Estrogen and Glucocorticoid Metabolism

Thérése Andersson
Sometimes we all have bad days, when everything feels far away...

Salem Al Fakir
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Background: Cardiovascular disease (CVD) is the leading cause of death among women in Sweden. The risk of CVD increases rapidly after the menopause. A major contributing factor may be the redistribution of adipose tissue, from the peripheral to central depots, associated with menopause. This change in body composition is commonly attributed to declining estrogen levels but may also be affected by tissue-specific alterations in exposure to other steroid hormones, notably glucocorticoids – mainly cortisol in humans. Indeed, adipose tissue-specific overexpression of the glucocorticoid-activating enzyme 11β-hydroxysteroid dehydrogenase type 1 (11βHSD1) induces central obesity, insulin resistance and hypertension in mice. Interestingly, estrogen may regulate this enzyme. The aim of this thesis was to investigate putative links between estrogen and glucocorticoid activation by 11βHSD1. Materials and Methods: 11βHSD1 expression and/or activity in adipose tissue and liver, and adipose estrogen receptor α and β (ERα and ERβ) gene expression, were investigated in lean pre- and postmenopausal women and ovariectomized rodents with and without estrogen supplementation. In lean women measures of 11βHSD1 were correlated to risk markers for CVD. The association between adipose 11βHSD1 and ER mRNA expression was investigated in both lean women and rats and in an additional cohort of obese premenopausal women. In vitro experiments with adipocyte cell lines were used to explore possible pathways for estrogen regulation of 11βHSD1. Results: Subcutaneous adipose tissue transcript levels and hepatic activity of 11βHSD1 were higher in postmenopausal vs. premenopausal women. In rodents, estrogen treatment to ovariectomized rats decreased visceral adipose tissue 11βHSD1, resulting in a shift towards higher subcutaneous (vs. visceral) 11βHSD1 mRNA expression/activity. Increased adipose and hepatic 11βHSD1 were associated with increased blood pressure and a disadvantageous blood lipid profile in humans. We found significant positive associations between 11βHSD1 and ERβ transcript levels in adipose tissue. The in vitro experiments showed upregulation of 11βHSD1 mRNA expression and activity with estrogen or ERβ-agonist treatment at low (corresponding to physiological) concentrations. Conclusions: Our studies show for the first time increased local tissue glucocorticoid activation with menopause/age in women. This may contribute to an increased risk of CVD. Estrogen treatment in rodents induces a shift in 11βHSD1 activity towards the subcutaneous adipose tissue depots, which may direct fat accumulation to this metabolically “safer” depot. The in vitro studies suggest that low-dose estrogen treatment upregulates 11βHSD1 via ERβ. In summary, estrogen - glucocorticoid metabolism interactions may be key in the development of menopause-related metabolic dysfunction and in part mediate the beneficial effects of postmenopausal estrogen treatment on body fat distribution.

Key words: 11β-Hydroxysteroid dehydrogenase type 1, estrogen, cortisol, adipose tissue, liver, menopause, ovariectomy, adipocyte, estrogen receptor β.
LIST OF PAPERS

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


*Joint first authorship

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### ABBREVIATIONS

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<th>Abbreviation</th>
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<tr>
<td>11βHSD</td>
<td>11β-hydroxysteroid dehydrogenase</td>
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<td>3αHSD</td>
<td>3α-hydroxysteroid dehydrogenase</td>
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<tr>
<td>AUC</td>
<td>Area under the curve</td>
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<tr>
<td>ACTH</td>
<td>Adrenocorticotropic hormone</td>
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<td>BMI</td>
<td>Body mass index</td>
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<tr>
<td>CRH</td>
<td>Corticotropin-releasing hormone</td>
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<td>CVD</td>
<td>Cardiovascular disease</td>
</tr>
<tr>
<td>CYP19A1</td>
<td>Cytochrome P450, family 19, subfamily A, polypeptide 1, (Aromatase)</td>
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<tr>
<td>DPN</td>
<td>diarylpropionitrile</td>
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<tr>
<td>ER</td>
<td>Estrogen receptor</td>
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<td>ERKO</td>
<td>Estrogen receptor knock-out</td>
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<td>GR</td>
<td>Glucocorticoid Receptor</td>
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<td>H6PDH</td>
<td>Hexose-6-phosphate dehydrogenase</td>
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<td>HDL</td>
<td>High density lipoprotein</td>
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<td>HOMA-IR</td>
<td>Homeostasis model assessment of insulin resistance</td>
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<td>HPA</td>
<td>Hypothalamic-pituitary-adrenal</td>
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<td>HSL</td>
<td>Hormone sensitive lipase</td>
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<td>IGF-1</td>
<td>Insulin-like growth factor-1</td>
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<td>IL-1β</td>
<td>Interleukin-1β</td>
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<tr>
<td>IL-6</td>
<td>Interleukin-6</td>
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<tr>
<td>Ka</td>
<td>Appearance rate constant</td>
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<td>LDL</td>
<td>Low density lipoprotein</td>
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<td>Lipoprotein lipase</td>
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<td>mRNA</td>
<td>Messenger ribonucleic acid</td>
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<td>NADPH</td>
<td>Nicotinamide adenine dinucleotide phosphate</td>
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<td>PPARγ</td>
<td>Peroxisome proliferator activated receptor γ</td>
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<td>SAT</td>
<td>Subcutaneous adipose tissue</td>
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<td>SGBS</td>
<td>Simpson-Golabi-Behmel Syndrome</td>
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<td>THF</td>
<td>Tetrahydrocortisol</td>
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<td>THE</td>
<td>Tetrahydrocortisone</td>
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<tr>
<td>TNF-α</td>
<td>Tumour necrosis factor-α</td>
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<td>VAT</td>
<td>Visceral adipose tissue</td>
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INTRODUCTION

Cardiovascular disease (CVD) is the number one cause of death in both men and women in Sweden [1]. Women are generally affected ten years later in life than men, and the risk of myocardial infarction increases substantially in women after their mid-fifties [2]. The age for this risk increase coincides with the time for the menopausal transition and hence, with a decline in estrogen levels.

Menopause is associated with an increase in abdominal fat accumulation, which gives rise to a more central fat distribution [3-6]. Because CVD is associated with a central fat distribution, this may partly explain the elevated CVD risk with menopause [7, 8]. It is important to gain a better understanding of the mechanisms that underlie these changes in fat distribution in order to prevent disease and prolong life in women. This thesis focuses on investigating one putative way of altering fat distribution - the regulation of glucocorticoid metabolism by estrogen.

Fat distribution, metabolic risk, and menopause

Obesity has escalated into a world health problem, and incurs great costs on both the individual and the community. The development of obesity is governed by several factors, including environmental, like eating habits and lifestyle, and genetics. Studies with twins have shown that about 50% of obesity can be explained by genes [9, 10], but only a few cases of monogenic obesity have been described; e.g., leptin deficiency [11]. In the vast majority of cases, obesity is a polygenic trait, which makes it difficult to target [11].

Although obesity has a negative impact on health, not all obese individuals have health problems [12]. Some factors appear to increase the susceptibility to metabolic disease in an obese person. One of those factors is adipose tissue distribution [8]. Adipose tissue can be localised subcutaneously, or intra-abdominally, where it surrounds the internal organs (for further information on different adipose tissue depots see the Methods section). The two primary phenotypes of fat storage are referred to as female (or gynoid) fat distribution, with subcutaneous fat accumulation on the buttocks, hips and thighs, and male (or android) fat distribution with central fat accumulation, particularly intra-abdominally. Obesity with central fat accumulation has been shown to be particularly detrimental to health [13, 14]. In
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contrast, obesity with peripheral fat accumulation appears to be relatively benign. Thus, gender differences in adipose tissue distribution may partly explain the difference in risk for CVD observed between men and premenopausal women [15]. The importance of adipose tissue distribution as a risk factor for metabolic disease is acknowledged in the criteria for the metabolic syndrome. The metabolic syndrome is a summary of risk factors that predisposes for CVD and the development of type 2 diabetes. Recently, in a joint statement by a number of health organisations, the metabolic syndrome was redefined as the presence of at least three out of the five following risk factors: central obesity (increased waist circumference), high serum triglycerides, low HDL levels, insulin resistance, and high blood pressure (or current medical treatment for any of these conditions) [16].

Menopause and older ages are, as mentioned above, associated with increased abdominal fat deposition. Indeed, studies have shown that menopause/ovariectomy in humans and rodents, respectively, are associated with increased abdominal adipose tissue accumulation [3-5, 17, 18]. This redistribution of adipose tissue with menopause is generally attributed to the decline in circulating estrogen levels that comes with failing ovarian function, and can thus be counteracted with estrogen or hormone replacement therapy [17-22]. However, the exact mechanisms underlying estrogen regulation of body fat distribution have not been elucidated. Estrogen has multiple effects on both peripheral and central levels, in rodents estrogen reduced food intake and increased energy expenditure [23-25]. In adipose tissue derived from women, estrogen increased proliferation in preadipocytes [26, 27]. In adipose tissue derived from ovariectomised rats, estrogen increased proliferation in subcutaneous preadipocytes and increased differentiation in parametrial preadipocytes [28]. Estrogen also affects the balance of fat accumulation vs. mobilisation, although studies have presented contradictory findings. In human adipose tissue, estrogen increased the levels of anti-lipolytic α2A-adrenergic receptors specifically in subcutaneous adipose tissue (SAT) [29], thereby favouring subcutaneous fat deposition. However, in mature subcutaneous adipocytes derived from women, studies have shown that high estrogen concentrations increased lipolysis by stimulating hormone sensitive lipase (HSL) expression [30]. In vivo, estrogen has been found to reduce lipoprotein lipase (LPL) activity in rodents and humans [31, 32], and similarly, in vitro, inhibit LPL expression in subcutaneous adipocytes derived from women [30]. In contrast, lower estrogen concentrations stimulated LPL expression in the same study. Thus, this dose-dependent regulation of LPL may be involved in the increased fat accumulation in postmenopausal women.
The increased abdominal fat deposition with menopause/older age is associated with increased risk of metabolic disease. The incidence of CVD is higher in postmenopausal than in premenopausal women \cite{15, 33}. Over the years of menopausal transition there is also a worsening of the blood lipid profile, with increased triglycerides, and the risk of hypertension increases \cite{7, 34-37}. This paints a picture not unlike the definition of the metabolic syndrome. In fact, menopause has been suggested to be an independent predictor of the metabolic syndrome in women \cite{38}, although this is controversial \cite{7, 39}. However, the leap from idiopathic abdominal obesity and central fat distribution in postmenopausal women to disturbances in local glucocorticoid metabolism was inspired by similarities with the syndrome of chronic hypercortisolism, Cushing’s syndrome.

