Central obesity is associated with type 2 diabetes mellitus, hypertension and dyslipidaemia. This cluster of risk factors is known as the metabolic syndrome, and also occurs in people with primary glucocorticoid excess (Cushing’s syndrome). Exogenous glucocorticoid use also increases the risk of cardiovascular disease. Circulating glucocorticoid concentrations are tightly controlled by the hypothalamic-pituitary-adrenal axis, however tissue glucocorticoid levels are also enhanced by the enzyme 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1). Transgenic overexpression of 11β-HSD1 in either adipose tissue or the liver in mice causes components of the metabolic syndrome, while transgenic deletion of 11β-HSD1 prevents adverse metabolic complications of obesity. Although plasma glucocorticoid levels are not elevated in obesity, dysregulation of 11β-HSD1 is observed with decreased activity in the liver and increased activity in adipose tissue. 11β-HSD1 is highly regulated, and dietary composition may be a powerful determinant of activity. Polymorphisms in the 11β-HSD1 gene are also associated with components of the metabolic syndrome. Inhibition of this enzyme appears to be an attractive option to treat metabolic disease. Selective 11β-HSD1 inhibitors in rodents cause weight loss, improve insulin sensitivity and delay progression of cardiovascular disease. Trials in humans though will be the ultimate test to determine if inhibition of 11β-HSD1 offers a new tool in the treatment of metabolic disease.

Key words: Glucocorticoids - Obesity - Metabolic syndrome.
key role in the pathophysiology of the metabolic syndrome, although the determinants of insulin resistance are largely unknown. One area which has received much research interest in recent years has been the role of glucocorticoids, as those with primary glucocorticoid excess (Cushing’s syndrome) develop the components of the metabolic syndrome, and it has been postulated that dysregulation of glucocorticoid production or metabolism could link the features of the metabolic syndrome.3, 4

Glucocorticoid excess and cardiovascular disease

Cushing’s syndrome is characterised by high circulating cortisol levels, either secondary to endogenous production from a cortisol- or adrenocorticotropic hormone (ACTH)-secreting adenoma, or alternatively from exogenous iatrogenic administration to treat inflammatory conditions such as rheumatoid arthritis or asthma. Patients with Cushing’s syndrome, in an environment of caloric excess, develop visceral obesity,5, 6 hypertension, insulin resistance and dyslipidaemia. In addition after surgical cure, fat mass decreases with a disproportionate reduction in the visceral component.7 The glucocorticoid receptor antagonist RU38486 has been used to treat Cushing’s syndrome, and decreases blood pressure, plasma glucose and triglyceride concentrations.8, 9 These findings confirm that primary glucocorticoid excess can cause the features of the metabolic syndrome.

There is also evidence that glucocorticoid excess leads not only to risk factors for cardiovascular disease, but also to an increased prevalence of cardiovascular disease. Patients with Cushing’s syndrome have a higher prevalence of carotid atherosclerotic plaques, which decrease in size after surgical cure.10 Those on chronic glucocorticoid treatments have also been studied and show increased risk of cardiovascular disease.11-13 This increased risk is present in a dose-dependent manner and is irrespective of the disease being treated. Although observational data cannot prove causality, it is plausible to infer that glucocorticoids are atherogenic.

Mechanisms of glucocorticoid action

To determine how glucocorticoid excess causes features of the metabolic syndrome, numerous in vitro and in vivo studies have been performed. Glucocorticoids act on intracellular glucocorticoid receptors (GR) which are present in most cells. Once cortisol has bound to GR in the cell cytoplasm, GR move to the nucleus and bind to glucocorticoid response elements (GRE) or interact with other transcription factors to either promote or repress messenger RNA (mRNA) transcription of numerous genes.14 Glucocorticoids have diverse effects which promote insulin resistance, a cardinal feature of the metabolic syndrome.15 For example, glucocorticoids both acutely and chronically potently stimulate appetite, particularly for high fat energy dense foods which are especially prevalent in today’s environment.16 Glucocorticoids have effects on the liver, increasing hepatic gluconeogenesis in some17, 18 but not all 19, 20 studies. However, fasting insulin levels are raised by glucocorticoid treatment in these studies, potentially showing that increased insulin secretion is required to maintain suppression of hepatic gluconeogenesis. Glucocorticoids increase mRNA transcription of two key enzymes involved in hepatic gluconeogenesis, phosphoenolpyruvate carboxykinase (PEPCK) 21, 22 and glucose-6-phosphatase (G6Pase).23, 24

