Pathogenesis of Obesity and Effects of Treatment

Clinical and Molecular Studies on
Body Fat, Energy Balance, and Weight Loss

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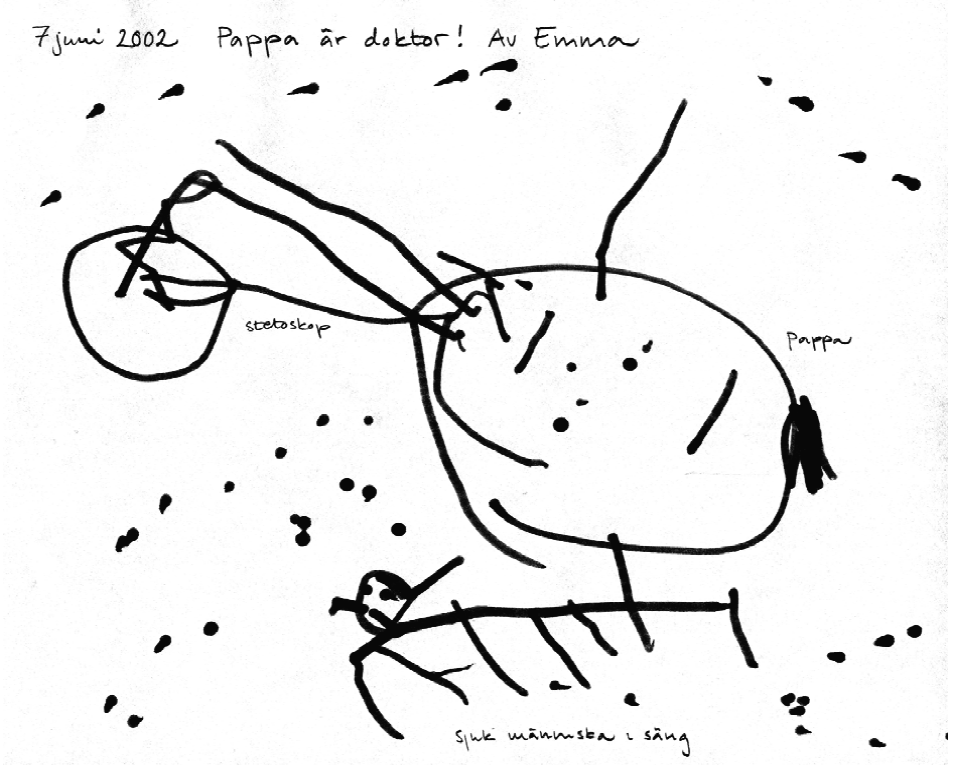
Dedicated to my family
Maria, Emma, Elias & Malte



ABSTRACT

Obesity is common and related to many health problems including various forms of cancer. The condition arises from the imbalance between food intake and energy expenditure, and is strongly influenced by genetic factors. Weight loss has several health benefits, but for many of the obesity-related diseases such as cancer, the impact of obesity treatment is not clarified. Unfortunately, weight loss is in most cases difficult to sustain, and obesity treatment today is insufficient. The adipose tissue and the gastrointestinal tract play active roles in the regulation of whole-body energy balance, and therapeutic targets for the treatment of obesity may be found within these sites. Also, these organs may be responsible for mediating some of the adverse effects of obesity. Special attention has been drawn to visceral adipose tissue, i.e. the fat surrounding the intestines, as being particularly harmful. The aim of this thesis was to increase our understanding of the mechanisms behind human obesity and the consequences of obesity treatment. We used population-based cross-sectional studies, as well as longitudinal intervention studies with short- and long-term weight loss.

CIDEA and CIDEC are two genes with putative functions in adipose tissue, and we therefore studied their transcriptional regulation in relation to energy balance and body composition as an attempt to elucidate their role in human obesity. The genes were predominantly expressed in adipose tissue as compared to other human tissues, both CIDEA and CIDEC gene transcription were highly responsive to changes in energy availability, and CIDEA correlated with body fat and insulin levels. CIDEA expression also correlated with basal metabolic rate and uncoupling protein 1, suggesting a role in the regulation of energy expenditure. In gene silencing experiments in cultured adipocytes, we showed that CIDEC is involved in the regulation of basal as well as stimulated lipolysis, and mitochondrial fatty acid oxidation. Together, our results support a role of CIDEC and CIDEA in human obesity.

There are indications that impaired intestinal barrier with increased passage of gut-derived antigens may drive visceral adipose tissue accumulation, and we therefore investigated if increased intestinal permeability is associated with visceral obesity in humans. Study subjects were recruited from a population-based cohort of Swedish women. Intestinal permeability was assessed using the urinary excretion of orally ingested sucralose and mannitol. We used computed tomography to measure visceral and liver fat. Intestinal permeability of the large intestine correlated with visceral fat area ($P=0.0003$) and liver fat content ($P=0.004$). The results indicate that gut leakiness should be further explored as a possible cause of visceral fat accumulation.

The Swedish Obese Subjects (SOS) study in combination with the Swedish National Cancer Register makes it possible to, for the first time, study the effects of bariatric surgery on cancer incidence in a prospective, controlled study setting. The SOS study started in 1987 and involves severely obese subjects, 2010 of which underwent bariatric surgery, and 2037 contemporaneously matched obese controls who received conventional treatment. Bariatric surgery resulted in a sustained weight reduction, whereas the average weight change in the control group was minimal. In women, the number of first-time cancers during on average 11 years after inclusion was lower in the surgery group compared to the control group (HR= 0.58, 95% CI: 0.44-0.77, $p<0.001$). In men, we could not detect any difference between treatment groups (HR=0.97, $p=0.91$).

In summary, the results of this thesis suggest that the CIDEA and CIDEC genes play a role in obesity, impaired intestinal barrier function contributes to visceral fat accumulation, and bariatric surgery reduces the risk of developing cancer in severely obese women.

CONTENTS

LIST OF PAPERS

This thesis is based on the following papers:

I **Relations of Adipose Tissue CIDEA Gene Expression to Basal Metabolic Rate, Energy Restriction, and Obesity: Population-Based and Dietary Intervention Studies.**
Gummesson A, Jernås M, Svensson PA, Larsson I, Glad CA, Schéle E, Gripeteg L, Sjöholm K, Lystig TC, Sjöström L, Carlsson B, Fagerberg B, Carlsson LM.
J Clin Endocrinol Metab 2007;92:4759-65.

II **Cell death-inducing DFF45-like effector C is reduced by caloric restriction and regulates adipocyte lipid metabolism.**
Magnusson B, Gummesson A, Glad CA, Goedecke JH, Jernås M, Lystig TC, Carlsson B, Fagerberg B, Carlsson LM, Svensson PA.
Metabolism 2008;57:1307-13.

III **Increased intestinal permeability is associated with visceral and hepatic fat accumulation.**
Gummesson A, Carlsson LM, Storlien L, Bäckhed F, Lundin P, Löfgren L, Stenlöf K, Fagerberg B, Carlsson B.
Submitted for publication.

IV **Effects of Bariatric Surgery on Cancer Incidence in Swedish Obese Males and Females.**
Sjöström L, Gummesson A, Sjöström CD, Narbro K, Peltonen M, Wedel H, Bengtsson C, Bouchard C, Carlsson B, Dahlgren S, Jacobson P, Karason K, Karlsson J, Larsson B, Lindroos AK, Lönroth H, Näslund I, Olbers T, Stenlöf K, Torgerson J, Carlsson LM, for the Swedish Obese Subjects Study.
Submitted for publication.

ABBREVIATIONS

BMI	Body mass index
BMR	Basal metabolic rate
Bp	Blood pressure
cDNA	Complementary deoxyribonucleic acid
CIDEA	Cell death-inducing DFFA effector A
CIDEC	Cell death-inducing DFFA effector C
cRNA	Complementary ribonucleic acid
CRP	C-reactive protein
CT	Computed tomography
DFFA	DNA fragmentation factor A
DXA	Dual x-ray absorptiometry
FSP27	Fat-specific protein 27
FTO	Fat mass- and obesity-associated
HDL	High density lipoprotein
HR	Hazard ratio
IPIVO	Intestinal permeability in visceral obesity
LDL	Low density lipoprotein
LRP10	LDL receptor-related protein 10
MC4R	Melanocortin 4 receptor gene
MetS	Metabolic syndrome
mRNA	messenger ribonucleic acid
NEFA	Non-esterified fatty acid
OGTT	Oral glucose tolerance test
PCR	Polymerase chain reaction
PGC-1alpha	PPARG coactivator 1 alpha
POMC	Pro-opiomelanocortin
PPARgamma	Peroxisome proliferator-activated receptor gamma
PPIA	Peptidyl-prolyl isomerise A
RIP140	Receptor-interacting protein 140
RT-PCR	Reverse transcription PCR
siRNA	Small interfering RNA
SOS	Swedish Obese Subjects
UCP1	Uncoupling protein 1
WHO	World Health Organization
WHR	Waist-to-hip ratio
VLCD	Very low calorie diet
VLED	Very low energy diet

INTRODUCTION

Obesity and the metabolic syndrome

Obesity can be described as the accumulation of adipose tissue to the extent that health may be impaired. An excess of body fat, and in particular of abdominal fat, is associated with multiple complications, leading to poor health. With increasing degrees of obesity there are increasing risks of a wide range of obesity complications (Table 1), and premature death [1-3]. The definition of obesity is based on the body mass index (BMI), which is calculated as weight in kilograms divided by height in meters squared (kg/m^2). Obesity is defined as a BMI greater than 30 kg/m^2, and overweight is defined as a BMI from 25 to 30 kg/m^2. In Europe, the prevalence of obesity in men range from 4-28% and in women from 6-36%, with a considerable geographic variation, with prevalence rates in Central, Eastern, and Southern Europe being higher than those in Western and Northern Europe (Fig. 1) [4]. In the USA, it is estimated that about one third of the adult population are obese [5]. The metabolic syndrome (MetS) is a term that refers to a collection of obesity-related metabolic abnormalities/risk factors that often co-occur in the same individuals [6]. MetS is defined in various ways, but the essential components are obesity, glucose intolerance, insulin resistance, lipid disturbances, and hypertension, all well documented risk factors for cardiovascular disease [7-10].

Table 1. Obesity-associated health problems [11-17]

Metabolic	Type 2 diabetes, insulin resistance, hypertension, high triglyceride levels, low HDL levels
Psychosocial	Depression, anxiety, social stigmatization, reduced quality of life, sick leave, early retirement
Cardiovascular	Coronary artery disease, stroke, heart failure, atrial fibrillation
Gynaecological	Infertility, polycystic ovary syndrome, obstetrical risks
Gastrointestinal	Gastroesophageal reflux, gallbladder disease, liver steatosis, non-alcoholic steatohepatitis
Respiratory	Sleep apnea, asthma, pulmonary embolism
Bone and joints	Osteoarthritis, gout, back pain
Dermatological	Striae, acanthosis nigricans, hirsutism, fungal infection
Cancer	Malignancies in the breast, endometrium, colon and rectum, oesophagus, pancreas, kidney, thyroid gland, gallbladder, hematopoietic system, malignant melanoma

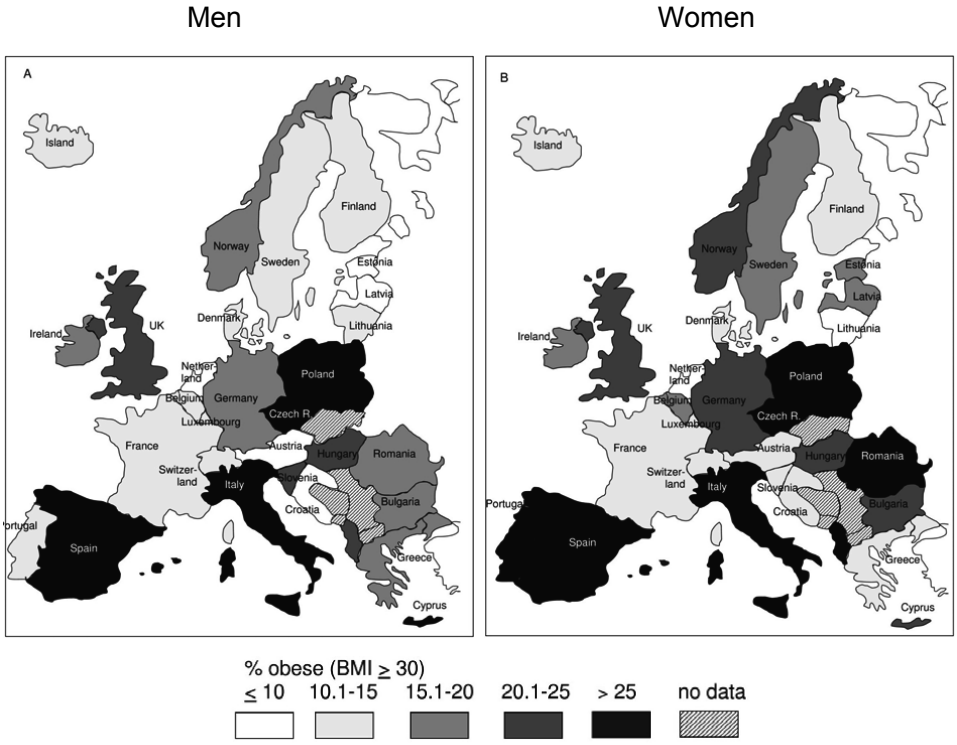


Figure 1. Prevalence of obesity (BMI ≥ 30 kg/m^2) among men and women in Europe.
Reprinted from Berghöfer et al. BMC Public Health 2008 8:200. Open access image.

