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GLUCOCORTICOID ADMINISTRATION – STUDIES ON WEIGHT REGULATION AND METABOLIC IMPLICATIONS

by

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Cover illustration:
"A man and a woman with big bellies", drawn by Alicia and Alexander Uddén
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"Vad är då egentligen viktigt i livet,
vad gör att vi varje dag vaknar med leende
hjärtan och öppna sinnen? Vad kommer vi att
minnas om livet vid dess slut?
Ja, svaret är enkelt – Kärleken!"
Imre Kertész

Dedicated to my parents
and to Alexander and Alicia

ABSTRACT

Long-term treatment with glucocorticoids induces weight gain and increased risk to develop obesity-related metabolic complications like insulin resistance, type 2 diabetes and cardiovascular disease. Glucocorticoids have been suggested to play a role in development of visceral fat accumulation. The similarities between conditions with cortisol excess, for example Cushing's syndrome and the metabolic syndrome are obvious.

In this thesis the main outcome variables are the glucocorticoid effects on eating behaviour and aspects of appetite regulation, adipose tissue secretion and distribution as well as cortisone/cortisol conversion in a clinical setting. The results are based on studies conducted on subjects treated with high doses of prednisolone for shorter periods of time (24 hours and seven days) as well as long-time treatment (twelve months). All subjects were their own controls, allowing disclosure of intra-individual variability.

The main findings were that:

- Long-term glucocorticoid treatment causes an increase in food intake in spite of elevated leptin levels. In addition, an association is suggested between unfavourable changes of the eating curve, probably indicating blunted satiety signals, and centralisation of fat depots.
- Short-term treatment with glucocorticoids also causes an increase in food intake, simultaneously with a rise in circulating leptin levels, indicating diminished satiation signalling.
- UCP2 mRNA expression decrease after short-term glucocorticoid exposure, that correlated with increased insulin levels, could promote adipose tissue accumulation. A causal link to the metabolic syndrome is therefore suggested.
- A significant increase in PAI-1 secretion from subcutaneous adipose tissue, following short-term glucocorticoid treatment suggests a possible mechanism for hypercortisolemia in mediating increased risk for cardiovascular disease.
- Impaired 11β-hydroxysteroid dehydrogenase 1 expression and an increase of glucocorticoid receptor expression, indicates a regulatory effect of glucocorticoids. An association with reduced feeling of satiation reinforces the suggested glucocorticoid mediated increase in appetite, occurring even after a short treatment period.

LIST OF PUBLICATIONS

The present thesis is based on the following studies, referred in the text by the Roman numerals I-V:

I. Uddén J, Barkeling B, Brismar TB, Björntorp P, Berlin M, Brismar K, Rössner S.
Effects of long-term prednisolone treatment of patients with pulmonary sarcoidosis; leptin levels, eating behaviour and body composition assessments.
Journal of Internal Medicine, submitted.

II. Uddén J, Björntorp P, Arner P, Barkeling B, Meurling L, Rössner S.
Effects of glucocortioicds on leptin levels and eating behaviour in women.
Journal of Internal Medicine 2003; 253: 225-231.

III. Uddén J, Folkesson R, Hoffstedt J.
Downregulation of uncoupling protein 2 mRNA in women treated with glucocorticoids.
International Journal of Obesity 2001; 25: 1615-1618.

IV. Uddén J, Eriksson P, Hoffstedt J.
Glucocorticoid-regulated adipose tissue secretion of PAI-1, but not IL-6, TNFα or leptin *in vivo*.
Hormone and Metabolic Research 2002; 34: 698-702.

V. Uddén J, Lönn M, Wake D, Walker BR, Barkeling B, Björntorp P.
Effects of cortisol/cortisone conversion in subcutaneous adipose tissue by 11β hydroxysteroid dehydrogenase in women after exposure to prednisolone.
Manuscript

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LIST OF ABBREVIATIONS

SAD	sagittal abdominal diameter
BMI	body mass index
GC	glucocorticoids
GR	glucocorticoid receptor
HDL	high density lipoprotein
LDL	low density lipoprotein
HSL	hormone sensitive lipase
LPL	lipoprotein lipase
DXA	dual energy x-ray absortiometry
NS	nervous system
mRNA	messenger ribonucleic acid
TG	triglycerides
WHR	Waist-to-hip ratio
11β-HSD	11beta-hydroxysteroid dehydrogenase
TNF-α	tumour necrosis factor-alpha
PAI-1	plasminogen activator inhibitor 1
UCP 2	uncoupling protein 2
IL-6	interleukin 6
RAS	renin-angiotensin system

1 BACKGROUND

1.1 OBESITY AND THE METABOLIC SYNDROME

Throughout most of human history, weight gain and fat storage have been viewed as signs of health and prosperity. Today, however, as standards of living continue to rise, weight gain and obesity are posing a growing threat to health of inhabitants from countries all over the world (WHO/NUT/NCD/98). In trying to define the problem of overweight and obesity, BMI (body mass index) is the most common classification. It is calculated as the weight in kilograms divided by the square of the height in meters (kg/m^2). Obesity is defined as BMI over 30 kg/m^2. Subjects above 25 kg/m^2 are defined as overweight.

The prevalence of obesity has increased by about 40-50% in most European countries in the past 15 years. The most dramatic increase has been observed in England, where it has more than doubled during this period. In Sweden, the prevalence is 12% for women and 10% for men, but the increase about 50% during a 10-year period is strikingly high (SBU rapport-02).

The health consequences of obesity are many and varied, ranging from an increased risk of premature death to several non-fatal but debilitating complaints. Obesity is a major risk factor of type 2 diabetes mellitus, cardiovascular disease (Groop -97), cancer, osteoarthritis, gallbladder disease and sleep apnoea, as well as hormonal disturbances such as infertility and polycystic ovary syndrome (Bjorntorp –88, WHO/NUT/NCD/98).

Obesity is not a recent phenomenon, but has never before reached such epidemic proportions as today. Individuals who have a genetic predisposition (Sakul -97) become obese more readily when they are exposed to unfavourable environment like excess intake of energy dense food combined with low physical activity, stress and smoking (Bjorntorp -93).

Excess abdominal fat is an independent predictor for type 2 diabetes (Boyko -00) and cardiovascular disease (Isomaa -01). Abdominal fat man can vary dramatically within a narrow range of total body fat or BMI. A high waist-hip ratio (WHR), more than 1.00

for men and 0.85 for women is the traditional method for measuring abdominal fat accumulation. However, recent evidence suggests that waist circumference alone (high-risk: men >102cm and women >88cm) is a more potent indicator of health risk than waist-hip-ratio (Poilot MC -94). Another important anthropometric predictor to visceral obesity is the sagittal abdominal diameter (SAD) (Kvist -88).

The metabolic syndrome was first described by Reaven et al in 1988 (Reaven -88). This initial definition included insulin resistance as a key factor in the development type 2 diabetes (Martin -92) and cardiovascular disease (Ginsberg -00). Although obesity was not included in the early definition by Reaven et al., abdominal obesity in particular is now generally considered an important part of the metabolic syndrome. WHO has previously suggested a definition including insulin resistance, but according to the Adult Treatment Panel III (Adult Treatment Panel III, ATP III -01) this definition does not include insulin resistance since it is hard to measure properly in a clinical setting. The Adult Treatment Panel III has provided a clinical definition of the metabolic syndrome according to the following criteria:

1. Abdominal obesity:
   waist circumference >102 cm for men and >88 cm for women.
2. HDL cholesterol:
   <1.04 mmol/l (<40 mg/dl) for men and <1.29 mmol/l (150 mg/dl) for women.
3. Triglycerides:
   >1.69 mmol/l (150 mg/dl) for both men and women.
4. Blood pressure:
   >130/85 mmHg for both men and women.
5. Fasting glucose:
   >6.1 mmol/l (110 mg/dl) for both men and women.

Although insulin resistance and obesity are not included in the definition, they are considered as key pathophysiological disorders in the metabolic syndrome. The components of the metabolic syndrome (impaired glucose tolerance or type 2 diabetes, hypertension and dyslipidemia) are all related to each other with frequent co-existing (Isomaa -01). Obesity is an independent risk factor for increased cardiovascular mortality and morbidity (Allison -99), but not necessarily for the metabolic syndrome. Furthermore, these factors link both obesity as well as the metabolic syndrome to the

increased risk of developing cardiovascular disease (National Task Force on the Prevention and Treatment of Obesity –00) (fig 1).

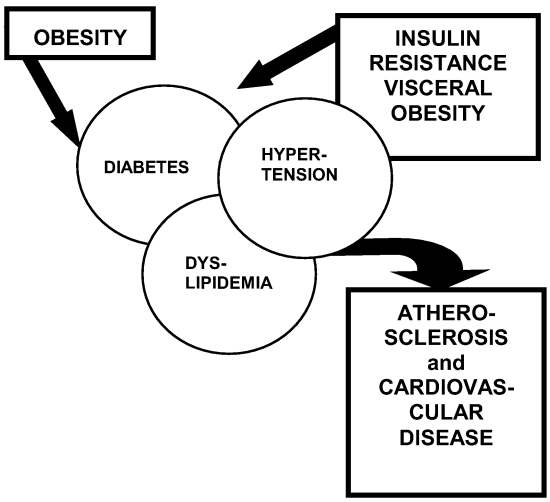

Fig 1.
Hypothetical overview of risk factors leading to atherosclerosis and cardiovascular disease.

1.2 STRESS AND THE METABOLIC SYNDROME

The individual reaction to the multitude of poorly defined factors that disturb and overload biological homeostasis is defined as "stress". Obviously, factors such as personality characteristics, competence, education and previous exposure are heavily involved in coping with stress. Henry and co-workers presented two principal types or stress reactions after experiments on rodents (Henry –77). One reaction went through activation mainly of the sympathetic nervous system the adrenal medulla. The sympathetic stimulation increases blood pressure and heart rate by increased cardiac output as well as elevated peripheral resistance (Goldstein –95). Catecholamines (adrenaline and noradrenaline) mobilises stored fuels in both adipose tissue, liver and skeletal muscle, by stimulation of lipolysis (activation of the hormone-sensitive lipase). In addition, adrenaline and noradrenaline suppress insulin and increase glucagon

secretion, which results in increased levels of both free fatty acids and glucose (Lafontan –93). This stress response was referred to as a "fight-flight" reaction.

The other reaction to stress defined by Henry and co-workers was a defeat reaction. The animals in this experiment were unable to cope with a stressful situation and became helpless, submissive and passive, caused by a stimulation of the hypothalamo-pituitary-adrenal axis (HPA-axis). This resulted in increased cortisol secretion from the adrenal cortex as the dominating reaction, but also decreased levels of sexual and growth hormones (Bjorntorp –99). This response to a stressful situation is akin to a surrender mechanism (Chrousos –92). If acute stress is repeated, the normal morning peaks of cortisol secretion are turned into an exaggerated cortisol response, with increased peaks after a given input (McEwen –98). However, if these signals are repeated frequently, early morning cortisol secretion tends to decline. Over time, this reaction may lead to chronic stress, resulting in a low, steady and rigid cortisol secretion with reduced reactivity to stressful stimulation, sometimes referred to as a "burned-out" HPA-axis.

In the hypothalamic origin of the metabolic syndrome it is suggested that stressful, environmental factors, along with a genetic susceptibility, arouse the HPA-axis and the central nervous system (fig 2) resulting in increased cortisol secretion as well as a decrease in sex and growth hormone secretion (Bjorntorp –99 and -01). Furthermore, in this hypothetical model, the feedback control of the HPA-axis also is diminished by a deficient responsitivity and sensitivity of the GR (Rosmond –02).

Stress has earlier been discussed with regard to eating behaviour among obese (Slochower –81), and it has been suggested that some individuals will respond to stressful emotions as if they were hungry (Bruch –61). Only some obese individuals respond with eating on stressful situations (Greeno –94). Furthermore, high cortisol reactivity in response to stress was followed by increased eating, compared to low cortisol reactivity in postmenopausal women (Epel –00).

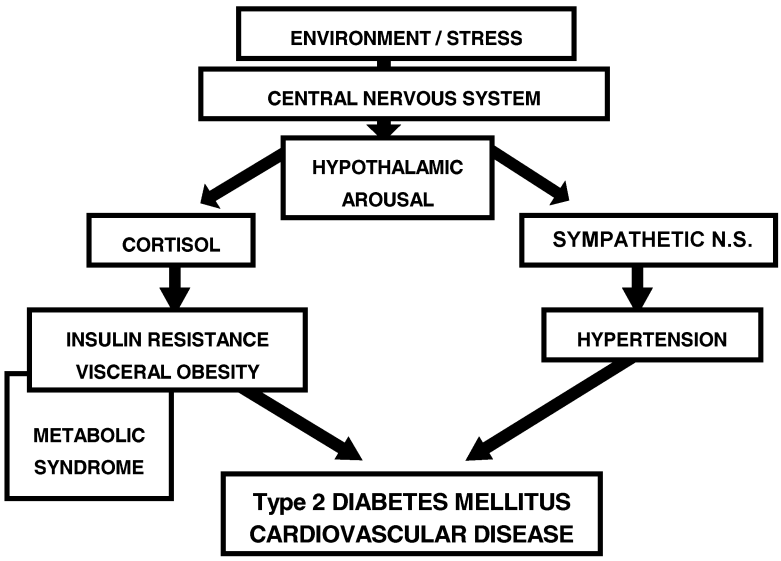

Fig 2

An overview of the hypothalamic arousal hypothesis, and its potential role in the aetiology of the metabolic syndrome. Environmental stress causes a HPA-axis stimulation, which increases cortisol secretion and induces visceral obesity. This will result in hypertension, insulin resistance, dyslipidemia (the metabolic syndrome), and increased risk for diabetes mellitus type 2 as well as cardiovascular disease (Bjorntorp –01).

1.3 GLUCOCORTICOIDS AND OBESITY

Long-term glucocorticoid medication can result in severe weight gain (cases of up to 30 kg have been reported), with increased risk of developing obesity-related metabolic complications (Jacobs –96). This medication side-effect poses a severe health threat. Indeed, the subsequent weight gain may often aggravate the underlying disease that was the reason for glucocorticoid treatment in the first place (Sartori –99).

1.3.1 Cortisol

Cortisol, which is the main form of glucocorticoids, is synthesized in the adrenal cortex under the control of the hypothalamic-pituitary-adrenal axis. The term glucocorticoids refer to their ability to stimulate hepatic and renal gluconeogenesis (Long -40). All steroid hormones are synthesized from cholesterol; aldosterone in the peripheral zona glomerulosa, while cortisol and androgens are produced in the more central layers called zonae fasciculata and reticualris.