**Cortisol**

**General effects**

Cortisol exerts its effects mainly via the activation of glucocorticoid receptors (GR), which are nuclear receptors that act as ligand-gated transcription factors \cite{40}. GRs activate and repress genes involved in many processes, including inflammation, gluconeogenesis, and adipocyte differentiation \cite{40, 41}. The beneficial anti-inflammatory and immunosuppressive effects of pharmacological cortisol treatments have been recognised for over half a century \cite{42}. Biologically, cortisol is essential for energy homeostasis during fasting and stress, and it exerts effects on the intermediary metabolism of glucose, lipids, and protein \cite{43}. However, high-dose and long-term cortisol treatments have several negative side effects. This is evident in patients with Cushing’s syndrome, which is caused by chronic excess of endogenous (due to a cortisol- or ACTH-producing tumour) or exogenous cortisol. Cushing’s syndrome manifests as central obesity, hypertension, glucose intolerance, and hyperlipidemia \cite{43}.

The role of cortisol in the stress response explains some of its metabolic effects - it maintains or increases blood glucose and increases blood pressure in order to protect the energy supply to the brain. To that end, cortisol induces insulin resistance in both adipose tissue and muscle by impairing insulin-dependent glucose uptake \cite{41, 44-46}. It also increases gluconeogenesis in the liver, which could further aggravate insulin resistance \cite{43}. To fuel gluconeogenesis, cortisol has catabolic effects on
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muscle and adipose tissue, where it increases lipolysis to elevate circulating free fatty acids.

**Effects on fat distribution and lipid metabolism**

**Adipose tissue**

Cortisol has multiple effects that regulate lipid metabolism and impact adipose tissue distribution. In preadipocytes, glucocorticoids impede proliferation and induce differentiation [47-49]. This activity of glucocorticoids is commonly used in cell culture, where, together with insulin, it induces differentiation of preadipocytes into mature adipocytes [50]. Glucocorticoids have been found to affect lipid accumulation and lipid turnover in a depot-specific manner. In isolated rat adipocytes, cortisol induces lipolysis by stimulating HSL [51]. However, in isolated human adipocytes, cortisol has been shown to reduce lipolysis [52]. This apparent discrepancy between species may actually arise from gender or depot differences, because the rat adipocytes were extracted from epididymal fat (male rats), and the human adipocytes were mainly derived from the subcutaneous depot of women. Glucocorticoids also increase LPL activity *in vitro*, with more marked effects on omental adipose tissue (VAT) compared to SAT [53-55]. This may be related to the higher GR expression in VAT compared to SAT [56, 57]. In addition, cortisol has been found to modulate the effects of insulin and thereby cause increased food intake [58-60]. Taken together, these effects of cortisol might, in part, explain the central obesity in Cushing’s syndrome.

**Liver**

Glucocorticoids have lipogenic effects on the liver that may contribute to metabolic disease. Glucocorticoid therapy is known to stimulate hepatic fat accumulation; it has also been shown that high doses induce steatohepatitis [41, 61]. However, few studies have investigated the possible pathways for these effects. Glucocorticoid injections in mice reduced lipolysis and increased biosynthesis of intracellular triglyceride in hepatocytes; this most likely contributed to hepatic fat accumulation [62]. In addition, glucocorticoids increased the activity of acetyl-coenzyme A carboxylase, which is important for fatty acid synthesis [63]. Glucocorticoids also promote apolipoprotein B secretion in the liver, which is associated with increased risk of atherosclerosis [64]. Furthermore, hepatic fat accumulation has been strongly associated with hepatic insulin resistance; this might be related to glucocorticoid-mediated stimulation of the enzymes involved in gluconeogenesis [65, 66].
Regulation and metabolism

Secretion and regulation
Cortisol is the main glucocorticoid in humans (corticosterone in rodents), and is secreted from the two innermost layers of the adrenal cortex. This secretion is driven by the feed forward mechanisms in the hypothalamic-pituitary-adrenal (HPA) axis [43] (Figure 1). Thus, corticotropin-releasing hormone (CRH) from the hypothalamus stimulates the pituitary to release adrenocorticotropic hormone (ACTH), which, in turn, stimulates cortisol secretion from the adrenal glands. Secreted cortisol then exerts negative feedback on both the pituitary and hypothalamus to regulate circulating levels. Cortisol levels vary diurnally with the highest levels in the morning and the lowest levels at midnight.

Figure 1. Regulation of cortisol in the circulation and in peripheral tissues by the Hypothalamic-Adrenal-Pituitary-axis and 11βHSD1.
Hepatic catabolism
Glucocorticoids are extensively catabolised and conjugated in the liver. Only small amounts of free unchanged glucocorticoids are excreted in the urine \[^{67}\]. Most glucocorticoids are irreversibly inactivated by A-ring reductases, 5α- and 5β-reductase, and then further converted into 5α- and 5β-tetrahydrocortisol (THF) and 5β-tetrahydrocortisone (THE) by 3α-hydroxysteroid dehydrogenase (3αHSD, Figure 2) (5α-reductase is also present in adipose tissue \[^{68}\].) Some glucocorticoids are converted to cortols and cortolones, and a small fraction is degraded in other pathways.

![Figure 2. Schematic overview of the catabolism of glucocorticoids in the liver.](image)

11β-Hydroxysteroid dehydrogenases
The striking similarities between the metabolic syndrome and Cushing’s syndrome has lead to the speculation that cortisol might also be important in idiopathic obesity. Obesity is associated with increased cortisol production and a subtle perturbation in negative feedback of the HPA-axis; however, this appears to be compensated by increased cortisol clearance and excretion \[^{69-72}\]. Thus, circulating cortisol levels are typically normal in idiopathic obesity, which is also the case in the postmenopausal state; furthermore, early morning cortisol levels may even be slightly reduced in obesity, with reduced diurnal variations \[^{73-76}\]. This apparent paradox can be resolved by understanding that cortisol availability is affected by
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both circulating levels and the local tissue activity of pre-receptor enzymes 11β-hydroxysteroid dehydrogenase types 1 and 2 (11βHSD1 and 2). The 11βHSDs can interconvert glucocorticoids between the active and inactive states (cortisol/cortisone in humans and corticosterone/11-dehydrocorticosterone in rodents, respectively).

11βHSD2

11βHSD2 converts active cortisol to inactive cortisone, primarily in the typical aldosterone target tissues, like the kidney [77]. This protects mineralocorticoid receptors (MR) from nonspecific cortisol binding. Inhibition or loss of 11βHSD2 function leads to the syndrome of apparent mineralocorticoid excess (AME), where increased MR signalling leads to sodium retention, hypertension, and hypokalemia [77]. Renal conversion of cortisol to cortisone is the major source of cortisone in humans [78]. Recently, a few studies also suggested a role for 11βHSD2 in adipose tissue [79-81]. 11βHSD2 was primarily expressed in the stromal fraction of adipose tissue, however, at levels several-fold lower than adipose 11βHSD1 expression.

11βHSD1

11βHSD1 is expressed in many tissues, but at high levels in liver, adipose tissue, and lung [67, 82, 83]. 11βHSD1 mainly converts inactive cortisone into active cortisol in vivo and in intact cells in culture. This has been shown repeatedly in different tissues, particularly liver and adipose tissue [84-89]. Further studies have shown that 11βHSD1 is the sole enzyme responsible for the reactivation of glucocorticoids in mice; thus 11βHSD1 null mice can not convert 11-dehydrocorticosterone into corticosterone [90, 91]. However, in tissue homogenates or purified preparations, when the enzyme is liberated from its intracellular location within the endoplasmic reticulum membrane [92], 11βHSD1 functions as a bi-directional enzyme that performs both inactivation of cortisol and activation of cortisone [84, 93]. Although 11βHSD1 was first purified from rat liver and cloned in the late 1980’s [93, 94], the two HSD isoforms were not properly characterised until the mid 1990’s [95, 96]. Thus, the dual function of 11βHSD1 caused some confusion in the interpretation of results in the early studies; for example, mRNA expression conflicted with activity data [97]. The reversibility of 11βHSD1 activity in tissue homogenates is due to the fact that the reductase activity of 11βHSD1 is highly dependent on the co-factor, nicotinamide adenine dinucleotide phosphate (NADPH) [98-101]. NADPH is supplied
by the enzyme hexose-6-phosphate dehydrogenase (H6PDH), which is co-localised with 11βHSD1 inside the cell \[102\]. In fact, several studies have demonstrated that 11βHSD1 and H6PDH are co-expressed in several tissues and co-localised within the endoplasmic reticulum \[99, 101, 103\]. Furthermore, H6PDH knock-out mice were unable to activate glucocorticoids and had increased dehydrogenase activity, this suggested that H6PDH is vital for 11βHSD1 reductase activity \[104\].

11βHSD1 in obesity

**Humans**

Interestingly, 11βHSD1 is regulated in obesity; this provides a putative explanation for the similarities between Cushing’s syndrome and the metabolic syndrome/idiopathic obesity. Most studies in humans show upregulation of 11βHSD1 expression and activity in SAT with obesity (whole tissue samples or adipocytes) in both men and women \[75, 105-111\]. Recently, this was also confirmed by *in vivo* microdialysis in men \[112\]. However, one study has reported no differences in 11βHSD1 transcription levels in whole tissues of lean and obese humans \[113\]. There is less data for 11βHSD1 in VAT; however, most \[110, 111, 114, 115\], but not all \[113, 116\], studies showed a positive correlation with BMI and high 11βHSD1 mRNA expression and/or activity in VAT from obese women.

**Rodent models**

In rodent models of monogenic obesity (leptin deficiency/receptor dysfunction), 11βHSD1 activity was upregulated in the intra-abdominal fat depots compared to lean controls \[117, 118\]. In mice models of obesity there was also a down-regulation of 11βHSD1 in SAT \[118\]. The effect of increased 11βHSD1 activity in adipose tissue has also been studied in transgenic mice that overexpressed 11βHSD1 selectively in adipose tissue \[119\]. This was achieved by expressing 11βHSD1 under the control of the promoter/enhancer region of the adipocyte fatty acid binding protein gene. These mice displayed an obese phenotype with increased intra-abdominal adipose tissue depots, enlarged adipocytes, hypertension, dyslipidemia, and glucose intolerance \[119, 120\]. Adipose tissue-specific 11βHSD1 overexpression thus replicated the metabolic syndrome; this suggested a link between obesity, the Cushing’s syndrome, and cortisol. These findings showed that an increase in adipose corticosterone, in spite of normal circulating levels, was sufficient to produce metabolic alterations. In further support of a role of 11βHSD1 in metabolic disease and obesity, several studies
showed that 11βHSD1 knock-out mice resisted the metabolically detrimental effects of high fat feeding \[91, 121, 122\]. Moreover, adipose tissue-specific glucocorticoid inactivation in 11βHSD2 overexpressing mice, provided resistance to metabolic disease and reduced fat depot weight gain on a high-fat diet compared to wild type mice \[123\].

