ALTERATIONS IN PERIPHERAL GLUCOCORTICOID METABOLISM: EFFECTS OF WEIGHT CHANGES

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To my father (in memory):

i carry your heart with me (i carry it in my heart)
i am never without it (<...>)

Here is the deepest secret nobody knows
(here is the root and the bud of the bud
and the sky of the sky of a tree called life;
which grows higher than the soul can hope or mind can hide)

and this is the wonder that's keeping the stars apart

i carry your heart (i carry it in my heart)

[E.E. Cummings]
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ABSTRACT

Background: An important role has been suggested for tissue-specific glucocorticoid metabolism in the development of obesity and its complications. 11β hydroxysteroid dehydrogenase 1 (11βHSD1) is an enzyme that catalyzes the interconversion of biologically inactive cortisone to active cortisol, thereby regulating its access to glucocorticoid receptors in target tissues. Indeed, an unfavorable metabolic outcome has been associated with increased 11βHSD1 gene expression and activity in adipose tissue and liver in humans and rodents. Cortisol is an important regulator of phosphoenolpyruvate carboxykinase (PEPCK) a key enzyme in gluconeogenesis and lipid metabolism. In rodents, overexpression of PEPCK in adipose tissue leads to adiposity and increased fatty acid re-esterification. In human obesity, PEPCK has been positively associated with body fat, total cholesterol levels, and plasma triglycerides. However, few studies have addressed the putative reversibility of peripheral cortisol levels and disturbed fatty acid homeostasis that may accompany weight loss. The aim of this thesis was to investigate alterations in peripheral glucocorticoid metabolism in the context of obesity, and putative modulations of glucocorticoid metabolism in the context of weight changes in humans and a high-fat diet in rodents. Materials & Methods: 11βHSD1 expression/activity in different adipose tissue depots and liver, the expression of genes involved in adipogenesis and fatty acid homeostasis, and serum levels of adipose tissue-derived adipokines were investigated in severely obese women before and after surgically induced weight loss. The same parameters were measured in female Sprague-Dawley rats fed on high-fat and control diets. Results: In severely obese women, 11βHSD1 expression was higher in subcutaneous adipose tissue (SAT), while 11βHSD1 activity and PEPCK expression were higher in the omental depot. In a multivariate analysis, SAT 11βHSD1 activity was an independent predictor for central fat accumulation. Hepatic 11βHSD1 activity and levels of intra-abdominal fat storage correlated negatively, while 11βHSD1 correlated positively with PEPCK in adipose tissue and liver. Weight loss after gastric bypass surgery was followed by significant and metabolically beneficial reductions in subcutaneous 11βHSD1 and leptin gene expression, as well as reduced circulating leptin and increased adiponectin levels. In contrast, PEPCK gene expression did not change with weight loss. In rats, a high-fat diet did not affect body weight, but was associated with increased serum leptin and decreased adiponectin levels. Short-term, high-fat diet feeding resulted in the up-regulation of SAT 11βHSD1 expression, while chronic feeding led to its significant down-regulation (compared with the control diet and short-term, high-fat feeding). Interestingly, hepatic 11βHSD1 expression was constantly down-regulated in rats that were fed a high-fat diet. Conclusions: Severe obesity in women was accompanied by a metabolically adverse increase of 11βHSD1 in adipose tissue, with a concomitant decrease in the liver. Subcutaneous 11βHSD1 was an independent predictor for central fat accumulation. As weight loss was followed by significant down-regulation of subcutaneous 11βHSD1, we suggest that up-regulation of this enzyme was a consequence, rather than a cause of obesity. In rodents, a high-fat diet induced dynamic changes in 11βHSD1 in SAT and liver, both being down-regulated after chronic high-fat feeding without altered weight. In summary, weight changes and alterations in fat and liver glucocorticoid metabolism are closely linked. Moreover, a high-fat diet significantly influences 11βHSD1 expression/activity in adipose tissue and liver without affecting body weight.

Key words: 11β hydroxysteroid dehydrogenase 1, obesity, glucocorticoids, cortisol, phosphoenolpyruvate carboxykinase, adipose tissue, liver.
LIST OF PAPERS

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals.


*Kotryna Simonyte*, Ingegerd Söderström, Andreas Sjödin, Tommy Olsson. 2011. Short- and long-term effects of high-fat diet on 11ßHSD1 expression in subcutaneous adipose tissue and liver in female Sprague-Dawley rats. (Manuscript) (*Study III*)

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### ABBREVIATIONS

<table>
<thead>
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<th>Abbreviation</th>
<th>Full Form</th>
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<tr>
<td>11ßHSD1</td>
<td>11ß HYROxySTEROID DEHYDROGENASE 1</td>
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<tr>
<td>11ßHSD2</td>
<td>11ß HYROxySTEROID DEHYDROGENASE 2</td>
</tr>
<tr>
<td>ACTH</td>
<td>ADRENOCORTICOTROPIC HORMONE</td>
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<tr>
<td>BMI</td>
<td>BODY MASS INDEX</td>
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<tr>
<td>CRH</td>
<td>CORTICOTROPIN-RELEASING HORMONE</td>
</tr>
<tr>
<td>CT</td>
<td>COMPUTED TOMOGRAPHY</td>
</tr>
<tr>
<td>FA</td>
<td>FATTY ACID</td>
</tr>
<tr>
<td>HPA</td>
<td>HYPOTHALAMIC-PITUITARY-ADRENAL</td>
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<tr>
<td>HF</td>
<td>HIGH-FAT</td>
</tr>
<tr>
<td>H6PDH</td>
<td>HEXOSE-6-PHOSPHATE DEHYDROGENASE</td>
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<tr>
<td>L4</td>
<td>LUMBAR VERTEBRA 4</td>
</tr>
<tr>
<td>mRNA</td>
<td>MESSENGER RIBONUCLEIC ACID</td>
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<tr>
<td>NADPH</td>
<td>NICOTINAMIDE ADENINE DINUCLEOTIDE PHOSPHATE</td>
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<td>OmAT</td>
<td>OMENTAL ADIPOSE TISSUE</td>
</tr>
<tr>
<td>PEPCK</td>
<td>PHOSPHOENOLPYRUVATE CARBOXYKINASE</td>
</tr>
<tr>
<td>PPAR</td>
<td>PEROxisome proliferator-activated RECEPTOR</td>
</tr>
<tr>
<td>PPIA</td>
<td>PEPTIDYLPROLYL ISOMARASE A</td>
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<tr>
<td>SAT</td>
<td>SUBCUTANEOUS ADIPOSE TISSUE</td>
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<tr>
<td>SREBP</td>
<td>STEROL REGULATORY ELEMENT BINDING PROTEIN</td>
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<tr>
<td>SD</td>
<td>STANDRAD DEVIATION</td>
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<tr>
<td>SEM</td>
<td>STANDARD ERROR OF THE MEAN</td>
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<tr>
<td>VAT</td>
<td>VISCERAL ADIPOSE TISSUE</td>
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<tr>
<td>TNFα</td>
<td>TUMOR NECROSIS FACTOR-α</td>
</tr>
<tr>
<td>THF</td>
<td>TETRAHYDROCORTISOL</td>
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<tr>
<td>THE</td>
<td>TETRAHYDROCORTISONE</td>
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Världshälsoorganisationen (WHO) definierar övervikt och fetma som onormal eller överflödig fettnäring vilken medför en hälsosisk. Fetma kan sammankopplas med en rad olika metabola sjukdomar, vilka innefattar typ 2 diabetes och hjärt-kärlsjukdom. Den ökade förekomsten av fetma kräver ytterligare forskningsinsatser för att förstå mekanismerna bakom sjukdomen.


Syftet med avhandlingen var att undersöka förändringar i perifer kortisolmetabolism, dvs. 11ßHSD1 genuttryck/aktivitet, i samband med grav fetma och viktnedgång hos människor. Dessutom undersöka hur fettrik kost påverkar 11ßHSD1 i fettväv och lever hos Sprague-Dawley hontröttar.