Glucocorticoids play a major roll in regulating salt and water metabolism, blood pressure, immune function and metabolism (McEwan –97). They maintain physiological homeostasis under both basal and stress-related conditions. The secretion of cortisol is regulated by adrenocorticotropic hormone (ACTH) that is produced in the anterior pituitary gland (Chrousos –92b). The ACTH secretion is stimulated by corticotropin-releasing hormone (CRF) from the paraventricular nucleus (PVN) in hypothalamus. Coriosol exerts a negativ feedback effect that inhibits both synthesis and secretion of CRF and ACTH (fig 3). Prednisolone, which is a synthetic glucocorticoid, suppresses pituitary ACTH secretion that consequently also diminishes endogenous production of cortisol.

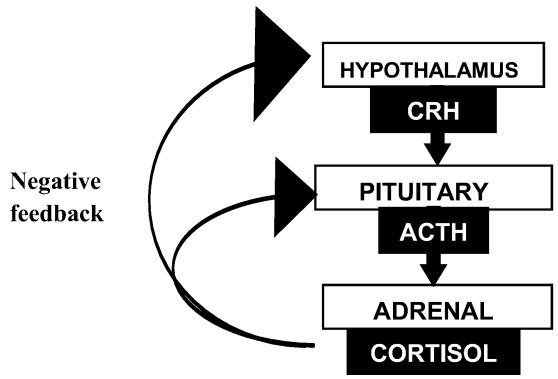

Fig 3.

The structure of the Hypothalamo-Pituitary-Adrenal axis and the secretion regulation of cortisol.

Blood levels of cortisol can increase within 30 minutes in response to a pulse of ACTH, and follow a constant intra-individual circadian rhythm with highest plasma cortisol levels in the morning between 6.00 and 10.00 h (Gallagher –73).

About 90% of the circulating cortisol is bound to the proteins corticosteroid-binding globulin (CBG, about 75%) and albumin, which leaves about 10% as unbound, free hormone. Cortisol is mainly metabolised in the liver by 5α-reductase in several steps (Tyrell –94).

The cortisol effect is mainly mediated via an intracellular glucocorticoid receptor (GR) (Rebuffe-Scrive –90), to form a complex, which in turn modulates transcription of GC-respondent genes (Yamamoto –85, Rosmond –02). There are two isoforms of the GR - the GRα isoform, which is the active form that responds to GCs, and the transcriptionally inactive GRβ isoform. Cortisol can also activate the mineralocorticoid receptor.

Glucocorticoid receptors are widely distributed, but the density of these receptors varies in different tissues. In adipose tissue the density even varies between different regions, with highest density in visceral fat and lowest in subcutaneous adipocytes (Rebuffé-Scrive –90, Ottosson –95).

An important factor regulating the access of active cortisol is the enzyme 11β-hydroxysteroid dehydrogenase (11β-HSD), which exists in two isoforms (fig 4). 11β-HSD type 1 activates cortisol from inactive cortisone and is widely distributed in many human tissues, including liver and adipose tissue. The second enzyme, 11β-HSD type 2, which converts active cortisol to inactive cortisone, is the predominant isoform in kidney, placenta and developing foetus (Tomlinson -01, Walker –01).

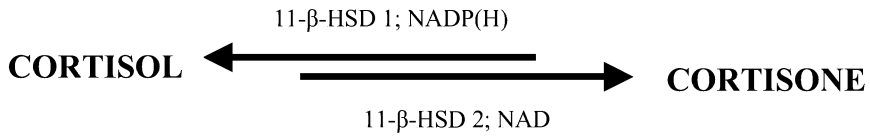

Fig. 4.

There are two isoforms of 11-β-hydroxysteroid dehydrogenase (11- βHSD). Type 1 is a reductase *in vivo*, activating cortisol from inactive cortisone. Type 2 functions as a dehydrogenase, inactivating cortisol into cortisone.

1.3.2 Physiological effects of cortisol

Glucocorticoids exert their effects on most systems of the body and are essential for life. Cortisol mediates the stress response by promoting an increase in glucose, mobilizes free fatty acids from adipose tissue, modulates cardiovascular as well as immune function. It also plays a key role during growth and maturation (McEwan –97).

By activating glycogen synthase in the liver and inactivate the glycogen mobilising enzyme, glucocorticoids enhance gluconeogenesis (Rooney –94, Rizza –82). Glucocorticoids also seem to impair insulin-dependent peripheral glucose uptake, as well as increase hepatic glucose release resulting in increased fasting plasma glucose (McMahon –88). In addition to the GC effect on insulin sensitivity, there is evidence of direct inhibition of insulin secretion from pancreatic β-cells (Delaunay –97, Ling –98). Increased lipolysis is also induced by glucocorticoids (Hojbjerg Gravholt C –02), and the elevated level of free fatty acids contributes to impaired insulin-dependent glucose uptake (Andrews –99). Glucocorticoids, in the presence of insulin, also stimulate the activity of lipoprotein lipase, which is the rate limiting enzyme for triglyceride uptake, leading to a lipogenic effect (Ottosson –95). After long-term glucocorticoid excess the adipose tissue depots redistribute to fat accumulation in the trunk, as well as visceral depots, whereas the extremities almost show a catabolic pattern. GC also regulates blood pressure and water metabolism. The endothelium-dependent vasodilatation, probably enhanced by insulin, is impaired by GC, which could contribute to the GC-induced hypertension (Walker –95). GC play an important roll of inflammatory reaction in tissue protection, by limiting the host defence response. Recent evidence, however, show that GC not only have an immunosuppressive role, but rather an immunomodulatory role (McEvans –97).

In Cushing´s syndrome, or other conditions with chronic cortisol excess, the clinical features (Kirk –00) resemble those of the Metabolic Syndrome (table 1). Weight gain, insulin resistance, hypertension, hyperlipidemia are only some of the factors leading to increased risk of developing cardiovascular disease. The similarities are remarkable, but have not fully been explained, since circulating cortisol levels are not elevated in the Metabolic Syndrome.

CLINICAL FEATURES	Cushing´s syndrome	Metabolic Syndrome
♦ Weight gain	X	X
♦ Central (Visceral) Obesity	X	X
♦ Hypertension	X	X
♦ Hyperlipidemia	X	X
♦ Insulin resistance	X	X
♦ Impaired glucose tolerance	X	X
♦ Diabetes Mellitus type 2	X	X
♦ Increased serum cortisol level	X	0

Table 1

Comparison between features of Cusing´s syndrome and the metabolic syndrome.

1.4 THE ADIPOSE TISSUE AS AN ENDOCRINE ORGAN

Adipose tissue has long been considered a passive tissue for energy storage. Various hormones regulate the size and distribution of the adipose tissue. Glucocorticoids are well-known modulators, and excess conditions, for example Cushing´s syndrome, results in centralisation of fat depots (Livingstone –00), as well as metabolic complications like hypertension, dyslipidemia, insulin resistance and diabetes (Jacobs –96, Sartori -99) (table 1).

The concept of adipocytes as passive cells has radically been revised with the discovery that they secrete hormones that affect other tissues (Arner –97). Moreover, adipocytes have both autocrine and paracrine functions (Mohamed-Ali –98). Visceral adipose tissue differs considerably from subcutaneous fat, mainly by its greater sensitivity to lipolytic stimuli. Free fatty acids (FFA) are thereby released directly to the portal circulation, which is considered as the first step in the development of the insulin resistance syndrome (Björntorp –99). The underlying mechanisms are decreased hepatic insulin clearance, increased hepatic glucose production and a reduction in peripheral glucose uptake by skeletal muscle (Andrews –99).

The enzyme lipoprotein lipase (LPL), which is secreted by adipocytes to the surface of capillary endothelia, plays a key role in lipogenic/lipolytic balance (Stralfors –87).

LPL regulates the FFA uptake, and is controlled by various hormones and conditions. After uptake in fat cells the FFA are bound to specific binding proteins and transferred for

triglyceride synthesis. The triglyceride accumulation (lipogenesis), via LPL, is mainly regulated by cortisol in the presence of insulin.
Regarding the breakdown of adipose tissue, i.e. lipolysis, it consists of a stepwise process where triglycerides are hydrolysed into FFA and glycerol. Hormone sensitive lipase (HSL) catalyses the process and is the rate-limiting step in the lipolytic cascade (Ramsay –96). Cathecolamines, noradrenaline and adrenaline, either stimulate lipolysis by activation of HSL via binding to β-adrenoceptors, or decrease the lipolysis rate by binding to α2-adrenoceptors. The rate of β- and α2-adrenoceptors thereby determines the cathecolamine action. The main anti-lipolytic hormone is insulin, which dephosphorylates HSL and thereby inactivates the enzyme.

Uncoupling proteins (UCP´s) have been suggested to be involved in regulating thermogenesis and energy expenditure by uncoupling the respiratory chain (Ricquier –00). UCP1 was discovered first, and was brown-adipocyte specific. UCP 2 and 3, however, have been discovered in white adipose tissue and muscle. Animal studies have shown that glucocorticoids may have a regulatory effect on uncoupling proteins, although different effects were seen in muscle and adipose tissue (Moriscot –93).

1.4.1 Hormones produced in the adipose tissue

Leptin, discovered 1994 (Zhang –94), is a polypeptide produced in adipocytes and acts both as a satiety factor but is also actively involved in the regulation of several endocrine axes though modulatory mechanisms (Friedman –98, Considine -01). It is produced in white adipose tissue, and the leptin secretion increases with the increase in body fat (Considine –96). In obese subjects, leptin production per unit of body fat is 75% higher in females, and pre-menopausal women have higher concentrations than post-menopausal (Faloia –00). Both estrogens insulin and glucocorticoids increase leptin levels. Leptin has a circadian rhythm, with a peak early in the morning, and a nadir late in the afternoon. In women this pulsatile secretion is synchronous with that of LH and estradiol. Furthermore, leptin seems to increase the release of GnRH. Starvation is also associated with decreased immune function, and leptin stimulates proliferation of CD4- and T-cells as well as cytokines (Lord –98). In addition to a regulatory role in regulation of food intake and energy expenditure, leptin seems to have a relationship with gonad function, puberty and the immune system.

Centrally in the hypothalamus, insulin and leptin inhibit the expression of orexigenic neuropeptides, and stimulate the secretion of anorexigenic peptides. The release of these

peptides is then proposed to regulate downstream neuronal pathways in the hindbrain. Afferent satiety signals like cholecystokinin, mainly via the vagal nerve, also arrive in the hindbrain mediating short term satiety signals in response to ingestion of a meal (Emond –99). The signals arriving from hypothalamus modulate these signals and alter the response of neurones that regulate the termination of meals (fig 5) (Porte –02).

A leptin threshold model has been suggested (fig 6), where the major biological responses regarding food intake are evoked by a decline in leptin levels (Porte –02). Caloric restriction leading to weight reduction also causes a decrease in leptin production, which lowers the metabolic rate. A compensatory hunger leads to increases in food intake to maintain an individual, genetically predisposed threshold. During this threshold the biological response is absent. The threshold model also implies that increased production of leptin is a consequence of impaired leptin signalling.

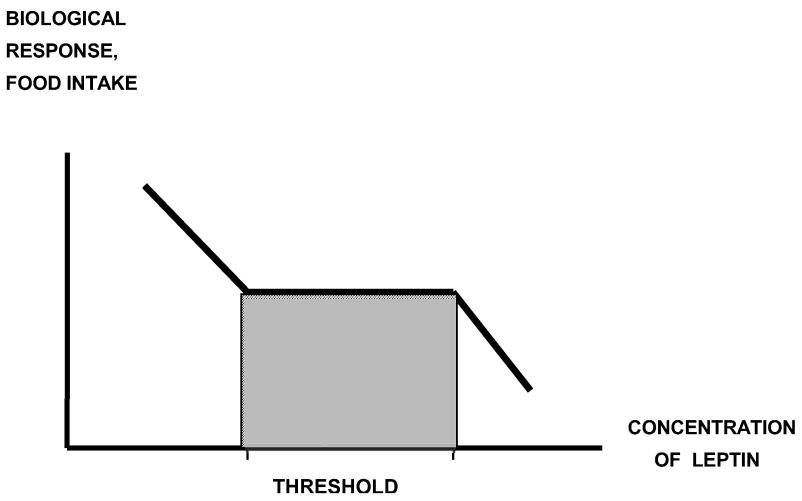

Fig 6

The hypothetical leptin threshold model. A genetically predisposed threshold determines the individual biological response to circulating leptin.

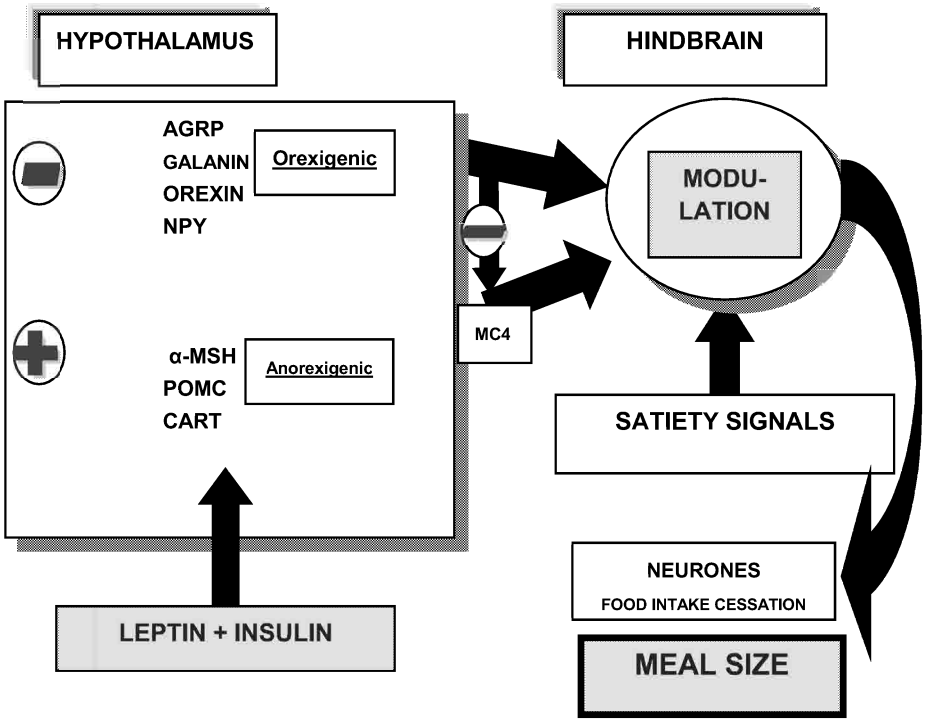

Fig 5

Hypothesized model of central pathways for integration of short- and long-term control of food intake (McMinn –00, Friedman –97). Leptin and insulin are adiposity signals and inhibit orexigenic signals like neuropeptide Y (NPY) and agouti-related peptide (AGRP) which promotes increased food intake. These stimulate the expression of pro-opiomelanocortin (POMC), cocain and amphetamine-regulated transcript (CART), which is the precursor of α-melanocyte stimulating hormone (α-MSH). The α-MSH signalling is then mediated via melanocortin 4 receptor (MC4R), which also is inhibited by AGRP being its antagonist. In the hindbrain these components of the long-term satiety system interact with short-term systems (Cholecystokinin, CCK, gastric tension etc.) and modulate the amount of food consumed during the meal.