Together, these data indicate that cortisol levels are increased in adipose tissue in obesity due to increased activity of 11βHSD1. This could, in turn, contribute to further adipose tissue accumulation. Indeed, it has been found that 11βHSD1 levels correlated with cell size in both visceral and subcutaneous adipose tissue depots \[80, 110, 114\].

**11βHSD1 in weight loss and weight gain**

Although it seems clear that 11βHSD1 is elevated in adipose tissue in obesity, it is not so clear whether 11βHSD1 levels are regulated with changes in adiposity. Thus in rodents, obesogenic diets have produced, at least initially, decreased levels of 11βHSD1 in SAT, VAT, and liver \[124-126\]. However, one study, in mice on a high-fat diet, showed that 11βHSD1 activity returned to normal in all tissues over time \[125\]. This is consistent with findings in SAT after weight loss in men; however, the same study found upregulation of 11βHSD1 when studying isolated subcutaneous adipocytes \[127\].

Surprisingly, food restriction also initially induced lower VAT 11βHSD1 levels in rodents \[128\]. However, once again, the 11βHSD1 expression returned to normal over time, and after two weeks, did not differ from that observed in controls. The circulating corticosterone levels were increased in these animals, and this was also found during weight gain \[126, 128\]. In line with this, omental adipose 11βHSD1 was not elevated in Cushing’s syndrome (where circulating glucocorticoid levels are elevated) \[129\]. This was thought to be due to a down-regulation of the enzyme as a reaction to long-term hypercortisolism.

All of the studies on diet alterations were conducted during, or shortly after, the dietary regimes were changed. Therefore, it is likely that the combined effects of obesity/leanness and the stress related to long-term dieting were investigated rather than the effects of weight loss or gain *per se*. Consequently, it may be more relevant
to study the effects of sustained changes in adiposity. Thus, a study in men, where weight was maintained for three months after the initial weight loss, showed down-regulation of 11βHSD1 expression in SAT\textsuperscript{[130]}.

11βHSD1 - implications for fat distribution

Rodent studies
Rodent studies that directly compare 11βHSD1 levels in different adipose tissue depots are essentially lacking. However, studies of 11βHSD1 activity in obese animals vs. lean animals implied that VAT expressed higher levels of 11βHSD1 than SAT\textsuperscript{[117, 118]}.

Human studies
In contrast to rodent data, the literature on 11βHSD1 in different adipose tissue depots in humans is extensive and quite heterogeneous. Importantly, the results are most likely influenced by differences in the stage/environment of the adipose material (preadipocytes/mature adipocytes/whole tissue), the health and sex of the subjects, and the level of the analyses (mRNA/protein/enzymatic activity).

Studies on 11βHSD1 mRNA expression in preadipocytes have shown higher levels in VAT than SAT\textsuperscript{[107, 131]} . Studies in mature adipocytes found higher 11βHSD1 activity in VAT than SAT in some cases, and no differences in other cases\textsuperscript{[86, 107, 131]}. In contrast, one study of 11βHSD1 protein expression, which was conducted in adipocytes from lean subjects, showed higher 11βSHD1 levels in SAT than VAT\textsuperscript{[131]} . In whole tissue samples, the picture gets even more complicated; the majority of studies showed no differences in 11βHSD1 transcript levels between the two fat depots\textsuperscript{[80, 110, 111, 115, 131-133]} . However, a few studies found higher 11βHSD1 levels in VAT than SAT\textsuperscript{[133-135]} and yet another found lower 11βHSD1 levels in VAT than SAT\textsuperscript{[116]} . Interestingly, it has been reported diurnal variations in 11βHSD1 expression with opposite patterns in SAT and VAT\textsuperscript{[136]} . Thus, this may contribute to the discrepancies in reported depot-specific 11βHSD1 levels.

Most investigations of 11βHSD1 activity, regardless of the adipose fraction, have found higher levels in VAT than SAT\textsuperscript{[85, 86, 114, 116, 132]}, with one exception that found no difference\textsuperscript{[80]} . The divergence between 11βHSD1 mRNA expression and activity in whole tissue may be related to the activity of H6PDH. In one study, H6PDH tended to be higher in VAT than SAT, thereby possibly giving rise to more abundant
supply of the cofactor NADPH \cite{132}. However, others have not found any depot differences in H6PDH levels \cite{80, 115, 133}. In summary, \(11\beta\text{HSD1}\) activity in whole tissue and mRNA expression in preadipocytes appear to be higher in VAT than SAT.

Of note, most studies have focused on subjects with varying BMI, but some studies that investigated healthy, normal weight, or pregnant subjects found that \(11\beta\text{HSD1}\) mRNA levels were higher in VAT than SAT\cite{133, 134}. However, these depot differences were not present in subjects with metabolic alterations or obesity. Furthermore, obese diabetic patients had higher \(11\beta\text{HSD1}\) mRNA levels in SAT compared to obese, healthy controls \cite{133}. SAT \(11\beta\text{HSD1}\) has been shown to correlate with reduced insulin sensitivity and elevated blood pressure \cite{106, 132, 137}. It is therefore most likely that variations in obesity and metabolic status within cohorts have impacted on the results from many studies that examined differences in \(11\beta\text{HSD1}\) expression between adipose tissue depots.

### \(11\beta\text{HSD1} in the liver\)

\(11\beta\text{HSD1}\) is highly expressed in the liver, where it is responsible for most of the activation of orally administered cortisone \cite{82}. Studies that investigated the proportional contributions of VAT, SAT, and liver to the circulating glucocorticoid pool found that splanchnic (VAT and liver) cortisol release was almost entirely accounted for by the liver \cite{138, 139}. In contrast to findings from adipose tissue in obesity, hepatic \(11\beta\text{HSD1}\) activity, measured by indirect methods, was downregulated with increasing adiposity \cite{75, 108, 140}. Recently, in vitro studies confirmed previous results by showing negative correlations between hepatic \(11\beta\text{HSD1}\) activity and measures of visceral obesity \cite{116}.

Similarly, in rodent models, obese animals exhibited decreased \(11\beta\text{HSD1}\) activity/expression in the liver compared to lean animals \cite{117, 118, 141}. However, in obese db/db mice (which lack a functional leptin receptor), hepatic \(11\beta\text{HSD1}\) expression correlated positively with insulin resistance \cite{142}. Furthermore, transgenic mice with liver-specific overexpression of \(11\beta\text{HSD1}\) has illustrated the powerful effects of increased glucocorticoid levels in the liver \cite{143}. In those mice, the \(11\beta\text{HSD1}\) gene was placed under the control of the promoter/enhancer region of the Apolipoprotein E gene. These mice had normal circulating corticosterone levels, but 2- to 5-fold increases in hepatic \(11\beta\text{HSD1}\) activity. Transgenic mice developed a
fatty liver, mild hyperinsulinemia, dyslipidemia, and hypertension, but retained normal body weight. Hence, it has been suggested that, in obesity, decreased hepatic 11βHSD1 levels may be a compensatory mechanism that protects against the hyperinsulinemic effects of elevated glucocorticoid released from adipose tissue [144].

**11βHSD regulation in adipose tissue and liver**

Adipose tissue was long considered as merely an inactive tissue for energy storage. Today, we know that it is quite the opposite; adipose tissue is an active endocrine organ that generates and releases several hormones, cytokines, and inflammatory factors [145, 146], including estrogen, leptin, and TNF-α (Figure 3). Many of these factors exert effects on central pathways in addition to peripheral pathways, and thus, may simultaneously regulate appetite and energy expenditure. In the quest for regulators of 11βHSD1, several studies have investigated the regulatory potential of adipokines, other endogenous factors, and synthetic agents. (Table 1) [67].

![Figure 3. Hormones and factors secreted from adipose tissue.](image-url)
Inflammatory factors
Inflammatory factors like IL-1β and TNF-α have been shown to exert upregulatory effects on adipose 11βHSD1, at least in vitro \cite{147, 148}. It is now well established that obesity is associated with a state of low-grade inflammation and macrophage infiltration \cite{149, 150}. Macrophages secrete pro-inflammatory factors, and the degree of macrophage infiltration is related to adipocyte size and adiposity in both mice and humans \cite{150}. Thus, it is possible that inflammation and macrophage accumulation in adipose tissue contribute to elevated 11βHSD1 in obesity.

PPARγ agonists, insulin and IGF-1
Insulin sensitising PPARγ agonists are reported to cause reductions in 11βHSD1 expression and activity both in vivo and in vitro \cite{151-153}. However, this result has been disputed on the basis that it might be a secondary effect \cite{154}. In vivo studies showed that insulin increased 11βHSD1 activity in SAT from humans and in epididymal adipose tissue from rats \cite{155, 156}. In vitro, in 3T3 cells, the results diverged, some studies showed up regulation, and others showed down-regulation of 11βHSD1 expression/activity with insulin treatment and also down-regulation with IGF-1 treatment \cite{147, 156-158}. In hepatocytes in vitro, insulin had down-regulatory or no effect on 11βHSD1 levels \cite{142}.

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<td>Estrogen</td>
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<td>Testosterone</td>
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<td>Progesterone</td>
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INTRODUCTION

**Leptin**
Leptin is an adipokine secreted by adipocytes \[^{167}\]. The circulating levels of leptin are in proportion to adipose tissue mass. Thus, leptin signals the state of energy stores in the body. Leptin acts on the central nervous system to reduce food intake. It also increases energy expenditure. Studies have shown that leptin can also upregulate 11βHSD1 expression in hepatocytes from the leptin deficient obese ob/ob mice \[^{166}\]. However, leptin did not affect 11βHSD1 activity in human hepatocytes \[^{147}\]. In adipose tissue, leptin appeared to have sex-specific effects; in omental preadipocytes, 11βHSD1 expression was increased in men, and decreased in women, with leptin treatment \[^{147, 163}\].