I kraftigt fetta kvinnor var aktiviteten av 11ßHSD1 i subkutan fettväv en oberoende riskfaktor för central fetma, medan aktiviteten av 11ßHSD1 i lever samt inlagring av bukfett var negativt korrelerade. Viktnedgång efter gastrisk bypass kirurgi följes av ett betydande och metabolt fördelaktigt minskat uttryck av 11ßHSD1 och leptin i det
subkutana fetter, samt minskade cirkulerande leptinnivåer och ökade adiponektinnivåer. Detta tyder på att en ökning av 11ßHSD1 är en konsekvens av, snarare än orsaken till, fetma. I råttor påverkades inte kroppsvikten av fettrik kost, men den var kopplad till ökande leptinnivåer samt minskade adiponektinnivåer i serum. Fettrik kost gav på kort sikt högre uttrycksnivåer av 11ßHSD1 i subkutant fett, medan kronisk fettrik kost ledde till en signifikant nedreglering (även i kontrollgruppen och fettrik kost under kortare tid). En intressant iakttagelse är att uttrycket av 11ßHSD1 var nedreglerat hela tiden i levern för råttor som åt fettrik kost.

Sammanfattningsvis, viktförändringar och förändringar i glukokortikoidmetabolismen i fettvävnad och lever har nära kopplingar till varandra. Dessutom påverkar en fettrik kost uttrycket av 11ßHSD1 i fettväv och lever signifikant utan att påverka kroppsvikten.
INTRODUCTION

The World Health Organization defines overweight and obesity as abnormal or excessive fat accumulation that presents a risk to health\(^1\). Obesity is associated with an array of metabolic pathologies, including type 2 diabetes and cardiovascular disease. The increasing prevalence of obesity spurs research efforts to understand the mechanisms underlying this disorder\(^2\).

The origins of obesity can be tracked back as many as 30,000 years. The ancient Greeks were the first to realize the dangers of obesity and its association with different diseases, infertility, and early death. However, acceptance of obesity as a medical problem has been slow. For thousands of years, overweight and obesity were exceptional, rarely seen and never studied. Moreover, in some cultures obesity has been (and in some cases still is) prized as an indication of status and wealth. In 1660, T. Venner first used the word 'obesity' in a medical context calling specifically for its treatment. In 1811, R. Thomas noted that not only the presence, but also the site of body fat is important, highlighting visceral fat as dangerous and metabolically active:

'Corpulency, when it arrives at certain height, becomes an absolute disease.'

[R. Thomas, 1811]

Gradually, a cluster of different conditions, including obesity, insulin resistance, glucose intolerance, hypertension, and dyslipidemia have been documented. In 1988, this cluster was presented by G. Reaven as a life-threatening syndrome – ‘Syndrome X’. Later, the concept of this cluster has been standardized as ‘the metabolic syndrome’, with proposed clinical guidelines\(^3\).

ADIPOSE TISSUE – AN ACTIVE ENDOCRINE ORGAN

Adipose tissue is a loose, connective tissue consisting of adipocytes (lipid-filled cells) surrounded by a matrix of collagen fibers, blood vessels, fibroblasts, and immune cells\(^4\). There are two types of adipose, which differ in their cell structure, location, color, and function. Brown adipose tissue contains multicolored adipocytes and has a large number of mitochondria; its main function is the regulation of thermogenesis. White adipose tissue is the quantitatively major adipose tissue and the primary site of triglyceride storage\(^5,6\), as each cell contains a single, large fat droplet. The capacity of adipocytes to store lipids is buffered by the growth of adipose tissue. Adipose tissue can undergo hyperplasia (increase in the number of adipocytes) and hypertrophy (increase in the size of individual adipocyte)\(^5\). The generation of adipocytes is a major factor underlying the growth of adipose tissue during childhood, and the number of
adipocytes is a major determinant of fat mass in adults. Increased fat storage in fully differentiated adipocytes, resulting in enlarged fat cells, has been suggested as the most important mechanism underlying increased adipose tissue in adults.

Adipose tissue depots

The distribution and location of adipose tissue relates to differences in functionality. The subcutaneous adipose tissue (SAT) depot, which is located directly under the skin and is mainly responsible for thermal isolation, contributes to the distinct body shapes of women (gynoid) and men (android). The abdominal SAT depot can be subdivided into two parts: the superficial and deep SAT, which have been suggested to have different metabolic activities. Visceral adipose tissue (VAT)/intra-abdominal fat fills space between the organs and can be subdivided into different depots according to its location (e.g. omental, mesenteric, retroperitoneal, etc). An increase in visceral fat mass has been suggested as highly unfavorable. Notably, central fat accumulation, whether visceral, abdominal subcutaneous, or both, may play a key role in the pathogenesis of insulin resistance, dyslipidemia, and glucose intolerance, and is also a risk marker for type 2 diabetes and cardiovascular disease. Figure 1 is a representative Computed tomoraphy (CT) picture demonstrating abdominal fat accumulation and depicting subcutaneous and visceral adipose tissue depots at lumbar vertebra 4 (L4).

Figure 1. Abdominal fat accumulation, shown by CT scan at L4 level
**Adipokines**

It took quite a while for adipose tissue to be acknowledged as an active endocrine organ that was not only used for triglyceride storage. Adipose tissue is now recognized as one of the major organs to control energy metabolism. This control is performed through the generation and release of a range of active molecules, called adipokines. Adipokines are compounds with specific biological functions, which can act as endocrine, paracrine, or autocrine signals and are important for different physiological processes (e.g., development and growth, appetite and energy homeostasis, angiogenesis, extracellular matrix reformation, steroid metabolism, and immune response). Figure 2 illustrates the roles of specific adipokines in different pathways, and emphasizes the importance of maintaining the healthy functionality of adipose tissue, which is severely disturbed in obesity.

![Figure 2. Different adipokines synthesized in and released from adipose tissue](image)

**Metabolic syndrome vs. Cushing’s syndrome**

The amount and distribution of adipose tissue as a risk factor for metabolic disease is emphasized in the criteria for the metabolic syndrome. Recently, in a joint statement by a number of health organizations, the metabolic syndrome was defined as the presence of at least three out of five factors (or current medical treatment for any of the
conditions), including central obesity (increased waist circumference), high serum triglycerides, low HDL levels, insulin resistance, and high blood pressure\textsuperscript{11,12}. Despite constantly increasing knowledge, it is still unclear whether a single abnormality triggers the cascade of the manifestation of other components of the metabolic syndrome, or whether they act synergistically to amplify the risk of developing metabolic disease. It is also important to mention that each component is an independent risk factor for the development of atherosclerotic cardiovascular disease and type 2 diabetes\textsuperscript{12}.

Cushing's syndrome is a constellation of symptoms and physical features caused by endogenous or exogenous hypercortisolism. Chronic glucocorticoid excess leads to increased morbidity and mortality through a variety of factors, including obesity, osteoporosis, hypertension, hyperglycemia, and impaired response to infection. Notably, most of the features of Cushing's syndrome are reversible upon removal of excess glucocorticoid\textsuperscript{13}.

Similarities between the features of Cushing's syndrome and those of the metabolic syndrome have led to the suggestion that subtle abnormalities in cortisol secretion or action may contribute to the pathogenesis of the metabolic syndrome. However, even though serum cortisol levels in idiopathic obesity are normal (or even low compared with the Cushing's syndrome), cortisol excretion levels are elevated\textsuperscript{14}; therefore, this conundrum has been suggested to be related to altered tissue-specific glucocorticoid metabolism.

\textbf{CORTISOL}

The abundant actions of glucocorticoids were acknowledged upon identification of the phenotypes of adrenocortical insufficiency and cortisol excess. Addison's disease, a state of adrenocortical deficiency, is characterized by hypoglycemia, weight loss, anorexia, and postural hypotension (Addison 1885). In contrast, elevated and sustained cortisol secretion results in the constellation of features described in Cushing's syndrome, with central obesity, hypertension, glucose intolerance, and dyslipidemia (Cushing 1912). Furthermore, enhanced rates of cortisol production in the periphery, despite normal circulating levels, are associated with obesity, high blood pressure, insulin resistance, and impaired glucose tolerance\textsuperscript{14}.