PAI-1 is the predominant regulator of plasminogen activation and has several functions mainly leading to progression of coronary atherosclerosis. Elevated plasma PAI-1 levels are found in subjects with high BMI as well as abdominal adiposity (Alessi –00, Juhan-Vague -00). A correlation has also been observed between the visceral fat area and plasma PAI-1 levels in women (Janand-Delenne -98). Moreover, patients treated with glucocorticoids after heart transplantations also show a significantly higher PAI-1 plasma level, as well as an impaired fibrinolytic potential, compared with controls (Patrassi GM -97).

Angiotensinogen is the precursor of angiotensin II, and well known to be involved in the control of blood pressure (Negrell –99, Guerre-Millo -02). Recent data also suggests an involvement of the rennin-angiotensin system (RAS) in adipose tissue growth. Furthermore, an interaction between insulin and RAS has also been suggested where elevated RAS activity in obese subjects was associated with a resistance to insulin antilipolytic action.

TNF-α and IL-6 are both cytokines produced in the adipose tissue, where circulating concentrations increase with fat mass (Negrell –99, Guerre-Millo –02). TNF-α has been discussed in the development of insulin resistance by inhibiting the insulin receptor signalling pathway via the insulin receptor substrate 1 (IRS-1) (Mohamed-Ali –98, Coppack –01). TNF-α has also been shown to increase leptin secretion, likely caused by an autocrine mechanism. Both TNF-α and IL-6 down-regulate LPL and are both likely, according to recent data, to trigger adipocyte cell differentiation (He –00, Grimble –02). Whether IL-6 is also involved in the development of insulin resistance syndrome is still unclear.

HORMONE/PROTEIN	EFFECT	PRODUCTION IN OBESITY
✓ LEPTIN	↓Appetite	↑
	↑Energy expenditure	
✓ PAI-1	↑Coagulation	↑
	→ assoc. with CVD	
✓ANGIOTENSINOGEN	↑Blood-pressure	↑
	↑ Lipolysis	
✓ADIPONECTINE	Protectory factor against	↓
	obesity / insulin resistance	
✓ACYLIATION STIMUL.	↑Lipid accumulation	?
PROTEIN (ASP)	↑Lipid mobilisation	
✓TNF-alpha	↑Insulin resistance	↑
✓IL – 1b, 6 and 8	Unclear	↑

Table 2

Adipose tissue secretion of hormones and their association to obesity. Increasing (arrow up) and decreasing (arrow down) effects are indicated (Guerre-Millo –02).

1.5 EATING BEHAVIOUR AND OBESITY

Eating behaviour is not only a determinant of what we eat, but also how we consume food. The "macro-structure" of eating describes the average intake of energy and macro-nutrients during a longer period of time, at least one day, and also how the nutrients are distributed during this period.

The "micro-structure" of eating on the other hand, describes the pattern of eating within one single meal. Kissilef and co-workers (Kissilef -80) developed a Universal Eating Monitor, a device consisting of a hidden scale built into a table. A plate with food is placed on top of the scale, and a computer stores readings over time when the subject eat. This information is then transferred, and total amount of eaten food, duration and average rate of eating is calculated. The linear coefficient represents hunger.

This eating curve is also fitted to a polynomial, and twice the quadratic coefficient represents the deceleration rate. A change of eating rate during a meal, normally a decrease is called a negatively accelerated intake curve or decelerated intake. This negatively accelerated, or decelerated, intake curve has also been called "biological satiation curve" (Meyer –72). Satiation is the process that brings a meal to an end. On the other hand, satiety refers to the time interval between meals as well as the initiation and size of the subsequent meal. Meyer and Pudel (Meyer –72) showed that normal weight subjects had a decelerated eating curve, while obese and latent-obese instead showed linear, or accelerating, eating curves. The authors concluded that deceleration of the eating curve reflects biological satiation, while an accelerating eating curve could be interpreted as a deficiency of satiation signals. Similar conclusions were made by Belliste and co-workers (Belliste -81) and Kissilef and colleagues (Kissilef -82). Accelerating eating curves are furthermore found in children with Prader Willi Syndrome (PWS; Lindgren -00). These results may be interpreted as enhanced eating regulation, and these children have a well-known appetite disturbance.

2 AIMS OF THE THESIS

The main aim of the thesis was to examine different metabolic aspects that could mediate the effects of glucocorticoid medication after both short-term as well as long-term treatment in adults.

The main outcome variables were eating behaviour, body composition and the effect on subcutaneous adipose tissue and circulating leptin levels, but also the interaction between measured outcome variables (studies I-V).

Secondary aims were to:

- Examine mechanisms that can mediate long-term glucocorticoid effect on weight gain, centralisation of fat depots and the development of the metabolic syndrome (study I).
- Compare circulating leptin levels and eating behaviour changes before and after glucocorticoid treatment (study II).
- Evaluate the glucocorticoid effect and regulation of adipose tissue hormones connected to insulin resistance and risk factors for cardiovascular disease (study III and IV).
- Measure the glucocorticoid effect on the activation and inactivation of cortisol in the adipose tissue after 24-hours glucocorticoid treatment (study V)
- Identify factors that may be of pathophysiological importance in the development of the metabolic syndrome.

3 MATERIAL AND METHODS

3.1 SUBJECTS AND INTERVENTION.

In study I, nine subjects, 8 women (four of them postmenopausal) and 1 man (median age 44, range 26-63 years,) were selected. They all had early pulmonary sarcoidosis and were awaiting treatment with prednisolone. Sarcoidosis is a multisystemic granulomatous disease of unknown aetiology (Muller –98). In its early stages it has a small affects on well-being, and our subjects were carefully selected by a physician specialised in lung diseases in order to decrease the possibility of disease interaction with the results of the study outcome variables. These patients were otherwise in a healthy general condition and had not decreased in weight within a year before treatment. They took no medications of known importance for the study, except one patient who took thyroxin substitution after a Hashimoto thyroiditis.

The treatment started with 60 mg prednisolone (Pharmacia-Upjohn, Astoli, Italy) every second day for two weeks, 50 mg every second day for another two weeks, 40 mg every second day for two more weeks, continuing with 30 mg every second day for three months, 20 mg every second day for six months, 10 mg every second day for one month, and finally 5 mg every second day for another month. Data was collected before starting prednisolone treatment, repeated after 2 weeks (not DXA), 3 months, and 12 months (only DXA). All subjects were on prednisolone treatment throughout the study.

The subjects participating in study II, III and IV were twelve postmenopausal women volunteers (mean age 56.9 years $\pm$1.0 SEM), recruited by an advertisement in the local paper. The subjects chosen for this study were postmenopausal, to avoid any effect that female sex hormone fluctuation could have on appetite. Chronic oestrogen substitution was the only accepted medication, and was used by ten of the subjects. In study II and IV all twelve were included. The participants had a mean BMI of 28.9 kg/m² $\pm$ 0.8 (SEM), range 25-34 kg/m².

In study III eight out of these 12 women were randomly selected to participate on the basis of adipose tissue availability in the previously performed subcutaneous biopsies, (mean age 57 years $\pm$1,0). The mean body mass index (BMI) of the study group was 28.9 kg/m^2 $\pm$ 0.8 (SEM), range 25-34 kg/m^2.

All subjects in study II, III and IV, were investigated before and after oral administration of 25 mg of prednisolone daily for 7 days. All the tablets were taken at the same hour in the morning. Compliance was controlled by determination of prednisolone in serum (Lasic – 89).

The subjects participating in study V were eight, healthy postmenopausal women. They were recruited by an advertisement in a local newspaper in Gothenburg. Low-dosage estrogen substitution was taken by five of the women, and one used thyroid hormone. Subjects on hormone replacement therapy were well substituted with the same dosage for at least six months. Therefore they were not excluded, since their substitution was not considered to confound the results. Four women were smokers. They were moderately overweight with mean BMI 26.7 $\pm$1.7 kg/m^2 (SEM) and a range of 18.0-30.7. Participants took 25 mg prednisolone (Pharmacia-Upjohn, Ascoli, Italy) at the clinic in front of the research assistant. The measurements were assessed before treatment, and 24 hours after prednisolone intake.

All subjects came to the clinic in the morning for data collection, after an overnight fast.

3.2 GLUCOCORTICOID TREATMENT AND COMPLIANCE

All subjects participating in the studies took prednisolone, a synthetical glucocorticoid per os (Tarchalska-Krynska –94). The tablets were given once a day, in the morning, to follow the natural, physiological pattern of adrenal glucocorticoid secretion. Twenty-five mg (study II-V) prednisolone daily suppresses the endogenous cortisol secretion (Livanou – 67), and provides plasma-concentrations that are of clinical importance. This dosage was chosen taking both ethical as well as scientific aspects into consideration.
In study II-IV compliance was controlled by determination of prednisolone in plasma. The concentration of plasma prednisolone was determined by means of reversed phase high performance liquid chromatography (HPLC) with UV-detection at 254 nm. Dexamethasone was used as an internal standard (Lasic -89). All women reported to have taken their tablets as instructed except one who took her tablets after the blood-samples on the 7th day of treatment. The prednisolone measurements in all other women confirmed compliance.

3.3 ANTHROPOMETRICAL MEASUREMENTS AND BODY COMPOSITION

Body weight was measured to the nearest 0.1 kg and height in cm, from which BMI (kg/m^2) was calculated. The same amount of indoor clothes was worn every time. Waist circumference was recorded in a standing, normal respiratory condition, horizontally halfway between the costal arch and the iliac crest, and the hip circumference over the widest part of the gluteal region. The ratio between these circumstances (WHR) was then calculated. The abdominal sagittal diameter, an estimation of intra abdominal fat, was measured as described (Kvist –88). Total body fat was determined by bio-impedance (Tanita TBF-305, Tokyo, Japan). Blood pressure was measured twice in the supine position after 10 minutes rest, and the mean values were recorded.

DXA measurements were performed in study I by using a Hologic QDR 2000 (Hologic Inc.) before treatment, and after three and twelve months of treatment, respectively. Fat and lean body mass were automatically calculated by the equipment. The amount of fat in trunk and legs were obtained by placement of regions of interest. The quotient between the amount of fat in the trunk and legs was also calculated.

3.4 BIO-CHEMISTRY

Serum leptin was determined by a commercially available radioimmunoassay (LINCO, St Charles MO, USA). The remaining biochemical analyses, i.e. serum insulin, plasma glucose, low density lipoprotein cholesterol (LDL), high density lipoprotein cholesterol (HDL), total cholesterol and triglycerides, were made by accredited methods in the hospital chemistry laboratory.

3.5 EATING BEHAVIOUR

3.5.1 The VIKTOR equipment (study I and II)

This equipment consists of a computer connected to a scale built into a table, which was made invisible to the participant by a table cloth (Barkeling –95, -90, Lindgren -00). A plate with a test meal is placed on the top of the hidden scale, allowing registration of the eaten amount of food bite-by-bite. The subjects were unaware of the purpose of the equipment, and were instructed to eat until pleasantly satisfied. The data was recorded graphically as an eating curve, allowing quantitative analyses and comparisons between eating curves. The measured eating variable was total intake of food in grams (g), duration of consumption in minutes (min) and the rate of consumption as grams per minutes (g/min). The stability of these eating variables has been tested previously and over five eating occasions. Both relative (test-retest) and absolute (F-test) stability has been found in both obese and normal weight men and women. The eating curve was fitted to a polynomial, and the square of the quadratic coefficient represents the rate of deceleration, and presented as grams/minute² (g/min^2). A negative eating curve coefficient illustrates a decelerating eating curve, and a positive coefficient illustrates an accelerating eating curve (Barkeling –95). The deceleration rate has proven to be stable within individuals by Westerterp-Plantega (-00).

3.5.2 Visual Analogue rating Scales (VAS) (study I, II and V)

Subjective motivation to eat, i.e. desire to eat, hunger, fullness and prospective consumption, was rated on 100 mm visual analogue rating scales. The questions asked were:

- How strong is your desire to eat?
- How hungry do you feel?
- How full do you feel?
- How much food do you think you could eat?
- How pleasant did you found your food? (This particular question was only asked after a test meal)

The subjects indicated with a cross on the rating scale how they felt, from 0 to 100 mm, where 0 represents “not at all” and 100 represents “very, very much” (Blundell –88, Hill -86).

The ratings were made before and after the test meal on VIKTOR in study I and II.
In study V, subjects did the eating behaviour ratings before a meal (i.e. breakfast, lunch and dinner) and before going to bed. They were given the VAS registration forms on 1) the day before coming to the clinic, 2) the same day as prednisolone intake prior to VAS, and 3) the day after prednisolone treatment (i.e. 24h after the prednisolone intake).

3.5.3 48-hour food-intake recall (study I)

Estimations of total energy intake as well as separate macronutrients (computer program: "Dietist", version 1.0; Kost och näringsdata AB; 167 67 Bromma, Sweden) the last 48 hours before coming to the clinic were made by food intake recalls. All interviews were performed in a standardised manner.

3.6 ADIPOSE TISSUE ANALYSES (STUDY III, IV AND V)

All adipose tissue analyses were performed on subcutaneous adipose tissue. A subcutaneous fat biopsy (0.5-1 g) was obtained from the umbilical region under local anaesthesia. Biopsies were taken before prednisolone treatment, after seven days of treatment (study III and IV), and 24 hours after prednisolone intake (study V).
In study IV, incubation of the tissue samples was made at 37°C (3.0 ml medium/300mg tissue) in a medium consisting of sterile Krebs-Ringer phosphate buffer (pH 7.4), endotoxin-free bovine serum albumin (4g/100 ml) and glucose (1 mg/ml), with air as the gas phase. Following a 2-h incubation, a 1-ml aliquot of medium was removed and stored at -70°C for subsequent analysis
In study III and V, the tissue samples were cut into small pieces (10-25 mg), and immediately frozen at –70 - –80°C for subsequent analysis.