Glucocorticoids also cause insulin resistance in muscle and adipose tissue. Insulin-stimulated glucose uptake in the muscle is decreased by glucocorticoid treatment in humans,17, 25 in part due to reduced translocation of the glucose transporter GLUT4 to the cell surface.26, 27 Glucocorticoids have direct but complex metabolic effects in adipose tissue, the major organ of lipogenesis and lipolysis. Rates of lipolysis are known to be increased in insulin resistance and obesity.28 In humans, in vivo measurements using microdialysis have shown that glucocorticoids increase subcutaneous adipose tissue
Furthermore, insulin resistance caused by glucocorticoids is improved by inhibition of lipolysis. Glucocorticoids regulate transcription of several important enzymes in adipose tissue, including increasing hormone sensitive lipase (HSL) expression in rat adipocytes. In contrast to their effects in the liver, glucocorticoids decrease PEPCK expression and activity in rat adipose tissue, leading to decreased glyceroneogenesis and higher plasma free fatty acid (FFA) concentrations. However, despite direct induction of lipolysis, glucocorticoids promote fat deposition, particularly in a central distribution. In vitro, glucocorticoids promote preadipocyte differentiation into adipocytes in the presence of insulin, potentially increasing adipocyte numbers. Glucocorticoids also increase lipoprotein lipase (LpL) activity, which hydrolyses circulating triglycerides prior to uptake in adipose tissue. This effect is observed in visceral rather than subcutaneous adipose tissue, thus promoting central fat accumulation.

In addition to promoting insulin resistance, glucocorticoids exert direct adverse effects on the pancreas. In vitro, glucocorticoids directly inhibit insulin release by beta-cells, an effect reproduced in vivo in transgenic mice selectively overexpressing GR in pancreatic β-cells. To summarise, in acute times of stress and starvation, glucocorticoids function appropriately to increase appetite to promote food seeking behaviour, and to decrease insulin secretion and cause insulin resistance in the liver, adipose tissue and muscle. Thereby, glucocorticoids increase gluconeogenesis and lipolysis in order to appropriately mobilise energy from food stores. However, in chronic glucocorticoid excess these alterations become maladaptive and result in increased appetite and insulin resistance, leading to unfavourable metabolic effects including hyperglycaemia and dyslipidaemia. Moreover, in the presence of a plentiful energy supply with subsequent high circulating insulin concentrations, glucocorticoids in fact have a net lipogenic effect to promote fat deposition particularly in the visceral depot, aggravating adverse metabolic consequences of glucocorticoid excess.

**Circulating glucocorticoids in obesity and the metabolic syndrome**

Several studies have examined whether there are abnormalities in circulating glucocorticoid levels in obesity and the metabolic syndrome. Morning fasting plasma cortisol levels are mildly elevated in people with components of the metabolic syndrome, including insulin resistance, hypertension, and hypertriglyceridaemia. Furthermore, increased activity of the hypothalamic-pituitary-adrenal (HPA) axis, as judged by the adrenal cortisol secreted in response to ACTH, correlates with hypertension and hypertriglyceridaemia. Higher 24-h urinary cortisol metabolite excretion, a measure of cortisol secretion rate, also associates with insulin resistance. Elevated plasma cortisol as a result of enhanced cortisol secretion rate may, therefore, link some features of the metabolic syndrome. Importantly, however, elevated plasma cortisol does not explain the association of features of metabolic syndrome with central obesity.

Increased HPA axis activity is also observed in people with simple obesity, measured either by corticotrophin-releasing hormone (CRH) or ACTH stimulation tests, or by the cortisol secretion rate. However, fasting morning plasma cortisol concentrations have been found to be either low or normal in obese subjects. These contrasting features can be accounted for by increased cortisol clearance. Cortisol is metabolised predominantly in the liver by the A-ring reductases (Figure 1), and indeed obesity is associated with increased hepatic 5α- and 5β-reductase activities.

Increased clearance by 5β-reductase is also seen in people with T2DM and those with fatty liver, the hepatic manifestation of the metabolic syndrome. As a result of increased clearance, plasma cortisol levels tend to fall, but intact negative feedback control of the HPA axis leads to a compensatory increase in cortisol secretion and a hyper-responsive HPA axis in response to other stimuli. However, since the net effect is that plasma cortisol is not elevated these alterations in glucocorticoid production rate and clearance

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do not account for the phenotypic similarities between central obesity and Cushing’s syndrome.

**Tissue regulation by the 11β-hydroxysteroid dehydrogenases**

While circulating glucocorticoid levels are tightly controlled by the HPA axis, tissue glucocorticoid levels are regulated by a separate system. The 11β-hydroxysteroid dehydrogenases (11β-HSDs) are enzymes which act to either amplify or decrease active tissue glucocorticoid levels (Figure 1). There are two isozymes from separate genes, termed type 1 (11β-HSD1) and type 2 (11β-HSD2) respectively. 11β-HSD2 is highly expressed in the kidney, placenta, colon, and salivary glands and utilises the co-factor nicotinamide adenine dinucleotide (NAD) to convert the active cortisol (Kendall’s compound F) to the inactive cortisone (E) in humans (corticosterone to 11-deoxycorticosterone in rodents). Glucocorticoids can bind and activate the mineralocorticoid receptor (MR) in addition to the GR. 11β-HSD2 is expressed in tissues with high levels of MR, where it functions to prevent MR inactivation by glucocorticoids and allows selective activation of MR by aldosterone. On of 11β-HSD2 by gene mutations leads to the syndrome of apparent mineralocorticoid excess (AME), which presents with hypertension and hypokalaemia but with low aldosterone levels.