\textbf{Regulation, secretion, \& catabolism}

Cortisol is the major glucocorticoid in humans (corticosterone in rodents) and is secreted from the adrenal cortex under the control of the hypothalamic-pituitary-adrenal (HPA) axis. Corticotropin-releasing hormone (CRH) from the hypothalamus stimulates the pituitary gland to release adrenocorticotropic hormone (ACTH), which
binds to the adrenal glands and increases both cortisol production and its secretion into the blood. Circulating levels of cortisol are regulated by negative feedback, where cortisol acts on the hypothalamus and pituitary. Central regulation of glucocorticoid secretion and metabolism is presented in Figure 3. Cortisol secretion is episodic and follows a circadian rhythm, with low levels in the evening and peak levels after six to eight hours of sleep. Approximately one-half of the total daily cortisol output is secreted during the morning, followed by a gradual decline during the course of the day. However, cortisol secretion is increased in response to eating and exercise. In the blood, cortisol circulates bound to plasma proteins (primarily corticosteroid-binding globulin and, to a lesser extent, albumin). Under basal conditions approximately 10% of circulating cortisol is free, and therefore active. Because there are no binding proteins in saliva, salivary cortisol reflects the levels of free cortisol in plasma. Glucocorticoids are catabolized in the liver. They are first irreversibly inactivated by A-ring reductases (5α- and 5β-reductase), and then further converted into 5α-tetrahydrocortisol (THF), 5β-THF, and 5β-tetrahydrocortisone (THE) by 3α-hydroxysteroid dehydrogenase. Conjugated cortisone and cortisol metabolites reenter the circulation to be excreted in the urine.
**Direct & intermediary effects of cortisol**

Cortisol is essential for survival. During periods of acute, severe stress (major injury, systemic infection), activation of the HPA axis results in elevated serum cortisol, which is essential for homeostatic adjustments, including energy homeostasis (effects on glucose and fatty acid metabolism), protection against shock (effects on blood flow/circulation and fluid balance), and innate immune response (anti-inflammatory effects).

Cortisol increases blood glucose concentrations by repressing the translocation of glucose transporter type 4 (GLUT4) to cell membranes, and in turn inhibiting peripheral glucose uptake in muscle and adipose tissue. It stimulates hepatic gluconeogenesis while increasing the expression of gluconeogenic enzymes, such as phosphoenolpyruvate carboxykinase (PEPCK). Glucocorticoids also increase hepatic responsiveness to the gluconeogenic hormone glucagon and increase the release of substrates for gluconeogenesis from peripheral tissues (particularly muscle). Furthermore, cortisol enhances hepatic glycogen synthesis and storage. However, these effects are insulin-dependent and may result in increased insulin secretion in states of chronic glucocorticoid excess.

Glucocorticoids affect fat metabolism by stimulating lipolysis, which leads to the release of free fatty acids (FAs) into the circulation, and by promoting the differentiation of preadipocytes to adipocytes. Moreover, it has been shown that glucocorticoids play a role in the redistribution of body fat from the periphery to visceral depots. In times of starvation or shortage of caloric intake, glucocorticoids promote the mobilization of energy substrates from peripheral fat through lipolysis, while fostering the accumulation of fat in abdominal depots, which can contribute to obesity over time.

**Effects of cortisol on other systems, tissues, & functions**

Glucocorticoids exert major effects on cardiovascular physiology; defiency and excess are associated with hypo- and hypertension, respectively. Glucocorticoids enhance sensitivity to vasopressors, such as norepinephrine and angiotensin II, and impair nitric oxide-mediated endothelial vasodilation. Furthermore, individuals exposed to exogenous glucocorticoids are at increased risk for cardiovascular events and atherosclerosis.

Chronic glucocorticoid excess has deleterious effects on the hippocampus, a part of the brain with a central role in forming long-term memories. High circulating cortisol levels in humans are linked to poor memory, hippocampal shrinkage, and neuronal loss. This process can be observed in normal aging, as well as in conditions like depression, Alzheimer's disease, and Cushing's syndrome.
Inflammation is a host defence process that involves the recruitment and activation of immune system cells in response to an infection or injury. Glucocorticoids are perhaps best known for their potent anti-inflammatory actions. However, endogenous glucocorticoids are immunomodulatory, rather than simply anti-inflammatory. Depending on concentration and timing, they can act as either enhancers or suppressors of immune reactions. During inflammation, the HPA axis is activated by an increase in circulating pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6), and leptin, which leads to increased production of serum cortisol, which acts in anti-inflammatory and immunomodulatory manner.

**11βHSDs – REGULATORS OF GLUCOCORTICOID AVAILABILITY IN THE PERIPHERY**

As previously mentioned, there are striking similarities between the metabolic syndrome (idiopathic obesity) and Cushing’s syndrome, and cortisol is highlighted as a putative key player in both. However, normal or even reduced circulating cortisol levels, but elevated excretion rates in idiopathic obesity/metabolic syndrome have led to questions regarding peripheral, tissue-specific control and responsiveness to this hormone. The specificity of tissue response depends on receptor availability. Glucocorticoid/mineralocorticoid receptors belong to the steroid receptor family and, under basal conditions, exist as cytoplasmic, multimeric complexes that include heat shock proteins. The receptor-glucocorticoid complex acts via two mechanisms: binding to specific sites in nuclear DNA; and interacting with other transcription factors. So far, no major alterations in glucocorticoid/mineralocorticoid receptor expression/sensitivity has been found in idiopathic obesity. In contrast, glucocorticoid metabolism at prereceptor level may be of major importance in obesity-related morbidity. Local, tissue-specific hormone concentration is determined by the presence of enzymes that either limit or amplify the availability of the ligand. For glucocorticoids, the relevant enzymes are 11β hydroxysteroid dehydrogenases (11βHSDs). This system of enzymes interconverts active cortisol with its inactive cortisone, regulating access to their receptors in target tissues.

Two 11βHSD isoforms have been described: 11βHSD type 1 and 2. 11βHSD2 is expressed in organs that are responsible for the regulation of water and salt balance. This dehydrogenase converts cortisol to inactive cortisone, and thereby protects mineralocorticoid receptors from nonspecific cortisol binding. Inhibition or loss of 11βHSD2 function leads to a syndrome of apparent mineralocorticoid excess in which the over-activation of mineralocorticoid receptors leads to sodium retention, hypertension, and hypokalemia. 11βHSD2 is also expressed in adipose tissue, but at several-fold lower levels than 11βHSD1.
In vivo, 11ßHSD1 predominantly acts as an oxo-reductase and amplifies local cortisol concentrations, thereby increasing glucocorticoid receptor activation in target metabolic organs, such as adipose tissue and liver. However, when the enzyme is liberated from its intracellular location within the endoplasmic reticulum (e.g., in tissue homogenates or purified preparations), it functions as a dehydrogenase, performing the inactivation of cortisol to cortisone.

The oxo-reductase activity of 11ßHSD1 is highly dependent on the co-factor nicotinamide adenine dinucleotide phosphate (NADPH), which is supplied by the enzyme hexose-6-phosphate dehydrogenase (H6PDH). 11ßHSD1 and H6PDH are co-expressed in several tissues, and are co-localized within the endoplasmic reticulum. H6PDH knock-out mice are unable to activate glucocorticoids and have increased 11ßHSD1 dehydrogenase activity, which suggests a vital role for H6PDH in 11ßHSD1 oxo-reductase activity. The regulation of peripheral glucocorticoid metabolism is presented in Figure 4.

![Diagram of Peripheral glucocorticoid metabolism](image)

**11ßHSD1 & human obesity**

Idiopathic human obesity has been associated with an up-regulation of adipose tissue 11ßHSD1. Most, but not all, studies have shown that 11ßHSD1 expression, enzyme activity, and protein levels in SAT are positively related to different measures of fat accumulation (e.g., body mass index (BMI), waist circumference and waist-hip ratio, in both men and women). Interestingly, the majority of studies have not shown any differences in 11ßHSD1 gene expression between SAT and VAT depots, although some studies have reported higher 11ßHSD1 gene expression in VAT. In keeping
with gene expression data, higher 11ßHSD1 activity in VAT depots than SAT, have been reported\textsuperscript{48,53-55}. In contrast, a negative association has been demonstrated between BMI and the hepatic cortisone conversion rate (which corresponds to 11ßHSD1 activity in the liver)\textsuperscript{41,42}. This down-regulation of hepatic 11ßHSD1 activity has been suggested as a compensatory/protective mechanism against the putatively deleterious effects of cortisol overexposure. Interestingly, a very recent study by Stimson et al. reported sustained hepatic 11ßHSD1 activity in obese individuals with type 2 diabetes\textsuperscript{56}.