3.6.1 UCP 2 mRNA level analysis

Total RNA was prepared using the Rneasy total RNA kit (QIAGEN, Hilden, Germany). The concentrations of RNA were determined spectrophotometriacally at 260 nm. The

absorption ratios at 260 to 280 nm were between 1.7 and 1.9. The RNA-samples were stored at -70°C. The levels of UCP2-mRNA and 18S ribosomal RNA (18S rRNA) in total RNA were measured using a reverse transcriptase (RT) competitive PCR assay (Auboeuf – 97). The assay involves the co-amplification of reverse transcribed RNA (cDNA) with known amounts of DNA-competitors. The expression of the 18s r RNA gene was presumed not to differ before and after glucocorticoid treatment, and therefore 18s mRNA was used as reference.

3.6.2 Adipose tissue secretion of IL-6, leptin, TNFα and PAI-1 (study IV)

These analyses were carried out using an enzyme-linked immunosorbent assay for PAI-1 (ELISA, TintELIZE PAI-1, Biopool), a radio-immunoassay kit for leptin (Linco) and ELISAs for IL-6 and TNF-α (HSTA 50; R&D Systems).

3.6.3 Taq Man mRNA PAI-1 analysis (study IV)

The primers and probes were designed using ABI PRISM™ Primer Express™ (Applied Biosystem, Foster City, CA, USA). Real-time quantitative PCR was performed using ABI PRISM™7000 Sequencing Detection System (Applied Biosystems,Foster City, CA, USA). For the amplification of the PAI-1 gene, the primers PAI-1F:5´-CGCCAGAGCAGGACGAA; and PAI-1R:5´-GGAGACATCTGCATCCTGAAGTT; and the probe PAI-1:6FAM5´-CGCCAATCGCAAGGCACCTCTG-3´TAMRA were used. The primers for ß-actin were: ß-actin-F:5´-CTGGCTGCTGACCGAGG and ß-actin-R:5´-GAAGGTCTCAA ACATGATCTGGGT and the probe was: ß-actin:6FAM5´-CCCTGAACCCCAAGGCCAACCG-3´TAMRA.

3.6.4 Adipose 11HSD1 activity (study V)

Adipose 11HSD1 activity was measured in thawed fresh frozen biopsies- by homogenising in Krebs buffer at pH 7.4, and incubating 750 µg/ml protein at 37^{O}C with NADP 2mM and 1,2,6,7-$^{3}H_4$-cortisol 100nM for 30 hours. Samples were withdrawn at 3, 6, 20 and 30 hours for separation of cortisol and cortisone by HPLC with on-line liquid scintillation detection. Although 11HSD1 act as a prominent reductase in vivo (in intact cells), we measured dehydrogenase activity in vitro (i.e. cortisol to cortisone conversion). This is the most stable and preferred reaction direction in homogenised cells and has been shown to be proportional to 11HSD1 protein levels.

3.6.5 Adipose mRNAs for 11HSD1 and GR-α (study V)

Approximately 500mg of fat was homogenised in 1.5 ml Trizol (registered trademark of Gibco) and RNA was purified using RNAid RNA binding matrix (Anachem, Luton,, UK), washed 3 times, and dissociated by addition of DEPC H_20/DTT/RNAsin. RNA was quantified using spectrophotometric analysis at OD_{260}. RNA integrity was checked by agarose gel electrophoresis. Oligo dT-primed cDNA was synthesized from 0.5 ug of RNA samples using Promega Reverse Transcription System. PCR amplification using GR primers confirmed successful cDNA synthesis. Transcript level quantification for 11HSD1 and GRα, was performed with Real Time PCR primer-probe sets using the ABI PRISM 7700 Sequence Detection System with the following primers and probes.

11HSD1:

5'GGAATATTCAGTGTCCAGGGTCAA3'(F), 5'TGATCTCCAGGGCACATTCCT3' (R), and 5'-6-FAM-CTTGGCCTCATAGACACAGAAACAGCCA-TAMRA-3' (probe).

GRalpha

5'CATTGTCAAGAGGGAAGGAAACTC3'(F),5'GATTTTCAACCACTTCATGCATA GAA 3'(R), and 5'-6-FAM-TTTGTCAGTTGATAAAACCGCTGCCAGTTCT-TAMRA-3' (probe)

Human cyclophyllin (Applied Biosystems, Cheshire, UK) primers/ probes were included in a multiplex reaction with the probes/primers for the gene of interest to normalise the transcript levels. Each sample was run in duplicate and the mean values of the duplicates were used to calculate transcript level. Values were calculated as a relative fold change in mRNA from an internal control sample. RT negative controls and intron spanning primers were used to examine for genomic DNA and prevent amplification.

3.6.6 Intra-Adipose Cortisol and Cortisone Levels

Following homogenisation in Trizol, the infranatant from the RNA extraction protocol (see above) was used to extract steroids. Approximately 0.3 pmol/ml (<1% final tissue concentrations) of 1,2,6,7-3H4-cortisone and 1,2,6,7-3H4-cortisol (Amersham, Little Chalfont, UK) were added to the homogenate to correct for steroid extraction efficiency. Samples was centrifuged to remove the lipid layer and extracted on a sep-pak (Waters (C18 cartridges), Watford, UK), further purified with hexane, and re-extracted with ethyl acetate. Sample extracts were assayed using a RIA for cortisone and a cortisol ELISA (Salimetrics LLC, State College PA). These methods produced similar results to RIAs following HPLC separation of cortisone and cortisol. Extraction efficiency for each sample was assessed by recovery of the 3H-steroid. Steroid concentrations are expressed per g of wet weight of adipose tissue after adjustment for extraction efficiency.

3.7 STATISTICAL METHODS

In general, non-parametric statistics have been used, due to the small sample size, which was not considered normally distributed. Statistical analyses were mostly carried out using Wilcoxon signed rank test. Spearman correlation test as well as Pearson correlation test, and simple regression analysis were also used. The results are presented as mean $\pm$standard error of mean (SEM). A two-tailed p-value of <0.05 was generally considered as statistically significant unless referred otherwise in the studies.

4 MAIN RESULTS

4.1 STUDY I

EFFECTS OF LONG-TERM PREDNISOLONE TREATMENT OF PATIENTS WITH PULMONARY SARCOIDOSIS; LEPTIN LEVELS, EATING BEHAVIOUR AND BODY COMPOSITION ASSESSMENTS.

In this study nine patients with newly diagnosed pulmonary sarcoidosis were administered prednisolone for one year. Anthropometrical, metabolic and eating behaviour variables were monitored. The patients took prednisolone during the whole study period.

Assessments of study variables were performed before and 2 weeks, 3 months and 12 months after the start of prednisolone intake.

Total energy intake increased about 10% ($p<0.05$), measured by 48-hour food-intake recall after two weeks treatment, but no changes in intake of separate macronutrients were observed.

Food intake (by VIKTOR) increased with 23% after 3 months of treatment ($p<0.05$) in spite of increasing s-leptin levels ($p<0.05$). Perceived desire to eat and hunger before meals also increased significantly compared to measurements after 2 weeks of treatment. Body weight increased between 2 weeks and 3 months. Total cholesterol as well as HDL cholesterol increased in comparisons with values before treatment. Triglycerides and the glucose levels remained unchanged.

A more unfavourable pattern of the eating behaviour parameter "deceleration" was also found ($p<0.05$). Body fat % measured by bio-impedance increased after 3 months treatment ($p<0.05$). The amount of abdominal fat in relation to the amount of fat on legs (by DXA) showed an increase in trunkal fat after 12 months ($p<0.05$). These results were reinforced by an increase in waist circumference ($p<0.05$) after three months. The redistribution of adipose after three months (by DXA) was also correlated to the change in eating behaviour parameter deceleration ($p<0.05$; $Rho=0.76$).

4.2 STUDY II

EFFECTS OF GLUCOCORTICOIDS ON LEPTIN LEVELS AND EATING BEHAVIOUR IN WOMEN

In this study twelve postmenopausal, overweight or obese women were taking 25 mg prednisolone for 7 days. All assessments were made before prednisolone treatment, and repeated on the seventh day of treatment.

Fasting serum leptin rose already on day 2 ($p<0.01$) and remained elevated on day seven ($p<0.01$). Single-meal food-intake on VIKTOR was also increased or unchanged in all the women, except for one who suffered from gastro-enteritis on the day of measurements after treatment. In the whole group the mean single-meal food intake increased significantly by 20% ($p<0.05$).

The micro-structure of eating, i.e. eating rate, duration and rate of deceleration, did not change, nor did any of the different appetite rating scales before or after the test meal.

In the 48-hour food-intake recall interview, there was a tendency for an increase in total energy intake, however, not statistically significant.

HDL-cholesterol increased and LDL-cholesterol decreased significantly ($p<0.05$), while total cholesterol and triglycerides remained unchanged. The anthropometrical values, blood-glucose and blood pressures did not change after treatment.

4.3 STUDY III

UNCOUPLING PROTEIN 2 mRNA DOWN-REGULATION IN WOMEN TREATED WITH GLUCOCORTICOIDS

This study was designed to evaluate the in vivo effect of glucocorticoids on the expression of UCP2 mRNA in subcutaneous adipose tissue in eight healthy postmenopausal women after seven days of treatment with prednisolone.

The value for UCP2 mRNA was markedly affected by prednisolone treatment. The level of UCP2 mRNA decreased in each subject following glucocorticoid administration. The value for UCP2 mRNA was about 50 % lower after prednisolone treatment, as compared to pre-treatment values ($p<0.05$). In contrast, no change of the 18S rRNA levels before as compared to after glucocorticoid treatment was demonstrated ($p=0.89$).

A significant increase in the plasma level of insulin before as compared to after glucocorticoid treatment was also observed, but no effect of glucocorticoid administration on either blood pressure levels or plasma values for glucose or FFA were found.

4.4 STUDY IV

GLUCOCORTICOID-REGULATED ADIPOSE TISSUE SECRETION OF PAI-1, BUT NOT IL-6, TNF-α OR LEPTIN, IN VIVO.

In this study the outcome variables were glucocorticoid regulation or not, of PAI-1, Leptin, IL-6 and TNF-α adipose tissue secretion in humans. Subcutaneous adipose tissue from healthy women was obtained and analysed, before and after 7 days of glucocorticoid treatment.

PAI-1 values were markedly affected by prednisolone treatment, as compared to pre-treatment levels, and increased by 80% in all but two subjects ($p<0.05$). In contrast, no difference in either TNF-α, IL-6 or leptin adipose tissue secretion was found comparing pre- and post glucocorticoid treatment.

The observed glucocorticoid mediated increase in PAI-1 adipose secretion was associated with an altered mRNA expression of PAI-1. However, no difference in adipocyte PAI-1 mRNA levels before, as compared to after, glucocortocoid treatment was found.

Finally, there were no significant correlations between changes in PAI-1 adipose secretion and changes in plasma levels of insulin and LDL-cholesterol, respectively, following prednisolone treatment.

The anthropometrical values including body weight, sagittal diameter, body fat percentage and waist/hip ratio, as well as blood pressures did not change after treatment. Insulin showed an increase of borderline significance ($p= 0.062$), but the plasma glucose level was unaffected by glucocorticoid exposure. The level of plasma HDL-cholesterol increased and LDL-cholesterol decreased significantly ($p<0.05$), while total cholesterol and triglycerides remained unchanged.

4.5 STUDY V

EFFECTS OF CORTISOL/CORTISONE CONVERSION IN SUBCUTANEOUS ADIPOSE TISSUE BY 11β HYDROXYSTEROID DEHYDROGENASE IN WOMEN AFTER EXPOSURE TO PREDNISOLONE

This study investigated the impact of 24 hours oral prednisolone treatment on healthy adult subjects. Peripheral cortisol metabolism via subcutaneous adipose tissue analysis of 11β-HSD 1 activity was linked to appetite parameters.

Real Time PCR showed a significant decreased in 11β-HSD1 mRNA expression ($p<0.05$), and glucocorticoid-receptor alpha mRNA was significantly increased ($p<0.05$) in subcutaneous adipose tissue after 24 hours glucocorticoid exposure.

11β-HSD1 bioactivity decreased after prednisolone treatment. The bioactivity decreased after prednisolone treatment. Comparing the pre-prednisolone bioactivity with post-prednisolone levels after 30 hours incubation, a significant decrease ($p<0.05$) was detected. Analyses of cortisol and cortisone secretion by RIA and ELISA in the adipose tissue showed no significant changes after glucocorticoid exposure (table 2).

There was a significant, positive, correlation on baseline between fasting insulin levels and 11β-HSD1 activity ($r=0.71$, $p<0.05$), and a negative correlation with GR mRNA expression ($r=-0.73$). Furthermore, a negative correlation was also found between GR mRNA and 11β-HSD1 mRNA ($r=-0.64$, $p<0.01$) as well as 11β-HSD1 activity ($r=-0.85$, $p<0.01$) before prednisolone exposure. There were no other significant correlations between 11β-HSD1 and anthropometrical/biochemical parameters.

Appetite assessments using the visual analogue rating scales one day before prednisolone intake, on the exposure day and 24 hours after treatment showed borderline significant increase in subjective feeling of hunger ($p=0.075$) and a statistically significant decrease ($p<0.05$) in perceived feeling of satiety before dinner 24 hours after glucocorticoid exposure.

5 DISCUSSION

Why are the metabolic and behavioural responses to glucocorticoid treatment of interest?
In the perspective of high relapse rate of most patients enrolled on obesity treatment programmes, a more comprehensive understanding of underlying pathophysiological mechanisms contributing to the development of obesity and the metabolic syndrome would be beneficial (Ginsberg –03).

Two main aspects of glucocorticoid treatment were studied in this thesis; One was the clinical problem of weight gain among patients reduced to chronic glucocorticoid intake for medical reasons (Jacobs –96, Palmer –91). These patients often re-distribute their fat depots into a more centralised distribution pattern, and develop a similar clinical profile as other patients suffering form hypercortisolemia (table 1) (Stokes –76, Kirk –00). The second aspect was the glucocorticoid modulation of adipose tissue metabolism and distribution, which could provide clues to understanding the role of endogenous cortisol in the development of the metabolic syndrome and cardiovascular disease risk factors (Bujalska –97, Walker –01, Tomlinson –01).

Generally, the similarities between conditions with cortisol excess, for example Cushing´s syndrome, and the metabolic syndrome are obvious (Kirk –00). Paradoxically, cortisol serum levels are usually not elevated. In trying to explain the origin of central and visceral obesity hypothetical models have been proposed, mainly: 1) the hypothalamic origin (Bjorntorp –01, Rosmond –98) 2) disturbances in adipose tissue secretion and function (Walker –01, Arner -97) and 3) dysregulation of cortisol/cortisone conversion (Donovan –99, Mazusaki -01).