Energy balance

All living organisms, including humans, obey the first law of thermodynamics which states that the amount of energy in a closed system must remain constant [18]. So any imbalance between food intake and energy expenditure results in a change in the amount of body energy stores, which is mainly fat.

The average human consumes one million calories per year, and an imbalance of only 1% may be enough to cause an annual weight change of 1-2 kg. Weight stability therefore depends on a very precise autonomous regulation of energy balance. If the regulation is not interacting optimally with environmental conditions, even minor differences in energy intake or expenditure may lead to weight change. Unfortunately, the regulatory systems that allow the maintenance of a relatively stable body weight throughout life could also be the most important threat to the capacity to overcome obesity, once it is established.

Genetics of obesity

Our unlimited access to food combined with a sedentary life style undoubtedly contributes to the increase in the incidence of obesity over time. However, the marked differences in adiposity between individuals seem to be explained mainly by our genes. Heritability estimates from twin studies show that as much as 70% of the individual variation in adiposity between people may be due to genetic factors [19-22]. There is also compelling evidence of a genetic variability in the response to changes in energy balance, with strikingly similar weight changes in response to overfeeding or exercise between identical twins, as opposed to marked differences between unrelated subjects [23,24].

A little more than a decade ago, important progress was made to identify genes that cause rare, monogenic forms of obesity. Common for most of these genes is their involvement in hypothalamic regulation of feeding behaviour, including the leptin gene [25], the pro-opiomelanocortin (POMC) gene [26], and the melanocortin 4 receptor gene (MC4R) [27,28]. Individuals with defects in these genes typically present with an increased drive to eat and early-onset obesity [29]. Large research efforts have started to reveal some of the low penetrance genes of obesity. There are many examples of genetic polymorphisms, i.e. genetic variants that appear in at least 1% of the population, that have been reported to be associated with obesity [30]. However, to date only a few genes have been identified in which common variants have been consistently associated with BMI in humans, e.g. the fat mass- and obesity-associated (FTO) gene [31-35] and the MC4R gene [34-36].

Obesity treatment

Obesity is a chronic condition that is difficult to treat. Unless adipose tissue is surgically removed (e.g. liposuction or omentectomy), the only way to lose fat is through negative energy balance. Theoretically this can be achieved by reduced food intake, reduced energy uptake, increased energy expenditure, or a combination of these. Here follows a summary of the main treatment options for obesity:

Dietary management and physical activity
Behaviour therapy in order to help patients adopt necessary life style changes is fundamental in obesity treatment. Many diet plans exist that focus on the relative amount of certain nutrients, such as low-carbohydrate diets, low-fat diets, high-protein diets, and low-glycemic-index diets. However, diet composition is less important than total calories consumed [37], which very much depends on the ability of patients to adhere to their diets. According to a meta-analysis, the effect of dietary counselling is modest, with an average decrease of approximately 0.1 BMI units per month and subsequent regain during the maintenance phase [38]. Exercise alone has a limited effect body weight [39], but the addition of physical activity to a dietary intervention increases the odds of successful long-term weight loss maintenance [38,40]. In addition, even modest increase in physical activity can produce favourable effects on cardiorespiratory fitness [41] and this seems to abolish much of the adverse effects of obesity [42].

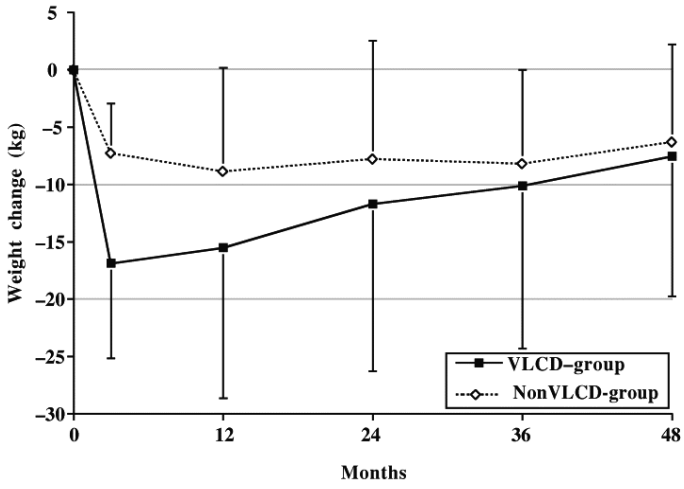


Figure 2. Weight changes among 55 patients (out of 113 patients at study start) who completed a 4 year obesity treatment program including a hypocaloric diet and behavioural support, either as single treatment (non-VLCD group) or following the VLCD period (VLCD group). The weight change compared with baseline was significant ($P < 0.01$) in both treatment groups, but there was no significant difference between groups after 4 years of treatment. Mean and 1 SD are shown.

Very low calorie diets

A very low calorie diet (VLCD) or a very low energy diet (VLED) is defined as a diet with an energy content of less than 800 kcal/day that contains adequate amounts of proteins, essential fatty acids, carbohydrates and the recommended daily allowances of vitamins and minerals [43]. Ordinary food is replaced by 3-5 VLCD meals together with 2-2.5 litres of non-energy fluid per day. At the end of the VLCD period, ordinary food is gradually reintroduced during 2-4 weeks. In medical treatment programs, VLCD is often used 12-16 weeks and result in average weight losses of 1.5-2.5 kg/wk [44,45]. VLCDs are mainly indicated in obese patients with disorders or risk factors that can be immediately improved by weight loss, e.g. type 2 diabetes, and when rapid weight loss is needed before a major surgical procedure. There is usually a rebound in weight after VLCD treatment programs (Fig. 2) which limits its use, and it is crucial that the VLCD phase is followed by active weight maintenance programs [45].

Pharmacological treatment

In Sweden there are currently two drugs available on the market for obesity treatment: Orlistat and Sibutramine. Until recently Rimonabant was approved for the European market, but it was withdrawn due to psychiatric side-effects, such as depression and anxiety [46,47]. Orlistat inhibits gastrointestinal lipase activity and reduces the uptake of dietary fat with about 30% [48]. Sibutramine is a reuptake inhibitor of serotonin and

norepinephrine that has weight reducing properties by increasing both satiety and energy expenditure [49]. A meta-analysis of clinical trials (1-4 years duration) on the long-term effect of anti-obesity drugs showed that the weight-reducing effects are in the order of 3 kg for Orlistat and 4 kg for Sibutramine [50]. Sibutramine treatment during the weight maintenance phase after an initial weight loss has been shown to improve the success rate [51], but the same study also demonstrated the difficulties in obesity treatment with weight regain and large drop-out rates in general.

Bariatric surgery

Obesity surgery provides the greatest degree of sustained weight loss for severely obese patients [52]. Several surgical procedures are used (Fig. 3). On average, surgical treatment of obesity results in 20–40 kg of weight loss and a 10–15 kg/m^2 reduction in BMI [53,54]. Weight changes during the first 15 years after bariatric surgery are shown in Fig. 6. Surgical obesity treatment is generally considered for adult patients if they have a BMI greater than 40 kg/m^2 or a BMI greater than 35 kg/m^2 with serious comorbid conditions, such as sleep apnea, diabetes mellitus, or joint disease [55].

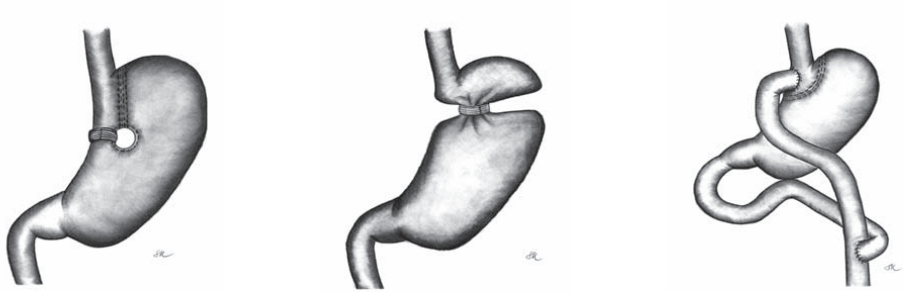


Figure 3. Examples of surgical procedures. Vertical banded gastroplasty involves a staple line paralleling the lesser curvature and a ring at the end of this narrowing to delay entry of food into the stomach. A less invasive method to narrow the opening between the upper and lower stomach is gastric banding, a laparoscopically-placed band around the stomach. Gastric bypass involves making a small pouch of the stomach just below the oesophagus that empties into a loop of jejunum.

Effects of obesity treatment

Although massive epidemiological data demonstrate that obesity increases the risk for numerous diseases and early mortality, it has not been easy to produce convincing evidence regarding the supposedly beneficial long-term effects of weight reduction. Observational data is obscured by the difficulty to separate intentional from

unintentional weight loss, the latter often being caused by disease or harmful health behaviours [56,57]. Several randomized non-surgical weight loss intervention trials have been performed, but these are often limited by the difficulty to achieve long-term weight reduction and by the high drop-out rates [58]. Bariatric surgery can achieve sustained weight loss [59], but is not a feasible intervention for a randomized trial design.

Despite these obstacles, the available evidence suggests that even modest weight reductions in obese people can lead to improvement in health outcomes [58,60,61]. Perhaps weight reduction has the most pronounced effects on diabetes risk; Both a Finnish and an American study have shown that intensive lifestyle modification can reduce the risk of developing diabetes with 58% in subjects with impaired glucose tolerance [61,62], and the Swedish Obese Subjects (SOS) study found a 75% diabetes reduction 10 years after bariatric surgery [59]. The SOS study has also reported beneficial effects of weight loss on cardiovascular risk factors [59], cardiorespiratory symptoms [63], sleep apnea [64], joint pain [65], and health-related quality of life [66]. We and others have shown that bariatric surgery increases the overall life expectancy of severely obese patients [67-69].

Most people who attempt to lose weight can achieve initial weight loss but then reach a plateau where further weight loss is difficult to achieve and initial weight loss difficult to sustain (Fig 2). Perhaps the most striking example is seen in patients treated with obesity surgery who despite this major alteration of the gastrointestinal anatomy start to regain body weight one year after the operation (Fig. 6). The difficulty to achieve sustained weight loss may be partly explained by compensatory responses which act to resist this weight change through a decrease in energy expenditure and an increase in hunger. For example, an obese person who goes from 120 kg to 80 kg would have to consume considerably fewer calories to maintain this weight loss than a person who started out at 80 kg [70].