**11ßHSD1 & monogenic rodent models of obesity/metabolic syndrome**

A series of studies of monogenic rodent models of obesity have elucidated the role of 11ßHSD1. Obese Zucker rats have elevated 11ßHSD1 levels in intra-abdominal adipose tissue but reduced hepatic 11ßHSD1 activity\textsuperscript{57-59}. Overexpression of 11ßHSD1 in adipose tissue in mice results in an obese phenotype with increased intra-abdominal adipose tissue depots, enlarged adipocytes, hypertension, dyslipidemia, and glucose intolerance\textsuperscript{60}. Hepatic 11ßHSD1 overexpression leads to a similar (although less severe) metabolic phenotype: these mice develop fatty liver, mild hyperinsulinemia, dyslipidemia, and hypertension, but retain normal body weight even when fed a high-fat (HF) diet\textsuperscript{61}. In contrast, the 11ßHSD1 deletion\textsuperscript{62,63} or inhibition\textsuperscript{64-67} result in beneficial effects on glucose and FA metabolism. Mice with genetic inactivation of 11ßHSD1 are protected from obesity, have a ‘cardio-protective’ phenotype and resist stress- and obesity-induced hyperglycemia\textsuperscript{62,63}. Additionally, they have lower serum triglycerides and increased adiponectin, but reduced intra-adipose TNF-α and resistin levels\textsuperscript{62,63}.

It is quite obvious that elevated adipose tissue and/or liver 11ßHSD1 are detrimental for metabolic control. Tissue-specific dysregulation of 11ßHSD1 in obesity, with high 11ßHSD1 activity levels in adipose tissue and low levels in the liver, has been suggested as a secondary compensatory mechanism that protects against the hyperinsulinemic effects of elevated glucocorticoid action\textsuperscript{14}. Whether changes in cortisol metabolism are primary or secondary to obesity, therapeutic intervention to reverse the tissue-specific alterations in peripheral glucocorticoid metabolism appears to be an attractive option.

**Regulation of 11ßHSD1**

Several factors, including cytokines, endogenous and synthetic glucocorticoids, growth factors, insulin, sex steroids, thyroid hormones, gonadotropins, peroxisome proliferator activated receptor (PPAR) agonists, CRH, and ACTH, have been identified as potential regulators of 11ßHSDs. Inflammatory cytokines are perhaps the most extensively studied group of mediators that regulate 11ßHSDs. Pro-inflammatory factors, such as TNF-α and IL-1β, have been shown to up-regulate 11ßHSD1 expression in vitro (i.e., primary cell cultures, matured adipocytes derived from human
Their effects on 11ßHSD1 activity are of great interest and relevance, as obesity is now recognized to be associated with a state of low-grade inflammation and macrophage infiltration in the adipose tissue\textsuperscript{71,72}. Associations between pro-inflammatory factors, the degree of macrophage infiltration, adipocyte size, and degree of adiposity have been demonstrated in both humans and mice\textsuperscript{72}.

PPARs (key regulators of glucose and lipid homeostasis) and PPAR agonists are increasingly used to treat type 2 diabetes (PPAR\textsubscript{\gamma}) and hyperlipidemia (PPAR\textsubscript{\alpha}). Therefore, it is of interest that 11ßHSD1 is down-regulated by these transcription factors both in vivo and in vitro\textsuperscript{73-75}.

Diverse effects of insulin on 11ßHSD1 have been reported: insulin alone may inhibit, stimulate, or have no effect on 11ßHSD1 expression/activity. Insulin can also antagonize TNF-\textalpha-mediated induction, and the induction of 11ßHSD1 by insulin can be counteracted by dexamethasone\textsuperscript{76,77}.

The growth hormone/insulin-like growth factor 1 axis has an inhibitory effect on adipocytes, but not on primary hepatocytes’ 11ßHSD1 activity\textsuperscript{68,69,78}.

In humans, cortisol itself has been shown to up-regulate 11ßHSD1 expression in adipose tissue\textsuperscript{29,38}. The role of glucocorticoids on hepatic 11ßHSD1 expression/activity have been explored in rodents, and both enhancing (in vitro)\textsuperscript{79} and suppressive (in vivo)\textsuperscript{80} effects have been observed.

The action of estrogen on 11ßHSD1 has been elucidated in the context of sexual dimorphism, with higher whole-body 11ßHSD1 activity in men compared with women\textsuperscript{81,82}. In rodent studies, a suppressive effect of estrogen on 11ßHSD1 expression/activity has been reported in adipose tissue and liver\textsuperscript{80,83-86}.

Dietary components are important in the etiology of the development of obesity. It has been suggested that glucocorticoid metabolism in non-adrenal tissues may be influenced by dietary macronutrients. In rodents, feeding a HF diet decreases 11ßHSD1 in adipose tissue and liver, with a concomitant increase in hepatic 5ß-reductase\textsuperscript{87-89}. In contrast to the effects of an HF diet, consumption of excess sucrose or a high-energy diet replicate the combination of increased 11ßHSD1 in adipose tissue and decreased 11ßHSD1 in the liver, as observed in idiopathic human obesity\textsuperscript{90,91}. However, whether and how dietary macronutrients contribute to tissue-specific dysregulation in idiopathic human obesity has not been extensively studied. Increased whole-body 11ßHSD1 activity after the consumption of a mixed meal\textsuperscript{92} and increased adipose tissue 11ßHSD1 activity after insulin and intralipid infusions\textsuperscript{83} have been reported in healthy, normal-weight individuals. Interestingly, in obese men a high-fat, low-carbohydrate diet enhances whole-body cortisol regeneration and reduces cortisol inactivation by A-ring reductases in the liver, without affecting SAT 11ßHSD1\textsuperscript{94}. 
11βHSD1 & weight loss

Clearly, the association between idiopathic obesity and disturbed peripheral glucocorticoid metabolism has been extensively explored. However, only a few human studies have addressed whether weight loss can reverse the alterations in glucocorticoid metabolism that are observed in obese individuals. Short-term studies of human weight loss, induced by high-fat, low-carbohydrate; moderate-fat, moderate-carbohydrate; and very low calorie diets, have demonstrated no changes in 11βHSD1 gene expression in whole adipose tissue, but a significant increase in 11βHSD1 mRNA levels in isolated adipocytes. A study by Purnell et al. reported a significant decrease in adipose 11βHSD1 gene expression in men after weight loss induced by a low-fat high-protein diet.

MANAGING OBESITY

The management of obesity has been directed toward reducing energy intake and increasing energy expenditure. However, due to the chronic and relapsing nature of obesity, the long-term efficacy of maintaining weight loss is of fundamental importance.

Diet

‘One cause which made it necessary to study the art of restoring lost health, was the great difference to be observed between the diet of the healthy and that of the sick.’

[Hippocrates]

Low-calorie diets incorporate various methods to restrict the intake of nutrients. Low-fat, high-carbohydrate diets seem to be effective at lowering energy density, and are associated with weight loss. Fixed energy deficit diets are based on the estimation of energy requirements by calculating basal individual metabolism, adjusting for physical activity, and subtracting an energy deficit (≈ 600 kcal/day) to induce ≈ 0.5 kg/week weight loss. The meal replacement approach leads to short-term weight loss. Notably, very low-calorie diets (up to 800 kcal/day) are often associated with fatigue, nausea, and occasionally diarrhea, and have more serious side effects than benefits. The long-term effects of dietary modulation are currently a matter of intense study.

