene expression in adipose tissue with DNA microarray. The smaller VLCD study (hereinafter “VLCD study II”) includes 10 individuals with obesity, 5 men and 5 women,

who were treated in the same way, but subcutaneous adipose tissue biopsies were only obtained before week 0, and at week 8 and 18. This study was used to measure *ITIH5* gene expression with real time polymerase chain reaction (PCR).

4.1.4 Lean and obese healthy women studies

Two cohorts of lean and obese healthy women are used in this thesis. The first cohort includes 95 women, 80 with obesity and 15 lean, who were all healthy and not on regular medication (hereinafter "Healthy women I"). Abdominal subcutaneous adipose tissue biopsies, blood samples, and anthropometric measurements were obtained from all subjects. This cohort was used to measure adipose tissue *ITIH5* gene expression using DNA microarray.

The second cohort includes 14 women, 7 individuals with obesity and 7 lean (hereinafter "Healthy women II"). Subcutaneous adipose tissue biopsies were obtained from all subjects. This cohort was used to measure ITIH5 protein expression in adipose tissue using Western blot.

4.1.5 Additional studies

The Depot study includes 10 healthy women undergoing elective surgery, from which subcutaneous and visceral adipose tissue biopsies were obtained. A human tissue panel was bought to determine tissue distribution of *ITIH5* and adipocytes and adipose tissue was collected from healthy volunteers.

4.2 Methodological considerations

4.2.1 The SOS study

General considerations

A study investigating long-term effects of bariatric surgery on mortality needs to include a large number of study participants being followed for a long time period, preferably several decades. The number of participants in the SOS study is based on a statistical power calculation for the primary endpoint mortality and the study protocol includes a follow-up time of 20 years. Ideally, the study should also be randomized, where study participants from a selected obese population are randomized to surgical or control treatment, creating similar and comparable groups at study start. However, due to high post-operative mortality at the start of the SOS study, randomization was not considered ethical and not approved. Instead,

individuals fulfilling study inclusion criteria and desiring surgical treatment formed the surgery group, and a matching procedure selected eligible individuals to a control group. The matching procedure was based on a specific matching algorithm aimed at creating equal group means for 18 specified variables, which were considered significant for the study [99]. For each new individual included in the surgery group, a control individual was selected based on this algorithm. Initially, the intention was to create a control group twice the size of the surgery group, but it was difficult to find that many matched controls. Finally, the inclusion procedure resulted in a surgery group of 2010 individuals and a control group of 2037 individuals with similar characteristics. Even though the matching procedure created similar characteristics of the groups, the time period between matching and baseline examination was prolonged due to long waiting lists for surgery, and at the baseline examination the two groups had diverged in characteristics. At baseline the surgery group was slightly heavier and had more co-morbidities and cardiovascular risk factors. However, at least regarding metabolic factors, these differences are in favor of the control group for metabolic endpoints of the SOS study. On the other hand, despite careful matching, there is a risk for allocation bias and participants who chose surgery may be more motivated to achieve weight loss.

The non-randomization design is not optimal, but it is questionable whether a randomized study would have been possible to keep running for decades. The control group would then probably have consisted largely of individuals rather having surgery, and the dropout rate and the number of controls having surgery would probably be quite high, especially as the evidence for positive effects of bariatric surgery increases. Indeed, in the SOS study, 287 control individuals have undergone surgery during follow-up, hence leaving their intended treatment protocol. It is also questionable whether a long-term randomized control trial for bariatric surgery would be ethical considering the great benefits from surgery that have been discovered, but also taking into account the emerging evidence for long-term side effects [70, 73, 100].

Regarding the surgical treatment, there has not been any standardization of the operational procedure in the SOS study. The surgical technique in each case was based on the operating surgeons' preferences and clinical traditions. Furthermore, the recruitment of study participants lasted 14 years and during that time there has been a shift in surgical techniques most commonly used. These circumstances resulted in three different techniques being used in the study. This is another issue of conducting a long-term study of effects of a surgical treatment as surgical techniques develop over time. Some of the techniques used in the SOS study might be considered "old" and not

representing today's techniques. This problem is hard to circumvent. However, all of them result in sustainable weight loss, which was the aim of the study (Figure 5).

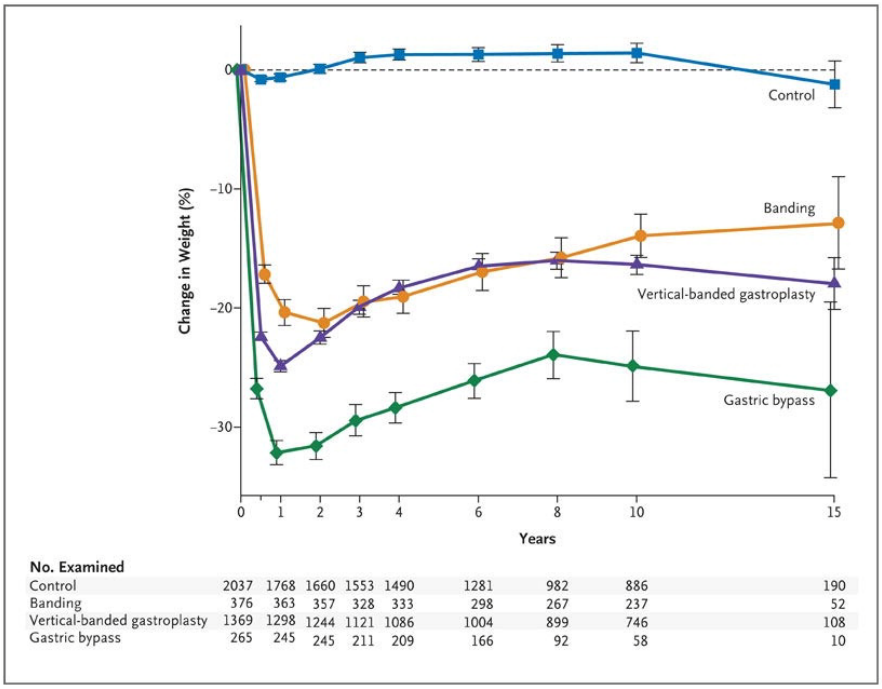

Figure 5 Mean Percent Weight Change during a 15-Year Period in the Control Group and the Surgery Group, According to the Method of Bariatric Surgery. I bars denote 95% confidence intervals. Banding = Gastric banding (GB). From Sjöström L et al., N Engl J Med 2007;357:741-752. © 2007 Massachusetts Medical Society. Reprinted with permission.

The choice of treatment in the control group is another issue. What treatment should the surgical intervention be compared to? The control group could for example receive no treatment at all, seeking to answer the question:"What happens to this population if we let them undergo bariatric surgery, as compared to doing nothing?" In the SOS study, the treatment of the control group was not standardized and they were given the conventional treatment at their primary health care center, mimicking the real situation for the obese population in Sweden at the time of the study. Most participants in the control group report that they have tried to lose weight (around 80 % at each

follow-up time point, unpublished data), but some did no attempt of losing weight.