11β-HSD1 is widely distributed in the body, but with high expression in the liver, adipose tissue and certain areas of the brain including the hippocampus. This enzyme is nicotinamide adenine dinucleotide phosphate (NADP(H))-dependent and can potentially
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Stimson

act bidirectionally to either increase or decrease tissue cortisol levels. In vitro, 11β-HSD1 acts as a dehydrogenase when cells are disrupted but in vivo 11β-HSD1 functions predominantly as a reductase, thus amplifying tissue glucocorticoid levels.

In vitro, 11β-HSD1 acts as a dehydrogenase when cells are disrupted but in vivo 11β-HSD1 functions predominantly as a reductase, thus amplifying tissue glucocorticoid levels. The reason for this disparity has recently been discovered, as the active catalytic region of 11β-HSD1 is known to sit in the lumen of the endoplasmic reticulum (ER). A second enzyme, hexose-6-phosphate dehydrogenase (H6PDH), is also present in the ER, and functions to generate the NAPDH required for reductase activity of 11β-HSD1. This hypothesis has been validated by the creation of transgenic mice with targeted deletion of H6PDH, which have very low reductase activities of 11β-HSD1.

Once it was known that 11β-HSD1 functioned primarily to increase glucocorticoid levels in tissues such as the liver and adipose tissue, the hypothesis arose that increased 11β-HSD1 activity could be a potential cause of the metabolic syndrome, increasing local glucocorticoid levels without necessarily altering circulating glucocorticoid concentrations. This led to a huge level of interest in 11β-HSD1 which consequently has become a research ‘hot topic’ in the past ten years. Although variations in 11β-HSD1 might be important in relation to T2DM and other conditions, the greatest amount of information concerns its role in obesity and this will be the focus for the remainder of this review.

Transgenic manipulations of 11β-HSD1

Transgenic mice have been created to determine whether increased 11β-HSD1 activity can directly cause obesity and the metabolic syndrome. Mice overexpressing 11β-HSD1 selectively in adipose tissue utilising the fatty acid binding protein AP2 promoter have 2-3 fold increased adipose tissue corticosterone levels but low or normal plasma corticosterone concentrations. These mice develop central obesity, insulin resistance, glucose intolerance, dyslipidaemia, and hypertension. These exciting findings showed for the first time that dysregulation of 11β-HSD1 could cause the metabolic syndrome. Mice which selectively overexpress 11β-HSD1 in the liver (2 and 5 fold) have also been generated using the ApoE promoter. These mice develop hypertension, potentially due to increased angiotensinogen levels with resultant activation of the renin-angiotensin-aldosterone system. They develop mild insulin resistance and dyslipidaemia, but interestingly do not become obese, indicating that increased adipose but not hepatic 11β-HSD1 activity is obesogenic.

Mice with selective disruption of 11β-HSD1 have also been created to determine if this is metabolically beneficial. These mice have a normal lifespan and, on a high fat diet, resist hyperglycaemia and weight gain, while they deposit fat in peripheral, not visceral, sites. Lipid profile is also improved, with decreased plasma triglycerides due to improved lipid oxidation and increased HDL-C. Corticosterone levels are increased in plasma in some strains, but are decreased in tissues which normally express 11β-HSD1. The phenotypes of the transgenic mice discussed above support the hypothesis that increased 11β-HSD1 activity can be metabolically disadvantageous and inhibition metabolically beneficial, but of course proof of this concept was required in humans.

11β-HSD1 activity in obesity

Whole body regeneration of cortisol from cortisone

In order to measure 11β-HSD1 activity in humans, 24 h urinary collections of cortisol metabolites were formerly considered a good non-invasive method of estimating whole body enzyme activity. Cortisol (F) and cortisone (E) are predominantly metabolised by the hepatic A-ring reductases, 5α- and 5β-reductase before further metabolism by 3α-hydroxysteroid dehydrogenase and excretion predominantly as their tetrahydro-metabolites 5α-tetrahydrocortisol (α-THF), 5β-tetrahydrocortisol (THF), and 5β-tetrahydrocortisone (THE) (Figure 1). The ratio of cortisol: cortisone metabolites excreted in the urine, (α-THF + THF)/THE, has been
used to infer total body 11β-HSD1 activity in many studies. Conversely, the ratio of urinary free cortisol: cortisone has been used to estimate renal 11β-HSD2 activity.86, 87

Several studies have examined global 11β-HSD1 activity using urinary ratios, but unfortunately the results are inconsistent. The (α-THF + THF)/ THE ratio has been found to be decreased,51, 88, 89 normal,45, 52, 90-92 or increased50, 59 in obese when compared with normal weight individuals (Table I 93-104). In addition, this ratio is no different between people with T2DM when compared to healthy volunteers.60, 105, 106 Some of the discrepancies observed between these studies may be because of gender differences in total body 11β-HSD1 activity, as the ratio is lower in females;90, 107, 108 However, the main problem with this method of assessment is the lack of specificity for 11β-HSD1 activity, because the ratio is affected by 11β-HSD2, 5α- and 5β-reductase activities in addition to 11β-HSD1. Consequently, alterations in any of these parameters will change the ratio and thus the 'estimated' 11β-HSD1 set point. For example, 5α- and/ or 5β-reductase activity are altered by obesity,50, 59 gender108, 109 and liver fat content.52