**Adiponectin**
Adiponectin is an adipokine secreted by mature adipocytes \[^{167}\]. However, unlike leptin, circulating adiponectin levels decrease with obesity. Low adiponectin levels are associated with insulin resistance, dyslipidemia, and atherosclerosis. Consistent with this, serum adiponectin levels decrease with increasing adipose 11βHSD1 expression in humans \[^{114, 133}\]. In contrast, local adiponectin mRNA expression in SAT increased with increasing 11βHSD1 expression in obese subjects \[^{133}\]. Direct effects of adiponectin on 11βHSD1 remain to be investigated.

**Glucocorticoids**
In humans, cortisol upregulates 11βHSD1 expression in adipose tissue \[^{79, 85, 113}\]. In rodent liver, glucocorticoids have been found to both decrease (in vivo) \[^{159}\] and increase (in vitro) \[^{142}\] 11βHSD1 levels.

**Testosterone and progesterone**
To date, the effects of testosterone and progesterone on 11βHSD1 activity have only been studied in liver, with somewhat mixed results. In gonadectomised rats, testosterone treatment increased hepatic 11βHSD1 activity \[^{164}\], but in intact animals, testosterone had no effect on hepatic 11βHSD1 activity \[^{159}\].

Only a few studies have investigated progesterone regulation of 11βHSD1 in liver. The results indicate that there may be species differences in the action of progesterone. Progesterone inhibited 11βHSD1 activity in human hepatocytes, but no effects were observed in rodents \[^{142, 165}\]. In another study, progesterone down-regulated 11βHSD1 activity in rodent liver \[^{159}\]. In summary, the effect of progesterone on hepatic 11βHSD1 activity is unclear, but may be down-regulatory.
Estrogen
Several studies point to a regulatory effect of estrogen on 11βHSD1. First, sexual dimorphism was observed in the excretion of glucocorticoid metabolites; this indicated higher whole-body 11βHSD1 activity in men than in women \[168-170\]. However, one study suggested that 11βHSD1 activity may be higher in postmenopausal women than in men \[171\]. Furthermore, in rat liver, 11βHSD1 activity was higher in males than in females \[97,161\]. Similarly, 11βHSD1 expression was higher in subcutaneous adipose tissue derived from men than in that derived from women (however, this gender difference disappeared in obese subjects) \[111\]. More subtle effects of endogenous estrogen were investigated in premenopausal women during different phases of the menstrual cycle \[172\]. However, no differences were found that might indicate changes in whole body or hepatic 11βHSD1 activity.

The *in vivo* effects of estrogen treatment on 11βHSD1 levels have been investigated in several rodent studies. These studies all showed that estrogen down-regulated 11βHSD1 in both VAT and liver \[18,97,159-161\].

Results from *in vitro* studies in both rat and human tissues are more heterogeneous, and remain inconclusive. In male rats estrogen down-regulated 11βHSD1 expression in isolated visceral adipocytes (and 3T3-cells) \[162\] but did not regulate 11βHSD1 expression in whole VAT \[18\]. In humans, estrogen upregulated 11βHSD1 mRNA expression in omental preadipocytes from women \[163\], but showed no regulation of 11βHSD1 expression in VAT and omental preadipocytes from men, in subcutaneous preadipocytes from women or in SAT from women \[18,79,148,163\].

Estrogen

*Production in women*
Estrogen compounds occur in three main forms: estradiol, estrone, and estriol \[173\]. Estradiol is the most biologically active form, and it is also the most abundantly produced form in premenopausal women. In postmenopausal women estrone is the most abundantly produced form. During the fertile years of a woman’s life, estrogen is synthesised in the ovaries under the control of the hypothalamic – pituitary – gonadal - axis. Circulating estrogen levels vary during the menstrual cycle, with low levels during menstruation in the early follicular phase and higher levels during the
late follicular and luteal phases (Figure 4). Progesterone levels also vary, with low levels during the follicular phase and high levels during the luteal phase.

![Figure 4. Variations in circulating hormones during the menstrual cycle.](http://creativecommons.org/licenses/by-sa/3.0/deed.en)

With menopause and the cessation of ovarian function, circulating estrogen levels drop and estrogen production is taken over by extragonadal tissues, mainly adipose tissue and liver. Estrogens are formed by the aromatisation of androgens with the enzyme aromatase (CYP19A1). Estradiol is formed from testosterone, and estrone is formed from androstenedione \(^{[173, 175]}\). In women, aromatase gene expression increases in SAT after menopause \(^{[176]}\). Similarly, in rats, aromatase activity in both subcutaneous adipose tissue and liver increases with time after an ovariectomy \(^{[177]}\). Aromatase activity is induced by several factors, e.g., glucocorticoids, IL-6, and TNF-\(\alpha\) \(^{[86, 178, 179]}\). In addition, estrone is inter-converted to estradiol by the enzyme 17β-hydroxysteroid dehydrogenase \(^{[175]}\). Hence, local estrogen levels in a given tissue in postmenopausal women are the net result of the abundance of an androgen substrate and the activity of metabolising enzymes. Thus, the circulating levels of estradiol in postmenopausal women merely reflect the peripheral production rate rather than affect local tissue levels, which can be considerably higher \(^{[175]}\).

**Signalling**

Estrogen signalling is mediated via \(\alpha\) and \(\beta\) estrogen receptors (ER). These nuclear receptors act as ligand-regulated transcription factors to induce or inhibit gene expression by binding to estrogen response elements or interacting with other
transcription factors [180, 181]. ERs may also have effects via non-genomic or ligand-independent pathways. ERα and ERβ often have opposite effects and when co-expressed ERβ may inhibit ERα signalling. Furthermore, ERs form dimers and act as homo- or heterodimers. Thus, the balance between the two receptor variants may be important for estrogen signalling, and this balance differs among tissues [181].

Both ERα and ERβ is expressed in a wide range of tissues. ERα is highly expressed in classic estrogen target tissues, like the female reproductive system, and e.g., in liver, heart and kidney; ERβ is found e.g., in ovary and lung [182]. The ERs are also co-expressed in a number of tissues and both ERα and ERβ are expressed in adipose tissue [183, 184]. In humans, ERβ expression was shown to be higher in SAT than VAT. In contrast, ERα expression was similar in SAT and VAT depots [184]. However, when receptor expression was determined in different cell types, only ERα expression was found in preadipocytes while expression of both receptors was found in mature adipocytes; moreover, ER expression was generally higher in mature adipocytes than preadipocytes [185, 186]. In rats, both receptors appeared to be expressed at overall higher levels in VAT vs. SAT [187]. Studies with ER knock-out mice (ERKO) suggested that ERα signaling has anti-obesity effects while ERβ signaling has an adipogenic effect [188]. Hence, αERKO mice, which retain intact ERβ signalling, had increased body weight, adipose tissue mass, adipocyte size, and adipocyte numbers [24, 189]. In addition, αERKO mice had considerably elevated plasma estrogen, which can potentially further enhance ERβ signalling [190]. Furthermore, ovariectomies in female αERKO mice caused weight reduction, which could be counteracted with estrogen treatment [188, 191]. On the other hand, βERKO mice were normal weight [189]. Double ER knock-out mice and aromatase knock-out mice lack estrogen signalling altogether, due to ER or estrogen deficiencies, respectively. These mice become obese with increased intra-abdominal fat depots [189, 192].
OBJECTIVES

The similarities between Cushing’s syndrome and the metabolic syndrome inspired the notion that glucocorticoids could be involved in idiopathic obesity. It has since been shown that adipose-specific 11βHSD1 overexpression could induce central obesity in rodents and that adipose 11βHSD1 was upregulated in human obesity [75, 108, 119, 120]. The facts that the Cushing’s and metabolic syndromes, and menopause are characterised by / associated with central obesity [3-6, 16, 43], and that 11βHSD1 could potentially be regulated by estrogen [18, 159, 162, 163] led us to hypothesise that 11βHSD1 might be important in the adipose tissue redistribution and the metabolic changes associated with aging in women.

The aim of this thesis was to investigate putative links between estrogen and glucocorticoid activation by the enzyme 11βHSD1.

Specific aims:

- To compare 11βHSD1 transcript levels and activity in SAT and liver between pre- and postmenopausal women of normal weight. (Paper I)

- To relate measures of 11βHSD1 to risk factors for CVD and diabetes. (Paper I)

- To investigate the effect of estradiol treatment on 11βHSD1 activity and gene expression in SAT, VAT, and liver in ovariectomised rats. (Paper II)

- To investigate further the putative estradiol regulation of 11βHSD1 gene expression via estrogen receptors by association- and in vitro studies. (Paper III)
SUBJECTS AND METHODOLOGICAL COMMENTS

This section is intended to describe briefly the study groups and methods that were central to these studies. Detailed descriptions are found in the different papers.

Study groups

These studies included two human female study groups and one female rodent study group. In addition, two different preadipocyte cell-lines were used for the *in vitro* studies: mouse 3T3-L1 cells and human Simpson-Golabi-Behmel Syndrome (SGBS) cells. All women gave written informed consent before entering the studies, and all studies were approved by the local Ethical Committees for animal or human studies, as applicable.

*Lean, healthy pre- and postmenopausal women (Papers I and III)*

Pre- and postmenopausal women (N=23 each) were recruited for this study. All subjects were healthy, normal weight, and did not use tobacco or hormonal contraceptives. Anthropometric data were collected at the initial health

![Diagram of study design for pre- and postmenopausal women]

Figure 5. Study design for the study of pre- and postmenopausal women.
checkup (Figure 5). Menopausal status or menstrual phase were confirmed by measuring plasma estradiol and progesterone levels. Postmenopausal women had reported no menstruation during the previous 12 months. Superficial, subcutaneous adipose tissue biopsies from the peri-umbilical region were taken under local anaesthesia. Hepatic cortisone-to-cortisol conversion tests were performed and urine was collected over 24 h. All procedures were performed on separate days. Venous blood samples were collected on all test days except in association with the urine collection. Premenopausal women were examined during both follicular and luteal phases of the menstrual cycle.

Ovariectomised rats with and without estradiol treatment (Paper II and III)

Eighteen Sprague-Dawley females were housed in standard laboratory cages (4 per cage) with free access to standard rat chow and water and lights on/off in a 12/12-hour cycle. Animals were handled for one week to ensure accommodation, then, an ovariectomy was performed and an estradiol or placebo pellet was implanted subcutaneously (Figure 6). Four weeks after the operation, rats were decapitated under stress-free conditions. Trunk blood, SAT, VAT (peri-renal), and liver samples were collected. The SAT and VAT depots were dissected and weighed. Samples from the SAT and VAT depots were also taken for cell size determinations.