In the first paper of this thesis, further sub-grouping of the SOS study was done based on today's criteria for bariatric surgery [75]. Specifically, participants with a BMI exceeding 40 kg/m^2 or BMI between 35 and 40 kg/m^2 in the presence of type 2 diabetes, dyslipidemia and/or hypertension were included in the eligible group. We used co-morbidities detectable at the biochemical or physical examination and from questionnaires at the time of matching examination. We did not use co-morbidities which were not easily defined, e.g. sleep apnea. We may therefore wrongly have categorized a few patients as non-eligible for surgery, but as most of the co-morbidities co-exist, this number is probably relatively low. An optimal study design would have been to recruit both eligible and non-eligible individuals according to current eligibility criteria and then randomize them to surgical or non-surgical treatment.

Outcome definitions

One outcome in this thesis is T2DM incidence. Today, T2DM can be diagnosed in four ways: A fasting plasma glucose (FPG) value exceeding 7 mmol/l, or by symptoms of diabetes accompanied by a random plasma glucose value exceeding 11.1 mmol/l, or by a plasma glucose exceeding 11.1 mmol/l after an oral glucose tolerance test (OGTT), or by a HbA1C exceeding 48 mmol/mol [101]. Regardless of method for diagnosis, the values should be confirmed the subsequent day with any of the first three variants. At the start of the SOS study, confirmatory measurements were not required to diagnose T2DM, hence we only have single values of blood glucose for each follow-up time point. Therefore, in the SOS study, T2DM is based on a single FPG exceeding 7 mmol/l and/or reporting use of antidiabetic pharmacological treatment. It has also been advised that, for epidemiological studies, a single value of FPG exceeding 7 mmol/l can be used [102]. Notable for the SOS study is also that until August 1 2009, glucose were measured in whole blood, in which the glucose concentration is lower, and these measurements have had to be converted to plasma glucose values. The outcome of T2DM would have been more precise if we have had repeated measurements of plasma glucose and/or measurements of HbA1C and/or plasma glucose measured after an OGTT. We may have wrongly given some participants a T2DM diagnosis, due to temporary high values or measurement error, but this would be as likely to occur in the surgery group as in the control group.

The aim of Paper II was to investigate whether bariatric surgery increases alcohol consumption and increases the risk for AUD, including alcohol dependence syndrome and alcohol abuse, endpoints that were not predefined in the SOS study. Nevertheless, the question "Do you think you have alcohol problems?" was included in the questionnaire, and from the validated food questionnaire [103], information on daily alcohol intake was available. To investigate the incidence of AUD in the SOS study, these two self-reported variables were used. For evaluating alcohol consumption, the WHO cut-off for medium risk consumption (40 g/day in men, 20 g/day in women) and above was used [104]. As a complement, the social security numbers of the SOS study participants were cross-checked with the Swedish National Patient Register of patients being hospitalized for any reason. We considered diagnoses specifically related to current or previous alcohol abuse in addition to diagnoses for alcohol dependence syndrome and alcohol abuse according to ICD-9/ICD-10 (International Classification of Disease). By including these diagnoses and having three different ways for defining AUD the chances of identifying all study participants having negative effects of alcohol use were increased. Ideally however, AUD should be based on evaluating study participants according to the Diagnostic and Statistical Manual of Mental Disorders (DSM) criteria, e.g. by using the Structured Clinical Interview for DSM disorders (SCID) [105], which is the gold standard for clinical trials. Another possibility to identify participants with harmful alcohol habits would be to use the Alcohol Use Disorders Identification Test (AUDIT), which is commonly used in the clinic today, but this tool was presented in 1993, after the initiation of the SOS study, and is a screening and not a diagnostic tool [106].

The outcome of the third paper, cancer incidence, was also not a predefined endpoint. It was previously investigated in 2009 [54] to follow-up the observation that cancer was the most common cause of death in the SOS study [57]. To study cancer incidence, the social security numbers of the study participants were cross-checked with the Swedish Cancer Registry, to which clinicians and pathologists report newly diagnosed tumors defined as malignant. The coverage of all cancers diagnosed in Sweden is 96 % [107], and this outcome should be most reliable. Diagnoses were selected similarly as in our previous report: All diagnoses found in the registry were used except for parathyroid adenomas, which may probably also represent ill-defined hyperplasia, and basal cell carcinoma which was not included in the registry until 2004 [108].

4.2.2 Gender and sex aspects of study populations

It is strongly recommended that both men and women are included in medical studies to produce representative results for both genders. By including both men and women in a study it is also possible to detect differences and similarities between genders. In the cohorts of this thesis, both men and women are represented but not of equal proportions, and most of the study participants are women. This is not in line with the proportion of women in the total global population, which is around 50 % [109], although there are slightly more women than men with obesity in the world [1]. In the SOS study, 70.8 % of the study participants are women. Thus, there are not equal proportions of men and women, and studying outcomes expected to differ between genders, especially rarely occurring outcomes, may be difficult. Also, the statistical power for separately studying effects in men is lower. This may lead to false conclusions for men for outcomes where the treatment effect is driven by an effect in women.

In the subpopulation of the Sib Pair study used in this thesis, 86.7 % are women. This cohort was used to quantify *ITIH5* gene expression of subcutaneous adipose tissue, and to correlate this gene expression with cardiovascular risk factors. To confirm the results on *ITIH5* gene expression, the first lean and obese healthy women cohort was used (Healthy women I). This cohort includes only women, and most of the participants have obesity (84.2 %). Furthermore, there were only women in the cohort used for measuring protein expression (Healthy women II). Hence, our expression measurements mainly represent the pattern in women. Meanwhile, the purpose of using the specific subpopulation of the Sib Pair study was to determine differences in gene expression between individuals with and without obesity, and this was statistically possible in both men and women. In the linear regression analysis in the Sib Pair study, sex was included as a variable in the model to account for any differences between the sexes. Regression analyses were also performed in Healthy women I. Optimally we would have done the same in a similar cohort of only men, but such a cohort was not available.

The gene *ITIH5* has previously not been studied in great detail and little is therefore known about sex differences in its regulation. To study gene expression changes during calorie restriction and weight loss, samples from the two VLCD studies were used. These studies differed from our other cohorts in proportion of men and women, with a dominance of men in VLCD study I (75 % men), and equal proportions of men and women in VLCD

study II. The VLCD study II was used to confirm the gene expression quantified with DNA microarray in VLCD study I, but expression data were not available for all corresponding time points. The gene expression pattern during the diet period may be different in the two studies, e.g. if the regulation is different between men and women. Meanwhile, the availability to look at expression changes during VLCD in both sexes and with two different techniques increases the robustness and reliability of our data.