Adipose tissue

Adipose tissue plays a key role in the development of obesity and metabolic complications, functioning both as an energy store and as a major endocrine organ. The adipocyte is the main cell type in adipose tissue, but the tissue is also comprised of adipocyte precursor cells, stromal-vascular cells, immune cells, and nerve cells [71]. In mammals, two types of adipose tissues are present: White adipose tissue which mainly serves as an energy storing tissue, and brown adipose tissue which is mainly a thermogenic tissue. White adipocytes are characterized by a large lipid droplet that occupies the major part of the cytoplasmic space, while brown adipocytes contain multiple and relatively smaller lipid droplets and a large number of mitochondria (Fig. 4). Brown adipose tissue is abundant in small mammals and in newborns of larger mammals, including humans [72]. In contrast to what was previously believed, a substantial fraction of adult humans possess some amount of active brown adipose [73]. What may also be of physiological significance, although not yet shown in humans, is that white adipocytes have the ability to acquire brown adipocyte features under various stimuli [74-77].

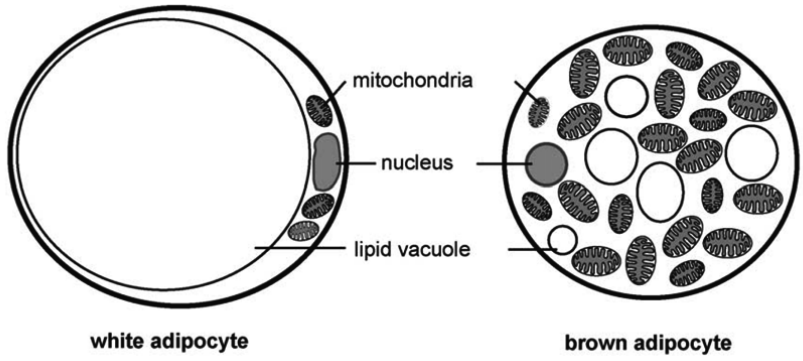


Figure 4. Morphological comparison of white and brown adipocytes.
Reprinted from Sell et al. The brown adipocyte: update on its metabolic role. Int J Biochem & Cell Biol. 36 (2004) 2098-2104, with permission from Elsevier.

The core function of the white adipocyte is to store excess energy and to provide other tissues with energy during periods of negative energy balance, by releasing fatty acids and glycerol from lipolysis of triglycerides stored in the adipocyte lipid droplet. The storage function of adipose tissue appears to be an important factor in obesity-related metabolic disorders. When the storing capacity of the adipose tissue is exceeded or when the adipose tissue is not functioning properly, fatty acids increase in the circulation and triglycerides accumulate in other organs such as liver, muscle, heart, and the beta cells of the pancreas [78-81]. This ectopic fat deposition may seriously affect the functions of these organs and contribute to the pathogenesis of obesity-related conditions such as insulin resistance, diabetes, non-alcoholic steatohepatitis and cardiovascular disease [78-81]. Disturbances in pathways of lipolysis (i.e. the conversion of triglycerides to fatty acids and glycerol) may contribute to the reduced capacity of adipocytes to keep lipids away from the circulation and peripheral tissues [82]. Key enzymes in adipocyte lipolysis are hormone sensitive lipase and adipose triglyceride lipase, and main hormonal regulators are catecholamines and insulin [82].

In addition to its storage function, adipose tissue also has endocrine, paracrine and autocrine activities. Adipocytes secrete various peptides that control a wide range of functions including whole-body energy balance, inflammation, insulin sensitivity, blood pressure regulation, angiogenesis and cellular growth [71,83]. Given the important role of adipose tissue in the pathogenesis of obesity, genes that regulate adipose tissue function may prove to be new therapeutic targets in the treatment of obesity and the metabolic complications of obesity. Drugs that interfere directly with adipocyte physiology include the glitazones, agonists for the adipocyte transcription factor peroxisome proliferator-activated receptor gamma (PPARgamma), which are used for the treatment of type 2 diabetes [84]. Other adipose related mechanisms that have been evaluated include beta-3 agonists and leptin. Both concepts have been very effective in animal models but not proven successful in man. There are currently no obesity drugs on the market that target adipocyte physiology.

Visceral obesity

Abdominal obesity is a stronger predictor for type 2 diabetes, cardiovascular risk factors and myocardial infarction, than is general obesity [85-87]. Abdominal fat includes the subcutaneous fat beneath the skin, and the visceral fat that surrounds the internal organs (Fig. 5). It seems as if the detrimental effects of abdominal obesity are largely explained by the visceral adipose tissue depot. Studies that have been able to differentiate between subcutaneous and visceral fat depots of the abdomen have demonstrated that visceral adipose accumulation is an independent risk factor associated with dyslipidaemia, insulin resistance, type 2 diabetes, liver fattening, cardiovascular disease, and all-cause mortality [88-93].

Visceral adipose tissue displays intrinsic properties that are different from subcutaneous adipose tissue. For example, the rate of lipolysis is higher in visceral than in subcutaneous fat depots, which may be explained by site variations in the function of receptors for insulin, catecholamines and adenosine [94]. One suggested explanation for the adverse effects of visceral obesity is an increased delivery of fatty acids from the visceral depot to the liver via the portal vein, leading to elevated hepatic triglyceride and glucose production and hyperinsulinemia [95]. Another important feature of visceral fat depots is the presence of lymphoid tissue such as lymph nodes in the mesenteric adipose tissue and milky spots in the omentum [96]. Compared with subcutaneous depots, visceral adipose tissue expresses higher levels of many cytokines, immunoglobulins and complement factors, suggesting a more active role in immune defence [97-100]. Furthermore, omental and mesenteric adipocytes interact strongly with immune cells such as dendritic cells and macrophages [96].

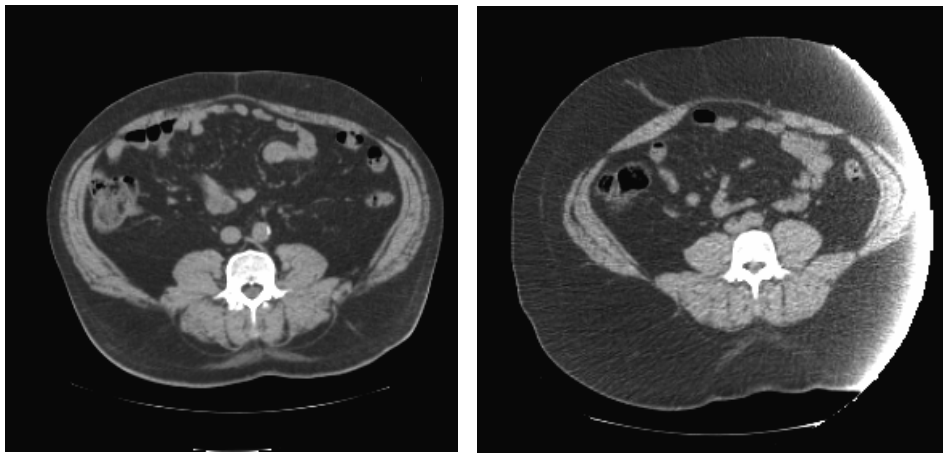


Figure 5. Computed tomography scan of the abdomen of two obese patients. The scans reveal the visceral adipose tissue that surrounds the internal organs within the abdominal cavity, and the subcutaneous adipose tissue beneath the skin. As seen in these pictures, there can be large differences in how fat is distributed between the two depots, with abundant visceral fat on the left CT-image, and a relatively high amount of subcutaneous fat on the right image.

Gut factors in obesity

The gastrointestinal tract plays an important role in the regulation of food intake and energy balance. Many peptides influencing eating behaviour, energy balance and glucose homeostasis are synthesised and released from the gastrointestinal tract [101,102]. Indeed, part of the beneficial effects of bariatric surgery is believed to be mediated by an altered secretion of gut peptides [103]. Vagal afferent nerves that supply the gastrointestinal tract are responsive to the amount and properties of ingested food and carry that information to the brain where it regulates food intake [102]. Gastrointestinal motility participates in the control of food intake by regulating the rates at which nutrients are processed and absorbed in the gut, and via mechanical and hormonal routes [104]. In addition, it has been shown that the relative proportions of microbial communities in the gut may influence fat accumulation, insulin resistance and inflammation in animal models [105].

Obesity and cancer

Obesity is associated with an increased risk of developing various forms of cancer. The World Cancer Research Fund and the American Institute for Cancer Research published in 2007 a large expert panel report that summarized the evidence [15]. The expert panel concluded that there is convincing evidence that obesity is associated with oesophagus cancer, pancreas cancer, colorectal cancer, postmenopausal breast cancer, endometrial cancer, and kidney cancer. The year after, Renehan *et al*. published a meta-analysis, including 282,000 incident cancer cases over more than 133 million person-years of follow up [106], showing that high BMI was associated with thyroid, renal, and colon cancers, oesophageal adenocarcinoma, multiple myeloma, leukaemia, and non-Hodgkin lymphoma in both genders. Furthermore, high BMI was associated with increased incidence of rectal cancer and malignant melanoma in males and with gallbladder, pancreas, endometrial, and postmenopausal breast cancers in women [106].

In other words, the link between excess body weight and cancer is not limited to a few forms of cancer, but instead positive associations seem to represent the rule rather than the exception. Since obesity is increasingly common throughout the world, its impact on cancer occurrence is considerable. In the United States, where obesity prevalence rates are well over 30% among adults [5] it has been estimated that overweight and obesity now could account for 14% of cancer deaths in men and 20% in women, and this would make obesity the largest avoidable cause of cancer besides smoking [107]. Emerging evidence shows that the distribution of body fat plays a role also in this context, with epidemiological data suggesting that abdominal obesity is a risk factor for colorectal cancer, pancreas cancer, endometrial cancer and postmenopausal breast cancer [15].

AIMS

The aim of this thesis was to increase our understanding of the mechanisms behind human obesity and the effects of obesity treatment.

Specific aims for the papers, as discussed in detail in the next sections, were the following:

- To study the adipose tissue regulation of the CIDEA (Paper I) and CIDEC (Paper II) genes with the aim to elucidate their role in human obesity.

- To study if increased intestinal permeability is associated with visceral obesity in humans (Paper III).

- To study the effects of bariatric surgery on cancer incidence in obese subjects (Paper IV).

SUBJECTS AND STUDY DESIGNS

The results in this thesis are mainly based on 4 human studies: 2 cross-sectional, population-based studies, and 2 longitudinal intervention studies with obesity patients undergoing short-term and long-term weight loss with VLCD and bariatric surgery, respectively. All studies were approved by the regional ethical review board at the University of Gothenburg, Sweden. In the multicenter SOS study, seven regional ethics review boards approved the study protocol. Informed consent was obtained from the study participants.

Very Low Calorie Diet (VLCD) study

The VLCD microarray study aimed to identify gene expression changes in adipose tissue of obese subjects undergoing weight loss from caloric restriction. Forty obese (BMI>30 kg/m^2) males and females 25–61 yr of age were recruited among patients treated at the Department for obesity, Sahlgrenska University Hospital, and by advertisement in the local press. Smokers and those with pharmacological diabetes treatment or lipid lowering medication or any serious medical condition were excluded. Two study groups were formed based on WHO metabolic syndrome criteria [7]. The WHO criteria were slightly modified in the sense that albuminuria and insulin resistance were not included as criteria. The metabolic syndrome (MetS+) group had diabetes, impaired glucose tolerance, or impaired fasting glucose according to WHO, and at least one of the following risk factors: (i) elevated arterial (systolic/diastolic) blood pressure, >140/90 mm Hg (either value) or use of blood pressure medication; (ii) raised triglycerides (≥1.7 mmol/L); (iii) low HDL cholesterol (<0.9 mmol/L in men and <1.0 mmol/L in women). All subjects in the MetS+ group had metabolic syndrome also according to International Diabetes Federation [9] and National Cholesterol Education Program criteria [10]. The MetS- group was formed by 19 obese subjects who were obese but metabolically unaffected according to above criteria. The MetS+ group and MetS- group were matched according to age, gender, and BMI.