![Figure 6. Study design for the rodent study.](image-url)
Obese, mostly premenopausal women (Paper III)
Thirty obese women, most premenopausal (three postmenopausal), were recruited from a series of patients undergoing gastric bypass surgery at the Örebro University hospital, Örebro, Sweden. Exclusion criteria were: body weight ≥ 160 kg, pregnancy, and untreated endocrine disorder. Women with well regulated thyroid disease, diabetes, hormonal contraceptives, and systemic gonadal hormone replacement therapy were accepted, but the medications were noted. Anthropometric data were collected on a separate occasion, at an initial health checkup. Blood samples and paired subcutaneous and visceral (omental) adipose tissue biopsies were taken peri-operatively, under general anaesthesia.

Anthropometric measurements
In human subjects, height and waist circumference measurements were recorded to the nearest 0.5 cm and weight was measured to the nearest 0.1 kg. Blood pressure was measured in the supine position with a mercury sphygmomanometer. Body fat percentage was estimated with bio-electric impedance analysis in lean women and with dual-energy X-ray absorptiometry in obese women.

In rats, body weight was recorded to the nearest gram and adipose tissue depot weight was recorded to the nearest 0.01 g.

Blood chemistry
Serum estrogen in papers I and II was measured by ultra-sensitive radioimmunoassays (CIS Bio International, France, and Diagnostics Systems Laboratories, TX, USA, respectively). All other blood parameters in the human studies were analysed with standard laboratory methods in the clinical laboratories at the Umeå University hospital, Umeå, Sweden or the Örebro University hospital, Örebro, Sweden.

Adipose tissue depots in humans and rodents
The major fat depots in humans are SAT and VAT. The main SAT depots is situated in the abdominal region (abdominal) and on the buttocks, hips and thighs (gluteo-femoral). Abdominal SAT can be further sub-divided into superficial subcutaneous and deep subcutaneous adipose tissues, separated by Scarpa’s fascia. VAT is the adipose tissues surrounding the internal organs in the abdominal cavity. The most
commonly investigated VAT in human studies is the omental adipose tissue, which tends to expand most of the VAT depots in obesity in humans.

Adipose tissue in rodents can be divided into the SAT and the intra-abdominal adipose depots. The latter consists of the retroperitoneal adipose tissue located dorsally in the abdominal cavity, the omental adipose tissue located on top of the stomach, the mesenteric adipose tissue, the peri-renal adipose tissue, and the gonadal adipose tissue depot located around the ovaries in the female and on the epididymis in the male.

**Cell size**

In paper II, subcutaneous and peri-renal adipocyte size was determined in single cell suspensions after collagenase digestion. Cells were photographed under a light microscope and cell size was measured with computerised image analysis software and expressed as the mean diameter of 100 cells.

**Measures of 11βHSD1**

*Urinary glucocorticoid metabolites – Whole body activity (Paper I)*

Urinary glucocorticoid metabolites were measured with electron impact gas chromatography mass spectrometry. As described in the introduction (Figure 3), cortisol and cortisone are, in part, metabolised by 5α- and 5β-reductases in adipose tissue and the liver. The whole body balance between 11βHSD1 and -2 activities have previously been estimated with the ratio of urinary \((5α-\text{THF} + 5β-\text{THF}) / (5β-\text{THE})\) \(^{[169-172]}\). However, this is a crude method for 11βHSD1 estimation, because it does not separate the activities of the 11βHSDs from the activities the A-ring reductases. Also, the ratio can be affected by other pathways for glucocorticoid degradation and the rate of excretion. The interpretation of the THF’s/THE ratio is aided by comparing the free cortisol/free cortisone ratio, which provides an appreciation of the 11βHSD2 activity in the kidney. Hence, if the cortisol/cortisone ratio does not differ, it is unlikely that it affected the results.

*Hepatic cortisone to cortisol conversion test (Paper I)*

Hepatic 11βHSD1 activity was estimated with a cortisone-to-cortisol conversion test. The participants ingested an oral dose of cortisone after an overnight dexamethasone suppression of endogenous cortisol production. The serum cortisol
levels were then measured every thirty minutes for four hours. This test is based on the fact that, with low endogenous production of cortisol, the first pass conversion of cortisone to cortisol in the liver provides a large contribution to serum cortisol after cortisone ingestion. Ideally, we would have been able to calculate $K_a$, the appearance rate constant, which is a more specific measure of hepatic 11βHSD1 activity than the area under the curve (AUC) or a crude serum cortisol value at a specific time point. The latter measurements are affected by both appearance rate and elimination, while $K_a$ gives a measure of the appearance rate only. However, as in the analyses of urinary glucocorticoid metabolites, individual differences in inactivation by 11βHSD2 could impact the $K_a$. In addition, the test assumes that all subjects have equal rates of cortisone absorption from the gut.

By combining the data from the measurement of urinary glucocorticoid metabolites and the hepatic cortisone-to-cortisol conversion test it is possible to add strength to the conversion test. Thus, if there are no differences in A-ring reduced metabolites it is unlikely that the activities of 5α- and 5β-reductase are affecting the conversion test data. Similarly, if the free cortisol/free cortisone ratio does not differ, 11βHSD2 is unlikely to affect the results.

**In vitro enzyme activity assay in homogenates - estimation of active protein (Papers I and II)**

In papers I and II, adipose tissue enzyme activity was measured in tissue homogenates with excess of the cofactor NADPH. This method measures the dehydrogenase activity of 11βHSD1, which is the preferred reaction direction for when the enzyme is liberated from the intracellular milieu (as discussed in the introduction) [94]. Tissues were homogenised and centrifuged. Protein concentration was determined with the Bradford technique and duplicate samples were incubated with excess cofactor and tritiated substrate for 24h (human adipose tissue, paper I), 1h (rat adipose tissue, paper II), or 30min (rat liver, paper II). In paper I, samples were withdrawn at four time-points. In paper II, only the indicated final time-points were sampled. Steroids were extracted and separated by thin-layer chromatography. The radiolabelled glucocorticoids were then detected by exposure to a tritium storage phosphor screen, scanned and quantified by computerized densitometry. Activity was expressed as the % conversion.

It should be noted that, because the cofactor, NADPH, and substrate, cortisol/corticosterone, were added in excess this method actually assessed the
amount of active 11βHSD1 protein, but not the \textit{in vivo} reductase activity. This has been recognised in the more recent studies that used this method \cite{118, 138, 193}. For convenience, the results of these analyses will be referred to as enzyme activity.

\textbf{In vitro enzyme activity assay in intact cells (Paper III)}

In cell cultures, 11βHSD1 activity was measured in the reductase direction in intact cells. Radiolabelled and cold corticosterone was added to the cultures, and triplicate reactions were incubated for 24h. Steroids were extracted from the incubation media, separated with high-performance liquid-chromatography, and quantified with on-line liquid scintillation counting. This method measured the \textit{in vivo} 11βHSD1 reductase activity, but is based on the assumption of free diffusion of glucocorticoids across the cell membrane.

\textbf{RNA extraction, cDNA synthesis and real time PCR (Papers I-III)}

RNA was extracted according to the manufacturer’s instructions with the Lipid Tissue RNasy kit (Qiagen, UK). The cDNA synthesis and real-time PCR were performed with TaqMan reagents (Applied Biosystems, CA, USA). Relative quantification was performed with the standard curve method. A standard curve made from adipose tissue was included on every reaction plate, this enabled comparisons between plates. The relative concentrations of the target gene and the endogenous control were inferred from the standard curves. In paper I, the endogenous control was chosen by comparing coefficients of variations and Normfinder stability values for three different genes, selected based on previous evaluations of endogenous controls in human adipose tissue \cite{194}.

\textbf{In vitro studies (Paper III)}

\textbf{SGBS and 3T3-L1 cells}

The human SGBS cells were originally isolated in 2001 from SAT derived from a male infant with Simpson-Golabi-Behmel Syndrome, a syndrome characterised by excess growth \cite{195}. These cells were found to be morphologically, biochemically, and functionally similar to human adipocytes \cite{196}. Compared to primary preadipocytes in culture, they grow well \textit{in vitro}, and retain the capacity for adipogenic differentiation for several generations \cite{195}. One of the great advantages of the SGBS cells is that they can be induced to differentiate in a chemically defined, serum-free medium \cite{197}. This allows a higher degree of control under experimental conditions, compared to e.g. 3T3-L1 cells, which are differentiated in
the presence of fetal calf serum. The fibroblast cell-line 3T3-L1 was originally derived from embryonic tissue from a Swiss mouse [198, 199]. 3T3-L1 cells differentiates to a adipocyte-like phenotype and are commonly used since more than 35 years [200].

**Manipulations**
For *in vitro* manipulations we used 17β-estradiol, and the synthetic ERβ-agonist diarylpropionitrile (DPN). The receptor-specificity of DPN is restricted to a limited concentration range, approximately between 1 and 10nM. In this range the binding preference for ERβ is more than 70-fold higher than for ERα [201]. In all experiments, we used phenol-red-free media in order to avoid the potential estrogenic effects of phenol-red [202].
RESULTS AND DISCUSSION

Details of the results can be found in the respective papers.

**Paper I**

In paper I, we aimed to determine whether pre- and postmenopausal women exhibited differences in the levels of 11βHSD1 in SAT, the *in vivo* 11βHSD1 activity in liver, and in the excretion of urinary glucocorticoid metabolites. Furthermore, we related measures of 11βHSD1 to risk factors for CVD. We chose to study healthy, normal weight individuals to avoid the influence of metabolic disease and obesity. In addition, we investigated both the follicular and luteal phases of the menstrual cycle in premenopausal women to evaluate the effects of hormonal fluctuations that occur during the menstrual cycle.

**Effect of menopausal status/age**

Postmenopausal women had higher SAT 11βHSD1 mRNA expression compared to premenopausal women in the follicular phase of the menstrual cycle (Figure 7A). There was a strong correlation between adipose 11βHSD1 enzyme activity and mRNA levels. However, we could not detect a difference in adipose enzyme activity between groups; at least partly due to a lack of statistical power with the limited number of samples available for these analyses.

Compared to follicular phase premenopausal women, postmenopausal women had higher serum cortisol levels at 30 min post oral cortisone in the hepatic conversion test. This suggested that they had higher 11βHSD1 activity in the liver. The analyses of urinary glucocorticoid metabolites, showed that postmenopausal women had higher \((5α-THF + 5β-THF)/5βTHE\) ratios than luteal phase premenopausal women. This indicated that postmenopausal women had an elevated whole body 11βHSD1 activity, consistent with elevations of both adipose and hepatic 11βHSD1 levels.