A more specific method of quantifying total 11β-HSD1 activity has been developed in humans. This has been achieved through intravenous infusions of non-radioactively labelled cortisol, in the form of deuterated 9,11,12,12-4[H]3-cortisone (d3E). This is then converted by 11β-HSD1 in the reductase direction with the addition of an unlabelled hydrogen to generate 9,12,12-4[H]3-cortisol (d3F). Once steady state is achieved, the ratio of d3F:d4F can be used to derive the rate of appearance of d3-cortisol, a specific measure of whole body 11β-HSD1 activity. Two studies have examined the effect of obesity on total body 11β-HSD1 activity using this technique, and both have shown no difference,89, 93 while the second study also found no change in those with T2DM. These results suggest strongly that obesity does not alter whole body 11β-HSD1 reductase activity in humans, and that changes in urinary cortisol metabolite ratios are influenced by confounding factors. This lack of difference in whole body 11β-HSD1 suggests that either the enzyme is unchanged in obesity, or that it is altered in a tissue-specific fashion such that an increase in one tissue is balanced by a decrease in another.

### Tissue-specific dysregulation of 11β-HSD1

Tissue-specific dysregulation of 11β-HSD1 has been observed in obese Zucker rats, which are leptin-resistant to a mutation in the leptin receptor. These rats have increased 11β-HSD1 activity in omental adipose tissue, but decreased mRNA expression and activity in the liver.111 Similarly, leptin deficient mice have decreased hepatic112 and increa-

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sed subcutaneous adipose 11β-HSD1 mRNA levels. Tissue-specific dysregulation of 11β-HSD1 has been recently investigated in humans in several studies.

**Liver**

The conversion of orally administered cortisone to cortisol has been used as a method to estimate hepatic 11β-HSD1 activity by first pass metabolism in humans. The oral cortisone is metabolised predominantly in the liver, and the results achieved are consistent, showing that hepatic 11β-HSD1 activity is decreased in obese individuals. Hepatic 11β-HSD1 activity has also been shown to be decreased, albeit to a lesser extent, in people with T2DM.

**Subcutaneous adipose tissue**

Adipose tissue, particularly from the subcutaneous compartment, has been investigated in detail to test for dysregulation of 11β-HSD1 (Table I). There is now a compelling body of evidence that 11β-HSD1 mRNA and activity are increased in subcutaneous adipose tissue in obesity, with mRNA levels correlating with activity when both are measured. However, the activities are measured *in vitro* in homogenised tissue so may not reliably represent what is occurring *in vivo*. Arteriovenous sampling across the subcutaneous adipose bed has been performed to address this, and shown cortisol extraction across the subcutaneous bed which is most likely due to 11β-HSD1 reductase activity as other metabolising enzymes of cortisone are not present in adipose tissue. In addition, *in vivo* 11β-HSD1 reductase has been quantified by microdialysis infusion of tritiated cortisone, which was increased in obesity. Somewhat surprisingly however, the two studies which have attempted to quantify adipose cortisol concentrations have not found any correlation between 11β-HSD1 mRNA or activity and tissue cortisol levels. This may be because analysis techniques are not sensitive enough at present, or that cortisol regeneration by 11β-HSD1 only contributes a small percentage of tissue cortisol compared to adrenal production. These adipose tissue biopsies are also generally taken in the morning when plasma cortisol concentrations are relatively high, and it may be that 11β-HSD1 is more important in the evening or overnight when levels are much lower. Subjects undergoing biopsies may also be stressed, increasing the contribution of plasma cortisol to the intra-adipose measurements.

In addition to being elevated in obesity, 11β-HSD1 mRNA and/or activity correlate with insulin resistance in most of the above studies, although interestingly the presence of diabetes does not lead to increased 11β-HSD1 activity over and above any effect of obesity. This may indicate that the presence of insulin resistance *per se* is not directly responsible for the increased transcription of 11β-HSD1.

**Visceral adipose tissue**

The results in subcutaneous adipose tissue show that dysregulation of 11β-HSD1 may be an important pathogenic mediator of adverse metabolic disease. Visceral adipose tissue though is often cited as a more important adipose bed as visceral volume correlates more strongly with insulin resistance. In addition, FFA and adipokines from visceral adipose tissue are released directly into the portal vein, where they could exert damaging metabolic effects on the liver. Glucocorticoid action may be more important in visceral fat, as the glucocorticoid receptor (GR) is more highly expressed in this site. Despite the technical difficulty of obtaining omental adipose tissue in humans, several studies have directly examined 11β-HSD1 in this adipose bed.
activity in cultured omental preadipocytes was decreased in the obese group.\textsuperscript{103} This study also found no increase in 11β-HSD1 mRNA and activity in subcutaneous adipose tissue in obesity. Another study also showed no change in 11β-HSD1 mRNA or activity in omental adipose tissue in obesity.\textsuperscript{102} However, several studies have since showed increased 11β-HSD1 mRNA \textsuperscript{100, 101, 104} in omental adipose tissue in obese compared with lean people, indicating that 11β-HSD1 may be dysregulated in both subcutaneous and visceral adipose depots in obesity.