The variety of diets, with no clear evidence of effectiveness, suggests that no single dietary approach will be suitable for all individuals, and this may be partly dependent on genetic/environmental interactions. Therefore, further studies should certainly take into account genetic background, and not concern with dietary modulation alone.
Physical activity

Regular physical activity has important physiological benefits independent of weight loss, including reducing blood pressure, improving atherogenic lipid profiles, and improving glucose tolerance. When physical activity alone is used to treat obesity, weight losses are modest (average 2-3 kg)\(^97\). The amount of daily physical activity that is currently recommended to maintain lowered weight and prevent weight regain is 45-60 minutes/day\(^{100}\).

Pharmacological treatment

Two types of anti-obesity drugs have been used: those that act on the gastrointestinal system (Orlistat) and those that act on the central nervous system, primarily to suppress appetite (Rimonabant and Subutramin). Randomized, controlled trials suggest that 12 months of treatment with anti-obesogenic drugs (orlistat and sibutramine) may lead to a 5-10% weight loss in 40-60% of patients\(^{101}\). Notably, appetite suppressors have recently been withdrawn from the market because of their unfavorable side effects.

Surgical intervention

Surgery (gastric restriction, gastric bypass surgery, or biliopancreatic diversion) can be used to treat obesity and type 2 diabetes\(^{102}\). Surgical interventions are successful in inducing substantial weight loss in the majority of obese patients; this outcome is primarily achieved by reduction in caloric intake. The Swedish Obesity Study (SOS) is, thus far, the largest intervention study, and compares the effects of bariatric surgery versus conventional treatment on overall mortality during an average follow-up of 10.9 years\(^{103}\). Maximum weight loss (up to 32% of pre-surgical weight) was observed 1 to 2 years after surgery. Ten years after surgery, weight loss stabilized at a 25% decrease compared with baseline. This study concludes that bariatric surgery for severe obesity is associated with long-term weight loss and beneficial effects on type 2 diabetes, different cardiovascular risk factors, decreased incidence rates for certain types of cancer, and marked reduction in overall mortality\(^{103}\).

11ßHSD1 inhibition

It has been hypothesized that decreased glucocorticoid activity in adipose tissue and liver might protect against the detrimental metabolic consequences of obesity. Two principal therapeutic strategies can be identified: antagonism of the receptor or its signaling pathway, and reduction of ligand availability\(^{104}\).

Administration of the glucocorticoid receptor antagonist RU38486 to Cushing’s syndrome patients and leptin receptor-deficient mice decreases plasma glucose
levels\textsuperscript{105,106}. The natural 11βHSD1 inhibitors glycyrrhetic acid and carbenoxolone are potent, but non-specific. Treatment with these compounds has demonstrated beneficial effects on several metabolic parameters, such as the improvement of hepatic insulin sensitivity and lowering cholesterol\textsuperscript{107,108}. Pharmacologically created compounds also efficiently inhibit 11βHSD1 in vivo and in vitro\textsuperscript{64-67} and result in increased insulin sensitivity, as well as reduced circulating levels of glucose and lipids. In addition, inactivation of glucocorticoids in adipose tissue with overexpression of 11βHSD2 leads to resistance against metabolic disease, with reduced fat accumulation even on an HF diet\textsuperscript{109}.

The inconsistent efficacy of 11βHSD1 inhibitors, which is probably due to differences in tissue-specificity, has been demonstrated previously. However, a recent study by Rosenstock and colleagues has, for the first time, demonstrated that INCB 13739 (an 11βHSD1 inhibitor) improves hyperglycemia in patients with type 2 diabetes that is inadequately controlled by metformin monotherapy\textsuperscript{110}. Moreover, sustained hepatic 11βHSD1 activity in obese men with type 2 diabetes has been reported, and it has been suggested that this group of patients might be more susceptible to 11βHSD1 inhibition\textsuperscript{56}. This very recent data emphasizes that more studies are needed to map tissue-specific 11βHSD1 activity in various physiological situations.
AIMS

Observations in humans and rodents have suggested that alterations in peripheral glucocorticoid metabolism, specifically tissue-specific alterations in 11ßHSD1 expression/activity, might be a key factor in increased fat accumulation and obesity-related morbidity. The aim of this research was to investigate the effects of morbid obesity and weight changes due to gastric bypass surgery or high-fat feeding on 11ßHSD1 expression/activity in adipose tissue and liver.

SPECIFIC AIMS:

• To map 11ßHSD1 expression/activity in different adipose tissue depots and liver in severely obese women (Study I)

• To investigate the putative reversibility of up-regulated 11ßHSD1 gene expression in subcutaneous adipose tissue after weight loss induced by gastric bypass surgery (Study II)

• To analyze the longitudinal effects of high-fat feeding on peripheral glucocorticoid metabolism in female Sprague-Dawley rats (Study III)
MATERIALS & METHODS

This section provides a brief description of the methods and procedures that were central to the work included in this thesis. Detailed descriptions are found in the corresponding papers.

STUDIES I & II

Subjects

Thirty-three obese women accepted for gastric bypass surgery and two years of follow-up were included in Studies I & II. Two women were excluded due to uncontrolled hypertension and malignant disease. Twenty-seven women finished the 2-year follow-up period. Four women did not participate in the follow-up part of the study. All participants gave written informed consent before the study began.

Study design

An overall view of how Studies I & II were performed is presented in Figure 5. Before baseline sampling, all women underwent a health examination. SAT biopsies were collected under local anesthesia during the preoperative evaluation and a follow-up visit.

Anthropometry & laboratory analyses

Blood parameters were analyzed in the accredited clinical laboratories at Umeå University Hospital or Örebro University Hospital. Body fat percentage was estimated using dual-energy X-ray absorptiometry (DXA). Distribution of adipose tissue was evaluated using an abdominal CT scan at the level of L4 and thigh. Liver fat content was estimated from CT scans. These procedures were performed at Örebro University Hospital.

In vitro 11ßHSD1 enzyme activity assay

In Study I, enzyme activity in the adipose tissue and liver were measured in tissue homogenates in the dehydrogenase direction with an excess of co-factor (NADPH) and substrate (cortisol). Notably, recent studies57,111,112 have recognized that this method assesses the amount of active 11ßHSD1 protein, but not the in vivo reductase activity. For convenience, I will continue to refer to this analysis as “enzyme activity”.

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Figure 5. Design of Studies I & II

**Gene expression analysis**

In Studies I & II, gene expression was analyzed using the relative quantification standard curve real-time PCR method. Commercially available assays with sets of primers and probes of genes of interest were used. The endogenous control genes PPIA (in Study I) and 18S (in Study II) were used for normalization. These genes were chosen after comparing the coefficients of variations of three (for Study I) and four (for Study II) different genes, selected based on previous evaluations of endogenous controls in human adipose tissue\(^\text{113}\).

**Statistical analyses**

Data are presented as mean ± SD or mean ± SEM. The nonparametric Wilcoxon test for paired samples was used to compare different depots (Study I) and variables before and after weight loss (Study II). Spearman’s rho-test was used to estimate bivariate correlations between different variables (Studies I & II) and corrected for multiple comparisons using the Bonferroni test. Multiple linear regression analysis was used to estimate independent predictors of variables (Study I). P values <0.05 were considered statistically significant (Studies I & II).
**STUDY III**

Ninety female Sprague-Dawley rats were randomly divided into two groups: 50 rats assigned to a high-fat diet (HFD) and 40 rats assigned to a control diet (CD) were included in the experiment. The experimental design and methods used in this study are presented in Figure 6.

![Figure 6. Experimental design for Study III](image)

**Gene expression analysis**

Gene expression levels were analyzed using the relative quantification Comparative CT Method (ΔΔ Ct), which employs real-time PCR. 18S was used as the endogenous control.

**Statistical analysis**

Data is presented as mean ± SD or mean ± SEM. Differences between groups were tested with one- and two-way ANOVA (for diet, time, and time & diet interaction effects). Post hoc tests for group differences were determined using Tukey’s HSD tests. Spearman’s rho-test was used to estimate bivariate correlations between different variables. P-values <0.05 were considered statistically significant.
RESULTS & DISCUSSION

This section summarizes the results and discussion for each study. See the corresponding papers for more details.