In total, our data suggested increases in both SAT and hepatic 11βHSD1 activities after menopause. This is in line with a previous study, where postmenopausal women had higher whole body 11βHSD1 activity, inferred from urinary metabolites, compared to men \(^{[171]}\); however, this is not a unanimous finding \(^{[169]}\). Of note, we were unable to separate the effects of age and menopausal status in this study.
Indications of estrogen regulation

Normal circulating cortisol levels but local increases in both SAT and hepatic cortisol reactivation seem to accompany menopause/older age in women. This picture differs from findings in idiopathic obesity where adipose 11βHSD1 was upregulated, but hepatic levels decreased compared to lean subjects \cite{75, 108}. Notably, the pre- and postmenopausal groups were well matched for BMI, which were roughly within the range of normal weight, and waist circumference did not differ between groups. Nevertheless, we found that waist circumference and menopausal status/older age both independently affected adipose 11βHSD1 expression.

Lower adipose and hepatic 11βHSD1 levels in premenopausal women compared to postmenopausal women indicates a down-regulatory effect of estrogen. This result concurred with earlier studies in rats \cite{18, 97, 160, 162}. Hence, the estrogen deficiency of postmenopausal women could lead to a relative upregulation of 11βHSD1 compared to levels found in premenopausal women. However, we did not find a significant correlation between SAT 11βHSD1 levels and serum estradiol levels; moreover, the negative correlation between serum estrogen and hepatic enzyme activity or urinary THF’s/THE did not persist after adjustments for menopausal group, waist circumference or BMI. In contrast, and quite unexpectedly, we found a positive association between 11βHSD1 levels and aromatase transcript levels in adipose tissue. This was independent of waist circumference, BMI, and menopausal status. Similar to a previous study \cite{163}, this suggested an upregulatory effect of estrogen in SAT, because higher aromatase expression indicates higher intra-adipose estrogen production. However, elevations in aromatase expression may be secondary to a rise in 11βHSD1 activity (and hence cortisol levels), because glucocorticoids induce promoter activity of the aromatase gene \cite{203}. In conclusion, these results do not provide a unified picture of estrogens effects on 11βHSD1 in women and further investigations are required.

Effect of menstrual phase

Previously, a study found no differences in urinary glucocorticoid metabolites or hepatic 11βHSD1 activity between the follicular and luteal menstrual phases in women \cite{172}. In contrast, we found a subtle, but significant increase in adipose 11βHSD1 mRNA expression in women in the luteal phase compared to women in follicular phase. Progesterone levels are high only in the luteal phase of the menstrual cycle, and therefore progesterone could be a potential cause for these differences. Progesterone might either upregulate 11βHSD1 or cancel part of the
RESULTS AND DISCUSSION

Estrogen effect. Although derivatives of progesterone have been shown to inhibit 11βHSD1 activity \(^{204}\), only a few studies investigated the potential regulation of 11βHSD1 by native progesterone. Hepatic 11βHSD1 activity decreased after progesterone treatment in vivo and in vitro \(^{159, 165}\). However, we did not detect any differences in hepatic 11βHSD1 activity during the different phases of the menstrual cycle. In summary, our results differed from previous studies and indicated that adipose 11βHSD1 expression is regulated during the menstrual cycle with higher levels in the luteal phase.

**Associations with metabolic risk**

Compared to premenopausal women, we found that postmenopausal women had significantly higher blood pressure and blood lipids, although both were within the normal ranges. It was previously shown that transgenic overexpression of 11βHSD1 in the liver or adipose tissue of mice could induce high blood pressure, dyslipidemia, and hyperinsulinemia/glucose intolerance \(^{119, 120, 143}\). Similarly, our study showed that, in women, hepatic 11βHSD1 activity (measured as serum cortisol levels at 30 min post oral cortisone) correlated positively with systolic blood pressure, total cholesterol and LDL cholesterol. Similar associations were found with adipose 11βHSD1 expression. However, no correlations were found between 11βHSD1 levels and serum insulin or glucose. In fact, postmenopausal women had lower fasting serum insulin levels and higher insulin sensitivity than premenopausal women, according to the HOMA-IR index. Insulin has been shown to have differential effects on 11βHSD1 activity in both adipose tissue and hepatocytes \(^{87, 155, 156}\). Therefore, in future studies, it will be interesting to include both lean and overweight postmenopausal women with varying degrees of insulin sensitivity/levels.

It should be noted that, by selecting normal weight postmenopausal women, we may have introduced a bias in the study; this is also suggested by the lower insulin levels in this group. Because there is generally a weight gain with age \(^{35}\), women who have remained lean after menopause are likely to have a beneficial genetic makeup and/or good diet and exercise habits. Indeed, macronutrient intake has been shown to affect 11βHSD1 \(^{205}\).

In summary, our data suggest that postmenopausal/older women have elevated 11βHSD1 levels in both SAT and liver. However, we did not find any convincing evidence for the role of estrogen in this regulation. Elevated 11βHSD1 was
associated with increased blood pressure and a less beneficial blood lipid profile. We therefore conclude that increases in adipose and hepatic 11βHSD1 with menopause/age could contribute to increased risk of hypertension and CVD, especially in combination with increased waist circumference.

Paper II
It has long been known that an ovariectomy leads to weight gain in rats and that subsequent estrogen administration can return body mass to normal [22]. Previous \textit{in vivo} studies on the estrogen regulation of 11βHSD1 in rats did not fully investigate the different adipose depots, and hepatic and adipose levels were only studied separately [18, 97, 159-161]. In paper II, we therefore extended the previous studies of the effects of \textit{in vivo} estradiol treatment on 11βHSD1 in ovariectomised rats to include SAT, VAT, and liver measurements in the same animals.

\textbf{Effect of estrogen treatment in vivo}
In agreement with previous studies in rats, we found that estrogen treatment in ovariectomised animals reduced 11βHSD1 mRNA levels and activity in both VAT and liver (Figure 7D) [18, 97]. However estrogen did not affect the SAT 11βHSD1 expression or activity. This resulted in a change in the balance between SAT and VAT 11βHSD1; estrogen shifted the balance from higher relative 11βHSD1 levels in VAT to higher expression and activity in SAT. This is a most interesting finding, because it implies that the change in body fat distribution with estrogen treatment in postmenopausal subjects may be partly mediated via regulation of 11βHSD1 [19-21].

However, estrogen treatment also resulted in leanness with smaller VAT depots, as expected. This reduction in body weight in the estrogen treated rats complicates the interpretation of the results. Others have attempted to circumvent this problem by pair-feeding ovariectomised rats with estrogen treated ovariectomised rats in order to attain a lean ovariectomised group [18]. These rats did not have an increased 11βHSD1 activity in VAT. However, this method may induce stress, due to the relative starvation in these animals, and stress can cause elevated corticosterone levels [128]. Of note, previous studies in rats have produced results similar to the human findings, with increased adipose 11βHSD1, but decreased hepatic 11βHSD1 levels in obesity [75, 108, 117, 125]. Thus, simultaneous increases in adipose tissue and
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Liver in obese ovariectomised animals argue for an additional effect of estrogen deficiency that cannot be explained by obesity alone.

There was no inter-group difference in adipocyte size in either the VAT or the SAT depots. Previous studies indicated that estrogen treatment in vivo would reduce cell size \[24, 206\]. However, in our study, the lack of change with estrogen administration, in combination with smaller VAT depots, suggested that adipocyte counts were lower in the estrogen treated animals. This should be confirmed in future studies. Consistent with human data, we found that adipocyte size correlated with 11βHSD1 mRNA expression in VAT in placebo treated/obese animals, independent of plasma estrogen levels and body weight \[110\]. VAT LPL transcript levels were also higher in the placebo treated animals and correlated with VAT 11βHSD1 activity. Elevated LPL levels would imply increased fat accumulation; this is consistent with the increased VAT observed in the ovariectomised rats. Furthermore, glucocorticoids are known to increase LPL activity, and estrogen to down-regulate LPL activity \[207\]. Interestingly, insulin resistance is strongly associated with increased cell size. Therefore, it would be of interest to investigate insulin sensitivity in these animals.

In summary, estrogen treatment in ovariectomised rats reduced both hepatic and VAT 11βHSD1 levels. Combined with the lack of effect on SAT 11βHSD1 activity, this caused a relative shift in the balance of 11βHSD1 activity between adipose tissue depots in favour of higher levels in SAT. This regulation could potentially drive the accumulation of fat in the SAT depot that is typically observed with estrogen treatment.

**Paper III**

In paper III, we further investigated potential regulation of 11βHSD1 by estrogen. This had not previously been investigated in adipose tissue. Signalling via ER α and ERβ would be the most likely pathway for direct regulation. We therefore analysed the mRNA expression of ERα and ERβ in adipose tissue and correlated this to 11βHSD1 expression in three different study groups; including lean pre- and postmenopausal women; obese, mostly premenopausal women; and ovariectomised rats with and without estrogen treatment. To extend our initial findings, we performed in vitro studies in differentiated adipocytes.
**ER expression in adipose tissue**

Differences relating to estrogen status
SAT ERα expression did not differ between pre- and postmenopausal women, but SAT ERβ expression was higher in postmenopausal compared to premenopausal women (Figure 7B and C). Previous studies did not find any differences between pre- and postmenopausal women in either SAT or VAT ER expression \[176, 208\]. The cause for the discrepancy with previous findings may be due to differences in BMI and age, or low statistical power in the previous studies.

In the ovariectomised rats, estrogen treatment increased ERα expression in VAT and ERβ expression in both adipose depots (Figure 7E). This could contribute to a change in the balance between ERα and ERβ towards a higher relative ERβ in the SAT depot. If ERα inhibit, and ERβ facilitate, fat accumulation, this change in ER balance would contribute to increased fat deposition in SAT with estrogen treatment \[182, 188\].

Depot-specific differences
ERα mRNA expression was higher in VAT than in SAT in premenopausal obese women (Figure 7H). This result differs from previous findings in normal weight subjects, where expression levels were equal between depots \[184, 185\]. Conversely, in ovariectomised rats with estrogen-treatment, ERα expression was higher in SAT than VAT (Figure 7E). Notably, although circulating estrogen levels are higher in these rats (cf. premenopausal women), they are also lean; the differences observed with humans may therefore be related to obesity.