Ideally, in vivo measurements would be made to determine if visceral adipose tissue is generating more cortisol in obese individuals. Portal venous cortisol concentrations have been found to be no different than peripheral cortisol levels in 6 morbidly obese patients,\textsuperscript{120} although extra-adrenal cortisol production is very difficult to assess during the stress of bariatric surgery. Deuterated cortisol infusions with the additional placement of catheters in the hepatic vein have been used to quantify splanchnic cortisol production by 11β-HSD1 in healthy subjects. These studies have shown that 11β-HSD1 in the splanchnic tissues generates a similar amount of cortisol to the adrenals.\textsuperscript{121, 122} Indirect estimates of the respective contributions of adipose tissue and liver approximate these to be 2/3 from visceral tissues and 1/3 from the liver.\textsuperscript{122} However, simultaneous portal and hepatic vein cannulation studies in dogs show that the liver and not adipose is responsible for all splanchnic 11β-HSD1 activity.\textsuperscript{123} Unfortunately, no studies have as yet been reported using portal vein cannulation with tracer infusion in humans to accurately quantify visceral adipose tissue cortisol production. One study has examined total splanchnic cortisol production in obesity, and found no change in either obese or diabetic individuals.\textsuperscript{93} However, any increased activity in visceral adipose tissue could potentially be counterbalanced by the decreased hepatic 11β-HSD1 activity observed in obesity.\textsuperscript{50, 51, 88}

Although there are reports that 11β-HSD2 is dysregulated in adipose tissue in obesity,\textsuperscript{97, 124} the transcript levels are extremely low and enzyme activity has yet to be convincingly demonstrated.

**Skeletal muscle**

Any role for 11β-HSD1 in skeletal muscle was initially neglected since expression in rodent muscle is very low. However, more substantial 11β-HSD1 mRNA and activity is present in human skeletal muscle,\textsuperscript{125} in both the slow and fast twitch fibres.\textsuperscript{126} There are conflicting reports on whether dysregulation of 11β-HSD1 in this tissue could mediate adverse metabolic effects. Although one study showed no association between 11β-HSD1 mRNA levels and body composition,\textsuperscript{99} a second found increased 11β-HSD1 mRNA expression in cultured myoblasts in association with body mass index (BMI), insulin resistance and systolic blood pressure.\textsuperscript{127} Isolated myotubes from people with T2DM reportedly have increased 11β-HSD1 mRNA levels,\textsuperscript{128} but another group found decreased skeletal muscle 11β-HSD1 activity in T2DM.\textsuperscript{129} Further work is needed to elucidate if this enzyme is in fact dysregulated in the metabolic syndrome in muscle.

**Mechanisms of altered 11β-HSD1**

Thus, there is good evidence that dysregulation of 11β-HSD1 can cause metabolic disease in rodents, and that tissue-specific dysregulation of 11β-HSD1 occurs in humans. It is, however, not known whether obese individuals for example have constitutively switched on 11β-HSD1 in adipose tissue from a young age potentially causing obesity/metabolic syndrome, or whether this is a secondary effect of obesity. Recent work has, therefore, focused on the genetics and on the regulation of 11β-HSD1.

**Genetics of 11β-HSD1**

11β-HSD1 expression and activity in adipose tissue has been shown to correlate prospectively over 2.5 years with changes in weight and insulin resistance, indicating that 11β-HSD1 may predict rather than result from
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by ANNE STIMSON

an adverse metabolic profile. Another method of determining whether dysregulation of 11β-HSD1 is a primary cause of obesity and/or the metabolic syndrome is to determine if mutations in the gene are associated with metabolic abnormalities. 11β-HSD1 is encoded by the HSD11B1 gene on chromosome 1, which contains 6 exons and is approximately 30 kilobase pairs in size. Single nucleotide polymorphisms (SNPs) in the HSD11B1 gene have been reported to be associated with reduced mRNA transcription, indicating these mutations may have functional significance. Several common SNPs and altered numbers of microsatellite repeats have been discovered in untranslated and intronic regions of the gene. Most studies though have not found a correlation between these variants in HSD11B1 and body composition. However, one study found that children homozygous for an intronic polymorphism had increased BMI, although only 11 homozygotes were studied and heterozygotes were no different from controls. The evidence on the whole suggests that SNPs in the HSD11B1 gene do not cause alterations in body composition.