In conclusion, all our cohorts would have been better designed with equal proportions of men and women. All our study participants have been recruited through local and media advertisements, and this recruitment strategy apparently appeals more to the female obese population. Meanwhile, unequal proportions of men and women are not unique to our studies, as several other large studies in obese populations have a higher proportion of women than men [41, 51, 55, 56, 64, 110].

4.2.3 Expression analyses

Nearly every cell in the human body contains the same genes, i.e. have the same genotype. But each cell does not use all of these genes, resulting in a specific phenotype. This is what makes cells different and determines the properties of different tissues. Measuring gene expression is a way of quantifying if and how much a certain gene is being used, i.e. “turned on”, in a certain tissue or certain types of cells. In this thesis, gene expression measurement has been used to characterize the use of the *ITIH5* gene in different tissues, different cells, in different human phenotypes, and under specific circumstances. An alteration in gene expression of a specific gene suggests that this gene is of importance to a certain cell, tissue, human phenotype, or under certain circumstances.

There are several methods for measuring gene expression. One of the methods used to quantify gene expression in this thesis is real time PCR. The first step in this analysis is conversion of mRNA to cDNA followed by a PCR reaction with two primers and a probe hybridizing to the sequence of interest. The detection is based on amplification of one specific mRNA sequence where a fluorescent reporter molecule is released from the probe for each amplification product. The fluorescence increases exponentially and the more abundant a certain mRNA is, the earlier the fluorescence level exceeds a certain threshold level. The time point when this happens is named cycle of threshold (C_T), and this value is linearly related to the logarithm of the initial number of copies of the target gene. This makes the quantification very precise and it is possible to detect genes expressed at low levels, i.e. low

amounts of mRNA. Optimally the primers and probes used to detect the specific mRNA transcript are designed to span exon borders, and should then specifically detect only the transcript of interest.

The other method used in this thesis, DNA microarray, is also based on hybridization technique, but this method can measure the expression of thousands of genes simultaneously. On the surface of a chip of glass, thousands of synthetic, short, single-stranded DNA sequences are placed in spots or squares forming an array system, where each spot/square or groups of spots/squares represent a gene. In this thesis, Affymetrix has been the Microarray system of choice. In this system, extracted RNA from samples is reversed transcribed to cDNA, which is then in vitro transcribed to biotin-labeled cRNA and fragmented. The fragmented cRNA is hybridized to the probes on the microarray chip. If a gene is highly expressed, more cRNA will be bound to its corresponding probe. Fluorescent molecules that bind to the biotin molecule are then added to the chip, and the fluorescence is measured at each spot of the microarray chip. The amount of hybridized cRNA for each gene sequence on each spot can then be calculated from the strength of the fluorescent signal. A higher gene expression is reflected by a stronger fluorescence signal, and the expression level is determined by the light intensity. However, the quantification of genes that are lowly expressed may be difficult due to difficulties in accounting for noise.

An older method, also based on hybridization but measuring only one gene at a time, is Northern blot. In this method, total RNA is separated on a gel, blotted to a membrane and bound to labeled RNA probes which are then detected, either by radioactivity or fluorescence. Apart from the use of radioactive material and other potentially harmful substances, it is very time consuming and less sensitive for mRNA quantification as compared to real time PCR. Meanwhile, it can detect splice variants of genes, and this would have been a good additional method for measuring *ITIH5* as several splice variants of the gene have been found [111].

A more modern method for measuring gene expression is RNA sequencing which is not, in contrast to the other methods, based on hybridization but rather reads cDNA sequences directly at the base pair level. This method is however expensive and generates vast quantities of data, making the data handling and data interpretation demanding.

The measured amount of mRNA does not necessarily correlate with the amount of protein produced from the specific mRNA. As a relevant step in investigating the presence and importance of a certain gene in samples, one

can quantify the protein expression. In this thesis quantification of protein has been done with Western blot and subsequent measurement of light intensity. This technique is based on the separation of proteins by electrophoresis, blotting to a membrane, the use of specific antibodies to detect the protein of interest and secondary antibodies with fluorescent or enzymatic properties, and quantifying the amount by measuring light density of specific bands on the blot. It is a widely used technique for protein expression, results in clearly visual results, and is a good option for measuring one specific protein in different samples to compare. Another possible method for detecting protein is enzyme-linked immunosorbent assay (ELISA). This method detects the protein of interest with the use of a primary specific antibody bound to a 96-well plate and a secondary antibody carrying an enzyme so that when the enzyme's substrate is added, a color shift occurs. This color shift is then measured with a spectrophotometer and used to quantify the expression of the protein. Unfortunately, this method was not an option when investigating ITIH5 expression, as no specific ELISAs were available at the time of investigation.

5 RESULTS AND DISCUSSION

5.1 Evaluation of current eligibility criteria for bariatric surgery

At the start of the SOS study, there were no official criteria for bariatric surgery, and the BMI cut-offs for inclusion in the study were based on a Norwegian study showing doubled mortality for the specific BMI values [112]. Hence, the study includes some individuals that would not have been eligible for bariatric surgery today. This offers an opportunity to analyze the effects of bariatric surgery in patients that are non-eligible for surgery and investigate if these effects differ from those seen in eligible patients.

Dividing the SOS cohort by eligibility for bariatric surgery resulted in 3814 individuals in the eligible group (1906 surgically treated and 1908 controls), and 233 individuals in the non-eligible group (104 surgically treated and 129 controls), (Table 1, Paper I). The low number of participants forming the non-eligible group is partly due to the BMI criteria for originally entering the SOS study: 34 kg/m^2 in men and 38 kg/m^2 in women, resulting in few participants having a BMI below 35 kg/m^2. The non-eligible group had, as expected, a lesser degree of obesity and lower rates of co-morbidities as compared to the eligible group, thus well representing a non-eligible obese population (Table 1, Paper I).

In both the eligible and the non-eligible groups, there were statistically significant improvements in anthropometric measures and cardiovascular risk factors 10 years after bariatric surgery (Table 3, Paper I). E.g. blood glucose levels were more decreased in surgically treated individuals than in controls in both groups. No difference in treatment effect was detectable, although for some variables, the improvements were slightly greater in the eligible group. A number of studies including individuals with BMI between 30 and 35 kg/m^2 show similar improvements of cardiovascular risk factors, especially in individuals with T2DM [61, 64, 76-79]. Altogether, this suggests that bariatric surgery has beneficial effects on cardiovascular risk factors in patients with lower degree of obesity that do not fulfill current eligibility criteria.