All subjects were treated with VLCD (450 kcal/d) for 16 weeks, followed by a 2-wk period when regular food was gradually reintroduced. Throughout the study, patients visited the Obesity clinic every second week for weight assessment. If weight loss was less than expected, patients were offered extra visits in order to encourage compliance to the diet regimen. Study assessments were performed at the start of VLCD treatment (wk 0), twice during the VLCD phase (wk 8 and 16), and 2 weeks after the end of VLCD treatment (wk 18). All examinations took place at the Department for obesity, Sahlgrenska University Hospital during 2002-2004. Anthropometrical measurements, blood pressure recording, blood sampling, oral glucose tolerance test, and abdominal subcutaneous adipose tissue biopsy were performed at each of the four time points. Four-day food records preceded the wk 0 and wk 18 visits. Of the 40 included subjects, 35 subjects completed the study. Complete series of adipose tissue biopsies large enough for microarray analysis were available from 24 of these subjects: 9 males and 3 females in each of the 2 groups.

Table 2. Characteristics of the 24 subjects in the VLCD microarray study that were analyzed with gene expression microarrays. Information on energy intake was obtained using a 4-day food registry before the visit. Values are given as mean ±SD.

	VLCD study			
	Wk 0 Baseline	**Wk 8 VLCD**	**Wk 16 End VLCD**	**Wk 18 Refeed**
N (males/females)	18/6	18/6	18/6	18/6
Age (years)	48±10			
Energy intake (kcal)	2307±568	450	450	1407±557 [a]
Body weight (kg)	119±20	101±17	91±16	91±16 [a]
BMI (kg/m^2)	37.6±4.9	31.8±4.1	28.6±4.1	28.9±3.9 [a]
Waist (cm)	123±12	110±12	101±13	101±13 [a]
Systolic Bp (mmHg)	138±17	121±12	117±14	124±16 [a]
Glucose (mmol/l)	6.0±1.6	4.5±0.7	4.5±0.7	5.0±1.0 [a]
OGTT 2-h glucose (mmol/l)	8.2±3.8	7.0±1.9	7.0±2.6	5.9±2.3 [a]
Insulin (mU/l)	16±7.4	7.0±4.1	4.3±2.2	6.3±3.7 [a]
HDL cholesterol (mmol/l)	1.4±0.4	1.2±0.3	1.4±0.4	1.4±0.3
Triglycerides (mmol/l)	1.8±1.0	1.0±0.2	0.9±0.2	1.2±0.5 [a]
CRP	5.3±5.8	4.6±5.4	2.4±1.5	2.4±2.2 [a]

[a] Significant ($P<0.05$) difference from baseline.

For verification of results obtained in the VLCD microarray study, we used adipose tissue biopsies from VLCD treated patients to perform real-time RT-PCR of specific transcripts. In paper I, subcutaneous adipose tissue samples were obtained from 10 obese subjects (5 males and 5 females age 27–57 yr) before (wk 0), during (wk 8), and after (wk18) VLCD treatment. These 10 subjects had an initial BMI of 38.8 ±5.1 kg/m^2, with an average weight loss of 28.4 kg after 18 weeks. In Paper II, biopsies were obtained from 28 obese subjects (8 men and 20 women, age 18-59 years, BMI of 36.3 ±3.7 kg/m^2) treated with VLCD for 12 weeks. These 28 patients lost on average 19.6 kg during the diet, and subcutaneous adipose tissue biopsies were obtained at the start of VLCD treatment (week 0) and 3 times during the VLCD phase (weeks 2, 6, and 12).

Mölndal metabolic study

The Mölndal Metabolic study aimed to elucidate the relation between body composition, energy expenditure, dietary intake, and risk factors for diabetes and cardiovascular disease in two age groups (27-31 years and 57-61 years) of 50 men and 50 women. Participants were recruited from a population-based sample of inhabitants in the city of Mölndal in Western Sweden. To ensure an even distribution of body size, the following stratified randomized protocol was used: A total of 1200 persons in each gender and age group were randomly selected from the SPAR register and asked to complete a questionnaire with health-related questions including self reported body

height and weight. Persons who responded (about 50% in each gender and age group) were divided into five groups according to ranks of height. Each of the five height groups was subsequently divided into five groups according to ranks of body weight. The median person for each of the 25 height- and weight-groups was then contacted and asked to participate in the study. All examinations took place at the Department for obesity, Sahlgrenska University Hospital during 1997-2000 and included anthropometry, blood pressure recording, blood sampling, OGTT, DXA, CT, abdominal subcutaneous adipose tissue biopsy, and measurement of BMR in a chamber of indirect calorimetry.

Intestinal Permeability in Visceral Obesity (IPIVO) study

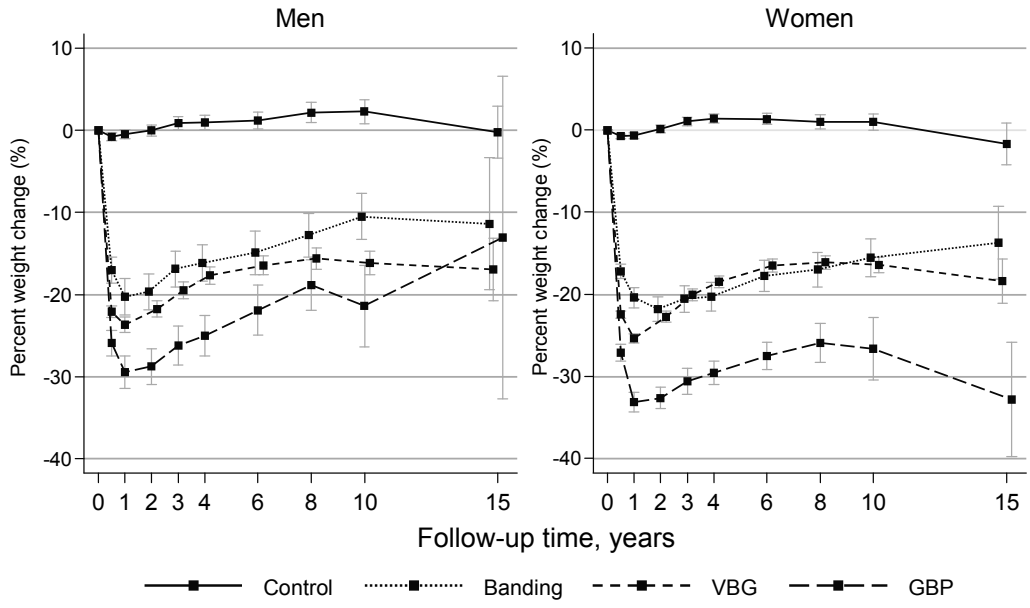
The IPIVO study aimed to investigate the relationship between intestinal barrier function and visceral fat accumulation in human subjects. The study subjects in the IPIVO study were recruited from a population-based cohort of 2595 Swedish women born in 1937-1940 who participated in a screening examination that took place in 2001-2004 at Sahlgrenska University Hospital [108]. A stratified random selection was used to include participants with varying degrees of visceral obesity: Selection of eligible study participants was done based on the anthropometrical measurements at the screening examination, with the aim to include women with either high waist and low BMI, or low waist and high BMI. Exclusion criteria were diabetes type 1 or insulin treatment, weight reducing drug therapy during the last month, previous bariatric surgery, history of post-surgical adhesions, active gastrointestinal disorders, gastroenteritis within one month prior to examination, ongoing infection, pancreatic disease, impaired renal function, heart failure, alcohol or substance abuse, or any serious systemic disease.

The IPIVO study included two study visits. The first visit was performed in 2006-2007 and included 67 women. This was a preliminary study in which we assessed intestinal permeability and used the waist circumference as a crude estimate of visceral obesity. Of the 67 women from the preliminary study, 55 agreed to participate in a second study one year later. This was the main study in which we assessed intestinal permeability and related it to visceral fat content and other fat depots as measured with CT and DXA. At the time of the second study visit, the age of the subjects ranged from 67 to 70 years.

Swedish Obese Subjects (SOS) study

The SOS intervention study is a prospective, matched, surgical interventional trial that was initiated in 1987 with the aim to elucidate the effects of long-term intentional weight reduction on mortality and morbidity rates. The recruitment process involved a large screening program with 6905 obese subjects that were examined (SOS registry study). Among those who underwent matching examination, 2010 eligible subjects wishing surgery were recruited to the surgery group. A computerized matching procedure, taking 18 variables into account, was used to select subjects for the control group (n=2037). Inclusion criteria for the interventional study were age 37 to 60 years and BMI of 34 or more for men and 38 or more for women. Exclusion criteria were minimal and aimed at obtaining an operable surgical group [109]. Identical inclusion

and exclusion criteria were used for both study arms. The surgically treated subjects underwent nonadjustable or adjustable banding (n=376), vertical banded gastroplasty (n=1369), or gastric bypass (n=265) operations. The obese, contemporaneously matched controls received the customary non-surgical obesity treatment for their given center of registration. Patients were recruited over 13.4 years during 1987-2001. The study is ongoing and follow-up examinations are performed regularly for up to 20 years and include anthropometric measurements, blood pressure recording, ECG, blood sampling and questionnaires.



No. Examined								
Control	590	446	287	59	1447	1044	599	131
Banding	116	103	75	20	260	230	162	32
VBG	398	312	223	35	971	774	523	73
GBP	76	55	20	3	189	154	38	7

Figure 6. Percent weight change in the Swedish Obese Subjects study over 15 years by gender in obese control subjects and in obese individuals treated with one of three surgical methods. VBG, vertical banded gastroplasty. GBP, gastric bypass.

METHODS

Anthropometry

Anthropometry is used to measure the size and proportions of the human body to assess total and regional body composition. For estimation of total body fatness, weight (kg) and height (m) are the basic measurements from which BMI (kg/m^2) can be calculated. An estimate of abdominal fat can be made by measuring the waist circumference. Abdominal obesity can also be expressed as the ratio of waist circumference to hip circumference (WHR). Yet another measurement of abdominal obesity is the sagittal diameter, which is closely related to the volume of visceral fat as measured with multi-scan [110] or single-slice computed tomography [111]. The sagittal diameter of the trunk is measured with subjects in the supine position, as the vertical distance between a firm examination table and a carpenter's level kept horizontally across the abdomen at the height of the iliac crest.

Computed tomography

To produce a computed tomography (CT) image, an x-ray source and a detector are rotated in an arc around a cross section of the subject. Data about the fraction of photons reaching the detector (i.e. attenuation) are collected throughout rotation. The data set of attenuation profiles is then computed to reconstruct a grayscale image of the tomographic section (Fig 5). Since the attenuation of fat tissue differs from that of other tissues in the abdomen, it is possible to define and calculate the areas of visceral and subcutaneous adipose tissue from the CT image. Increased content of fat in the liver reduces its density and thus lowers the attenuation of CT x-rays. CT can therefore be used as a non-invasive tool for investigating liver fat content [112,113]. CT imaging in the IPIVO study (Paper III) was performed at the Department for obesity, Sahlgrenska University Hospital, using a General Electric HiSpeed Advantage CT system. Subcutaneous and visceral fat areas of the abdomen were determined using a single-slice CT scan at the level of the lumbar 4 vertebrae. To assess hepatic fat content, the attenuation of the liver was determined within three circular regions of interest, avoiding blood vessels, artefacts and areas of tissue inhomogeneity.

Dual energy X-ray absorptiometry

Dual x-ray absorptiometry (DXA) is mainly used for bone density measurements, but can also be used to assess the amount of body fat. DXA measures the attenuation of two different x-ray energies. In projections where no bone is present, the ratio of the measured attenuation of the high and low energy level is proportional to the proportions of fat and lean tissue. Thus, the DXA examination measures bone mineral content, body fat and lean tissue. The fat-free mass is defined as the sum of lean tissue and bone mineral content. DXA measurements in the Mölndal Metabolic study (Paper I) and the IPIVO study (Paper III) were performed at the Department for obesity, Sahlgrenska University Hospital, using a LUNAR DPX-L scanner.