Polymorphisms in the HSD11B1 gene have been associated with other features of the metabolic syndrome. One specific SNP has been associated with insulin resistance, T2DM and hypertension, although the genotype did not correlate with 11β-HSD1 mRNA transcript levels in either adipose tissue or muscle. Meanwhile in lean patients with polycystic ovarian syndrome (PCOS), a SNP in HSD11B1 associated with decreased mRNA transcription predicts hyperandrogenism, potentially because of reduced cortisol regeneration by 11β-HSD1 with resultant compensatory activation of the HPA axis. These results may suggest that polymorphisms in the 11β-HSD1 gene do not determine whether obesity develops, but do determine whether associated metabolic abnormalities will occur if obesity is present.

CORTISONE REDUCTASE DEFICIENCY

Cortisone reductase deficiency (CRD) is a very rare PCOS-like disorder first reported in 1984 which is attributed to congenital lack of 11β-HSD1 activity. CRD most commonly presents in women, and is characterised by hirsutism, hyperandrogenism and oligomenorrhoea. However, one case has been reported in a 6 year old boy who presented with precocious puberty. Although these people do not appear to be protected from developing obesity, it is not an essential component. These patients all excrete a very high proportion of their urinary cortisol metabolites in the inactive 11-keto form, and consequently have very low (α-THF+THF)/THE urinary ratios. They are also unable to convert oral cortisone to cortisol. Their inability to regenerate cortisol presumably leads to lower tissue cortisol concentrations and hyperactivation of the HPA axis to normalise plasma cortisol levels, with resultant androgen excess. They do not appear to be protected from obesity but their susceptibility to insulin resistance and other cardiometabolic complications of obesity has not been tested systematically.

It was originally hypothesized that CRD patients would have severe mutations in the HSD11B1 gene. However, genetic testing of this gene has revealed no mutations in any of these patients. However, one group hypothesized that H6PDH, which confers directionality of 11β-HSD1 by generating the co-factor NADPH, could be mutated and cause CRD. They discovered that all three of their patients with CRD had polymorphisms in intron 3 of HSD11B1 and intron 5 of H6PD. Thus, it was hypothesized that defects in both genes were required to cause this disorder. However, several recent studies have shown that these polymorphisms are more common, with these specific SNPs found in combination in approximately 3-7% of the general population who lack any biochemical evidence of CRD. Further examination of these patients is needed to unpick the genetic cause of this interesting disease and confirm the role of H6PDH. Recent work has shown that conditions such as the glycogen storage disorders type 1a and 1b, which dramatically change the availability of the H6PDH substrate glucose-6-phosphate (G6P) required to generate
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NADPH, cause dramatic changes in 11β-HSD1 reductase activity. Although these patients have other metabolic features not seen in CRD, it shows that other enzyme deficiencies can significantly alter 11β-HSD1 activity.

Weight loss and nutrition

A good approach to determine reversibility of 11β-HSD1 in adipose tissue is to examine the effect of weight loss in humans. Total body 11β-HSD1 activity, measured by the urinary (α-THF + THF)/THE ratio, is unchanged after 10% weight loss on a very low calorie diet (VLCD), but this would not reveal tissue-specific changes. Two studies examining effects of weight loss on subcutaneous adipose tissue showed: 1) no change in cultured abdominal adipocyte 11β-HSD1 mRNA expression after 5% weight loss;97 and 2) no change in mRNA expression in gluteal tissue after 14% weight loss.152 These two studies certainly do not show any decrease in 11β-HSD1 after weight loss, and the latter study in fact found increased 11β-HSD1 mRNA in isolated adipocytes after weight loss.152

However, there may be confounding effects in weight loss studies. Changes in dietary constituents may have profound effects on 11β-HSD1, such that the different diets used to achieve weight loss in the above studies may account for any discrepancies. Mice fed a high fat diet show a dramatic decrease in 11β-HSD1 mRNA and activity in all fat depots after 2-20 weeks, while a similar decrease in 11β-HSD1 activity in adipose tissue and liver has been observed in rats. In contrast, a high fat diet increases 11β-HSD1 activity in the brain, potentially regulating appetite. In humans, in vivo studies have been performed in humans showing that 11β-HSD1 responds to nutritional factors. For example, infusion of intralipid (an aqueous suspension of lipid droplets) increases subcutaneous adipose tissue 11β-HSD1 activity. Moreover, eating a mixed meal acutely increases whole body but not splanchnic 11β-HSD1 activity measured using deuterated cortisol infusion, which may be accounted for by changes in subcutaneous adipose tissue. Intravenous insulin administration acutely increases whole body 11β-HSD1 activity, which may account for this effect. Nutrition has also been shown to have a more chronic effect on 11β-HSD1, as a ketogenic high fat diet increases total body 11β-HSD1 activity but this effect is not mediated through subcutaneous adipose tissue (Stimson et al., in press). More work is needed to determine whether diet is partly responsible for the dysregulation of 11β-HSD1 in obesity.