STUDY I

In this study, we aimed to investigate 11ßHSD1 expression/activity and the expression of key genes involved in fat metabolism in SAT and omental adipose tissue (OmAT) depots, as well as in the liver, in severely obese women.

Adipose tissue depot-specific differences

11ßHSD1 was expressed at a higher level in the SAT depot than the OmAT depot (P < 0.01), while enzyme activity was significantly higher in the OmAT depot (P < 0.01). This data strengthens earlier findings about the adipose tissue depot-specific 11ßHSD1 pattern, which exhibits higher enzyme activity in VAT depots than in SAT depots\(^4\). We found a positive correlation between 11ßHSD1 expression and activity in the OmAT (\(r = 0.47, P < 0.05\)) but not in the SAT depot, as has been previously reported\(^{40-48}\). The disagreement between expression and activity may suggest depot-specific post-transcriptional and/or post-translational regulation of 11ßHSD1, possibly involving mRNA degradation or the presence of putative activators or inhibitors.

Leptin expression was twice as high in SAT depots as in OmAT depot (P < 0.001), while the adiponectin gene did not differ between the depots. Increased adiposity is associated with altered leptin and adiponectin levels, and glucocorticoids have been proposed as potential up-regulators of leptin\(^{114,115}\). Although adiponectin expression did not differ between the depots, there was a negative association between adiponectin expression in OmAT and waist circumference, as well as insulin levels (data not shown). Increased leptin, but decreased adiponectin levels, in adipose tissue as well as in circulation, are accepted as a metabolically unfavorable profile and are predictive for the development of type 2 diabetes and cardiovascular disease\(^{116-118}\). SREBP, an important transcription factor involved in adipogenesis, insulin sensitivity, and FA homeostasis\(^{119,120}\), was expressed at a higher level in SAT depot than OmAT depot (P < 0.05), in keeping with previous data\(^{121}\). This finding suggests that in cases of severe obesity, the SAT depot may have higher rate of de novo FA synthesis, and therefore increased release of free FAs into the blood\(^{121}\).

PEPCK is a key enzyme involved in the regulation of FA release via the triglyceride–FA cycle within adipocytes\(^{122}\). The overexpression of PEPCK-C in adipose
tissue increases FA re-esterification, leading to obesity\textsuperscript{123-125} and a positive association has been reported between PEPCK expression in SAT and BMI\textsuperscript{126}. We found that PEPCK was expressed at higher levels in the OmAT depot than the SAT depot (P < 0.001), which could be due to a parallel increase in local cortisol levels (linked to higher 11βHSD1 activity in the OmAT depot). Moreover, we also observed a positive correlation between 11βHSD1 and PEPCK in both OmAT and SAT depots. These correlations suggest a positive role for glucocorticoids with respect to PEPCK in adipose tissue, possibly leading to an increased rate of FA re-esterification, and therefore FA uptake and storage.

**SAT 11βHSD1 is an independent predictor of central fat accumulation**

Despite higher 11βHSD1 enzyme activity in OmAT, the SAT 11βHSD1 correlated with measures of central fat accumulation (e.g., waist circumference), even though we could not verify previously reported associations between BMI and adipose tissue 11βHSD1 expression/activity\textsuperscript{40-48}. Moreover, multiple linear regression analysis revealed SAT 11βHSD1 activity as an independent predictor of central fat accumulation.

**Hepatic 11βHSD1**

In this study, we had a unique opportunity to evaluate hepatic 11βHSD1 and key genes involved in energy metabolism in human samples. Due to the lack of a control group of normal-weight subjects, it is not possible to estimate whether there was up- or down-regulation of different genes in the liver of severely obese subjects vs. normal-weight subjects. Notably, there was a negative correlation between hepatic 11βHSD1 activity and abdominal/OmAT areas ($r = -0.7$, $P < 0.01$ and $r = -0.6$, $P < 0.01$, respectively), supporting the hypothesis that obesity is associated with decreased hepatic 11βHSD1 activity\textsuperscript{41}.

**In summary**, we suggest that in severely obese women, glucocorticoid generation in SAT, rather than OmAT, is associated with central fat accumulation. Abdominal obesity correlated negatively with hepatic 11βHSD1. Severe obesity was accompanied not only by disturbances in peripheral glucocorticoid metabolism, but also by major changes in FA recycling pathways in adipose tissue.
**STUDY II**

In this study, we aimed to investigate changes in anthropometric and biochemical characteristics, as well as 11ßHSD1, leptin, adiponectin, and PEPCK in SAT and serum after significant weight loss induced by gastric bypass surgery.

**Anthropometrics, biochemical characteristics, & imaging**

At the two-year follow-up, all women had a significantly decreased BMI and waist circumference (P < 0.0001, for both measures). These outcomes were associated with a two-fold reduction of the SAT depot volume and four-fold reduction of the intra-abdominal fat depot volume. A pronounced decrease in intra-abdominal vs. SAT volume may be of great importance to the decreased risk for type 2 diabetes, cardiovascular disease, certain types of cancer, and overall mortality after gastric bypass surgery-induced weight loss. Importantly, CT scans also revealed reduced hepatic fat deposition after weight loss, reflecting decreased liver steatosis.

The significant weight loss was followed by metabolically favorable biochemical profiles with markedly reduced fasting insulin and glucose levels, and therefore increased insulin sensitivity (P < 0.0001, for all). A nearly two-fold increase in circulating adiponectin with a concomitant decrease of leptin (by 37%) followed the weight loss.

**Gene expression in the SAT depot**

Postoperative weight loss was accompanied by a four-fold decrease in 11ßHSD1 gene expression in the SAT depot (P < 0.0001). Interestingly, previous studies have reported no changes or increased adipose tissue 11ßHSD1 expression levels after weight loss of ≤ 5 BMI units, and decreased levels after a BMI reduction of 6 units. These discrepancies suggest that it might be important to take into account the degree of weight loss when evaluating the effects of weight loss on 11ßHSD1 expression/activity. We investigated the transition from severe obesity to overweight, whereas other studies were limited to changes within the overweight range. In addition, the duration of follow-up may also have contributed to different outcomes.

The previously reported linear correlation between SAT 11ßHSD1 and BMI was absent before surgery, but was observed after weight loss. This finding suggests that there might be a BMI threshold where the association between 11ßHSD1 and BMI disappears, and disturbances in glucocorticoid metabolism may be influenced by other factors aside from weight per se. Disturbances in glucocorticoid metabolism in adipose tissue seem to be more a consequence or adaptation, rather than a cause of obesity.
Consistent with previous studies, we found a significant reduction in leptin gene expression and circulating leptin levels after weight loss\textsuperscript{127}. There was also a positive correlation between \(11\beta\)HSD1 mRNA and circulating leptin levels after weight loss. This finding supports the notion that the inhibition of endogenous cortisol biosynthesis results in reduced circulating leptin levels\textsuperscript{114}. As expected, weight loss was followed by a metabolically beneficial increase in serum adiponectin.

\textit{In summary}, weight loss after gastric bypass surgery was followed by metabolically favorable reductions in \(11\beta\)HSD1 and leptin gene expression in the SAT depot, which were in turn linked to lower leptin and increased adiponectin levels in the circulation. A significant reduction of \(11\beta\)HSD1 expression in adipose tissue due to surgically induced weight loss suggested that up-regulation of this enzyme was a consequence, rather than a cause, of obesity.

\textbf{STUDY III}

In this study, we aimed to longitudinally evaluate the effects of an HFD on glucocorticoid metabolism in the adipose tissue and liver of female Sprague-Dawley rats.

\textit{Gene expression analysis in adipose tissue}

Differences in SAT \(11\beta\)HSD1 gene expression between the diet groups throughout the experiment are presented in Figure 7. After two weeks, \(11\beta\)HSD1 was more highly expressed in rats fed HFD than in controls. However, the increase of \(11\beta\)HSD1 mRNA levels (observed at week 2) in the HFD group gradually decreased, and from week 16 onwards \(11\beta\)HSD1 mRNA levels were significantly lower in the HFD group than in the controls (diet effect; \(P < 0.05\)). Therefore, a short-term HFD had an enhancing effect on SAT \(11\beta\)HSD1 expression levels compared with CD, while a long-term HFD had an opposite, suppressive effect. Notably, in contrast to the dynamic changes of SAT \(11\beta\)HSD1 expression seen in the HFD group, \(11\beta\)HSD1 expression in the CD group was rather equal.