Indirect calorimetry

Basal metabolic rate (BMR) is the amount of energy expended while at rest in a neutrally temperate environment, in the post-absorptive (fasting) state. BMR is measured under very restrictive circumstances when a person is awake, but at complete rest. In the Mölndal metabolic study, BMR was assessed in standardized conditions after an overnight stay in a chamber of indirect calorimetry at the Department for Obesity, Sahlgrenska University Hospital. At the time of the examination the temperature in the chamber was 25 ºC and the humidity was 40%. The subject was awaked at 7.30 am. During the coming 60 minutes the subject was instructed to lie in bed in complete silence, awakened and relaxed, with arms and legs stretched along the body. During the examination period the subject was checked upon every 10 minutes so he or she had not fallen asleep. Before analysing the collected data in each subject, the first and last 15 minutes were withdrawn. The BMR from the remaining 30 minutes assessment was extrapolated to 24-h values.

Blood samples, oral glucose tolerance test, and adipose tissue biopsies

Venous blood samples were obtained in the morning after a 10- to 12-hour fast and analyzed at the Central Laboratory, Sahlgrenska University Hospital or at the Wallenberg laboratory, Sahlgrenska University Hospital (both accredited according to European Norm 45001). The oral glucose tolerance test (OGTT) was performed in the morning (before 11 a.m.). Participants had been asked to fast overnight and to avoid heavy physical activity during the previous day and to avoid smoking the morning before the test. The participants drank a solution containing water and 75 g sugar. Fasting and 2-hour post load glucose levels were measured in capillary blood (VLCD study) or in venous blood samples (Mölndal Metabolic study). Abdominal subcutaneous biopsies were taken from the paraumbilical area of the abdomen in local anaesthesia using a syringe with manually applied vacuum. The biopsies were immediately frozen in liquid nitrogen and stored at -80 ºC until analysis.

DNA microarray

Microarray technology makes it possible to measure the expression level of thousands of genes simultaneously. The microarray consists of a coated glass surface on which synthetic probes for different gene transcripts have been synthesized. Each probe is a 25-mer oligonucleotide. A probe pair consists of two almost identical oligonucleotides: the perfect match probe that is complementary to the gene of interest, and the mismatch probe that differs at the central position. The mismatched probe is used as a control for non-specific binding. For the assessment of a defined transcript, there are 12-16 different probe pairs randomly distributed over the microarray. The process from biopsy to gene expression data includes extraction of total RNA from cells or tissue samples, cDNA synthesis, transcription into biotin-labeled cRNA, fragmentation of the cRNA, hybridization to microarrays, staining with fluorescent dye linked to streptavidin, scanning of arrays with a confocal laser scanner, calculation of relative gene expression levels from probe set signal intensities, and normalization of data using the mean signal intensity of all probesets.

Real-time reverse transcription polymerase chain reaction

Real-time reverse transcription polymerase chain reaction (real-time RT-PCR) is a method that quantifies specifically amplified gene transcripts [114]. The first step in real-time RT-PCR is reverse transcription of RNA into cDNA. The cDNA is used in a PCR reaction with fluorescent reporter molecules that enables the monitoring of amplification products during each cycle of the PCR. The higher the starting copy number of the nucleic acid target, the sooner a significant increase in fluorescence is observed. The threshold cycle (Ct) value represents the point in time during cycling when the instrument can first reliably detect fluorescence derived from the amplification reaction. Since there is a linear relationship between the Ct value and the log of the initial target copy number, the level of the target mRNA is quantified using a standard curve based on serial dilutions of standard cDNA. To adjust for differences in total cDNA amount among samples, the mRNA level of a gene of interest is divided by the mRNA level of a reference gene (or "house-keeping gene"). In our experiments we used LDL receptor-related protein 10 (LRP10) mRNA expression to normalize the expression levels between adipose tissue samples, and peptidyl-prolyl isomerise A (PPIA) mRNA expression was used as reference in the tissue distribution panels. These genes have consistently shown small inter-individual and inter-tissue variation, respectively [115].

Small interfering RNA–mediated gene silencing in cultured cells

Small interfering RNA (siRNA)-mediated gene silencing provides a method to turn off the expression of a specific gene in cells by introducing interfering RNA that targets the gene transcript, and initiates its degradation [116]. In paper II we studied the effect on lipid metabolism after knockdown of the CIDEC gene in 3T3-L1 mouse adipocytes. Either siRNA against CIDEC, or scrambled control siRNA, was introduced into differentiated adipocytes by electroporation. In each experiment, 2 separate anti-CIDEC siRNA oligos were used to control for nonspecific siRNA effects. Knockdown was confirmed 24 h post electroporation using real-time RT-PCR, showing >90% decrease of CIDEC mRNA compared with cells electroporated with control siRNA. Experiments were performed with and without the β-adrenergic agonist isoproterenol to study stimulated and basal lipolysis, respectively. Lipolysis was determined by assaying release of non-esterified fatty acids (NEFA) into the medium, and oxidation of fatty acids was assessed by analyzing oxidation products in media after the cells had been labeled with radioactive palmitic acid.

Intestinal permeability test

The degree of intestinal permeability can be estimated by measuring the urinary excretion of the orally ingested carbohydrate markers sucralose and mannitol. After oral intake, a proportion of these sugars pass the intestinal barrier. The carbohydrates are not metabolized in the body, and are excreted in the urine in proportion to the amount that has been absorbed. Sucralose is a disaccharide with relatively large molecule size that is normally excluded by the epithelial cells of the intestinal mucosa, and instead the permeation of this molecule occurs primarily through the paracellular route [117]. An important property of sucralose is that it is resistant to bacterial

fermentation and can be found in high concentrations in the colon following oral administration, which makes it suitable for assessing large intestinal permeability [118-120]. Mannitol is a monosaccharide that is believed to permeate through the epithelial cells (transcellular route), and was included in the permeability test to serve as a reference used to adjust for factors that we want to leave out of the equation such as differences in intestinal transit, blood flow, and completeness of urine collection [117]. In the present study, the subjects drank a solution containing 1.0 g sucralose and 5.0 g mannitol in 200 ml tap water in the morning and urine was collected for up to 12 hours. Before that, subjects had been given detailed instructions to avoid products that contain these specific carbohydrates 24 hours prior to the visit and during the urine collection period. A control urine sample was collected immediately before administering the carbohydrates. For measurement of each carbohydrate, a selective and sensitive method based on gas chromatography - mass spectrometry was used. The recovery of carbohydrates in the urine was calculated from the urine sample weight and urine concentration of the carbohydrate, and expressed as percent of the orally ingested amount.

Register information

The Swedish National Cancer Register was founded in 1958 and aims to register all cancers in Sweden. According to Regulations by the National Board of Health and Welfare, all physicians in hospitals and other establishments for medical treatment under public or private administration in Sweden must report all cases of cancer to the Cancer Register. Furthermore, pathologists and cytologists separately report every cancer diagnosis on surgically removed tissues, biopsies, cytological specimens, bone marrow aspirates and autopsies. Thus, the majority of cases are notified twice, in separate reports. It is estimated that the register is 98% complete [121].

RESULTS AND DISCUSSION

CIDEA and CIDEC in human adipose tissue (Paper I and II)

Cell death-inducing DFF45-like effectors

The cell death-inducing DNA fragmentation factor alpha-like effector (CIDE) family consists of three highly homologous proteins: CIDEA, CIDEB and CIDEC [122]. The CIDE proteins were originally identified by their sequence homology to the N-terminal region of the DNA fragmentation factor A (DFFA/DFF45) [122], which triggers DNA fragmentation during apoptosis. All three CIDE proteins have been found to activate apoptosis in mammalian cells [122,123].

Several studies have revealed important roles of the CIDEs in metabolism. Mice deficient of CIDEA are lean and resistant to diet-induced obesity and diabetes [124], and CIDEA appears to be involved in controlling thermogenesis and lipolysis [124,125]. In addition, a polymorphism in the CIDEA gene has been associated with obesity in one population [126]. CIDEB has been suggested to play an important role in the development of obesity, liver steatosis, and insulin sensitivity by controlling lipogenesis and fatty acid oxidation in the liver [127]. CIDEC has been shown to enhance adipocyte triglyceride storage [128]. In paper I and II we studied the regulation of CIDEA and CIDEC human adipose tissue in order to find support for a role of these genes in human energy balance and obesity [129,130].

Tissue distribution of CIDEA and CIDEC

We investigated the tissue distribution of CIDEA and CIDEC and found that both genes were predominantly expressed in adipose tissue and adipocytes as compared to other human tissues (Fig. 7). More recent studies have confirmed that adipose tissue is the major expression site for CIDEA and that methylation seems to play a crucial role in establishing and maintaining tissue- and cell-specific transcription of the CIDEA gene [131]. Fat-specific protein 27 (FSP27) is the mouse homolog of human CIDEC, and as the name implies there is an adipocyte-specific expression of FSP27 in mice [132]. The adipose tissue specific expression of CIDEA and CIDEC strongly suggests that they play their main role in adipose tissue. We also observed that CIDEC expression was lower in visceral than in subcutaneous adipose tissue.

Regulation of CIDEA and CIDEC during VLCD treatment

It has been reported that CIDEA adipose mRNA expression is increased by weight reduction from an energy-restricted diet [133], and from bariatric surgery [125]. However, CIDEA was not regulated by a moderately calorie-restricted diet in another study [134]. Previous reports have not discriminated between the effects of caloric restriction and the effects of weight loss. In the VLCD study, there was a strong induction of CIDEA gene transcription during diet, and when regular food was reintroduced, CIDEA gene transcription dropped (Fig. 8). In all 24 subjects, the average CIDEA expression was higher during the VLCD phase (mean of wk 8 and 16),

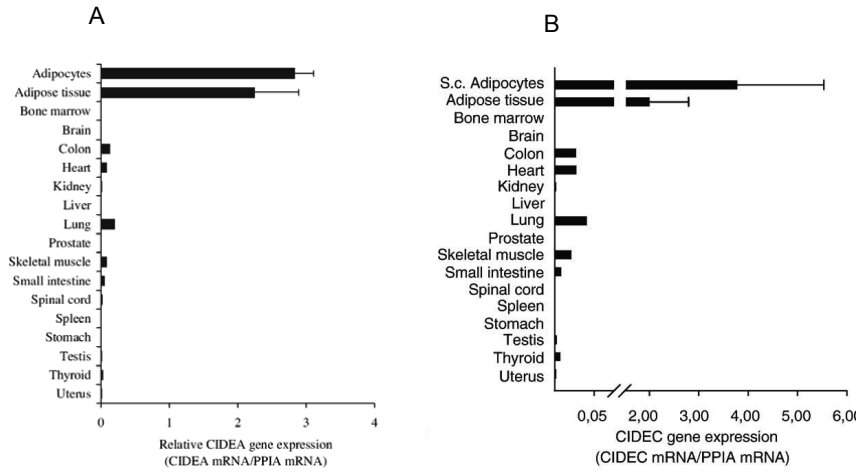


Figure 7. Validation of CIDEA (panel A) and CIDEC (panel B) tissue distribution using real-time RT-PCR of isolated adipocytes (n = 3), adipose tissue (n = 3) (mean ± SEM), and a human tissue panel.
7A: Reprinted from Paper I with permission. Copyright 2007, The Endocrine Society. 7B: Reprinted from Paper II with permission from Elsevier.

compared with time points of regular food intake (mean of wk 0 and 18). The observation that the induction of CIDEA expression during caloric restriction was reversed when calories were reintroduced (while body weight was stable) suggests that it is the caloric restriction *per se* that has the major impact on CIDEA expression, rather than the actual weight change.