The beneficial effect of bariatric surgery was also evident when investigating T2DM incidence: In both eligible and non-eligible participants, bariatric surgery reduced the risk for T2DM during 15 years follow-up (Figure 6). The

effect was strong in the eligible group (Hazard Ratio [HR] 0.26, 95 % Confidence Interval [CI] 0.21-0.32, p < 0.001) as well as in the non-eligible group (HR 0.34, 95 % CI 0.14-0.86, p = 0.022), and we could not detect any difference in treatment effect (p for interaction 0.568). In the non-eligible group, the number needed to treat (NNT) to prevent the incidence of T2DM in one patient was 6.9, as compared to 4.1 in the eligible group, both reflecting the strong effect of bariatric surgery in preventing T2DM as has previously been reported [50]. Booth and colleagues also found a similar risk reduction for T2DM after bariatric surgery, but in the subgroup of participants with BMI of 30-35 kg/m^2, they could not detect a preventive effect [51].

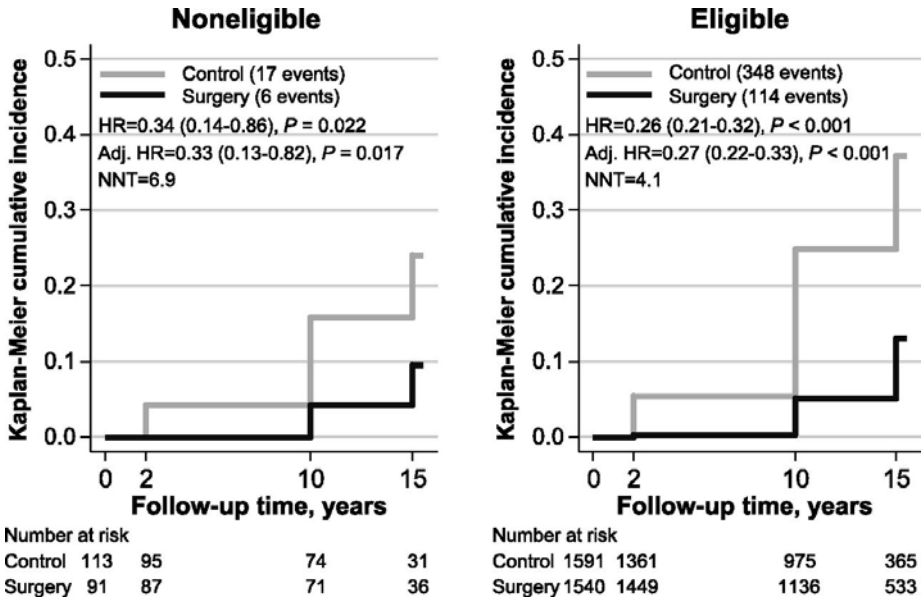

Figure 6 The Kaplan-Meier cumulative incidence of T2DM over 15 years by treatment in non-eligible and eligible groups. Both unadjusted HR and HR adjusted for confounders (sex and age) are shown. Unadjusted interaction *P* value = 0.568 and adjusted interaction *P* value = 0.713. Note that only patients without diabetes at matching and baseline were included in the analysis. NNT, number needed to treat. From Sjöholm et al., Diabetes Care 2013;36:1335-1340. ©2013 by American Diabetes Association. Reprinted with permission.

Nevertheless, our results indicate that patients with a lower degree of obesity and/or without co-morbidities may also benefit from bariatric surgery. Today, these patients are never considered for bariatric surgery – the only effective obesity treatment today – as they do not fulfill current eligibility criteria. As the BMI-based criteria may not select the patients that would benefit the most from bariatric surgery, other variables for selection could be valuable.

Measures of glucose homeostasis could be one option, as serum insulin levels predicted treatment effect on both T2DM and cardiovascular events in the SOS study [50, 52]. A high level of serum insulin is commonly associated with obesity and insulin resistance and is a good marker of metabolic disturbances. The strong effects of bariatric surgery on T2DM, both for remission [59, 62-64], and as prevention [50, 51], have resulted in the treatment more frequently being referred to as "metabolic surgery". Using the term "metabolic surgery" highlights the treatment not solely as a means of losing weight, but rather a surgical treatment to prevent and treat metabolic disease associated with obesity.

5.2 Alcohol consumption and alcohol problems after bariatric surgery

Anecdotes of increased alcohol consumption following bariatric surgery have been frequent in daily media, and an increased sensitivity to alcohol, especially for GBP patients, have been reported [71, 72]. We therefore investigated the effects of bariatric surgery on alcohol habits in the SOS study and identified three different outcomes that were possible to evaluate: incidence of at least medium risk alcohol consumption according to the WHO, incidence of self-reported alcohol problems, and incidence of AUD diagnoses during hospitalization for any reason.

The daily alcohol intake was investigated up to 10 years after bariatric surgery in the control group and in groups that underwent different surgical techniques. In all treatment groups, both in men and women, the mean alcohol consumption during follow-up never exceeded 20 g/day, which is equal to one can of beer (5 % alcohol by volume [ABV]) or slightly less than two glasses of wine (12 % ABV).

Specifically in the GBP group, the mean alcohol consumption was not alarmingly high at any follow-up time point, and never exceeded the level defined as medium risk consumption by the WHO [104]. Even though the mean alcohol consumption during follow-up remained within the low-risk range ($\leq$ 40 g/day in men, 20 g/day in women, WHO [104]), participants operated with GBP had a higher risk of ever reporting at least medium risk consumption (alcohol consumption of $\geq$ 20 g/day in women and $\geq$ 40 g/dag in men, WHO) as compared to the other treatment groups (Table 2). The results were similar when adjusting for baseline confounders (sex, age, daily smoking, alcohol consumption, and total calorie intake) and when analyzing per protocol (Table 1, Paper II). One study reported that a large proportion of

patients with high-risk drinking behavior improved their drinking behavior one year after GBP surgery, but two years after surgery half of them had relapsed [113]. This early decrease in high risk drinking behavior following bariatric surgery is consistent with our consumption data, where all treatment groups reduce their daily intake of alcohol one year after study start (Figure 1, Paper II). This reduced intake during the first year is probably associated with the major lifestyle changes that the surgical patient is recommended, including the strict diet and advice not to consume alcohol. The long-term follow-up in the SOS study is a strength that allows us to investigate changes that occur several years after surgery. This has been important for the investigation of alcohol consumption because our results suggest that the changes occur after more than two years post surgery.

Table 2 Unadjusted HR with 95 % CI and p values for AUD-related outcomes.

	Self-reported medium risk alcohol consumption, n = 3980[a]		**Self-reported alcohol problems, n = 4009[b]**		**Alcohol abuse diagnosis during hospitalization**	
	HR (95 % CI)	**P value**	**HR (95 % CI)**	**P value**	**HR (95 % CI)**	**P value**
GBP vs. Controls	2.63 (1.71-4.03)	< 0.001	5.13 (2.99-8.82)	< 0.001	4.29 (2.37-7.79)	< 0.001
GBP vs. GB	1.60 (0.95-2.67)	0.075	3.54 (1.68-7.45)	0.001	2.79 (1.26-6.21)	0.012
GBP vs. VBG	1.93 (1.26-2.95)	0.003	2.49 (1.51-4.13)	< 0.001	2.15 (1.21-3.82)	0.009
VBG vs. Controls	1.36 (1.00-1.86)	0.051	2.06 (1.32-3.22)	0.002	2.00 (1.27-3.14)	0.003
GB vs. Controls	1.64 (1.07-2.52)	0.022	1.45 (0.71-2.95)	0.303	1.54 (0.75-3.13)	0.237

[a]Excluding participants with missing values or medium risk alcohol consumption at study start.
[b]Excluding participants with missing values or alcohol problems at study start.