On the other hand, ERβ expression was higher in SAT than VAT irrespective of species (Figure 7F and I). These findings concurred with human studies of ERβ expression in whole tissue \[184\], but not with ERβ expression in mature human adipocytes, where no difference was detected between SAT and VAT \[185\]. In addition, a previous study also showed that ERβ expression in intact (not ovariectomized) animals was higher in VAT than SAT; this may indicate an effect of ovariectomy per se, which cannot be reversed with estrogen treatment \[187\].
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Figure 7. Gene expression of 11βHSD1, ERα, and ERβ in lean women (A-C), ovariectomised rats with and without estrogen treatment (D-F), and obese women (G-I). *P<0.05, **P<0.01 and ***P<0.001 vs. VAT or premenopausal group in women, and vs. the ovariectomised group in rats, #P<0.05, ###P<0.001 vs. VAT. SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; ER, estrogen receptor.

ER associations with 11βHSD1

Interestingly, there was a strong positive correlation between ERβ and 11βHSD1 expression in all three study groups. Hence, 11βHSD1 and ERβ expressions were correlated in SAT from pre-and postmenopausal women (VAT not examined), in SAT and VAT from obese women, and in SAT, but not VAT, from estrogen treated rats. This implied, in contrast to the findings in paper II, that estrogen would
upregulate 11βHSD1 via ERβ in adipose tissue, regardless of menopausal status, obesity, or species. This result was consistent with the positive correlation to aromatase expression observed in paper I. Conversely, 11βHSD1 expression did not correlate to ERα expression in any study group. This suggested that the down-regulation of 11βHSD1, observed in this and other previous studies, was not mediated by direct ERα- or ERβ-signalling, but rather, by other pathways; e.g. the 11βHSD1 down-regulation may have occurred secondary to the weight reduction caused by circulating estrogen. This notion is supported by a recent study in 3T3-L1 cells and rodents, which concluded that the reduction of 11βHSD1 activity with estrogen treatment was not mediated via ER-signalling [162].

**In vitro estrogen regulation of 11βHSD1**

Preliminary tests in mouse 3T3-L1 adipocytes indicated that low doses of estrogen, that were comparable to physiological concentrations, and treatment with the ERβ agonist, DPN, increased adipocyte 11βHSD1 expression (although not significantly) [209]. These results were confirmed in human SGBS cells, where low concentrations of DPN upregulated 11βHSD1 mRNA, protein, and enzyme activity. Importantly, DPN is specific for ERβ in the lower concentration range (~1 to 10nM) [201], i.e. at concentrations similar to those that induced 11βHSD1 expression. At higher concentrations of DPN the binding to ERα increases. Earlier in vitro studies reported mixed findings with estrogen manipulations in adipocytes. In rodent VAT or 3T3-L1 cells, estrogen either reduced or did not affect 11βHSD1 levels [18, 162]. In human studies, either no effect [18, 79, 148, 163] or upregulation of 11βHSD1 was observed with estrogen treatment [163]. The discrepancy between our results and those from other studies may be due to a number of differences in experimental conditions; e.g., cell differentiation stage, gender- and depot-specificities etc. However, upon examining the previous studies more carefully, estrogen dose appeared to be of crucial importance. Thus, our in vitro studies showed maximal upregulation of 11βHSD1 expression at 0.1 and 1nM estrogen, but the mRNA expression decreased at higher estrogen concentrations. Similarly, 11βHSD1 upregulation was observed in female samples by Dieudonné et al. (2006), who used similarly low estrogen concentrations [163], but no regulation of 11βHSD1 expression was observed by others that used higher estrogen doses [18, 79].
In summary, our findings suggested that 11βHSD1 was upregulated by physiological concentrations of estrogen in adipose tissue via ERβ. Our data also indicated that this could occur irrespective of menopausal status/age, obesity, adipose depot, and species (rats and humans).
GENERAL DISCUSSION & FUTURE DIRECTIONS

Based on the current knowledge, several different mechanisms may underlie the regulation of fat distribution and accumulation in women. The complex nature of the putative hormones and factors involved can give rise to multiple actions. For example, cortisol and estrogen, which are focused on in this thesis, have multiple effects, both in the local adipose tissues and in the central nervous system. We propose that tissue-specific interactions between cortisol and estrogen can contribute to the regulation of fat distribution and that this regulation merits further investigation.

At first glance, the results of the studies presented here may appear contradictory. The first two papers suggested that circulating estrogen down-regulated both hepatic and adipose 11βHSD1; conversely, paper III suggested that low dose estrogen treatment at the local level could increase adipose 11βHSD1 activity via ERβ-signalling. The key factors that might explain these apparently contradictory results include hormonal status, effect of obesity, and differences between adipose tissue depots.

Estrogen status or adiposity – what governs 11βHSD1?
One important unanswered question is whether hormonal status (circulating or local estrogen levels) or adiposity is the main contributor to the results in papers I and II. Thus, two separate scenarios appear plausible regarding estrogen and tissue-specific glucocorticoid metabolism: 1) low circulating estrogen elevates adipose 11βHSD1, which then promotes obesity, or 2) low circulating estrogen leads to obesity with concomitant increases in adipose 11βHSD1. Conversely, high circulating estrogen could either, a) reduce 11βHSD1 which would contribute to leanness, or b) primarily promote leanness with the secondary effect of reduced 11βHSD1 levels.

In support of the first alternative, we found a positive correlation between mRNA expressions of 11βHSD1 and ERβ in adipose tissue and an up-regulatory effect of low estrogen levels on 11βHSD1 in vitro. Furthermore, increased aromatase gene expression was associated with higher 11βHSD1 activity and expression in paper I; this suggested the importance of local estrogen production in adipose tissue. With
increased ERβ expression in SAT in the postmenopausal state this regulation may be of relatively greater importance in postmenopausal women.

However, in accordance with alternative 2), the higher adipose 11βHSD1 levels observed in ovariectomised animals and postmenopausal women may also be caused by differences in fat mass per se, as suggested previously [18]. Although the postmenopausal women in our study were of normal weight according to the BMI’s, the significantly higher body fat percentage indicated that they may have undergone a relative increase in fat mass, presumably abdominal, with menopause or age. An increase in fat mass alone could, potentially upregulate 11βHSD1, due to an increased release of adipokines [147, 148, 163]. Estrogen deficiency in rats causes obesity and, conversely, treating estrogen-deficient rats with estrogen restores them to normal weight [22]. The use of high fat feeding or caloric reduction to create control groups may be questionable, because diet has been reported to affect 11βHSD1 levels [126, 127]. Therefore, it is not entirely straightforward to separate the effects of estrogen and obesity in an experimental situation. To offset these limitations in our experiments, it may have been helpful to include control groups of SHAM-operated rats with and without estrogen treatment. Similarly, in our human study, these limitations may have been offset by comparing the lean women to groups of obese pre- and postmenopausal women, and postmenopausal women on hormone replacement therapy.

In the premenopausal state, with high circulating estrogen levels, the net effects of circulating estrogen on adipose tissue in women are the promotion of female fat distribution and leanness [17-20]. These are achieved by local regulation of lipolysis, lipogenesis, energy expenditure, and central effects on food intake [23-25, 29-32]. This argues for alternative b), because a reduction of adiposity, in parallel with the arguments presented above, is per se associated with lower 11βHSD1 levels. However, our data could also imply that down-regulation of 11βHSD1 may be part of the anti-obesity effects of estrogen, in agreement with alternative a). A recent in vitro study found that adipose 11βHSD1 activity is inhibited by estrogen by a non-ER-dependent, non-transcriptional pathway [162]. Hence, based on the evidence presented here, and by others [162], we suggest that high circulating estrogen is associated with reduced adipose and hepatic 11βHSD1 activity; but, that this down-regulatory effect of estrogen is unlikely to be mediated by direct ER-signalling. Lastly, and perhaps most likely, hormonal status and adiposity may have a combined effect on 11βHSD1 activity. This is implied by the finding that adipose
11βHSD1 expression was affected independently by both menopausal status/age and waist circumference in lean women.

Estrogen regulation of hepatic 11βHSD1 requires different considerations. Results from studies on estrogen regulation of hepatic 11βHSD1 are much more consistent than the data from adipose tissue studies. Both in vivo and in vitro studies have showed that estrogen down-regulated 11βHSD1 activity in the liver [97, 159-161]. Furthermore, most previous studies showed elevated adipose expression and reduced hepatic 11βHSD1 activity in obese compared to lean individuals [75, 108, 117]. This disagrees with our findings in estrogen-deficient rats and humans where 11βHSD1 levels were concomitantly increased in both adipose tissue and liver. Thus, we propose that estrogen exposure had an additional effect in the liver that could not be explained by adiposity alone; i.e., estrogen may directly lower 11βHSD1 activity in the liver. To test this hypothesis, it would be interesting to investigate the potential regulation of 11βHSD1 via ER’s in liver. Moreover, studies in mouse models showed that overexpression of 11βHSD1 in the liver gave rise to risk factors for CVD and diabetes, including fatty liver, dyslipidemia, insulin resistance, and hypertension, but without general obesity [143]. This association showed similarities with observations in humans with myotonic dystrophy [210]. Thus, some of the adverse effects of estrogen-deficiency that lead to increased risk of CVD may be mediated by upregulation of hepatic 11βHSD1 activity.

**Depot-specific estrogen-regulation of 11βHSD1 in adipose tissue – fine tuning fat accumulation?**

Interestingly, our results suggested a role for 11βHSD1 in estrogen regulation of fat distribution. In ovariectomised rats, estrogen-treatment resulted in a shift in 11βHSD1 expression from higher levels in VAT to higher levels in SAT. This was accompanied by increased transcript levels of ERβ in SAT and ERα in VAT. Of note, ER’s dimerise after ligand binding and ERβ can inhibit ERα-signalling. Thus, the relative expression of ERα and ERβ may be important for the final local effects of estrogen-treatment [181]. Hence, if ERβ mediates adipogenic effects [182, 188], higher relative levels of ERβ in SAT than VAT might direct fat accumulation into the metabolically safer SAT depots. Furthermore, if ERβ signalling increases 11βHSD1 expression, as suggested by the data in paper III, the resulting increases in glucocorticoid levels might mediate this adipogenic effect. Thus, assuming that rat data will translate into humans, part of the effects of estrogen on adipose tissue distribution may be mediated by regulation of intra-adipose glucocorticoid
metabolism. It would be most interesting to investigate VAT and gluteo-femoral SAT in pre- and postmenopausal women to gain a more detailed evaluation of the importance of 11βHSD1, and the putative regulation via ERs, in the redistribution of adipose tissue with menopause/older age.