In contrast to CIDEA, CIDEC was downregulated during VLCD. In the VLCD microarray study, CIDEC was downregulated 25% after the first 8 weeks of VLCD treatment (P=0.0001), and 10% below baseline after 16 weeks of VLCD treatment (P=0.028). At wk 18, 2 weeks after reintroduction of normal food, CIDEC levels no longer showed a significant difference from baseline (P=0.22). Real-time RT-PCR verification experiments in adipose tissue of obese subjects undergoing VLCD treatment confirmed a downregulation of CIDEC during VLCD and showed that CIDEC expression decreased within the first 2 weeks of VLCD but did not decrease any further during 12-16 weeks of treatment despite continuous weight loss throughout the treatment period (Fig. 9A). The finding indicates that caloric restriction is an important inhibitor of CIDEC transcription, which fits well with a suggested role of CIDEC as a promoter of triglyceride accumulation [128]. PPARgamma, a key transcription factor in the regulation of adipocyte differentiation and function [135], has been suggested to regulate CIDEC transcription [136]. We observed a positive correlation between changes in PPARgamma mRNA and CIDEC mRNA during diet (Fig. 9B), a finding that supports the laboratory data of possible role of PPARgamma in regulating CIDEC transcription. A more recent study showed that PPARgamma binds to the CIDEC promoter and induces CIDEC transcription [137].

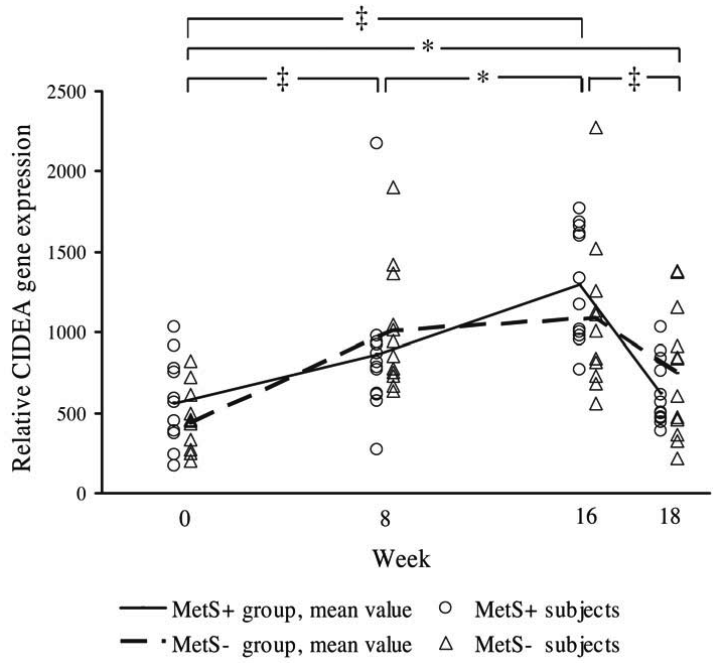


Figure 8. CIDEA gene expression in adipose tissue from 12 obese subjects with metabolic syndrome (MetS+) and 12 obese subjects without metabolic syndrome (MetS-), treated with VLCD for 16 wk, followed by a 2-wk gradual reintroduction of regular food. * P<0.05 and ‡ P<0.01 with Wilcoxon signed-ranks test using all subjects. CIDEA expression increased 1.9-fold increase after 8 weeks of diet (P<0.0001) and 2.4-fold after 16 weeks of diet (P<0.0001), as compared to baseline. Between wk 16 and 18 when regular food was gradually reintroduced, the average body weight was unchanged, whereas CIDEA expression decreased (P<0.0001) to a level that was still higher than baseline (1.4-fold increase from wk 0; P=0.028).

CIDEA and CIDEC in lipolysis

CIDEA has been shown to negatively regulate lipolysis in human adipocytes [125]. Our study showed that VLCD induced a pronounced increase in adipose tissue CIDEA transcription. This upregulation during caloric restriction did not fit well with CIDEA's putative role as an inhibitor of lipolysis, since adipocytes respond to caloric restriction by mobilizing and releasing fatty acids. Recent studies have shown that CIDEA in addition to its role in lipolysis may function as a regulatory protein which facilitates the preferential oxidation of fatty acids instead of glucose in white adipocytes in order to optimize glucose recycling [138]. These findings may help to explain the increased CIDEA under conditions of high fatty acid mobilization such as caloric restriction.

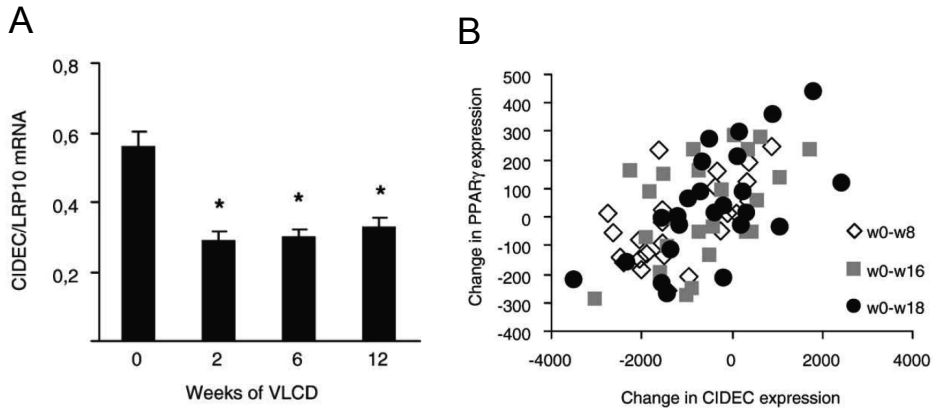


Figure 9. A) Real-time PCR verification of VLCD microarray data. CIDEC expression in subcutaneous adipose tissue during a 12-week VLCD was analyzed. Mean value ±SEM. * P ≤ 0.0001 (paired t tests vs. week 0). No significant differences between weeks 2, 6, and 12 (paired t tests). B) Correlation between changes in PPARgamma and CIDEC mRNA levels during the microarray VLCD study (week 0-8 and week 0-16) and 2 weeks after reintroduction of normal food (week 0-18). Differences are shown for each subject. P<0.0001 for correlation (Wald test).
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In Paper II we showed that downregulation of CIDEC/FSP27 in a 3T3-L1 mouse adipocyte cell line resulted in increased basal NEFA release, decreased responsiveness to adrenergic stimulation, and increase in oxidation of endogenous fatty acids (Fig. 10). Recent studies of mouse FSP27 have indicated that a central role of FSP27 is to maintain the large unilocular lipid droplet structure within the adipocyte, and the mechanism by which FSP27 depletion stimulates lipolysis may to be fragmentation of lipid droplets which may stimulate lipolysis through enhanced lipase action on the larger surface area per unit volume of triglyceride [128,132]. While CIDEC/FSP27 depletion in 3T3-L1 adipocytes resulted in increased basal lipolysis in our experiments, isoproterenol-stimulated lipolysis was blunted. Studies of isolated white adipocytes from FSP27-deficient mice have also suggested an impaired isoproterenol-stimulated lipolysis in the absence of CIDEC/FSP27, but this could not be verified in vivo [132]. Hence, the role of CIDEC in stimulated lipolysis remains unclear. Given the suggested role of CIDEC as inhibitor of lipolysis, it is interesting to note that CIDEC is expressed to a lesser extent in visceral than in subcutaneous adipose tissue. It is possible that this depot difference may contribute to the increased lipolysis in visceral adipose tissue, but this remains to be elucidated.

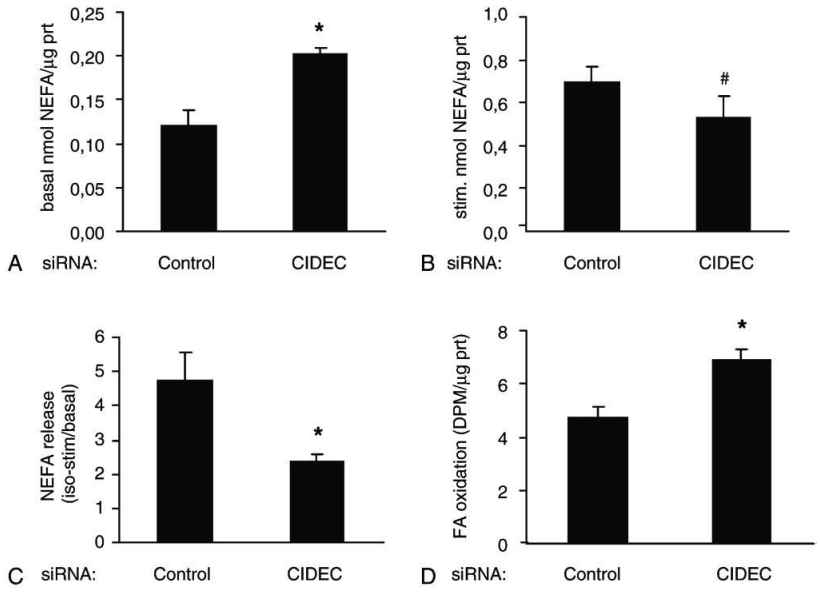

Figure 10. CIDEC silencing in the 3T3-L1 adipocyte cell line. Differentiated 3T3-L1 cells were electroporated with antimouse CIDEC or scrambled control siRNA. A) Basal release of NEFA. B) The NEFA release after stimulation with β-adrenergic agonist isoproterenol. C) Responsiveness to isoproterenol stimulation, expressed as NEFA release after isoproterenol stimulation/basal NEFA release. D) Oxidation products from labeled endogenous fatty acids. n = 4. Mean ±SD. *P ≤ 0.004 and #P = 0.042 (2-sample T test). Iso indicates isoproterenol.
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CIDEA and CIDEC in energy expenditure

Some observations suggest that CIDEA may play a role in energy expenditure. The expression of the CIDEA gene is regulated by PPARgamma coactivator 1α (PGC-1α) and receptor-interacting protein 140 (RIP140) [139,140], two important regulators of mitochondrial biogenesis and cellular programs that promote energy expenditure [77,141,142]. CIDEA-null mice are resistant to diet-induced obesity and diabetes, and have elevated metabolic rate [124]. In brown adipocytes, uncoupling protein-1 (UCP1) is responsible for brown adipocyte heat production by diverting energy from ATP synthesis to thermogenesis in the mitochondria. *In vitro* observations using immunoprecipitation techniques suggested that CIDEA interacts with UCP1 [124], but these findings were challenged by a later study that could not find CIDEA protein in mitochondria [143].

We studied CIDEA in adipose tissue of subjects in the cross-sectional Mölndal Metabolic study, and found that the CIDEA gene expression was inversely associated with BMR independently of body composition, age, and gender (P=0.014). We also

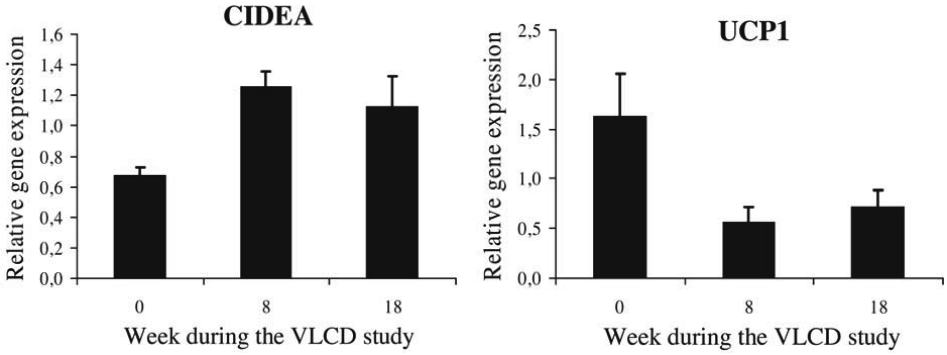

Figure 11. Adipose tissue gene expression (mean ±SEM of normalized ratios with LRP10) of UCP1 and CIDEA in 10 subjects treated with VLCD for 16 wk, followed by a gradual reintroduction of regular food, with adipose tissue biopsies obtained at wk 0, 8, and 18. Both CIDEA and UCP1 were regulated by diet (P= 0.020 and P=0.0026, respectively, using an exact binomial test to assess expressional regulation from wk 0), and there was a negative association between CIDEA and UCP1 gene expression (P= 0.0014, combining all time points with a generalized Wald test).

showed that the transcriptional regulation of CIDEA and UCP1 went in opposite directions during VLCD (Fig. 11) and that there was an inverse association between the two (P=0.0014). These observational findings place CIDEA in the context of energy expenditure in humans, and thus give support to the animal data. A downregulation of UCP1 in adipose tissue during diet has not been described before in humans. Although UCP1 expression is very low in white adipose tissue, a downregulation during diet may be viewed as a marker of the compensatory responses that act to decrease energy expenditure in order to resist weight loss.