In this thesis the main outcome variables were the glucocorticoid effects on eating behaviour and aspects of appetite regulation, adipose tissue secretion and cortisol/cortisone conversion in a clinical setting.

5.1 LIMITATIONS OF THE STUDIES

Having the intention of studying glucocorticoid medication effects in a clinical setting there were obvious limitations. For ethical reasons, the treatment period as well as the given dosage of glucocorticoids was limited, and sample sizes restricted. In our studies healthy subjects were not treated for more than seven days, and only as their own controls in a within-subjects design. This design limitation could be regarded as a weakness, since no placebo-treated control group was included. A bias, which however is difficult to circumvent, is that all subjects knew they were taking an active drug. The participants were informed that the purpose of the study was to examine effects of the medication (glucocorticoids) on biochemical aspects and hormonal changes. Simultaneously, several studies were performed in the same laboratory on other drugs, for the purpose of decreasing appetite and reduce body weight. Therefore, the study design should not invalidate the observations on appetite and eating behaviour.

In study 1, we wanted to examine long-term effects of glucocorticoids. Since glucocorticoids cannot be given on a long-term basis to normal subjects for experimental purposes, we selected patients with newly diagnosed early pulmonary sarcoidosis, in whom long term treatment with corticosteroids is a clinically established routine. All subjects were healthy apart from their sarcoidosis, and the disease itself was essentially stabile.

Another limiting aspect of the studies, which has to be elucidated, is the potential interaction of measured outcome variables (fig 7), and omissions from the measurement protocol. As discussed previously, glucocorticoids exert their effect on several different systems in the body, which makes it problematic to distinguish direct or indirect glucocorticoid effects in a clinical setting. From this point of view, the design of the studies made it possible to detect intra-individual differences presumably indicating glucocorticoid effects.

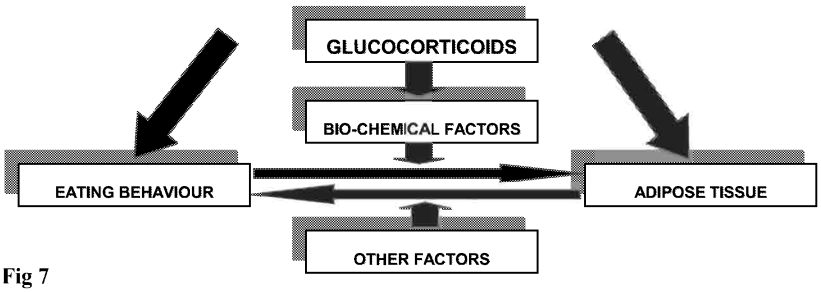

Fig 7

Studying the glucocorticoid effect needs some consideration concerning both interactions between chosen variables as well as the potential regulatory effect of confounders.

5.2 APPETITE REGULATION AND GLUCOCORTICOIDS

Under normal circumstances energy balance oscillates from meal to meal, and from day to day, without persistent changes of body weight or fat stores. Social and behavioural factors can influence the control of body weight to a certain extent, but a series of physiological processes are primarily responsible for body weight regulation. It is thought that the body exerts a stronger defence against undernutrition and weight loss than it does against overconsumtion and weight gain (WHO –98, Friedman -02). A range of mechanisms within the intestine, adipose tissue and brain, sense the inflow if dietary nutrients, and modulate behavioural changes in eating, physical activity, body metabolism and energy storage.

As stated previously, weight gain is one of the major consequences of long-term treatment with glucocorticoids, and increased cortisol secretion one of the characteristics of central obesity. In order to gain weight, a positive energy balance is required, with a deviation from energy balance. Centrally, insulin and leptin inhibit the expression of orexigenic neuropeptides, and stimulate the secretion of anorexigenic peptides in order to influence the termination of a meal (McMinn –00, Friedman –02).

Circulating leptin levels rose significantly after glucocorticoid administration in both study I and II. This rise occurred already after two days treatment in study II, but the increase was maintained after seven days of treatment. Study II compared leptin-levels after 3 months GC exposure, and also resulted in a significant elevation. In statistical path analyses of humans, elevated cortisol secretion is followed directly by increasing body mass index with an associated leptin elevation (Rosmond –98)

In study I, single-meal food-intake was increased after three months of glucocorticoid treatment under controlled conditions as measured by VIKTOR. During this period subjects gained weight significantly. The increase in food intake was also observed after seven days of glucocorticoid intake in study II. Food intake after glucocorticoid treatment has not been measured previously, apart from a study by Tataranni et al (Tataranni –96) where intake increased. However, a food dispenser was used, which can overestimate ordinary energy intake.

The VIKTOR measurements allowed not only registrations of food intake after prednisolone treatment by a standardised method, but also an opportunity to investigate

which part of the microstructure of the eating behaviour was affected (Barkeling –90, 95). Measurements indicate that the deceleration phase of eating, in study I, was more unfavourably affected. The most common type of curve is the decelerated, negative, eating curve, sometimes referred to as the "biological satiation curve" (Meyer–72). In study I, there was an accelerating, positive, curve after three months of glucocorticoid treatment, which could indicate a reduced signalling of satiety signals.

An increase in circulating leptin levels in study I and II also occurred. In study I leptin levels rose significantly after three months of treatment and in study II this increase was noted after two days, still being significantly increased after seven days. Administration of various glucocorticoids to humans has generally been followed by elevated leptin concentrations (Newcomer –98, Dagogo-Jack –97, Larsson –96, Miell –96, Berneis –96). Furthermore, elevated plasma leptin levels have been found in patients with Cushing´s syndrome (Mazusaki –97).

Animal experiments on glucocorticoid administration and food intake regulation support our findings. Zakrzewska and co-workers showed that adrenalectomy on mice was followed by diminished food intake, which was gradually increased with corticosterone (mice glucocorticoid analogue) substitution (Zakrzewska –97). With high doses of the hormone, food intake became excessive, resulting in obesity. This was paralleled by an increased secretion of the satiety hormone leptin, and obesity with elevated leptin levels occured. In another rodent study, mice with overexpression of adipose tissue cortico-sterone, exhibited pronounced hyperphagia despite hyperleptinemia (Masuzaki –01). These animal studies suggest that glucocorticoids induce overeating in spite of increased leptin production, creating a resistance to the satiety signals of leptin.

Leptin and insulin counteract centrally as anorexigenic mediators (McMinn –00, Friedman –01). Glucocorticoids probably block intracellular, hypothalamic transcription factors postreceptorally (Campfield –95), the so-called JAK-STAT pathway, which is used by leptin (fig 8), thereby also inhibiting the leptin satiety signals (Madiehe -01). Furthermore, a downregulation of leptin receptor expression after glucocortiocoid exposure has also been suggested (Smith –02). These models could propose explanations to a hypothetical inhibition of satiety signals due to glucocorticoid excess.

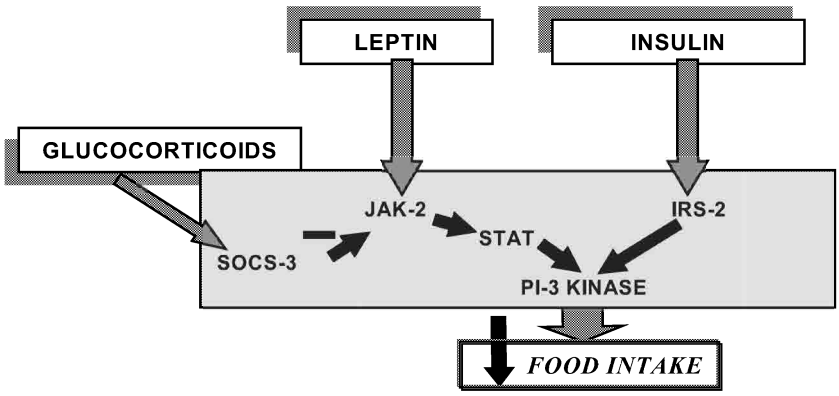

Fig 8

Overview of leptin and insulin action centrally as anorexigenic factors. Their effect is counteracted by glucocorticoids by inhibition of the so called JAK-STAT pathway.

5.3 GLUCOCORTICOIDS AND ADIPOSE TISSUE DISTRIBUTION AND SECRETION

Intra-abdominal fat accumulation is, as stated previously, a recognised component of the metabolic syndrome. To our knowledge, there has been no previous investigation into when the redistribution of adipose tissue to the abdominal area actually begins, once glucocorticoids have been administered.

In study I the patients suffering from sarcoidosis were followed for twelve months and taking prednisolone during this entire period. Even though the increase in total weight occurred after 3 months treatment, it was significant only due to a small weight decrease after 2 weeks of treatment, consequently not significantly different compared to the pre-treatment weight. Interestingly, body fat percent, measured by bio-impedance, increased steadily, and rose significantly after 3 months of treatment compared to pre-treatment values. The DXA measurements did not indicate increased trunkal fat contents. On the other hand, comparing total fat contents in both legs to trunkal fat contents indicated shifting fat depots. The index between trunkal fat and legs fat contents increased significantly after 12 months prednisolone treatment, which

indicated a change in adipose tissue distribution from peripheral into more centralised locations.

Mechanisms supporting this centralisation of fat depots during glucocorticoid exposure are not fully understood. As mentioned previously, abdominal fat depots contain a higher density of GR, which contributes to the increased degree of fat accumulation in adipocytes via LPL. Mediating mechanisms for the centralisation of fat depots during GC exposure could be of clinical interest.

Uncoupling proteins have been implicated in both lipid oxidation and thermogenesis as an influence in weight regulation. UCP2 is expressed in all tissues (Mattson –03), thus found also in white adipose tissue. In study III there was a significant down-regulation in UCP2 mRNA expression in all subjects after seven days of GC treatment. Interestingly, serum insulin levels also increased, and a positive correlation between UCP2 mRNA expression and s-insulin levels was found after GC treatment. In animal studies, UCPs have been associated with insulin resistance and diabetes (Bao –98, Krook –98, Pinkney –00), which is one of the main consequences of central obesity as well as chronic glucocorticoid treatment. We showed that glucocorticoids may be involved in regulating UCP2 expression, but also hyperinsulinemia, or insulin resistance. However, there is no evidence yet that UCP´s are involved in thermogenesis regulation in humans (Dulloo –01), although recent data suggest that UCP´s are involved in regulating lipids as metabolic fuel. Given this putative role to UCP´s, they are still involved in body-weight regulation via the control of food intake and substrate balance (Moriscot –93). Considering that leptin has modulated the expression of uncoupling proteins (Scarpace –00, Gong –97, Gullicksen PS -02), it is of interest that the subjects in study III participated also in study II, where glucocorticoid exposure elevated serum leptin levels. However, the FFA plasma level, measured in study III, was not found to be regulated by glucocorticoid administration, which has been shown previously (Ricquier –00). Nevertheless, other intermediate variables affected by predinsolone treatment may be involved in regulating UCP2 expression in man.

Leaving different aspects of abdominal fat accumulation during glucocorticoid excess, and instead focusing on associated metabolic complications, the endocrine function of adipose tissue may also be important. Adipose tissue secretes a number of proteins out of which plasminogen activator inhibitor 1 (PAI-1), tumour necrosis factor alpha (TNF-

α), leptin and interleukin-6 (IL-) have been implicated in both obesity and its co-morbidites (Arner –01, Alessi –00). In study IV all subjects except two showed increased secretion of PAI-1 from subcutaneous adipose tissue, after seven days of glucocorticoid treatment. In contrast to the glucocorticoid effect of increased PAI-1 secretion, TNF-α, IL-6 and leptin adipose tissue secretion did not change after prednisolone exposure. These findings may seem conflicting compared to previous studies, showing increased leptin secretion and decreased IL-6 secretion after glucocorticoid exposure. Most such studies were, however, conducted *in vitro*, or for a relatively short study period of only two days, and on diverse populations (such as obese and male) (Masuzaki –97, Fried –98, Halleux –98, Papaspyrou-Rao –97).

The underlying mechanisms explaining the observed increase in PAI-1 secretion following glucocorticoid exposure is unclear, but two glucocorticoid responsive elements within the human PAI-a promoter have been identified (von Zonneveld –98). A direct effect of glucocorticoids on adipose tissue secretion of PAI-1 is further reinforced by the fact that neither anthropometrical nor biochemical effects were observed in this study. The increase in PAI-1 secretion is in agreement with previous *in vitro* studies (Halleux –99, Sartori –99). Furthermore, the assumption that the increased PAI-1 secretion is originating from adipocytes is supported by findings that stromal cells did not increase PAI-1 secretion after glucocorticoid exposure, while human adipocytes responded significantly with increased PAI-1 and PAI-1 mRNA (Morange –99, Seki -01). In study IV, however, the PAI-1 mRNA level remained constant suggesting that mechanisms other than gene transcription could be influential, including PAI-1 protein translation/stability or specific effects on the secretion machinery. The relevance of glucocorticoids in PAI-1 regulation is also emphasised by clinical studies showing that Cushing´s disease and steroid treatment are associated with increased PAI-1 plasma levels (Patrassi –92, -97). Since elevated PAI-1 levels have been associated with increased risk of atherosclerosis (Sartori –99) and diabetes mellitus (Mohamed-Ali –99), as well as abdominal obesity (Eriksson –98, Alessi –00), which are essential features of hypercortisolemia. The findings from study IV suggest a possible link between hypercortisolemia and the metabolic syndrome.

An involvement of 11β-HSD1 in the development of the metabolic syndrome has been suggested. As discussed previously, concentrations of cortisol in central fat depots are increased, although the circulating concentrations of plasma cortisol are not elevated,

which was illustrated by the term “Cushing´s disease in the omentum” coined by Bujalska, Kumar and Stewart (Bujalska –97). Transgenic mice, overexpressing 11β-HSD1 in adipose tissue, developed central obesity, as well as symptoms of the metabolic syndrome – increased insulin resistance, impaired glucose tolerance and hyperlipidemia (Mazusaki –01). In a study by Rask et al. obese men had increased 11β-HSD1 activity in subcutaneous adipose tissue, and decreased liver 11β-HSD1 activity, suggesting impaired activation of cortisone to cortisol. Consequently lower circulating plasma levels of cortisol among these obese men was also found in spite of increased adipose tissue cortisol levels (Rask –01).