A suppressive effect of both short- and long-term HFDs on \(11\beta\)HSD1 activity in adipose tissue (with no changes in gene expression) have been previously reported in rodents\textsuperscript{87,88,94}, and suggest the possibility of post-transcriptional \(11\beta\)HSD1 inactivation. The adipose tissue depot-specific role of an HFD on \(11\beta\)HSD1 expression/activity is of major interest.
We did not observe any effect of HFDs per se on 11ßHSD1 expression in the OmAT depot (although there were significant changes with time; P < 0.001 in both groups, data not shown). However, other studies have reported a down-regulation of 11ßHSD1 activity in visceral/omental, epididymal and peri-renal depots.

![Figure 7. 11ßHSD1 gene expression in SAT depot. (HFD – black squares, solid line; CD – white squares, dotted line)](image)

The present results support observations that long-term HFDs have a suppressive effect on 11ßHSD1 expression/activity in adipose tissue. This longitudinal investigation revealed, for the first time, dynamic changes in 11ßHSD1 gene expression where both time and diet are crucial determinants in the regulation of 11ßHSD1 gene expression in adipose tissue.

We also investigated whether changes in SAT 11ßHSD1 expression could be explained by putative changes in H6PDH. Indeed, H6PDH gene expression followed the same pattern as 11ßHSD1, and genes correlated significantly in both groups (P < 0.0001 for the HFD group, and P < 0.001 for the CD group). It has been suggested that H6PDH activity is dependent on intracellular glucose levels. However, as the composition of the diets did not change during the experiment, the reasons for H6PDH changes in the HFD group are unclear and needs further investigations.

Increased FA uptake and storage in adipose tissue in the context of obesity have been associated with altered PEPCK activity. Glucocorticoids have been shown to regulate PEPCK in fat and liver. Indeed, 11ßHSD1 and PEPCK correlated positively in SAT and OmAT depots in both dietary groups. This suggests that the link between 11ßHSD1 and PEPCK is independent of diet, weight, and adipose tissue depot. Further studies are needed to clarify the role of glucocorticoids with respect to PEPCK in different tissues, possibly at different stages of obesity.
Hepatic glucocorticoid metabolism

In obesity, the inactivation of hepatic 11ßHSD1 has been suggested as a protective response to avoid tissue overexposure to glucocorticoids. We found an immediate, strong, and persistent suppressive effect of HFD on hepatic 11ßHSD1 expression, reflecting observations in idiopathic human obesity and rodent models of obesity. Hepatic 11ßHSD1 expression was constantly down-regulated throughout the experiment in the HFD group compared with the control group, which emphasizes the effect of diet (P < 0.001) on 11ßHSD1 expression levels in the liver. In the CD group, hepatic 11ßHSD1 expression decreased gradually during the experiment (time effect, P < 0.01; Fig. 8). These time-related changes in the CD group point to putative changes in hepatic glucocorticoid metabolism due to age/development, and express the need for better knowledge about peripheral glucocorticoid metabolism in the context of aging under normal biological conditions.

![Figure 8. Hepatic 11ßHSD1 gene expression according to the diet (HFD – black squares, solid line; CD – white squares, dotted line)](image)

The gene expression of enzymes involved in glucocorticoid clearance (A-ring reductases) was lower in the HFD than in the control group (diet effect, P < 0.001). This finding is reasonable, as HFD led to the down-regulation of 11ßHSD1 in both SAT and liver. Previous findings, with higher expression levels during high-fat feeding or in idiopathic human obesity, suggest that glucocorticoid clearance in the context of high-fat feeding is regulated differently depending on the presence or absence of obesity.

Body weight & serum analyses

We hypothesized that chronic high-fat feeding would lead to weight gain, disturbed peripheral glucocorticoid metabolism, and unfavorable metabolic outcome. In contrast to previous reports, our HFD-fed rats did not gain more weight than control animals. This might be because those studies used rodents with a known susceptibility to obesity.
and its metabolic consequences. Because HFD did not induce weight gain in our model, we were able to investigate the role of diet per se, whereas in other studies it has been difficult to independently differentiate between the effects of increased fat mass (obesity) and diet (diet composition/energy intake) on metabolic phenotype.

Even without weight changes, we observed disadvantageous effects of the HFD on serum metabolic markers. Increased leptin and decreased adiponectin levels were observed in the HFD group compared with the CD group (diet effect: $P < 0.05$ for leptin and $P < 0.001$ for adiponectin). This is of primary interest, as this adipokine pattern has repeatedly been associated with an unfavorable metabolic balance, which is in turn linked to an increased risk of developing type 2 diabetes and cardiovascular disease in humans$^{1,16,117}$.

In this model we chose to use Sprague-Dawley rats, a strain known for interindividual variability, which might provide a better reflection of the heterogenous genetic background in the human population$^{131}$. Moreover, we used female rats because the majority of human studies are performed in women, while rodent studies primarily tend to use males. Additionally, because previous experiments had only taken into account single time points when examining the effect of HFDs on glucocorticoid metabolism, we decided to complement this information by increasing the time resolution. Therefore, we performed a longitudinal study, following the effects of different diets over time.

In summary, we report dynamic changes in 11βHSD1 expression in the SAT depot during high-fat feeding in female rodents, with a concomitant down-regulation of hepatic 11βHSD1, increased serum leptin, and decreased serum adiponectin levels; however, weight was unaffected.
GENERAL DISCUSSION

‘Life expectancy has been improving for centuries; advances in hygiene, science, public health and medicine have allowed longer and more productive lives.

Obesity threatens to undo many of these gains...’

[D. Haslam, 2006]

The main challenge when studying obesity is to constantly keep in mind the complexity of this condition. Most cases of obesity can be explained by lifestyle, with excessive caloric intake and lack of physical activity. However, genetic background can play a significant role when explaining incidences of obesity\textsuperscript{132,133}, although a very limited number of individuals with monogenic obesity have been reported (e.g. leptin deficiency)\textsuperscript{134,135}. Some medications are known to affect weight (including glucocorticoids, antipsychotics, antidepressants, and some forms of contraceptives). It is important to mention that the studies included in this thesis are focused on idiopathic obesity.

CENTRAL FAT ACCUMULATION – SAT VS. VAT

While peripheral fat accumulation has been suggested to be relatively safe, visceral adiposity has been named as particularly detrimental to health\textsuperscript{9,10,136}. The effects of glucocorticoids are determined by the availability of the hormone and its receptors. Glucocorticoid receptors are highly expressed in VAT\textsuperscript{137}, and it was therefore suggested that circulating glucocorticoids may have a greater impact in the VAT on general metabolic responses (i.e., insulin sensitivity, lipolysis) and on the expression and release of different adipokines through the portal vein to the liver\textsuperscript{138}. However, while circulating glucocorticoid levels remain unaltered or even slightly decreased in idiopathic obesity, the local tissue concentrations of glucocorticoids are dysregulated by the enzymatic interconversion of active and inactive hormones\textsuperscript{31}. Here, adipose tissue depot-specific 11ßHSD1 activity comes into the picture. Almost uniformly, with only one exception\textsuperscript{38}, higher 11ßHSD1 expression/activity in the SAT depot has been reported in obese people compared with normal-weight controls, regardless of sex\textsuperscript{40-42,45,47,48,50,139}.

Studies regarding adipose tissue depot-specific differences in cortisol metabolism in idiopathic obesity are few and partly conflicting, as obesity and its related complications have been associated with increased 11ßHSD1 gene expression/activity in both SAT and VAT\textsuperscript{45,48,49,83}, suggesting that both depots may be involved in metabolic alterations. We report that severely obese women have higher 11ßHSD1 expression in SAT but higher activity in the OmAT depot. However, despite the higher enzyme activity in the visceral depot, 11ßHSD1 in SAT correlated with measures of central fat accumulation.
and blood pressure, while no associations were found for OmAT 11ßHSD1. This central difference highlights the importance of the subcutaneous depot.