FSP27-deficient mice are protected from diet-induced obesity and insulin resistance and display increased metabolic rate due to increased energy expenditure in the white adipose tissue [132,144]. FSP27 deficiency results in the acquisition of a brown adipocyte-like morphology in white adipocytes, with the appearance of smaller lipid droplets, increased mitochondrial size and activity, and upregulation of UCP1 [132,144]. We did not investigate the relationship between CIDEC and BMR in humans. However, the suggested role of CIDEC/FSP27 as inhibitor of mitochondrial activity [132,144] fits well with our *in vitro* findings of an increased oxidation of endogenous fatty acids after CIDEC/FSP27 downregulation (Fig. 10D).

CIDEA and CIDEC expression in relation to body composition and metabolism

We found that CIDEA expression in adipose tissue was negatively associated with all measurements of body fatness, including DXA body fat. There was no dysregulation of CIDEA or CIDEC expression in subjects with the metabolic syndrome when compared with BMI -matched controls, but we did find a negative correlation between

CIDEA expression and insulin levels that was independent of adiposity. As opposed to CIDEA, the CIDEC expression did not correlate significantly with BMI.

Summary

Our observations that both CIDEA and CIDEC transcription are highly responsive to changes in energy availability suggest that they are involved in the adipocyte adaptation to these changes. Taken together, our data obtained mainly from human subjects support a role for CIDEA and CIDEC in human energy balance and obesity via the regulation of adipose tissue physiology. Our findings are consistent with data obtained in rodents.

Intestinal permeability in visceral obesity (Paper III)

The accumulation of visceral fat is influenced by age, gender, and hormones such as sex steroids, cortisol and growth hormone [145]. In paper III we studied intestinal permeability as a potential factor in visceral fat accumulation.

There are indications that the gut microbial flora, and the intestinal barrier that forms a protection against these microbes, could be factors that influence fat accumulation. The gastrointestinal tract has a single contiguous layer of epithelial cells that separates the inside of the body from the external environment. Much of the epithelial barrier is formed by the rigid lipid bilayer of the enterocyte brush border, and the tight junctions between cells. The epithelial cells form a barrier towards the complex bacterial ecosystem that resides in the gastrointestinal tract. Bacteria and bacterial constituents that do enter this barrier are primarily handled by the lymphatic tissues that surround the intestines, and by the liver. An interesting example of impaired intestinal barrier function is Crohn disease. Increased intestinal permeability is believed to initiate and maintain the mucosal inflammation in Crohn disease by allowing abnormal presentation of luminal constituents to the surrounding immune system [117,146]. A striking finding in patients with Crohn disease is the increased accumulation of visceral fat [147].

Compared with subcutaneous adipose tissue, visceral adipose tissue contains more immune cells and expresses higher levels of inflammatory mediators, suggesting a more active role in immune defence [97-100]. This immunological activation may be linked to the anatomical location of visceral adipose tissue close to the gastrointestinal tract, as there are various environmental agents in the lumen of the intestine that can initiate or maintain immune responses if they penetrate the intestinal wall [148]. As a result of an impaired intestinal barrier, translocation of non-self antigens across the gastrointestinal wall may challenge the lymph nodes and adipose tissue that surround the viscera. Adipocytes express receptors that mediate cellular responses to bacterial endotoxins [149] as well as receptors for immunoglobulins that, when activated, potently stimulate adipocyte lipid accumulation [97]. Chronic low-grade immune stimulation has been shown to induce local hypertrophy of adipose tissue in both mice and rats [150,151], and studies in mice have suggested a chain of events where increased intestinal permeability causes metabolic disorders [152].

The aim of the IPIVO study was to investigate the relationship between gut permeability and visceral obesity in humans. In a preliminary study of 67 healthy women with varying degrees of abdominal obesity, we assessed intestinal permeability using sucralose and mannitol followed by a 9 h urine collection period, and used waist circumference as a crude estimate of visceral obesity. The preliminary data showed a positive association between the sucralose/mannitol ratio and waist circumference (r=0.27, P=0.024). Analysis of the different urine collection periods revealed that significant associations were only found for the late (6-9 h) urine sample (r=0.28, P=0.022), a time frame consistent with barrier function at the level of the colon. The correlation was not explained by differences in factors known to influence gut barrier such as alcohol consumption, smoking, psychological stress, irritable bowel symptoms, or use of anti-inflammatory drugs.

Encouraged by these preliminary findings, we performed a second study visit in which we analyzed the sucralose/mannitol ratio for an early (0-6 h) and a late (6-12 h) urine sampling period in relation to CT and DXA measurements of visceral, liver, subcutaneous abdominal, and total fat depots. Consistent with what we observed in the preliminary study, the sucralose/mannitol ratio from the late (6-12 h) urine sample was significantly associated with visceral fat area (r=0.48, P=0.0003). No associations were found between the sucralose/mannitol ratio and fat depots when the early (0-6 h) urine sample was used, suggesting that upper gastrointestinal tract is not involved. An alteration of colonic permeability is consistent with our hypothesis, given that the majority of bacteria are present in the colon, and thus an increased permeability at that level is likely to augment the entry of microbial derived factors. As predicted, the sucralose/mannitol ratio was not correlated to subcutaneous or total body fat.

Table 3. Pearson correlations between markers of intestinal permeability and CT/DXA measurements of various fat depots. The analysis was divided into an early (0-6 h) and a late (6-12 h) urine collection period in order to separate between intestinal permeability of the small and large intestine, respectively. Ratios of large and small carbohydrates (sucralose/mannitol) were used in order to reduce pre- and post-absorptive variance, and to allow for comparisons between individuals.

	Sucralose/mannitol in the 0-6 hour urine		Sucralose/mannitol in the 6-12 hour urine	
	r	*P-value*	r	*P-value*
Visceral fat area[1]	0.16	*0.23*	0.48	*0.0003*[2]
Subcutaneous fat area[1]	-0.05	*0.74*	0.17	*0.23*
Liver fat	0.21	*0.13*	0.39	*0.004*
Total body fat[1]	-0.05	*0.73*	0.24	*0.081*

[1]The influence of body size on these fat depot measurements was removed by calculating ratios with total body mass before statistical analysis. [2]P=0.0014 without adjustment for total body mass.

The IPIVO study was primarily designed to study intestinal permeability in relation to visceral obesity, but we also found that the sucralose/mannitol ratio of the late urine sample was associated with liver fat content as assessed with CT (r=0.39, P=0.004). An association between intestinal permeability and liver fat content has not been reported before in humans. Gut-derived antigens that enter the portal vein as a result of increased permeability are primarily handled by the liver, and studies in rodents have shown that endotoxin can induce liver fattening [151]. In non-alcoholic steatohepatitis, liver fattening and inflammatory liver damage are the major culprits, and an impaired barrier seems to contribute to the inflammatory liver damage [153]. The ability of a healthy liver to handle portal endotoxin before it enters the systemic circulation may explain why we failed to detect any alterations in endotoxin levels in peripheral blood samples from this study population.

Summary

Several animal studies have demonstrated the importance of the gut microbiota and endotoxemia in driving fat accumulation [105,151,152]. We found a positive association between intestinal permeability, assessed as the urine recovery of orally administered carbohydrates, and visceral obesity and liver fattening. Our report represents the first human study in a new and exciting field of research about the interplay between the intestinal barrier function and visceral obesity, and opens up new possibilities for intervention in an area where specific treatment has been lacking. We suggest that therapeutic agents aiming to improve intestinal barrier function should be explored for prevention or treatment of visceral and hepatic fat accumulation.

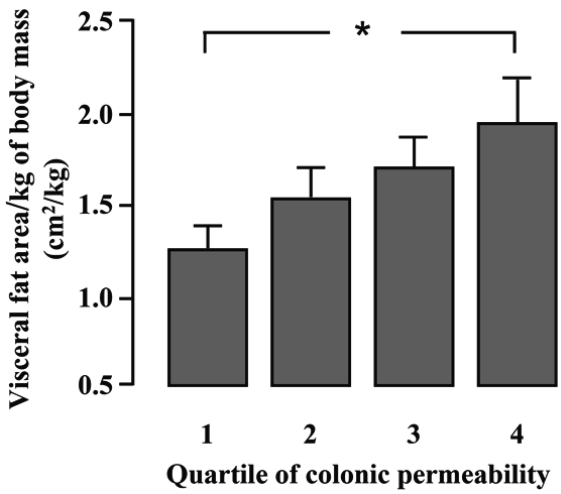

Figure 12 Relative size of the visceral fat area (mean ±SEM) for each quartile of intestinal permeability (lowest to highest) using the sucralose/mannitol in the 6-12 h urine sample as the measure of colonic permeability. Subjects within the highest quartile of colonic permeability had on average a 56% larger visceral fat area as compared to subjects in the lowest quartile. *p=0.023 between 1st and 4th quartile (t test) and P=0.010 for trend across groups (ANOVA).

Effects of obesity treatment on cancer incidence (Paper IV)

Since the association between obesity and cancer is well established, a key question is whether obesity treatment can reduce the risk of developing cancer. Observational studies that address this question are obscured by the difficulty to separate intentional from unintentional weight loss, and by the difficulty to achieve long-term weight loss. To date, the best evidence that weight loss can prevent cancer comes from two very recent, retrospective studies of obese subjects treated with bariatric surgery [154,155], both reporting significant reductions in overall cancer incidence among surgically treated subjects, as compared to controls.

The SOS study is the first prospective, controlled intervention study that investigates the impact of long-term weight loss on disease and death rates [59,109]. In the SOS study, bariatric surgery was used to achieve weight loss, since such surgery was and still is the only available technique with established long-term effects on weight loss. Mortality data in the SOS study was published in 2007, showing that bariatric surgery was associated with long-term weight loss and significantly reduced overall mortality, as compared with conventional treatment [69]. Cancer was the single most common cause of death, but the number of events was not sufficient to evaluate the risk reduction for death from cancer or other specific causes. To study the effects of bariatric surgery on cancer, we performed a follow up study in which we investigated incident cancer in the SOS study. Information on cancer incidence was obtained from the National Cancer Register in Sweden which aims to register all cancers in Sweden.

In women, the number of first-time cancers after inclusion was lower in the surgery group (n=79) compared to the control group (n=130) (HR=0.58, 95% confidence interval 0.44 to 0.77, p=0.001), whereas there was no effect of surgery in men (38 vs. 39 cases, HR=0.97, p=0.91). Kaplan-Meier curves of the overall cancer rates in the two groups are shown in Fig. 13. Important to note is that there are only about half as many men as there are women in the SOS study and hence a lower statistical power.

To get a more detailed picture of the data, we also separated the different types of cancer and calculated hazard ratios for each (Fig. 14). These analyses had a limited statistical power because of few cases in each site. In women, most of the common cancer types such as colorectal, breast and endometrial cancer occurred to a lesser extent in the surgery group than in the control group. However, none of the differences were significant, except for malignant melanoma and haematopoietic cancers (Fig. 14).

The main limitation of the SOS study was that the surgical intervention could not be randomly assigned. Instead a computerized matching procedure was adopted to make the surgery and control group as similar as possible based on 18 matching variables. To make certain that the reduction in cancer incidence in the surgery group as compared with the control group was not explained by uneven distributions of potential covariates between the two groups, multivariate analyses were performed. The hazard ratio for bariatric surgery was not affected by these adjustments (see Paper IV), which shows that the difference in cancer incidence between treatment groups was not explained by any of these variables.