The GBP group is also more likely than the other treatment groups to report having alcohol problems, with a HR for self-reported alcohol problems as high as 5.13 as compared to the control group (Table 2). The formulation of the question, on which this outcome is based, makes multiple interpretations possible, but regardless of the interpretation a positive reply indicates a

problematic relation to alcohol. Thus, the GBP group is at greater risk to experience alcohol problems. One possible mechanism behind this increased risk is the alteration in pharmacokinetics of alcohol observed after GBP [114-118]. Studies have consistently reported higher maximum alcohol concentrations after GBP surgery; compared to pre-surgical measurements [114, 118], to non operated controls [71, 72, 118] and to expected in a non operated population [117]. The blood alcohol concentrations have been disproportionately high for the consumed amount, and within minutes after drinking it exceeded the legal driving limit [117, 118]. This faster and increased alcohol absorption could be the reason that GBP operated individuals are more sensitive to alcohol after surgery [71, 72, 118], and this may be problematic in social contexts when drinking reasonable amounts leads to disproportionate intoxication symptoms. Pepino and colleagues, who investigated alcohol metabolism and alcohol sensitivity, both as compared to pre-operative measurements and to non surgical controls, referred to the difference as two drinks ingested by an operated individual equals four drinks ingested by a non operated individual [118].

The only objective variable for AUD in the SOS study was the registry based incidence of AUD diagnosis during hospitalization. For the GBP group, there was an increased risk for AUD diagnosis during hospitalization as compared to the control group (HR 4.29), GB group (HR 2.79), and the VBG group (HR 2.15) (Table 2). There was no difference in hospitalization frequency between the surgery groups and the control group (data not shown). This variable on AUD is probably the most reliable, as it depends on physicians having set a diagnosis of alcohol abuse or alcohol dependence syndrome, or deemed that serious medical conditions were due to abusive alcohol consumption. Conason and colleagues reported an increased risk for substance use two years after bariatric surgery, and especially an increased risk for alcohol use after GBP surgery, but their follow-up rate was low [119]. King and colleagues reported a greater prevalence of AUD two years after bariatric surgery, and GBP was independently predicting increased risk of AUD [120]. In Sweden, Östlund and colleagues investigated in-hospital treatment for alcohol abuse and found that GBP patients had a higher risk for admission as compared to VBG and GB operated patients analyzed as a group [121]. A recent review on the existing literature concluded that GBP patients have an increased risk for AUD after surgery, but the general prevalence of AUD is low in this group and more prospective, longitudinal studies using validated assessment instruments are needed [100].

Altogether, these effects of bariatric surgery on different AUD outcomes are indeed of clinical significance as AUD implies a potential health risk, not

only for the affected patient but also for its surroundings. Furthermore, GBP is the most common procedure for bariatric surgery today. The GBP technique is principally different from the VBG and GB since it is both restrictive and malabsorptive, and the passage and metabolism of alcohol probably differs depending on surgical technique. In GBP, the bypassing of the main ventricle implies a bypass of the first-passage metabolism of alcohol by alcohol dehydrogenase and an almost immediate delivery to the jejunum after ingestion. GBP has been associated with higher peak alcohol levels [114-116] and longer time for alcohol elimination [114, 116], while GB has not been associated with such changes [122], and data on VBG are lacking. Meanwhile, the increased risk is not restricted to only GBP participants as we also detected an increased risk for self-reported alcohol problems and AUD diagnosis during hospitalization in the VBG group as compared to the control group (Table 2). Other explanations for the increased risk such as addiction transfer, where an excessive food intake is replaced by excessive alcohol consumption, have been postulated, but this needs to be studied further. The GBP group is the most successful treatment group with regards to weight loss [44], and as alcohol is a high calorie product participants in the control group may avoid it in their attempts to lose weight. It is also possible that the surgery group is more socially active and more frequently dine out with friends or have a drink after work with colleagues. An increase in such social activities may also increase the risk of consuming more alcohol, as this is a common part of such activities.

5.3 Cancer incidence up to 26 years after bariatric surgery

Increased BMI and obesity have consistently been associated with an increased risk for cancer [25, 28]. Evidence for reversibility of this association, i.e. a decreased risk for cancer following weight-loss, is scarce. A few studies have investigated the association between bariatric surgery and cancer showing similar risk reductions for overall cancer, but different results for specific cancers, and the effect appears to be exclusive to women [55, 56, 123]. In 2009, we reported a reduced incidence of cancer after bariatric surgery in women [54]. One of the aims of this thesis was to extend these findings with a longer follow-up and investigate incidence of specific cancers in the SOS study cohort.

During up to 26 years following bariatric surgery, there was a reduced risk for overall cancer in the surgery group as compared to the control group with a HR of 0.80 (95 % CI 0.68-0.93, p=0.03) (Figure 7). This risk reduction is similar to that observed by Adams and colleagues for overall cancer (HR=0.76) [55] but slightly less than that observed in the SOS study previously (HR=0.67) [54]. As overall cancer includes sex-specific cancers, such as endometrial and prostate cancers, the same analysis was performed excluding all sex-specific cancers. In this analysis there was not a reduced risk for cancer in the surgery group as compared to the control group. The number of events is markedly lower in this analysis, and consequently the statistical power to detect a treatment effect is lower. Meanwhile, as the large number of all female cancers is excluded in this analysis, one might speculate if the treatment effect on overall cancer is driven by a specific treatment effect on these cancers.