In study V we found a marked downregulation of 11β-HSD1 mRNA expression in subcutaneous adipose tissue after 24 hours exposure to prednisolone. In previous studies, glucocorticoids regulate both 11β-HSD1 and 2, but generally up-regulate the expression of 11β-HSD1 (Masuzaki –01, Bujalska –99, Hauner –89). However, the results are conflicting (Low –94, Weidenfield –82, Napolitano –98, Jamieson –99), and, generally, these studies were conducted for longer periods than study V. Making a hypothetical parallel between glucocorticoid treatment, i.e. glucocorticoids administered exogenically, to the increased secretion of cortisol following the metabolic syndrome, we would expect an increase in 11β-HSD1 expression in study V. The down-regulation that was actually found cannot be explained entirely by the short duration of the study. A down-regulation of 11β-HSD1 gene expression was also found in another study using Affymetrix micro-array system (un-published data), with subjects treated with prednisolone for seven days. A compensatory decrease due to glucocorticoid excess is of course a possible explanation, but also divergent study populations, exposure time to glucocorticoid excess and differences between in vivo and in vitro findings. Taken together these findings suggest that exogenous glucocorticoids regulate the expression of 11β-HSD1.
An increased expression of glucocorticoid receptor alpha was also found in study V. In the seven-days micro-array study mentioned above, an up-regulation of immunophilin (involved in the activation of steroid receptors) was found, which, supports the findings of study V.

An increased expression of 11β-HSD1 in subcutaneous adipose tissue correlating with increased BMI was also found by Rask and co-workers (Rask –02). We found no correlation between 11β-HSD1 and BMI, though, which could be due to the short

prednisolone exposure time. However, a significant, positive correlation before prednisolone treatment between fasting insulin levels and 11β-HSD1 activity, was detected. This correlation has been shown previously (Andrews –99), and could hypothetically indicate a role for 11β-HSD1 in mediating insulin resistance. 11β-HSD1 levels at baseline was analysed as a predictor of changes in other outcome variables. The data supported that 11HSD1 may indeed be involved in downregulation of LPL, HSL (trend), and in regulation of intra-adipose cortisol and cortisone. A reduced angiotensinogen mRNA level was also observed post-prednisolone exposure, contradictory to most previous studies (Luft –01) instead indicating an elevation. Results diverge, though, with lower angiotensinogene gene expression in obese subjects (Gorzelniak –02).

5.4 INTERACTION BETWEEN MEASURED OUTCOME VARIABLES

Understanding how the problem of overweight and obesity develops is complex and several factors interact to create a positive energy balance. The interaction between a number of these influences, rather than any single factor acting alone, is probably causing obesity. Glucocorticoids seem to play a role in the development of obesity (Walker –01), in particular central obesity. This thesis was therefore designed to investigate different aspects of potential mechanisms contributing to the mediation of glucocorticoid effects (fig 9).

In study I the alteration of fat contents after three months (by DXA) was correlated to the change in eating behaviour parameter deceleration ($p<0.05$). Due to the small sample size, a cautious interpretation in needed. Nevertheless, the data suggest which part of the micro-structure of eating behaviour that may contribute to the development of central obesity. In a recent study of Kral and co workers, severe obese subjects suffering from metabolic co-morbidity, increased their eating rates (Kral –01). Furthermore, valuing the results, one should also bear in mind that the subjects were eating unconsciously of the purpose of the study until pleasantly satisfied, unaware of any behavioural alterations.

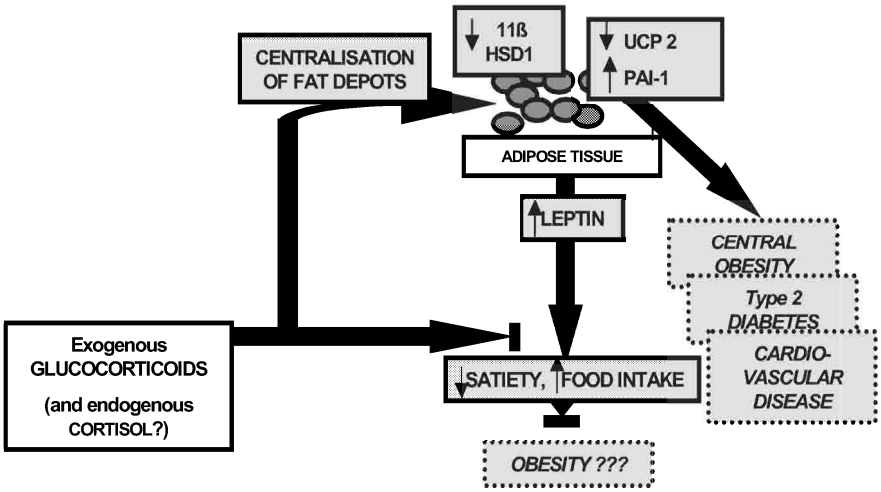

Fig 9

Overview of main findings of the thesis. Dotted boxes are speculative consequences founded partly on thesis results.

Exogenous glucocorticoids may hypothetically exert their effect in a similar way as endogenous cortisol. Due to increased glucocorticoid levels, leptin signals may be blunted, resulting in decreased feeling of satiety and consequently also increased food intake. The redistribution of fat depots resulted in adipose tissue centralisation. Metabolic alterations in the subcutaneous adipose tissue are suggested to be of pathophysiological importance in development of obesity as well as central obesity, type 2 diabetes and cardiovascular disease – features of the metabolic syndrome.

The fact that food intake increased by 23 % during the test meal, but feeling of fullness remained unchanged, suggests that the prednisolone exposure altered the relationship between intake and satiation. This satiation, that regulates the meal size, is a hypothetical leptin effect in the human body (Friedman –02), implying decreased leptin signalling. An attenuated feeling of satiety rated on visual rating scales was also found after 24 hours prednisolone exposure in study V. However, leptin levels were not monitored in study V. In study I and II a marked increase of circulating leptin levels were observed following prednisolone treatment already after two days of treatment.

This observation remained after three months. Rising leptin levels after glucocorticoid exposure, which is a well known phenomenon in the literature, could be caused by a compensatory release due to blunted satiety effects (Zakrzewska –97, Bjorntorp –01). The increased intake of food after glucocorticoid treatment in both study I and II as well as a more accelerating, unfavourable, eating curve, in spite of elevated serum leptin levels, may be an indication of decreased satiation signalling.

Speculating in the increased accumulation of central fat depots, the findings of UCP2 down-regulation in study III is interesting from the point of view that it was performed on eight of the patients from study II. Given UCP2 a putative role in thermogenesis and/or lipid oxidation involvement all women decreased their UCP2 mRNA expression after glucocorticoid exposure, and this decrease was correlated to an insulin increase. Insulin resistance is one of the features of Cushing´s syndrome, hypercortisolemia, but also the metabolic syndrome. All women in study II were included in study IV, which evaluated some of the endocrine aspects of adipose tissue secretion after prednisolone treatment. PAI-1 secretion increased significantly, and this protein represents a risk factor for both type 2 diabetes and cardiovascular disease. Paradoxically the adipose tissue secretion of leptin did not change, although circulating leptin levels increased significantly. This could be explained by alterations in leptin clearance, or maybe by the fact that adipose tissue biopsies were repeated only after seven days of treatment making it possible to miss the adipose tissue secretion peak.

HDL-cholesterol and LDL-cholesterol both increased in study I, and LDL-cholesterol decreased while HDL-cholesterol values were elevated in study II after prednisolone exposure. This might be at least partly explained by an up regulation of LDL-cholesterol receptor density, which has been reported previously after glucocorticoid and adrenocorticotropin administration(Berg –94, -99).

The impaired expression of 11β-HSD1 in subcutaneous adipose tissue in study V, and the increased GR alpha expression were linked to an attenuated feeling of satiety after 24 hours prednisolone exposure. The down-regulation of 11β-HSD 1 activity, and in parallel up-regulation of GR alpha in our study, could hypothetically be the result of compensatory negative feedback mechanisms after glucocorticoid excess (Whorwood –01). A significant pre-treatment correlation between GR alpha and 11β-HSD 1 mRNA

furthermore reinforces this suggested explanation model linking the expression of these two parameters to each other and to the presence of glucocorticoids.

The 11β-HSD1 bio-activity also decreased after prednisolone treatment indicating a profound regulatory effect of glucocorticoids on the adipose tissue function.
Interestingly, a recent study also showed leptin administration activated 11β-HSD 1 in hepatocytes of *ob/ob* mice as well as resulted in weight decrease (Liu –03). Supposing that glucocorticoids are involved in appetite regulation and central fat accumulation (Benediktsson –93, White –97, Walker –01) data shown by Liu and co-workers could hypothetically present an explanation model for glucocorticoid-mediated blocking of leptin signalling, weight gain, and modulation of 11β-HSD 1 expression. In previous studies increased 11β-HSD1 expression was found to parallel increased BMI (Rask –02). It is unclear, though, if the hypothesis of the hyperactive HPA-axis causes the increased secretion and thus enhances the development of visceral adiposity, or if a susceptibility or predisposition promotes an increased 11β-HSD1 expression, thus increasing local levels of cortisol in the central adipose tissue, thereby promoting fat accumulation.

5.5 FUTURE PERSPECTIVES

The role of glucocorticoids in the development of obesity and the metabolic syndrome is not a new knowledge, but linking different aspects of eating behaviour, adipose tissue distribution and function to each other gives a more in-depth view of contributing mechanisms mediating the impact of glucocorticoids in humans.

To increase understanding of eating behaviour aspects further, it would be beneficial if randomised, placebo controlled intervention studies with larger sample sizes could be performed. The impact of the discussed bias concerning open-label prednisolone treatment procedures in this thesis, is unknown, why comparisons with a placebo-treated control group could be of interest. In previous studies sex differences concerning both eating behaviour and prednisolone effects have been detected. Male subjects seem to have more stabile eating patterns than women (Barkeling –95), and also respond differently to glucocorticoid exposure regarding leptin levels (Newcomer –98, Dagogo-Jack –97, Larsson –96, Miell –96, Berneis –96). We have only examined leptin secretion and serum levels, but a variety of hormones regulate short-term and long-term hunger and satiety signalling (McMinn –00). In study I an arginin-stimulation test was also performed, allowing further hormonal analyses which will be performed.

The identification of genes predisposing for glucocorticoid-mediated obesity could be thoroughly investigated. Apparently there are glucocorticoid-responsive elements in genes involved in the development of the metabolic syndrome (van Zonneveld –88). Genes regulated after GC exposure has been performed by Affymetrix micro-array system, and will be further analysed. Since glucocorticoids are suggested to be involved in the metabolic syndrome genesis, a genetic susceptibility could be of interest. Our clinical observations indicate a differentiation, which is not gender or age specific, between those who gain weight during glucocorticoid treatment and those who do not. Examining these two patient categories could add several clues to the understanding of glucocorticoid-mediated obesity. Are those who do not gain weight restrained eaters i.e. do they finish their meal before they are full? Epel and co-workers showed that subjects responding with elevated cortisol secretion after exposure to stress also overate (Epel –00). Are different patterns of responding to stress contributing to the development of

obesity during glucocorticoid treatment? Individual glucocorticoid sensitivity in humans has been suggested (Knutsson, thesis –00) as well as intra-individual glucocorticoid receptor responses (Knutsson & Stierna unpublished data). Possible dose-response effects of glucocorticoids on appetite regulation, metabolic implications and eating behaviour could therefore be of interest. Due to the individual responsiveness to glucocorticoid treatment, the optimal dosages, with best clinical effects and with medication side-effects reduced to a minimum, could be beneficial. Another aspect is of course glucocorticoid involvement in the metabolic syndrome. Understanding individual patterns of responses to glucocorticoids could contribute with additional pathophysiological clues to the development of central obesity.

Cytokines, and other components of the immune system, have been discovered as components contributing to the development of obesity (Guerre-Millo –02). Many of these factors are produced in the adipose tissue. In this thesis we only examined the secretion of TNF-α and IL-6, but considering the well-established effects of glucocorticoids on the immune system, further analyses of factors involved in the immune system could be of interest.

Adipose tissue hormones are also involved in vascular homeostasis, and one well-known side effect of glucocorticoid treatment is hypertension. Angiotensinogen plays a critical role in the regulation of blood pressure (Negrell –99), and overproduction elevates blood pressure in obese subjects. Therefore, examining glucocorticoid effects on angiotensinogen could maybe contribute to the understanding of hypertension in central obesity.

A more accurate evaluation of the range of factors contributing to glucocorticoid-mediated obesity should be defined to enable specific therapy development.
More profound knowledge about the adipose tissue as an endocrine organ opens up possibilities for explanation models regarding the aetiology of the metabolic syndrome. The regulation of adipose tissue secretion and distribution, and the modulation of local glucocorticoid levels, as well as factors involved in glucocorticoid sensitivity, is therefore important to evaluate. Novel therapeutic approaches are needed to develop specific agents that reverse and treat glucocorticoid-mediated obesity and the metabolic syndrome.

6 CONCLUSIONS

- Long-term glucocorticoid treatment causes an increase in food intake despite elevated leptin levels. In addition, an association is suggested between unfavourable changes of the eating curve, probably indicating blunted satiety signals, and centralisation of fat depots.

- Short-term treatment with glucocorticoids also causes an increase in food intake, simultaneously with a rise in circulating leptin levels (after prednisolone treatment), indicating diminished satiation signalling.

- Giving UCP2 a putative role in thermogenesis and/or lipid oxidation involvement the UCP2 mRNA expression decrease after glucocorticoid exposure could promote adipose tissue accumulation. This decrease was correlated with increased insulin levels, suggesting that hyperinsulinemia is involved in the regulation of UCP2 expression. A causal link to the metabolic syndrome is therefore suggested.

- A significant increase in PAI-1 (but not TNF-α, IL-6 or leptin) secretion from subcutaneous adipose tissue *in vivo*, after short-term treatment with glucocorticoids suggests a possible mechanism for the well-known increase in plasma PAI-1 in clinical hypercortisolemia, which in turn represents a risk factor for type 2 diabetes mellitus and cardiovascular disease.

- Impaired subcutaneous adipose tissue 11β-hydroxysteroid dehydrogenase 1 expression and increased glucocorticoid receptor alpha expression following glucocorticoid treatment for 24 hours, indicates a regulatory effect of glucocorticoids. An association with reduced feeling of satiation reinforces the hypothetical glucocorticoid mediated increase in appetite occurring even after a relatively short treatment period.

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8 REFERENCES

Alessi MC, Morange P, Juhan-Vague I. Fat cell function and fibrinolysis. Horm Metab Res 2000; 32: 504-508.

Allison DB, Fontaine KR, Manson JE, Stevens J, VanItallie TB. Annual death attributable to obesity in the United States. JAMA 1999; 282: 1530-1538.