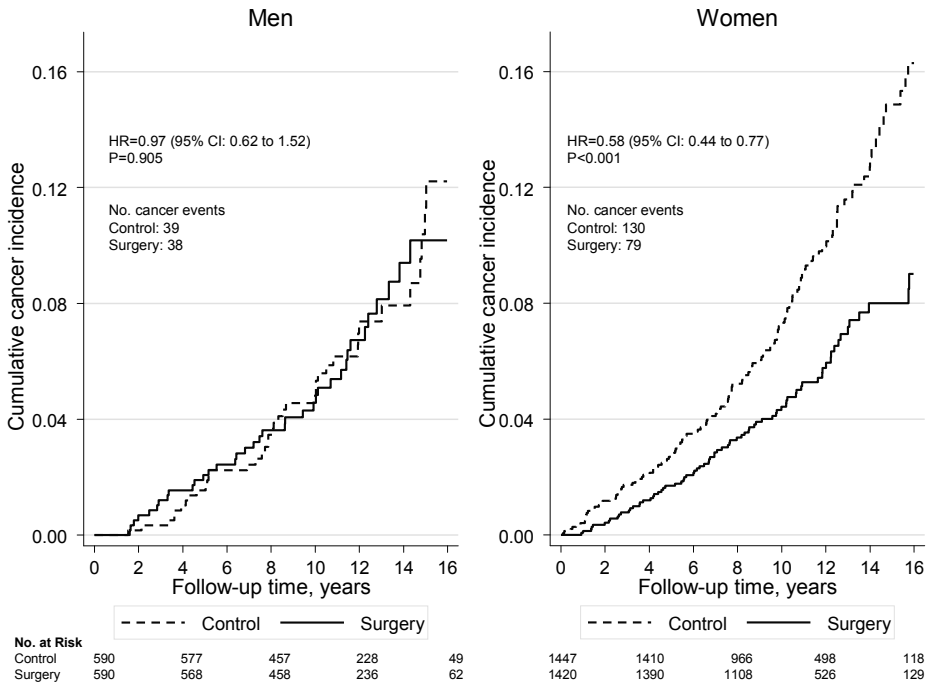

Figure 13. The unadjusted cumulative fatal plus non-fatal cancer incidence by gender in surgically treated obese subjects and in obese control individuals. At bottom, number of subjects at risk over 16 years.

Other than age and smoking, there were few predictors for cancer among the matching variables, but in women the sagittal diameter, which serves as an estimate visceral obesity, was independently associated with cancer risk (P<0.001). The treatment effect was not affected by menopause, diabetes, BMI, age or smoking (Fig. 15).

In addition to the main analysis of surgery treatment and cancer incidence, we also analyzed how the magnitude of weight loss was related to cancer incidence. The univariate analysis showed that female patients with the largest weight losses during the first year after inclusion had a significantly lower incidence of cancer as compared to the female patients with the least weight loss. However, weight loss did not come out significant in a multivariate model that included treatment group. This observation could be due to the fact that treatment and weight loss are so closely associated in the SOS study; therefore just one of the correlated variables becomes significant while we are trying to analyse both of them together. While taking this limitation into account, one might also speculate about the possibility that it could be factors other than the actual number of kilograms lost that explain the lower cancer incidence in the surgery group.

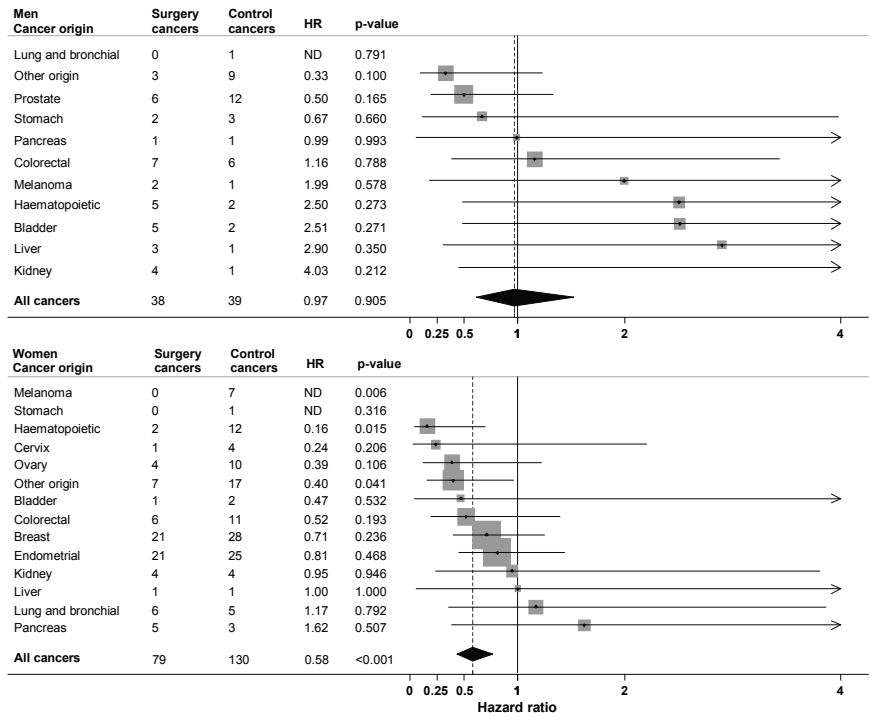

Figure 14. Number of first time cancers by gender and origin. Males in upper and females in lower panel. Dots with bars represent the unadjusted HR and its 95% confidence interval for each cancer type. Areas of squares are proportional to the number of cancer cases within each group of origin. The rhombi at the bottom of the two panels illustrate the total treatment effect (HR and 95% CI) taking all males and all females, respectively, into account.

The reduced statistical power in men compared to women may help to explain why we did not find any treatment effect in men. On the other hand, while the treatment effect is very strong in women there was not even a tendency in men. In addition, there was a borderline significant P-value in gender-treatment interaction analysis. Observational studies of intentional weight loss from Williamson *et al.* suggested that there might be a gender difference in this context, since self-reported intentional weight loss was found to be associated with reduced cancer mortality in women but not in men [156,157]. A recent retrospective study by Adams *et al.* is, beside our study, the only bariatric surgery study to date that has analyzed incident cancer for men and women separately. Similar to our results, they found a highly significant HR of 0.73 for overall cancer in surgically treated women as compared to controls (P=0.0004), but no difference in men (HR 1.02, P=0.91) [155].

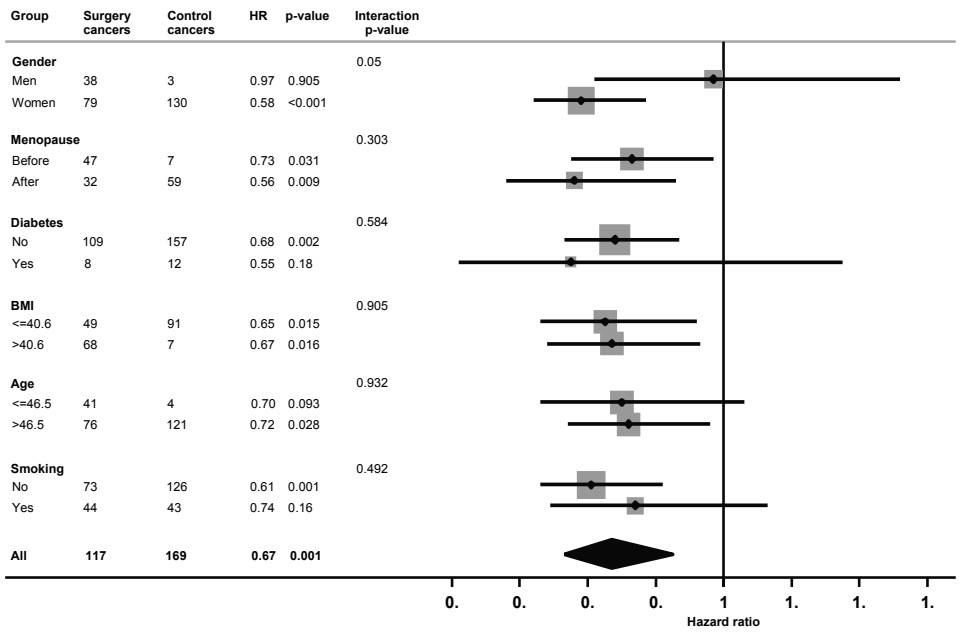

Figure 15. Unadjusted subgroup-treatment interactions with respect to overall cancer incidence. Dots with bars represent the HR and its 95% confidence interval for subgroups (based on matching data). Areas of squares are proportional to the number of cancer cases within subgroups. The rhombus at the bottom of the figure illustrates the total treatment effect (HR and 95% CI) taking all subjects into account.

Summary

The conclusion of Paper IV is that in severely obese women, obesity surgery is associated with a reduced cancer risk of developing cancer, and the data suggest that 4 out of 10 cancers could be avoided in severely obese women. When it comes to men, it is still difficult to draw conclusions.

CONCLUSIONS AND FUTURE PERSPECTIVES

Obesity treatment today is insufficient. Bariatric surgery can help some patients, but there is clearly a need for a viable pharmacological treatment for obesity. There is no doubt that disturbances in appetite regulation in the brain can cause overeating and severe obesity. However, an important obstacle in the pharmacological targeting of energy balance in the brain is that many of the signalling systems that regulate appetite and food seeking behaviour seem to overlap with neuronal systems that influence psychological well-being. Several drugs trials over the past years have had to be terminated due to psychiatric side effects. A more attractive alternative may be to target peripheral mechanisms that influence energy balance. Adipose tissue related mechanisms have a huge pharmacological potential that has to be exploited in attempts to find novel therapeutic areas in the management of obesity. From a pharmacological point of view, there are two main reasons to search for adipose tissue-specific genes: 1) They most likely function in adipose tissue physiology and thus they are potential drug targets for the treatment of obesity, and 2) If they are not expressed in other organs, drugs that interfere with their function are less likely to cause side-effects. Inducing white adipose to adapt brown adipocyte-like features and consume more energy may be one approach to treat obesity or obesity-related co-morbidities. Also, pathways of lipolysis may become targets for the treatment of obesity-related metabolic derangements. Hence, the pathways that involve CIDEA and CIDEC have therapeutic potential for the treatment of obesity and obesity-related metabolic complications. Like adipose tissue, the gastrointestinal tract may provide clues regarding how to treat obesity. Our findings provide evidence of a possible interaction between intestinal permeability and visceral fat accumulation, and thus improve the prospects for intervention studies in humans.

Despite the accumulating evidence of obesity as a risk factor for cancer, not much effort has been made to develop programs to prevent cancer by weight control. One reason for this lack of interest may be that it has not been convincingly shown that weight loss can prevent cancer. Our data, in combination with previous observation studies and data from retrospective bariatric surgery studies, indicate that much could be gained from obesity treatment in women. Future research and clinical management recommendations should take into account cancer as an important complication to obesity. However, there are still many questions that need to be answered, and our findings need to be confirmed by additional studies. The possibility of a gender differences in this context is very intriguing and should be specifically addressed in coming research. To what extent obesity treatment should be used in the clinical management of obese cancer survivors, or in obese patients with a strong hereditary risk of developing cancer, has not yet been clarified. Our data suggest that visceral obesity (as estimated with the sagittal diameter) may be an independent predictor of cancer incidence, and this finding merits further investigation. The mechanisms underlying the association between obesity, energy balance and cancer are not well understood. Obesity, caloric restriction and weight loss exert a broad impact on mediators of cellular growth, inflammation, oxidative stress, angiogenesis, cell adhesion, all of which may influence carcinogenesis [158]. A possible strategy to study such complex effects of energy balance on cancer could be to use microarray

techniques to study the transcriptional responses that follow caloric restriction. For many relevant tissues, such studies can be performed in humans.

Finally: An important mission today is to alleviate the stigma of obesity. We have to acknowledge that severe obesity is a chronic disease that is difficult to treat, and not a personal choice. We have learnt from genetic studies that human food intake cannot be considered as an entirely voluntary act, but instead driven by powerful biological signals. Even more difficult to control is the body's response to, and handling of, energy excess and energy deficit. Yet so many people (including the obesity patients themselves) firmly believe that it is all a matter of discipline, which it is certainly not [159].

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