Hormonal regulation

11β-HSD1 is regulated by numerous hormones, and only a small proportion of this work will be presented here: for a detailed review see Tomlinson et al. Several studies have examined the regulation of 11β-HSD1 in the liver and adipose tissue in an attempt to elucidate which factors may be responsible for the tissue-specific dysregulation of this enzyme in obesity. For example, in vitro, cortisol increases 11β-HSD1 expression and activity in human adipose stromal cells and adipocytes. However, plasma cortisol is not elevated in obesity, while patients with Cushing’s syndrome have normal adipose 11β-HSD1 mRNA levels. Obesity has been recently termed a ‘pro-inflammatory state’, with increased plasma and adipose tissue cytokine levels. The cytokines IL-1β, IL-6 and TNFα increase 11β-HSD1 mRNA or activity in pre-adipocytes or adipocytes but interestingly not in hepatocytes, highlighting tissue-specific regulation. Furthermore, 11β-HSD1 is increased in adipose tissue in patients with human immunodeficiency virus (HIV) treatment-associated lipodystrophy, a condition associated with marked intra-adipose inflammation. More work is needed to determine whether increased inflammation in adipose tissue is responsible for the increased 11β-HSD1 in obesity.

Conversely, several hormones and transcription factors decrease 11β-HSD1. Growth hormone (GH), through insulin-like growth factor-1 (IGF-1), decreases whole body 11β-HSD1 activity in humans in vivo and in

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adipocytes in vitro. The decreased activity in humans is at least partly due to down-regulation of 11β-HSD1 in adipose tissue. Although 11β-HSD1 may have an important role in hypothalamic obesity, and obese individuals tend to have low GH levels, treatment with GH does not decrease fat mass despite lowering 11β-HSD1 in obese subjects. The effects of peroxisome proliferator-activated receptor (PPAR) agonists have also been examined, showing that PPARγ-agonists decrease 11β-HSD1 in the liver, while PPARα-agonists decrease 11β-HSD1 mRNA in vitro and in vivo in rodent adipose tissue. However, the effects of PPAR agonists on 11β-HSD1 have not been reproduced in humans, indicating that species specific differences exist in the regulation of 11β-HSD1. The role of insulin in the regulation of 11β-HSD1 is complex. In vitro, insulin has been shown to decrease 11β-HSD1 in rat hepatocytes, but does not alter adipose 11β-HSD1 expression. However, in vivo acute intravenous insulin administration in humans has been shown to either increase or decrease 11β-HSD1 activity in subcutaneous adipose tissue, while increasing total body activity. Obesity is associated with chronically elevated insulin concentrations, and it remains possible that high insulin levels contribute to the decreased hepatic 11β-HSD1 activity in human obesity. Indeed, hepatic 11β-HSD1 is only minimally decreased in T2DM, a condition of relative insulin insufficiency.

There are at least 2 different promoters for 11β-HSD1, with the P1 promoter specifically active in lung. Although separate promoters could allow differential regulation of 11β-HSD1 in distinct tissues, both liver and adipose tissue are under the control of the P2 promoter. The CCAAT/ enhancer binding proteins (C/EBPs), which are transcription factors with specific binding sites in the 11β-HSD1 promoters, increase 11β-HSD1 mRNA expression in hepatocytes, and it is thought that most of the effects of the above hormones and transcription factors are mediated through the C/EBPs. Potentially the balance of the various C/EBPs may result in the relative activation or repression of 11β-HSD1 expression in different tissues, mediating such tissue-specific regulation.

Inhibition of 11β-HSD1 in the metabolic syndrome

The potential of glucocorticoid excess to cause the metabolic syndrome in humans leads to the hypothesis that inhibiting glucocorticoid action might treat this condition. The glucocorticoid receptor antagonist RU38486 for example improves insulin resistance in both high fat fed and streptozotocin-induced diabetic rats, while RU38486 also acutely decreases hepatic gluconeogenesis in humans. However, the more chronic metabolic effects of RU38486 have not been studied in humans and there is some concern that glucocorticoid receptor antagonism could potentially precipitate an adreno-cortical crisis in times of stress when increased concentrations of cortisol are required. Moreover, chronic administration of RU38486 induces compensatory activation of the HPA axis, potentially overcoming the blockade.

The powerful effects of transgenic manipulation of 11β-HSD1 and the dysregulation of 11β-HSD1 observed in human obesity have made inhibition of 11β-HSD1 an attractive proposition for the treatment of metabolic disease. 11β-HSD1 inhibitors would have the potential metabolic benefit of decreasing tissue cortisol concentrations chronically, but would also not interfere with acute increases in glucocorticoid secretion by the adrenal or glucocorticoid action in times of stress (Figure 2). In contrast to many areas of drug development, most of the early work in this field was performed in humans. Carbenoxolone, the hemi-succinate derivative of glycyrrhetinic acid, was licensed in humans for the treatment of peptic ulcer disease in the 1950s. Carbenoxolone is an inhibitor of both 11β-HSD1 and 11β-HSD2 activity, and has consequently been utilised extensively to study the effects of 11β-HSD1 inhibition in humans. Carbenoxolone improves hepatic insulin sensitivity in lean healthy men and decreases glycolysis in lean men with
T2DM. However, carbenoxolone does not improve insulin sensitivity in obesity or improve glycaemic control in overweight/obese diabetics, while its metabolic effects are largely limited to the liver as carbenoxolone does not inhibit 11β-HSD1 in adipose tissue. This may explain the lack of effect on insulin sensitivity in obesity, as hepatic 11β-HSD1 activity is decreased in these individuals. Inhibition of 11β-HSD1 in adipose tissue has greater potential though, as of course this enzyme is increased in adipose tissue.