Andrews RC, Walker BR. Glucocorticoids and insulin resistance: old hormones, new targets. Clin Sci 1999; 96: 513-523.

Arner P, Eckel RH. Adipose tissue as a storage organ. In: Handbook of obesity, eds. GA Bray, C Bouchard & WPT James, 1997; pp 379-395, New York: Marcel Dekker, Inc.

Arner P. Regional differences in protein production by human adipose tissue.Biochem Soc Trans 2001; 29: 72-75.

Auboeuf D, Vidal H. The use of the reverse transcription-competitve polymerase chain reaction to investigate the in vivo regulation of gene expression in small tissue samples. Ann Biochem 1997; 245: 141-148.

Bao S, Kennedy A, Wojciechowski B, Wallace P, Ganaway E, Garvey WT. Expression of mRNAs encoding uncoupling proteins in human skeletal muscle: effects of obesity and diabetes. Diabetes 1998; 47: 1935-1940.

Barkeling B, Rössner S, Björvell H. Effects of high-protein meal (meat) and a high-carbohydrate meal (vegetarian) on satiety measured by automatic computerized monitoring of subsequent food intake, motivation to eat and food preferences. Int J Obes 1990; 14: 743-51.

Barkeling B, Rössner S, Sjöberg A. Methodological studies on single meal food intake characteristics in normal weight and obese men and women. Int J Obes 1995; 19: 284-90.

Belliste F, LeMagnen J. The structure of meals in humans: eatiang and drinking patterns in lean and obese subjects. Physiol Behav 1981; 27: 649-658.

Benediktsson R, Lindsay RS, Noble J, Seckl JR and Edwards CRW. Glucocorticoid exposure in utero: new model for adult hypertension. Lancet 1993; 341: 339-341.

Berg AL, Nilsson-Ehle P, Arnadottir M. Beneficial effects of ACTH on the serum lipoprotein profile and glomerular function in patients with membranous nephropathy. Kidney Int 1999; 56: 1534-43.

Berg AL, Nilsson-Ehle P. Direct effects of corticotropin on plasma lipoprotein metabolism in man – studies in vivo and in vitro. Metabolism 1994; 43: 90-7.

Berneis K, Vosmeer S, Keller U. Effects of glucocorticoids and of growth hormone on serum leptin concentrations in man. Eur J Endocrinol 1996; 135: 663-5.

Bjorntorp P, Rosmond R. Visceral obesity and diabetes. Drugs 1999; 58: 13-18.

Bjorntorp P. Do stress reactions cause abdominal obesity and comorbidities? Obes Rev 2001; 2: 73-86.

Bjorntorp P. The association between obesity, adipose tissue distribution and disease. Acta Med Scand Suppl 1988; 723: 121-134.

Bjorntorp P. Visceral obesity: a civilization syndrome. Obes Res 1993; 6: 8-17.

Blundell JE, Rogers PJ, Hill AJ. Evaluating the satiating power of foods: Implications for acceptance and consumption. In: Solms J, Ed. Chemical composition and sensory properties of food and their influence on nutrition. Academic Press, London 1988; 205-219.

Boyko EJ, de Courten M, Zimmet PZ, Chitson P, Toumilehto J, Alberti KG. Features of the metabolic syndrome predict higher risk of diabetes and impaired glucose tolerance: a prospective study in Mauritius. Diabetes Care 2000; 23: 1242-1248.

Bujalska LJ, Kumar S, Hewison M and Stewart PM. Differentiation of human adipose stromal cells: the roles of glucocorticoids and 11β-hydroxysteroid dehydrogenase. Endocrinology 1999;140:3188-3196.

Bujalska LJ, Kumar S,and Stewart PM. Does central obesity reflect "Cushing´s disease of the omentum?". Lancet 1997;349:1210-1213.

Campfield LA, Smith FJ, Guisez Y, Devos R, Burn P. Recombinant mouse OB protein: evidence for a peripheral signal linking adiposity and central neural networks. Science 1995; 269: 546-9.

Chrousos GP, Gold PW. The concept of stress and stress system disorders. Overview of physical and behavioural homeostasis. JAMA 1992; 267: 1244-1252.

Chrousos GP. Regulation and dysregulation of the hypothalamic-pituitary-adrenal axis; The corticotrophin-releasing hormone perspective. Endocrinol Metab Clin North Am 1992; 21: 833-858.

Considine RV, Sinha NK, Heiman ML, Kriauciunas A, Stephens TW, Nyce MR, et al. Serum immunoreactive leptin concentrations in normal-weight and obese humans. N Engl J Med 1996; 334: 292-5.

Considine RV. Regulation of leptin production. Rev Endocr Metab Disord 2001; 2: 357-363.

Coppack SW. Pro-inflammatory cytokines and adipose tissue. Proc Nutr Soc 2001; 60: 349-356.

Dagogo-Jack S, Selke G, Melson AK, Newcomer JW. Robust leptin secretory responses to dexamethasone in obese subjects. J Clin Endocr Metab 1997; 82: 3230-3.

Delaunay F, Khan A, Cintra A et al. Pancreatic beta cells are important targets for the diabetogenic effects of glucocorticoids. J Clin Invest 1997; 100: 2094-2098.

Donovan JS. 11β-hydroxysteroid dehydrogenase: a link between the dysregulation of cortisol metabolism and hypertension. Br J Biomed Sci 1999; 56: 215-225.

Dulloo AG, Samec S. Uncoupling proteins: Their roles in adaptive thermogenesis and substrate metabolism reconsidered. Br J Nutr 2001; 86: 123-139.

Emond M, Schwartz G, Ladenheim E, Moran T. Central leptin modulates behavioral and neural responsivity to CCK. Am J Physiol 1999; 276: R1545-R1549.

Epel E, Lapidus R, McEwen B, Brownell K. Stress may add bite to appetite: A laboratory study of stress-induced cortisol and eating behavior. Psychoneuroendocrinology 2000; 26:37-49.

Eriksson P, Reynisdottir S, Lonnqvist F, Stemme V, Hamsten A, Arner P. Adipose tissue secretion of plasminogen activator inhibitor-1 in non-obese and obese individuals. Diabetologia 1998; 41: 65-71.

Faloia E, Camilloni MA, Giacchetti G, Mantero F. Adipose tissue as an endocreine organ? A review of some recent data. Eat Weight Disord 2000; 5: 116-123.

Fried SK, Bunkin DA, Greenberg AS. Omental and subcutaneous adipose tissues of obese subjects release interleukin-6: Depot difference and regulation by glucocorticoid. J Clin Endocrinol Metab1998; 83: 847-850.

Friedman JM, Halaas JL. Leptin and the regulation of body weight in mammals. Nature 1998; 395: 763-770.

Friedman JM. The alphabet of weight control. Nature 1997; 385: 119-120.

Friedman JM. The function of leptin in nutrition, weight and physiology. Nutr Rev 2002; 60: S1-S14.

Gallagher TF, Yoshida K, Roffwarg HD et al. ACTH and cortisol secretory patterns in man. J Clin Endocrinol Metab 1973; 36: 1058-1068.

Ginsberg HN. Insulin resistance and cardiovasuclar disease. J Clin Invest 2000; 106: 453-458.

Ginsberg HN. Treatment for patients with the metabolic syndrome. Am J Cardiol 2003; 91: 29E-39E.

Goldstein DS. Stress response patterns. In: Goldstein DS, ed. Stress, catecholamines and cardiovascular disease. New Yourk: Oxford University Press 1995; 287-328.

Gong DW, He Y, Karas M, Reitman M. Uncoupling protein-3 s a mediator of thermogenesis regulated by thyroid hormone. Beta 3-adrenergic agonists and leptin. J Biol Chem 1997; 272: 24129-24132.

Gorzelniak K, Engeli S, Janke J, Luft F, Sharma A. Hormonal regulation of the human adipose-tissue renin-angiotensin system: relationship to obesity and hypertension. J Hypertens 2002; 20: 965-973.

Grimble RF. Inflammatory status and insulin resistance. Curr Opin Clin Nutr Metab Care 2002; 5: 551-559.

Groop LC, Tuomi T. Non-insulin-dependent diabetes mellitus – a collision between thrifty genes and an affluent society. Ann Med 1997; 29: 37-53.

Guerre-Millo M. Adipose tissue hormones. J Endocrinol Invest 2002; 25: 855-861.

Gullicksen PS, Flatt PW, Dean RG, Hartzell DL, Baile CA. Energy metabolism and expression of uncoupling proteins 1,2 and 3 after 21 days of recovery form intracerebroventricular mouse leptin in rats. Physiology & Behavior 2002; 75: 473-482.

Halleux CM, Declerck PJ, Tran SL, Detry R, Brichard SM. Hormonal control of plasminogen activator inhibitor-1 gene expression and production in human adipose tissue: Stimulation by glucocorticoids and inhibition by catecholamines. J Clin Endocrinol Metab 1999; 84:4097-4105.

Halleux CM, Servais I, Reul BA, Detry R, Brichard SM. Multihormonal control of ob gene expression and leptin secretion from cultured human visceral adipose tissue: Increased responsiveness to glucocorticoids in obesity. J Clin Endocrinol Metab 1998; 83: 902-909.

Hauner H et al. Promoting effects of glucocorticoids on the differentiation of human adipocyte precursor cells cultured in chemically defined medium. J Clin Invest 1989; 84: 1663-1670.

He G, Pedersen SB, Bruun JM, Richelsen B. Regulation of Plasminogen activator inhibitor-1 in human adipose tissue; Interaction between cytokines, cortisol and estrogen. Horm Metab Res 2000; 32: 515-520.

Henry J, Stephens P. Stress, health and the social enviroment. A sociobiological approach to medicine. New York: Springer-Verlag, 1977.

Hill AJ. Investigation of some short-term influences on hunger,satiety and food consumtion in man. Thesis. Department of Psychology, University of Leeds, England, 1986.

Hojbjerg Gravholt C, Dall R, Sandahl Christiansen J, Moller N, Schmitz O. Preferential stimulation of abdominal subcutaneous liolysis after prednisolone exposure in humans. Obes Res 2002; 10: 774-781.

Isomaa B, Almgren P, Tuomi T, Forsen B; Lahti K, Nissen M, Taskinen MR, Groop L. Cardiovascular morbidity and mortality associated with the metabolic syndrome. Diabetes Care 2001; 24: 683-689.

Jacobs U, Klein B, Miersch WD, Molitor D, Klehr HU. Dilemma: Maintenance therapy enhances sclerogenic risk profile. Transplant Proc 1996; 28: 3227-30.

Jamieson PM; Chapman KI and Seckl JR. Tissue- and temporal-specific regulation of 11 beta-hydroxyxteroid dehydrogenase type 1 by glucocorticoids in vivo. J Steroid Biochem 1999; 68: 245-250.

Janand-Delenne B, Chagnaud C, Raccah D, Alessi MC, Juhan-Vague I, Vague P. Visceral fat as a main determinant of plasminogen activator inhibitor 1 level in women. Int J Obes 1998; 22: 312-317.

Juhan-Vague I, Alessi MC, Morange PE. Hypofibrinolysis and increased PAI-1 are linked to atherosclerosis via insulin resistance and obesity. Ann Med 2000; 32: 78-84.

Kirk LF, Hasch RB, Katner HP, Jones T. Cushing's disease: clinical manifestations and diagnostic evaluation. Am Fam Physician 2000; 62: 1119-27.

Kissilef HR, Thornton J. Facilitation and inhibition in the cumulative food intake curve in man. In: Morrison AJ, Strick P, ed. Changing concepts of the nervous system. New York: Academic Press, 1982: 585-607.

Kissileff HR, Klingsberg G, Van Itallie TB. Universal eating monitor for continous recording of solid or liquid consumption in man. Am J Physiol 1980; 238: R14-R22.

Knutsson U. Individual glucocorticoid sensitivity in the human. Thesis. Karolinska Institutet, Stockholm 2000.

Kral JG, Byckley MC, Kissileff HR, Schaffner F. Metabolic correlates of eating behavior in severe obesity. Int J Obes 2001; 25: 258-264.

Krook A, Digby J, O`Rahilly S, Zierath JR, Wallberg-Henriksson H. Uncoupling protein 3 is reduced in skeletal muscle of NIDDM patients. Diabetes 1998; 47: 1528-1531.

Kvist H, Chowdhury B, Grangard U, Thylen U and Sjostrom L. Total and visceral adipose-tissue volumes derived from measurements with computed tomography in adult men and women: predictive equations. Am J Clin Nutr 1988; 48: 1351-1361.

Lafontan M, Berlan M. Fat cell adrenergic receptors and the control of white and brown fat cell function. J Lipid Res 1993; 34: 1057-1091.

Larsson H, Ahrén B. Short-term dexamethasone treatment increase plasma leptin independently of changes in insulin sensitivity in healthy women. J Clin Endocr Metab 1996; 81: 4428-32.

Lasic S, Bobarevic N, Nikolin B. Simultaneous determination of prednisone, prednisolone, cortisol and dexamethasone in plasma by high-performance liquid chromatography. J Pharm Biomed Ann 1989; 7: 777-82.

Lindgren AC, Barkeling B, Hägg A, Ritzén, Marcus C, Rössner S. Eating behaviour in Prader-Willi syndrome, normal weight and obese control groups. J Pediatr 2000; 137: 50-55.

Ling ZC, Khan A, Delauneay F et al. Increased glucocorticoid sensitivity in islet beta –cells: effects on glucose 6-phosphatase, glucose cycling and insulin release. Diabetologia 1998; 41: 634-639.

Liu Y, Nakagawa Y, Wang Y, Li R, Li X, Ohzeki T and Fredman TC. Leptin activation of corticosterone production in hepatocytes may contribute to the reversal of obesity and hyperglycemia in lepitn-deficient ob/ob mice. Diabetes 2003;52:1409-1416.

Livanou T, Ferreiman D, James VHI. Reovfery of hypothalamic-pituitary-adrenal function avter corticosterid therapy. Lancet 1967; 2; 865-859.

Livingstone DEW, Jones GC, Smith K et al. Understanding the role of glucocorticoids in obesity: tissue-specific alterations of corticosterone metabolism in obese Zucker rats. Endocrinology 2000; 141: 560-563.

Long CNH, Katzin B, Fry EG. The adrenal cortex and carbohydrate metabolism. Endocrinology 1940; 26: 309-344.

Lord G. Leptin modulates the T-cell immune response and reverses starvation induced immunosupression. Nature 1998; 394: 897-901.

Low SC, Moisan MP, Noble JM et al. Glucocorticoids regulate hippocampal 11beta-hydroxysteroid dehydrogenase activity and gene expression in vivo in the rat. J Neuroendocrinol 1994; 6:285-290.