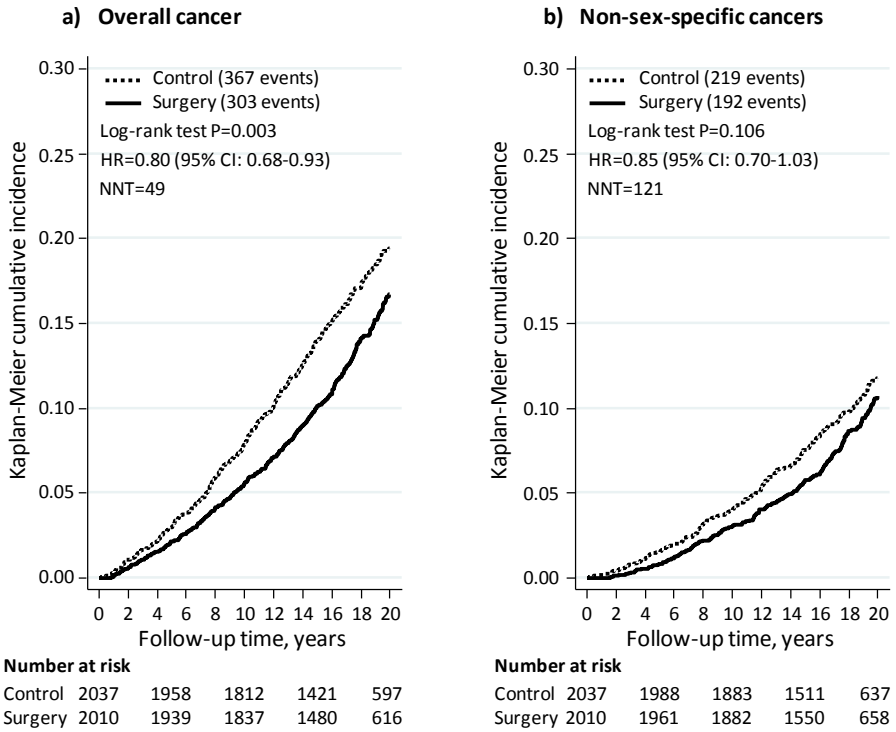

Figure 7 The Kaplan Meier cumulative incidence of (a) overall cancers and (b) overall cancers excluding sex-specific cancers, during 20 years, in surgically treated and control individuals of the SOS study. All hazard ratios (HR) are unadjusted. NNT = Number needed to treat. P for sex*treatment interaction was 0.12 in both analyses.

The same analysis was then performed stratified by sex to specifically investigate the treatment effect in men and women. In women, bariatric surgery leads to statistically significant risk reductions for overall cancer, female cancers, and all cancers excluding female cancers (Figure 8). Analyzing different female cancers independently showed a protective effect of bariatric surgery on endometrial cancer (HR=0.62, 95 % CI 0.39-0.97, p=0.038), but this finding was not confirmed in all our sensitivity analyses (Supplementary Table 1, Paper III). The protective effect on female cancers is an interesting finding considering the hypothesis that increased sex steroids in obesity causes cancer [124]. In obesity, there is an increased peripheral conversion of androgens by aromatase in the adipose tissue, and reduced level of sex hormone binding globulin in the circulation, both resulting in increased amounts of circulating estrogens [124, 125]. Many female cancers are hormone responsive, and our results suggest a reversibility of the association between obesity and female cancers, which could be due to reduced levels of sex hormones. It is also an important finding, as female cancers are among the most common in the world [126] and especially in the obese population [25-27, 124]. The preventive effect on female cancers as a composite endpoint, has not been shown previously, but one study has shown a preventive effect specifically on endometrial cancer [127].

In men, bariatric surgery did not lead to significant risk reduction for overall cancer, for male cancers or for overall cancer when excluding male cancers (Figure 3, Paper III). The number of men in the SOS study is less than half the number of women, and the statistical power is lower to detect a treatment effect, but there is not even a tendency to an effect on overall cancer incidence in men. However, we cannot state that there is a difference in treatment effect between men and women, as we did not detect a statistically significant interaction between sex and treatment effect (Figure 7). Cancer is the only outcome where the treatment effect of bariatric surgery seems to differ between sexes, and we have not been able to detect any difference for T2DM prevention, cardiovascular events or mortality in the SOS study [50, 52, 57]. This probably is an illustration of the complexity of malignant disease and its association with obesity and metabolic disturbances.

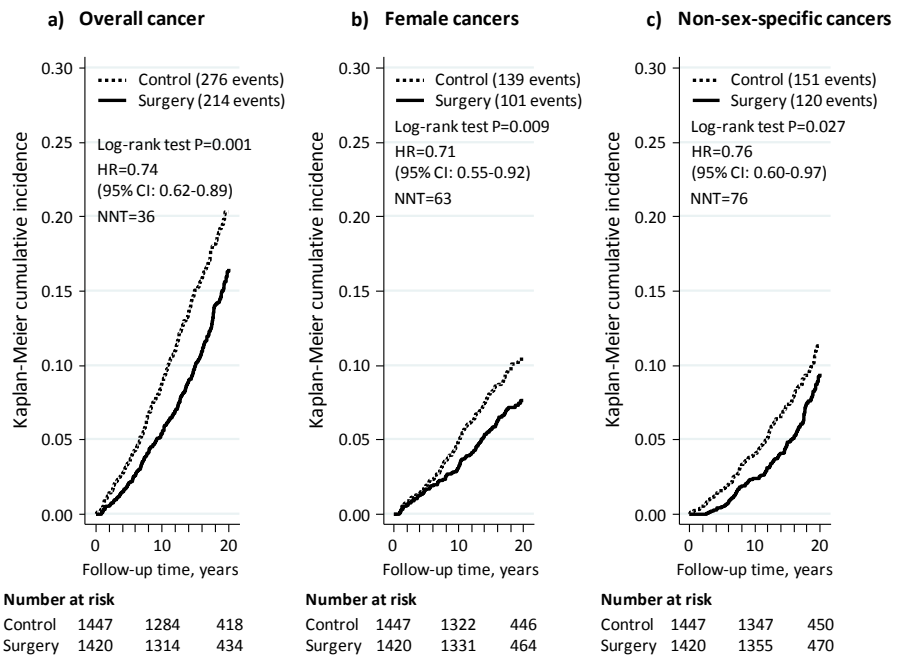

Figure 8 The Kaplan Meier cumulative incidence of (a) overall cancers, (b) female cancers and (c) overall cancers excluding female cancers, during 20 years of follow-up in women, in surgically treated and control individuals. All hazard ratios (HR) are unadjusted. NNT = Number needed to treat.

In the whole cohort, there was a preventive effect of bariatric surgery on skin cancers, as well as on malignant melanoma alone (Table 2, Paper III). We detected a similar effect when analyzing women only, but no effect was found in men. In men, there were few skin cancer events but fewer events in the surgery group than in the control group, and it is possible that the low number of events is the reason for not finding statistical significance. Apart from the sex steroid hypothesis mentioned above, many hypothesis regarding the mechanisms linking adiposity with cancer have been postulated, including effects of adipokines, and increased levels of insulin and IGF-1 [124, 125]. The reduced risk for skin cancers could be associated with the improved glucose homeostasis after bariatric surgery with reduced levels of insulin [128], which is hypothesized to stimulate tumor growth [124, 125]. In women we also found a decreased risk for hematopoietic cancers (HR=0.40, 95 % CI 0.20-0.82, p=0.012) but in men, the number of events was actually higher in the surgery group than in the control group. This result was not statistically significant however, but the association between bariatric surgery and hematopoietic cancers does seem to differ between men and women, with a significant interaction for sex and treatment (p=0.01). One might

speculate that the association between obesity and hematopoietic cancers differ between men and women due to a stronger impact by sex steroids and particularly estrogen. For cancers of the urinary tract, there were more events in the surgery than in the control group in the whole cohort (35 versus 20 events), as well as in men and women separately, but we could not detect an increased risk for these cancers after bariatric surgery.