Carbenoxolone cannot be used long-term due to concurrent inhibition of 11β-HSD2, leading to hypertension and hypokalaemia, but it proves the potential of 11β-HSD1 inhibition. The pharmaceutical industry has recently become highly interested in this area, and developed several novel selective inhibitors of 11β-HSD1 (summary of effects in Table II). For example, the arylsulphonamidothiazole drug BVT.2733 was the first published selective inhibitor of 11β-HSD1. This compound decreases plasma glucose, insulin, and cholesterol concentrations, while it improves hepatic insulin sensitivity in hyperglycaemic mice. BVT.2733 also reduces food intake and weight gain in mice, although inhibition of 11β-HSD1 in adipose tissue has not been demonstrated. Some compounds have been developed which do inhibit adipose 11β-HSD1 activity in rodents, which show extremely promising results. For example, these drugs decrease visceral fat accumulation, by inhibiting lipogenesis and improving β-oxidation in mesenteric fat. In apolipo-protein E knockout

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mice, selective 11β-HSD1 inhibition decreases cholesterol accumulation in atherosclerotic plaques, showing that these drugs can potentially delay the progression of cardiovascular disease. The data in rodents are certainly encouraging, although several potential concerns exist over the use of these inhibitors. Decreasing plasma glucocorticoid levels may lead to compensatory activation of the HPA axis, as in people with cortisone reductase deficiency. This could potentially cause hyperandrogenism in humans, which was not observed in the mice as they lack the capacity to secrete adrenal androgens. Glucocorticoids are potent anti-inflammatory agents, and inhibition of 11β-HSD1 may increase inflammation in the body. This has not been detected in any of the rodent trials though, while mice with selective disruption of 11β-HSD1 do not develop spontaneous inflammatory disease. Trials of new selective 11β-HSD1 inhibitors in humans are keenly awaited which will answer these questions.

Conclusions

Dysregulation of glucocorticoids can cause obesity and the metabolic syndrome, while glucocorticoid excess is associated with increased cardiovascular disease in humans. Concentrations of circulating glucocorticoids are controlled by the HPA axis, but tissue levels are further controlled by the 11β-HSDs. Tissue-specific dysregulation of 11β-HSD1 is observed in human obesity, although the causes for this are not well understood. Inhibition of 11β-HSD1 shows promising effects in rodents, and results with selective inhibitors in humans will be available in the next few years. Selective 11β-HSD1 inhibitors may potentially become a valuable tool in the treatment of the metabolic syndrome.

Riassunto

Glicocorticoidi e 11β-idrossisteroide deidrogenasi tipo 1 nell’obesità e nella sindrome metabolica

L’obesità centrale è associata con il diabete mellito di tipo 2, l’ipertensione e la dislipidemia. Questo insieme di fattori di rischio è noto come sindrome metabolica e compare anche in soggetti con eccesso primitivo di glicocorticoidi (sindrome di Cushing). Anche la surreale è associata con il rischio di malattia cardiovascolare. Le concentrazioni di glicocorticoidi circolanti sono strettamente correlate, tuttavia la surreale può rivelare anche aumentati dall’enzima 11-α-idrossisteroide deidrogenasi tipo 1 (11-α-HSD1). L’iperespressione transgenica di 11-α-HSD1 è stata notificata nel tessuto adiposo che nel fegato, nei topi favorisce le componenti della sindrome metabolica, mentre la surreale transgenica di 11-α-HSD1 favorisce le complesse metaboliche avverse dell’obesità. Sebbene nel l’obesità i livelli plasmatici di glicocorticoidi non siano elevati, la disregolazione di 11-α-HSD1 viene osservata sotto forma di una diminuita attività a livello epatico e a un’aumentata attività nel tessuto adiposo. L’attività di 11-α-HSD1 è strettamente regolata e la composizione della dieta può rappresentare una determinante molto potente della sua attività. Anche i polimorfismi del gene 11-α-HSD1 sono associati a componenti della sindrome metabolica. L’inibizione di questo enzima sembra rappresentare un’attrattiva opzione per il trattamento della malattia metabolica. Nei roditori, gli inibitori selettivi di 11-α-HSD1 provocano perdita di peso, migliorano la sensibilità all’insulina e ritardano la progressione della malattia cardiovascolare. Gli studi sull’uomo saranno il test definitivo per determinare se l’inibizione di 11-α-HSD1 possa rappresentare un nuovo strumento per il trattamento della malattia metabolica.

Parole chiave: Glicocorticoidi - Obesità - Sindrome metabolica.

References


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