Luft FC. Molecular genetics of salt-sesitivity and hypertension. Drug Met Dispos 2001; 29: 500-504.

Madiehe A, Lin L, White C, Braymer DH, Bray GA, York DA. Constitutive activation of STAT-3 and downregulation of SOCS-3 expression induced by adrenalectomy. Am J Physiol Regulatory Integrative Comp Physiol 2001; 281: R2048-R2058.

Martin BC, Warram JH, Krolewski AS, Berman RN, Soeldner JS, Kahn CR. Role of glucose and insulin resistance in development of type 2 diabetes mellitus: results of a 25-year follow-up study. Lancet 1992; 340: 925-929.

Masuzaki H, Paterson J, Shinyama H, Morton NM, Mullins JJ, Seckl JR, Flier JS. A transgenic model of visceral obesity and the metabolic syndrome. Science 2001; 294: 2166-2170.

Mattson MP, Liu D. Mitochondrial potassium channels and uncoupling proteins in synaptic plasticity and neuronal cell death. Biochem Biophys Res Communic 2003; 304: 539-549.

Mazusaki H, Ogawa Y, Hosoda K, Miyawaki T, Hanaoka I, Hiraoka J, Yasuno A, Nishimura H, Yoshimasa Y, Nishi S, Nakao K. Glucocorticoid regulation of leptin synthesis and secretion in humans: Elevated plasma leptin levels in Cushing´s syndrome. J Clin Endocrinol Metab 1997; 82: 2542-2547.

Mazusaki H, Ogawa Y, Hosoda K, Miyawaki T, Hanaoka I, Hiraoka J, Yasuno A, Nishimura H, Yoshimasa Y, Nishi S, Nakao K. Glucocorticoid regulation of leptin synthesis and secretion in humans: Elevated plasma leptin levels in Cushing´s syndrome. J Clin Endocrinol Metab 1997; 82: 2542-2547.

Mc Ewan BS, Biron CA, Brunson KW, Bulloch K, Chambers WH, Dhabhar FS, Goldfarb RH, Kitson RP, Miller AH, Spenser RL, Weiss JM. The role of adrenocorticoids as modulators of immune function in health and disease: neural, endocrine and immune ineteractions. Brain Res Rev 1997; 23: 79-133.

McEwen BS. Protective and damaging effects of stress mediators. N Engl J Med 1998; 338:171-179.

McMahon M, Gerich J, Rizza R. Effects of glucocorticoids on carbohydrate metabolism. Diabetes Metab Rev 1988; 4: 17-30.

McMinn JE, Baskin DB, Schwartz MW. Neuroendocrine mechanisms regulating food intake and body weight. Obes Rev 2000; 1: 37-46.

Meyer JE, Pudel V. Experimental studies on food intake in obese and normal weight subjects. J Phychosom Res 1972; 16: 305-308.

Miell JP, Englaro P, Blum WF. Dexamethasone induces an acute and sustained rise in circulating leptin levels in normal human subjects. Horm Metab Res 1996; 28: 704-7.

Mohamed-Ali V, Pinkney JH, Coppack SW. Adipose tissue as an endocrine and paracrine organ. Int J Obes 1998; 22: 1145-1158.

Mohamed-Ali V, Pinkney JH, Panahloo A, Cwyfan-Hughes S, Holly JMP, Yudkin JS. Insulin-like growth factor binding protein-1 in NIDDM: relationship with the insulin resistance syndrome. Clin Endocrinol 1999; 50: 221-228.

Morange PE, Aubert J, Peiretti F, Lijnen HR, Vague P, Verdier M, Negrel R, Juhan-Vague I, Alessi MC. Glucocorticoids and insulin promote plasminogen activator inhibitor 1 production by human adipose tissue. Diabetes 1999; 48: 890-895.

Moriscot A, Rabelo R, Bianco AC. Corticosterone inhibits uncoupling protein gene expression in brown adipose tissue. Am J Physiol 1993; 265: E81-87.

Muller-Quernheim J. Sarcoidosis: immunopathogenetic concepts and their clinical application. Eur Respir J 1998; 12:716-738.

Napolitano A, Voice MW, Edwards CR et al. 11 beta-hydroxysteroid dehydrogenase 1 in adipocytes: expression is differentiation-dependent and hormonally regulated. J Steroid Biochem 1998; 64: 251-260.

National Institutes of Health. Third Report of National Cholesterol Education Program Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Bethesda Md: National Institutes of Health; 2001. NIH Publication 01-3670. In.; 2001.

Negrell R. Paracrine/autocrine signals and adipogenesis. In.: Progress in obesity research 8, eds: Guy-Grand B and Aillaud G. 1999; pp 55-63. John Libbey & Co Ltd.

Newcomer JW, Selke G, Melson AK, Gross J, Vogler GP, Dagogo-Jack S. Dose-dependent cortisol-induced increases in plasma leptin concentration in healthy humans. Arch Gen Psychiatry 1998; 55: 995-1000.

Obesity. Preventing and managing the global epidemic. Report of a WHO Consultation on Obesity, Geneva. WHO/NUT/NCD/98.

Ottosson M, Lonnroth P, Bjorntorp P, Eden S. Effects of cortisol and growth hormone on lipolysis in human adipose tissue. J Clin Endocrinol Metab 2000; 85: 799-825.

Ottosson M, Marin P, Karason K, Elander A, Bjorntorp P. Blockade of the glucocorticoid receptor with RU486: effects in vitro and in vivo on human adipose tissue lipoprotein lipase activity. Obes Res 1995; 3: 233-240.

Overweight, obesity and health risk. National Task Force on the Prevention and Treatment of Obesity. Arch Intern Med 2000; 160: 898-904.

Palmer JBD. Inhaled beclomethasone diproprionate: systemic effects reviewed (abstract 454). J Allergy Clin Immunol; 87: 253.

Papaspyrou-Rao S, Schneider SH, Petersen RN, Fried SK. Dexamethasone increases leptin expression in humans in vivo. J Clin Endocrinol Metab 1997; 82: 1635-1637.

Patrassi GM, Sartori MT, Livi U, Casonato A, Danesin C, Vettore S, Girolami A. Impairment of fibrinolytic potential in long-term steroid treatment after heart transplantation. Transplantation 1997; 64: 1610-1614.

Patrassi GM, Sartori MT, Viero ML, Scarano L, Boscaro M, Girolami A. The fibrinolytic potential in patients with Cushing´s disease: a clue to their hypercoagulable state. Blood Coag Fibrinol 1992; 3: 789-793.

Pinkney JH, Boss O, Bray GA, Bulmer K, Coppack SW, Mohamed-Ali V. Physiological relationships of uncoupling protein-2 gene expression in human adipose tissue in vivo. J Clin Endocriol Metab 2000; 85: 2312-2317.

Porte D, Baskin DG and Schwartz MW. Leptin and insulin action in the central nervous system. Nutrition Reviews 2002; 60: S20-29.

Pouliot MC, Despres JP, Lemieux S, Moorjani S, Bouchard C, Tremblay A, Nadeau A, Lupien PJ. Waist circumference and abdominal sagittal diameter: best simple anthropometric indexes of abdominal visceral adipose tissue accumulation and related cardiovasuclar risk in men and women. Am J Cardiol 1994; 73: 460-468.

Ramsay TG. Fat cells. Endocrinol Metab Clin North America 1996; 25: 847-870.

Rask E, Olsson T, Soderberg S et al. Tissue-specific dysregulation of cortisol metabolism in human obesity. J Clin Endocrinol Metab 2001; 86: 1418-21.

Rask E, Walker BR, Soderberg S et al. Tissue-specific changes in peripheral cortisol metabolism in obese women: Increased adipose 11β-hydroxysteroid dehydrogenase type 1 acitvity. J Clin Endocrinol Metab 2002; 87: 3330-3336.

Reaven GM. Banting lecture 1988. Role of insulin resistance in human disease. Diabetes 1988; 37: 1595-1607.

Rebuffe-Scrive M, Bronnegard M, Nilsson A, Eldh J, Gustafsson JA, Bjorntorp P. Steroid hormone receptor in human adipose tissues. J Clin Endocrinol Metab 1990; 71: 1215-1219.

Rebuffé-Scrive M, Bronnegard M, Nilsson A, Eldh J, Gustafsson JA, Bjorntorp P. Steroid hormone receptors in human adipose tissues. J Clin Endocrinol Metab 1990; 71: 1215-1219.

Ricquier D, Bouillaud F. The uncouplingt protein homologues: UCP1, UCP2, UCP3, StUCP and AtUCP. Biochem J 2000; 345: 161-179.

Rizza RA, Mandarino LJ, Gerich J. Cortisol-induced insulin resistance in man: impaired suppression of glucose production and stimulation of glucose utilization due to a postreceptor defect of insulin action. J Clin Endocrinol Metab 1982; 54: 131-138.

Rooney DP, Neely RDG, Cullen C, Ennis CN, Sheridan B, Atkinson AB. The effect of cortisol on glucose/glucose-6-phosphate cycle activity and insulin action. J Clin Endocrinol Metab 1994; 77: 1180-1183.

Rosmond R, Björntorp P. The interactions between hypothalamic-pituitary- adrenal axis activity, testosterone, insulin-like growth factor I and abdominal obesity with metabolism and blood pressure in men. Int J Obes 1998; 22:1184-1196.

Rosmond R, Björntorp P. The interactions between hypothalamic-pituitary- adrenal axis activity, testosterone, insulin-like growth factor I and abdominal obesity with metabolism and blood pressure in men. Int J Obes 1998; 22: 1184-96.

Rosmond R. The glucocorticoid receptor gene and its association to metabolic syndrome. Obes Res 2002; 10: 1078-1086.

Sakul H, Pratley R, Cardon L, Ravussin E, Mott D, Bogardus C. Familiality of physical and metabolic characteristics that predict the development of non-insulin-dependent diabetes mellitus in Pima Indians. Am J Hum Genet 1997; 60: 651-656.

Sartori MT, Patrassi GM, Pontarollo S, Livi U, Angelini A, Theodoridis P, Fioretti M, Girolami A. relation between long-term steroid treatment after heart transplantation, hypofibrinolysis and myocardial microthrombi generation. J Heart Lung Transplant 1999; 18: 693-700.

SBU rapport nr 160, 2002. Fetma – problem och åtgärder: en systematisk litteraturöversikt. Elanders Graphic Systems: Göteborg; 2002. (in Swedish)

Scarpace PJ, Matheny M, Moore RI, Kumar MV. Modulation of uncoupling protein 2 and uncoupling protein 3: regulation by denervation, leptin and retinoic acid treatment. J Endocrinol 2000; 164: 331-337.

Seki T, Miyasu T, Noguchi T, Hamasaki A, Sasaki R, Ozawa Y, Okukita K, Declerck PJ, Ariga T. Reciprocal regulation of tissue-type and urokinase-type plasminogen activators in the differentiation of murine preadipocyte line 3T3-L1 and the hormonal regulation of fibrinolytic factors in the mature adipocytes. Cell Physiol 2001; 189: 72-78.

Smith JT, Waddell BJ. Leptin receptor expression in the rat placenta: changes in Ob-Ra, Ob-Rb, and Ob-Re with gestational age and suppression by glucoocorticoids. Biol Reprod 2002; 67: 1204-1210.

Stokes GS. Drug-induced hypertension: pathogenesis and management. Drugs 1976; 12: 222-230.

Stralfors P, Olsson H, Belfrage P. Hormone-sensitive lipase. In the enzymes.1987; Vol XVIII, Academic Press, Eds PJ Boyer, J Krebbs.

Tarchalska-Krynska B. [Glucocorticosteroids: mechanisms of action, pharmacological effects, pharmacokinetics and adverse effects] Otolaryngol Pol 1994; 48: 41-48 (article in Polish).

Tataranni PA, Larson DE, Snitker S, Young JB, Flatt JP, Ravussin E. Effects of glucocorticoids on energy metabolism and food intake in humans. Am J Physiol 1996; 271: E317-25.

Tomlinson JW and Stewart PM. Cortison metabolism and the role of 11β-hydroxysteroid dehydrogenase. Res Clin Endocrinol Metab 2001; 15 :61-78.

Tyrell JB, Aron DC, Forsham PH. Glucocorticoids and adrenal androgenes. In: Basic and clinical endocrinology. Ed. Greennsan FS and Baxter JD, Appleton & Lange, 4 ed. Norwalk, 323.359.

van Zonneveld AJ, Curriden SA, Loskutoff DJ. Type 1 plasminogen activator inhibitor gene: functional analysis and glucocorticoid regulation of its promoter. Proc Natl Acad Sci USA 1988; 85: 5525-5529.

Vincennati V, Vottero A, Friedman C, Papanicolaou DA. Hormonal retulation of interleukin-6 production in human adipocytes. Int J Obes 2002; 26: 905-911.

Walker BR, Swan PJH, Phin P et al. Mechanisms of glucocorticoid-induced hypertension: inhibition of endothelium-dependent vasodilatation in rat and human vessels. J Endocrinol 1995; 144 (Suppl), OC34 (Abstract).

Walker BR. Activation of the hypothalamic-pituitary-adrenal axis in obesity: cause of consequence? Growth Horm IGF Res 2001; suppl A: S91-95.

Weidenfeld J, Siegel RA, Levy J and Chowers I. In vitro metabolism of cortisol by human abdominal adipose tissue. J Steroid Biochem 1982; 17: 357-360.

Westerterp-Plantega MS. Eating behaviour in humans, characterized by cumulaitve food intake curves – a review. Neuroscience and Biobehavioral Reviews 2000; 24: 239-248.

White PC et al. 11β-hydroxysteroid dehydrogenase and the syndrome of apparent mineralocorticoid excess. Endocrin Rev 1997;18:135-136.

Whorwood CB, Donovan SJ, Wood PJ, Philips IW. Regulation of glucocorticoid receptor α and β isoformsand type 1 11 β-Hydroxysteroid dehydrogenase expression in human skeletal muscle cells: a key role in the pathogenesis of insulin resistance? J Clin Endocrinol Metab 2001;86:2296-2308.

Yamamoto KR. Steroid receptor regulated transcription of specific genes and gene networks. A Rev Genet 1985; 19: 209-252.

Zakrzewska KE, Cusin I, Sainsbury A, Rohner-Jeanrenaud F, Jeanrenaud B. Glucocorticoids as counterregulatory hormones of leptin: toward an understanding of leptin resistance. Diabetes 1997; 46: 717-19.

Zhang Y, Proenca R, Maffei M et al. Positional cloning of the mouse obese gene and its human homologue. Nature 1994; 372: 425-432.