Altogether, our results indicate associations between bariatric surgery and reduced cancer incidence and, although not significant, the associations seem to differ between men and women, and may be dependent on type of cancer. This highlights the diversity of malignant disease and calls for further research into the association between obesity and cancer, and between bariatric surgery and cancer.

5.4 ITIH5 expression in human adipose tissue is increased in obesity

Many genes that are important for adipose tissue function and its association with obesity have been shown to be specifically expressed in adipocytes, such as leptin [82]. Our group has done genome wide searches for adipocyte-specific genes, and *ITIH5* was one of the genes we found.

In our extended analyses of the *ITIH5* expression we found that *ITIH5* was almost exclusively expressed in adipocytes of the human adipose tissue (Figure 9). It was not expressed in the same manner as the bikunin gene, which protein is known to form a complex with the other members of the ITIH gene family, suggesting that ITIH5 does not form a complex with bikunin and is functioning on its own in the adipose tissue. *ITIH5* mRNA levels were also higher in the subcutaneous adipose tissue as compared to the omental adipose tissue, which is considered to be more associated with metabolic disease. Hence, its gene expression pattern suggests *ITIH5* is an adipocyte specific gene.

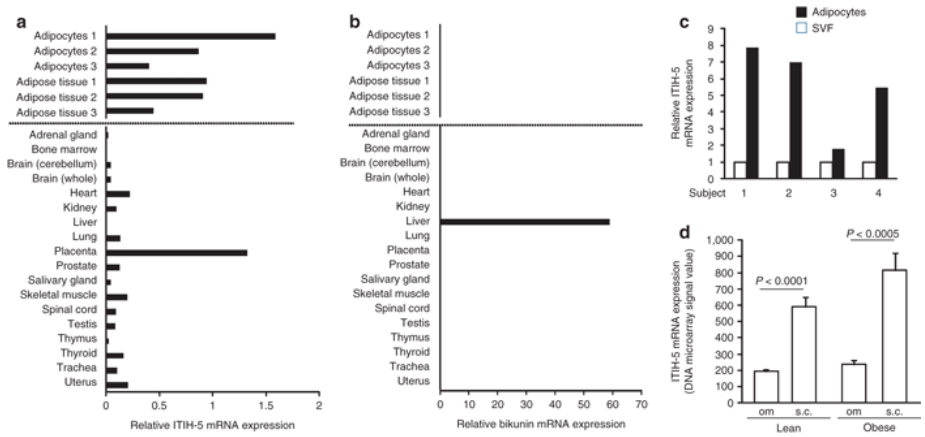

Figure 9 *ITIH5* and bikunin mRNA expression in human tissues. mRNA expression of (a) *ITIH5* and (b) bikunin in adipocytes, sc adipose tissue and a panel of human tissues (below the dashed line) analyzed by real-time PCR. (c) *ITIH5* mRNA expression analyzed by real-time PCR in sc adipocytes and stromal vascular fraction cells (SVF) isolated from four subjects. (d) *ITIH5* mRNA expression analyzed by DNA microarray in paired abdominal sc and om adipose tissue biopsies. SVF, stromal vascular fraction cells. From Anveden et al., Obesity. 2012 Apr;20(4):708-14. © The Obesity Society. Reprinted with permission.

In subcutaneous adipose tissue of individuals with obesity, especially women, higher levels of both *ITIH5* mRNA and ITIH5 protein were found, as compared to individuals without obesity (Figure 10), thus suggesting that ITIH5 expression is associated with adiposity. During a VLCD treatment of individuals with obesity, the expression of *ITIH5* mRNA in subcutaneous adipose tissue was reduced, in both men and women at 16 weeks and at 18 weeks when regular food had been reintroduced. This expression pattern suggests that the *ITIH5* gene expression is not associated with the caloric restriction, but rather with obesity/BMI, or adipose tissue rearrangements following weight loss. Altogether the results support the hypothesis that ITIH5 is associated with obesity and reduced by weight-loss. However, it is unclear whether or not ITIH5 plays a role in the development of obesity or obesity-related disease.

To further investigate the association with obesity driven morbidities, the *ITIH5* mRNA expression in the Sib Pair study was examined in relation to several anthropometrical measures and cardiovascular risk factors (Table 1, Paper IV). In both individuals with and without obesity, measures of obesity (such as BMI), and glucose homeostasis (such as insulin) correlated with mRNA expression of *ITIH5*. Similar correlations were found when repeating

the analyses in Healthy women I, (Table 2, Paper IV). Thus, the gene expression of *ITIH5* in subcutaneous adipose tissue is positively associated with measures of obesity, and markers of metabolic disease. Speculatively, this association may be causative, and ITIH5 could play a role in the disease development in obesity, but the association may also be non causative.

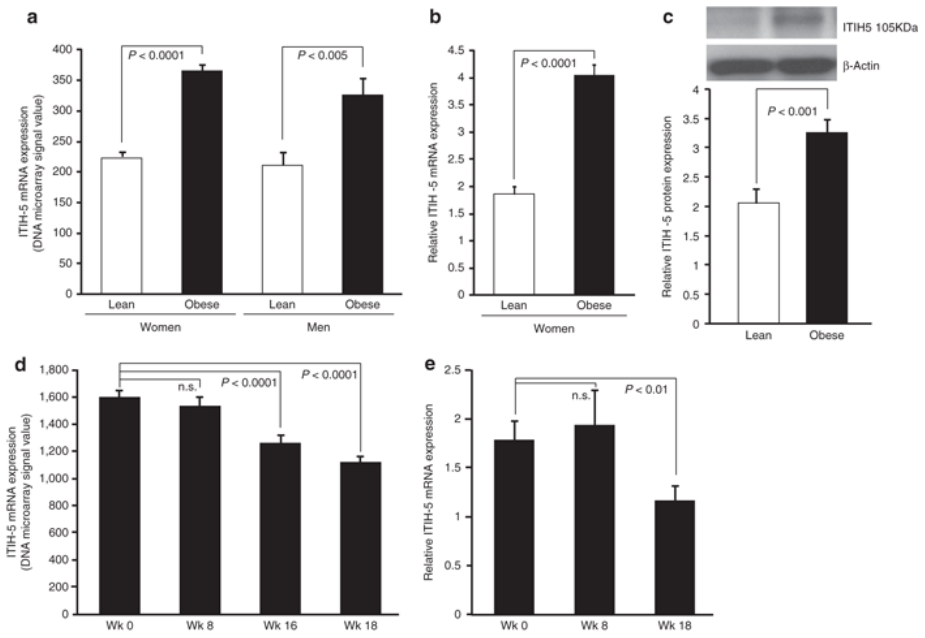

Figure 10 ITIH5 expression in sc adipose tissue from lean and obese subjects. (a) *ITIH5* mRNA expression in the SOS Sibpair study (n = 90) analyzed with DNA microarray. (b) *ITIH5* mRNA expression in Healthy women I, lean (n = 15) and obese (n = 80), analyzed with real-time PCR. (c) Western blot analysis of ITIH5 protein expression in obese (n = 7) and lean (n = 7), Healthy women II. *ITIH5* mRNA expression in (d) VLCD study I (n = 24) and (e) VLCD study II (n = 10). Data are presented as mean + s.e.m. n.s., not significant; VLCD, very low calorie diet; wk, week. From Anveden et al., Obesity. 2012 Apr;20(4):708-14. © The Obesity Society. Reprinted with permission.