Moreover, SAT and VAT depots have been shown to contribute differently to the circulating free FA pool, and it has been suggested that SAT may have a dominant role\(^{140}\). Unfortunately, we did not have more detailed biochemical characteristics (e.g., circulating triglyceride/free FA levels, HDL/LDL, etc.) and therefore were unable to evaluate the contribution of different depots per se, as well as depot-specific 11ßHSD1 activity, to the overall lipid profile.

The importance of the SAT depot regarding metabolic disturbances may be related to the finding of higher leptin and SREBP expression in the SAT depot than the OmAT depot. Leptin production in SAT correlates with circulating levels\(^{141}\) (which in our case likely means increased serum levels), and therefore an increased risk of developing cardiovascular disease\(^ {117,118,142}\). SREBP has previously been shown to associate inversely with obesity\(^ {119,120}\) and to be expressed at a higher level in SAT than in VAT\(^ {122}\). We did not observe any association between SREBP and different measures of obesity or 11ßHSD1; however we confirm that in severely obese women this enzyme is expressed at higher levels in SAT than in OmAT, indicating putatively higher rates of de novo lipogenesis. Interestingly, PEPCK (the enzyme responsible for FA re-esterification) was more highly expressed in the OmAT depot than the SAT depot, which might suggest a disturbance in the FA-triglyceride cycle within OmAT depot. Notably, glucocorticoids have been reported to negatively regulate PEPCK in adipose tissue in vitro\(^ {17}\), while we report positive associations between 11ßHSD1 expression and PEPCK expression in both fat depots in severely obese women.

Despite depot-specific differences in glucocorticoid metabolism, adipogenesis, and FA homeostasis, it is quite clear that both SAT and VAT depots can contribute significantly to unfavorable metabolic profiles. We have also demonstrated that gastric bypass-induced weight loss is accompanied by a four-fold decrease in intra-abdominal adipose area, while the subcutaneous adipose area decreased by approximately one-half. For comparison, subcutaneous fat accumulation was almost three times higher than intra-abdominal accumulation. This finding may be very important for the reduction of obesity-related morbidity and mortality after gastric bypass surgery. However, it is important to remember that subcutaneous fat deposition is virtually limitless and to ignore its role in the development of obesity-related complications would therefore be thoughtless.
**11ßHSD1 & OBESITY – CAUSE VS. CONSEQUENCE**

As most of the features of Cushing’s syndrome are reversible upon removal of glucocorticoid excess, the question of whether peripheral glucocorticoid concentrations due to weight changes are reversible is of primary interest. We found that significant weight reduction was followed by a dramatic decrease in 11ßHSD1 expression within the SAT depot, suggesting that overexpression of this enzyme in adipose tissue in severely obese women is a consequence rather than a cause. It would be of major interest to investigate putative gastric bypass surgery-induced changes in glucocorticoid metabolism in the VAT depot, as well as in the liver.

Although weight loss was followed by a favorable reduction of 11ßHSD1 (and leptin) in SAT, a concomitant increase in serum adiponectin levels, and improved insulin sensitivity, one should keep in mind that the patients in this study remained overweight even after significant weight loss. Under no conditions can these results be extrapolated to the physiology of normal-weight individuals.

The major limitation of Studies I & II is the absence of control groups. Lean and overweight (non-operated and matched for BMI after gastric bypass surgery) control groups would allow us to learn more about the linkage between alterations in 11ßHSD1 expression/activity (in adipose tissue and liver) and obesity. Additionally, it would be interesting to observe whether dietary interventions and surgery have the same effects on peripheral glucocorticoid metabolism and overall metabolic profile. A recent report that demonstrated sustained hepatic 11ßHSD1 activity in obese patients with type 2 diabetes suggests that intervention studies including these patients would be of particular interest.

**LOST IN TRANSLATION**

Despite the available data, many questions remain unanswered when attempting to explain the linkage between obesity and 11ßHSD1. Human studies are valuable and important, but take a long time to perform and are very expensive. Different rodent models have been used to investigate this link. The overexpression of 11ßHSD1 in adipose tissue and liver leads to a metabolic syndrome, regardless of whether or not fat accumulation increases. Meanwhile, high-fat feeding in rodents leads to obesity and a disturbed metabolic profile, but down-regulates 11ßHSD1 expression/activity in adipose tissue and liver.

Because only cross-sectional/single-time point information was available regarding the effects of obesity/HFD on peripheral glucocorticoid metabolism in both humans
and rodents, we decided to complement previously published data by performing a longitudinal study to follow the effects of different diets in rats over time. We found that a short-term HFD had an up-regulatory effect, while a long-term HFD had a down-regulatory effect on SAT 11ßHSD1 expression levels compared with control rats. Interestingly, HFD had an immediate, strong, and persistent suppressive effect on hepatic 11ßHSD1. This finding mirrors observations in idiopathic human obesity and rodent models of obesity, but concomitantly raises the question of whether the down-regulation of hepatic 11ßHSD1 is secondary to obesity, as has been suggested. As there were no changes in hepatic 11ßHSD1 expression over time in the HFD group, the observed down-regulation can be explained by diet.

Although there was no difference in body weight between the dietary groups, the HFD led to an unfavorable adipokine pattern with increased circulating leptin and decreased adiponectin levels. Therefore, in our model we were able to investigate the role of diet (over time), compared with previous rodent studies in which it was difficult to independently evaluate the effects of increased fat mass (obesity) and diet (diet composition/energy intake) on metabolic phenotype.

Therefore, feeding rodents an HFD may lead to two different outcomes: (1) obesity and down-regulation of 11ßHSD1 in adipose tissue, suggesting that the development of obesity may be independent of changes in adipose tissue 11ßHSD1 level; and (2) down-regulation of 11ßHSD1 in fat without affecting weight, suggesting that alterations in peripheral glucocorticoid metabolism can occur regardless of absence of obesity. In both scenarios, HFD has a down-regulatory effect on hepatic 11ßHSD1 (reflecting findings in human obesity). Comparing rodents to humans, high-fat feeding/obesity is clearly associated with a disturbed metabolic serum profile and has similar effects on 11ßHSD1 in the liver, but different effects on 11ßHSD1 in adipose tissue.

Obesity is a state of disturbed functionality of adipose tissue that results in increased risk of developing type 2 diabetes, cardiovascular disease, and cancer. The mechanisms underlying obesity are unclear; however, disturbances in peripheral glucocorticoid metabolism due to alterations in 11ßHSD1 expression/activity have been put forward as a putative key player. The results from this thesis add further pieces of information in this very complex puzzle.
SUMMARY & CONCLUSIONS

- Severe obesity is accompanied by a metabolically adverse increase of 11ßHSD1 in adipose tissue, but a decrease of 11ßHSD1 in the liver. SAT 11ßHSD1, but not OmAT 11ßHSD1, was an independent predictor for central fat accumulation. Severe obesity was accompanied by disturbances in peripheral glucocorticoid metabolism, as well as by major changes in FA recycling pathways in adipose tissue.

- Weight loss after gastric bypass surgery was followed by metabolically favorable reductions in 11ßHSD1 and leptin gene expression in the SAT depot, which were linked to reduced leptin and increased adiponectin levels in the circulation. A significant reduction of 11ßHSD1 expression in adipose tissue due to surgically-induced weight loss suggested that up-regulation of this enzyme was a consequence, rather than a cause, of obesity.

- In female rodents, chronic feeding of an HFD induces dynamic changes in 11ßHSD1 expression in the SAT, with concomitant down-regulation of hepatic 11ßHSD1, increased serum leptin, and reduced serum adiponectin; however, weight was unaffected.

In conclusion, weight changes and alterations in peripheral glucocorticoid metabolism are closely linked. Severe human obesity is accompanied by increased 11ßHSD1 in adipose tissue, but decreased hepatic 11ßHSD1, while weight loss is followed by down-regulation of SAT 11ßHSD1. Moreover, HFD has a significant effect on the 11ßHSD1 expression pattern in adipose tissue and liver, even without affecting weight.
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[Bo Kaspers Orkester; Söndag i sången (1993).]

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