Noteworthy, in relation to hormone replacement therapy in postmenopausal women, a couple of earlier studies pointed to depot-specific estrogen regulation of ERα and ERβ expression, and that this regulation may differ with age/menopausal status (Table 2) [183, 185]. ERα expression was thus down-regulated, while ERβ expression was upregulated, by estrogen in SAT from older women. The opposite pattern was found in visceral adipocytes; i.e., estrogen treatment down-regulated ERβ but upregulated ERα expression in VAT. This suggested that estrogen replacement therapy could further promote increased ERβ signalling, specifically in the SAT depot in postmenopausal women.

Table 2. Combined results of estrogen manipulations in adipocytes in vitro by Anwar et al. and Dieudonne et al.[183, 185].

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Therapeutic implications

The difference in adipose 11βHSD1 levels between pre- and postmenopausal women observed in our studies was relatively subtle and the biological relevance of these changes requires further examination. However, with a life expectancy for Swedish women of about 83 years [211], the average woman will be postmenopausal for over 30 years. Hence, even subtle changes may have dramatic effects over time. It is also important to note that the women in paper I were all normal weight. The slight increase in 11βHSD1 levels observed in that study may be aggravated by obesity.
Due to the putative importance of 11βHSD1 activity in the development of obesity-related disorders, it has gained a lot of attention from pharmacological companies as a potential drug-target. A large number of 11βHSD1 inhibitors have been tested in preclinical studies, and some in early clinical trials; but, to date, none has been approved for medical treatment [212]. One class of 11βHSD1 inhibitors that includes carbenoxolone and other derivatives of glycyrrhetinic acid (a bioactive constituent of liquorice) have been shown to have beneficial effects on several metabolic parameters, including the blood lipid profile and hepatic steatosis; these studies have demonstrated the beneficial effects of 11βHSD1 inhibition. However, carbenoxolone is a non-specific 11βHSD inhibitor; therefore, high-dose treatment might cause electrolyte disturbances and hypertension. Thus, it is vitally important to develop inhibitors that are specific for 11βHSD1. Furthermore, it might be advantageous to develop inhibitors with tissue-specific action. General inhibition of 11βHSD1 could produce adverse effects, for example impairment of inflammatory responses and reduced negative feed-back on the HPA-axis; the latter might arise from inhibition of brain 11βHSD1 amplification of glucocorticoid action. Interestingly, Berthiaume and colleagues recently reported that, in rodents, a triazole compound with tissue-specific 11βHSD1 inhibition reduced intra-abdominal fat depots, promoted peripheral fat accumulation, and reduced fasting triglyceridemia [213]. Similar effects have been shown for other triazole and thiazole compounds [212]. These effects remain to be confirmed in humans. However, postmenopausal women with increased abdominal obesity and elevated metabolic risk may putatively benefit from treatment with such an 11βHSD1 inhibitor.
SUMMARY AND CONCLUSIONS

- Postmenopausal women had higher 11βHSD1 mRNA expression in SAT compared to premenopausal women in the follicular phase of the menstrual cycle. Both waist circumference and menopausal status independently affected SAT 11βHSD1 expression. Postmenopausal women also had increased 11βHSD1 activity in the liver compared to premenopausal women. Increased hepatic and adipose 11βHSD1 expression/activity were associated with a less beneficial blood lipid profile and higher blood pressure.

- Estrogen treatment of ovariectomised rats reduced 11βHSD1 expression and activity in VAT and resulted in a shift towards higher 11βHSD1 expression and activity in SAT. Estrogen treatment also reduced hepatic 11βHSD1 expression and activity in these rats.

- Gene expressions of ERβ correlated with that of 11βHSD1 in both SAT and VAT from obese women, in SAT from lean pre- and postmenopausal women, and in SAT from ovariectomised estrogen treated rats. In addition, low concentrations of estrogen or an ERβ agonist induced 11βHSD1 expression and activity in vitro. This suggested that estrogen directly upregulated 11βHSD1 expression and activity via local ERβ-signalling in adipose tissue. Transcript levels of adipose ERα were not correlated with 11βHSD1 expression in either female rats or women.

In conclusion, our data suggest that low circulating estrogen levels are associated with elevated 11βHSD1 levels in both adipose tissue and liver. This effect could be counteracted with estrogen treatment, at least in rats, and may be influenced by the degree of adiposity. We suggest that adipose tissue depot-specific regulation of glucocorticoid metabolism via ERβ mediates part of the estrogen effect on maintenance of female fat distribution. In the liver, there may be an independent effect of estrogen on 11βHSD1 activity that merits further studies.
Figure 8A. Summary of suggested interactions between estrogen and 11βHSD1 in adipose tissue and liver in premenopausal women including the importance of local and circulating estrogen levels, and ER expression. Due to high circulating estrogen levels, the general effects of estrogen dominate. Central effects of estrogen on appetite and voluntary exercise, in combination with local effects in muscle and adipose tissue, produce leanness with decreased fat mass and low 11βHSD1 levels. These effects are most likely mediated via ERα, which has previously been shown to have anti-obesity effects in transgenic mice. However, inhibition of adipose 11βHSD1 activity at high estrogen levels may be mediated in an ER-independent manner, i.e. estrogen acts as a non-competitive inhibitor. There may also be depot-specific effects as physiological levels of estrogen upregulate 11βHSD1 via ERβ-signalling in adipose tissue. ERβ is more highly expressed in SAT than VAT and ERβ-mediated upregulation of 11βHSD1 may therefore contribute to maintenance of female fat distribution. In liver the data presented so far implies an additional down-regulatory effect of estrogen on 11βHSD1, possibly mediated via ERα.
Figure 8 B, Summary of suggested interactions between estrogen and 11βHSD1 in adipose tissue and liver in postmenopausal women, including the importance of local and circulating estrogen levels and ER expression. Due to low circulating estrogen levels, the general anti-obesity effects of estrogen are reduced and local effects of estrogen dominate. Thus, producing increased fat accumulation. Increased fat mass is associated with elevated adipose 11βHSD1, and may contribute to a general increase in 11βHSD1 levels. Conversely, lack of 11βHSD1 inhibition by estrogen may lead to increased 11βHSD1 activity and increased fat accumulation. Within the SAT depot, ERβ is more highly expressed in postmenopausal than premenopausal women. (Notably, we have not investigated the VAT depot.) However, if both ERβ and 11βHSD1 expression is higher in VAT than SAT in postmenopausal women this could contribute to increased intra-abdominal fat accumulation and central fat distribution. In the liver, the 11βHSD1 reducing effect of estrogen, possibly mediated via ERα-signalling or non-ER-dependent inhibition of 11βHSD1 activity, is reduced in the estrogen-deficient state. 11βHSD1 is therefore upregulated. This may be further aggravated by an upregulatory effect of ERβ-signalling, if ERβ is elevated in liver as well as in adipose tissue in postmenopausal women.

Östrogenbehandling till postmenopausala kvinnor kan förhinda omfördelningen av fettdepåerna. En möjlig bidragande faktor till dessa effekter är ändrad vävnadsspecifik kortisolomsättning. Den förändring i fettansamling som sker vid klimakteriet liknar nämligen det som sker hos patienter med Cushing’s syndrom, ett tillstånd som beror på kroniskt förhöjda kortisolnivåer i blodet. Kortisol produceras i binjurarna men de aktiva nivåerna i vävnaderna påverkas påtagligt av enzymen 1β-hydroxysteroid dehydrogenase (11βHSD) typ 1 och 2. 11βHSD1 aktiverar kortison till kortisol medan 11βHSD2 gör det omvänd. 11βHSD1 finns i vävnader som lever och lever som lever, fettväv och lungor medan 11βHSD2 återfinns i bland annat njurarna. Denna avhandling har fokuserat på enzymet 1βHSD1 och möjlig reglering via östrogen dvs. enzymets möjliga roll i den förändring av fettfördelning som är associerad med menopaus.

I studie I jämfördes pre- (yngre) och postmenopausala (äldre) kvinnor avseende genuttryck och aktivitet av 11βHSD1 i underhudsfett och lever, samt öndringen av kortisol och dess nedbrytningsprodukter i urinen. Mätt på11βHSD1 aktivitet korrelerades till riskfaktorer för hjärt-/kärlsjukdom. Våra resultat tyder på att postmenopausala/äldre kvinnor har högre nivåer av 1βHSD1 i fettrav och lever som kan aktivera kortisol och bidra till ökad fettinlagring och risk för hjärt-/kärlsjukdom.

I studie II studerades kastrerade (har därmed ingen egen östrogenproduktion) honrättor som gavs östrogen- eller placebobehandling. Dessa användes som en
modell för postmenopausal östrogenbehandling till kvinnor. Vi fann att östrogenbehandlingen var associerat med lägre 1βHSD1 i bukfett och lever, vilket bidrog till en förändring i balansen av 11βHSD1 nivåerna mellan fettdepåerna; från högre nivåer i bukfett till högre nivåer i underhudsfett. Om dessa resultat visar sig överförbara på människa indikerar det att 11βHSD1 kan regleras viaöstrogen och bidra till en gynnsammare fettfördelning.

I studie III intresserade vi oss för möjliga vägar för östrogen att påverka 11βHSD1. Vi undersökte därför genuttrycket av östrogenreceptorerna (ER) och β och den eventuella kopplingen till 11βHSD1. Vi fann att ERβ och 11βHSD1 korrelerade i underhudsfett från både normalviktiga pre- och postmenopausala kvinnor, underhudsfett och bukfett från överviktiga, huvudsakligen premenopausala kvinnor samt i underhudsfett från kastrerade råttor med östrogenbehandling. Ytterligare studier på odlade fettceller visade att låga nivåer av östrogen samt en ERβ -specifik aktivator ökade 11βHSD1-aktivitet och genuttryck. Dessa resultat tyder på att låga doser östrogen genom signalering via ERβ kan uppreglera 11βHSD1 i fettväv.

Sammantaget visar denna avhandling att låga östrogennivåer är associerat med ökad kortisolproduktion lokalt i fettväv och lever hos både människa och råttor. Våra resultat visar också på möjligheten att en del av den gynnsamma effekten av östrogenbehandling på fettfördelningen hos postmenopausala kvinnor kan förmedlas via reglering av det kortisol-aktiverande enzymet 11βHSD1. Ytterligare studier behövs dock för att verifiera de resultat som presenterats här. Dessa studier ökar kunskapen runt de faktorer som styr fettfördelningen hos kvinnor efter menopause och öppnar för ytterligare studier rörande effekten av vävnadsspecifik hämnning/aktivering av enzymet 11βHSD1.
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