Very recently, a study verified our findings that ITIH5 expression in adipose tissue is associated with obesity [129]. Interestingly, they also show that methylation sites (GpC sites) close to the *ITIH5* gene were associated with BMI, suggesting that this gene is strongly regulated by epigenetic mechanisms. In addition, another study found up-regulation of the *ITIH5* gene in adipose tissue, as well as higher expression in the subcutaneous depot as compared to the visceral [130]. Thus, ITIH5 is potentially important for adipose tissue function, but further studies are needed to determine the role of

the *ITIH5* gene and its association with obesity and metabolism. In our research group, we have continued the work with ITIH5 by stimulating 3T3-L1 cells with different metabolically relevant substances (e.g. insulin and TNF-α), but these results did not provide novel insights into ITIH5 function in adipose tissue (data not shown) Furthermore, the *ITIH5* gene expression was analyzed in adipose tissue depots of the mouse, but the expression pattern was not similar to that seen in humans, although the ITIH5 protein level in serum was higher in mice fed with a high fat diet as compared to controls (data not shown). In evaluating the function of ITIH5, establishing an animal model where the *ITIH5* gene is knocked-down or overexpressed in adipose tissue would be a relevant next step. This would probably give insights into ITIH5 function in adipose tissue and its putative role in metabolism. However, given the difference in regulation of adipose tissue ITIH5 in mice and human, mice models may not be optimal in this case.

6 CONCLUDING REMARKS

The main focus of this thesis has been to assess bariatric surgery as an intervention for obesity with regards to positive effects on obesity co-morbidities but also negative side effects in individuals with obesity regardless of eligibility for surgical treatment. Main findings of this thesis are summarized in Figure 11.

Bariatric surgery is today the only effective treatment of obesity with substantial long-term effects on weight loss as well as positive effects on hard endpoints such as T2DM, cardiovascular events, cancer, and mortality. Through extensive research, that may include characterization of the adipose tissue or investigation of physiological mechanisms of bariatric surgery, it is likely that new potential pharmacological therapy for obesity will arise. The work on ITIH5 in this thesis adds to the characterization of adipose tissue and to this research field. However, new pharmacological therapies will probably not eliminate surgery as a therapeutic option. Furthermore, the patients that we operate today will be using health care for at least five decades on. The importance of further research on obesity and bariatric surgery for millions of individuals worldwide is evident.

Balancing positive and negative effects of bariatric surgery as well as investigating factors predicting these effects is important in elucidating who will benefit most from surgical obesity treatment. Bariatric surgery has potential side effects and the increased risk for alcohol use disorders described in this thesis is one example. Caregivers and caretakers need to be aware of potential side effects, and follow-up care should be structured and designed to early discover patients displaying them. Still, it is generally considered that the positive effects of bariatric surgery outweigh the side effects. This thesis' finding that bariatric surgery has positive metabolic effects even in a non-eligible obese population adds to the growing literature supporting a revision of current criteria for bariatric surgery. According to current eligibility criteria, individuals with obesity are treated as a very homogenic group with BMI as a primary selection criterion, but this thesis supports an increased individualization of obesity management. Another important positive effect observed after bariatric surgery is that it prevents incidence of overall cancer, and specifically female cancers. The association between bariatric surgery and cancer risk seems to differ between different types of cancers, as well as between the sexes and further research is needed to understand the mechanisms behind the association between obesity and cancer.

On the whole, bariatric surgery is the best currently available treatment of obesity. It can improve the lives of millions of people worldwide and most of them will not be affected by serious side effects. However, obesity appears to be a chronic disease and should be considered as such, also for those having lost most of their excess weight after bariatric surgery. Consequently, bariatric surgery should be considered a life-long treatment, rather than the "quick fix" it is sometimes referred to. Individuals with obesity, as well as operated individuals formerly having obesity should probably have a life-long follow-up, and be given individualized care. In the future, I wish to see further research on how bariatric surgery can be an individualized treatment – who should be given this treatment, when should they receive it, and how do we give them best possible care after this treatment?

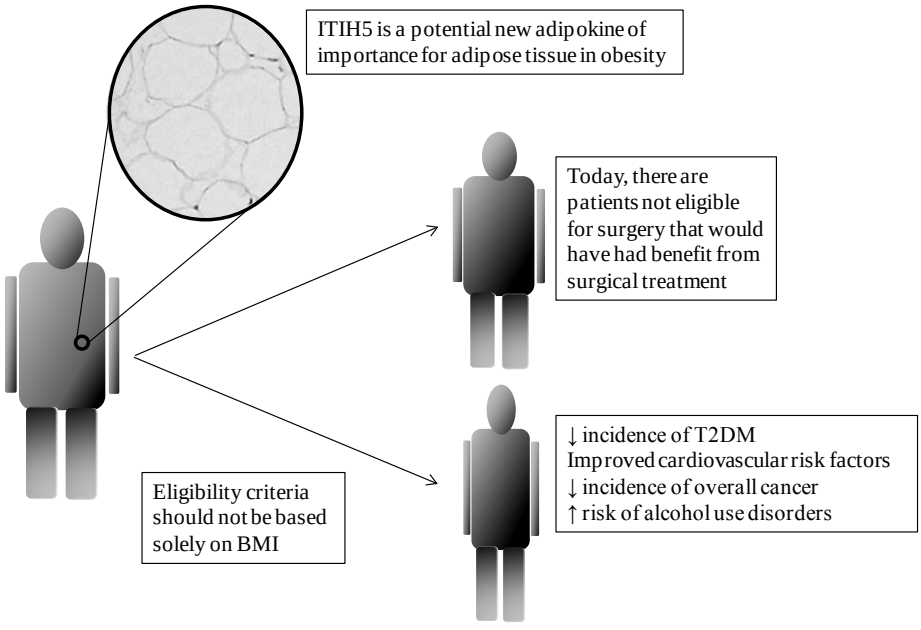

Figure 11 Summary of the main findings of this thesis. Illustrations by Per-Arne Svensson.

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