Glucocorticoids and 11beta-Hydroxysteroid Dehydrogenase in Adipose Tissue

JONATHAN R. SECKL, NIK M. MORTON, KAREN E. CHAPMAN, AND BRIAN R. WALKER

Endocrinology Unit, School of Molecular and Clinical Medicine, University of Edinburgh, Western General Hospital, Edinburgh EH4 2XU, Scotland, United Kingdom

ABSTRACT

The highly prevalent metabolic syndrome (insulin resistance, type 2 diabetes, dyslipidemia, hypertension, along with abdominal obesity) resembles Cushing's syndrome. However, in simple obesity, plasma cortisol levels are not elevated. 11beta-hydroxysteroid dehydrogenase type 1 (11β-HSD1), at least in mature adipocytes and hepatocytes, converts inactive circulating 11-keto steroids into active glucocorticoids, amplifying local glucocorticoid action. 11β-HSD1 is elevated in adipose tissue in obese humans and rodents, suggesting that adipose tissue glucocorticoid excess may explain the conundrum. Indeed, transgenic mice overexpressing 11β-HSD1 in adipose tissue faithfully replicate the metabolic syndrome. Conversely, 11β-HSD1$^{-/-}$ mice resist the metabolic consequences of stress and high-fat feeding via insulin sensitisation and other advantageous effects in the liver and adipose tissue. Adipose 11β-HSD1 deficiency contributes to a protective metabolic phenotype, supporting its role as a therapeutic target for the metabolic syndrome.

"I am resolved to grow fat and look young till forty, and then slip out of the world with the first wrinkle." — John Dryden, *The Maiden Queen*, 1668

I. Preamble

Dryden's witty 17th-century view of obesity as a convenient disguise for ageing reflects an era when the average life expectancy of a child surviving infancy was only half of today's eight decades or more in western countries. Millions of years of vertebrate evolution and most of human history are reflected in spectacular metabolic adaptations to periodic starvation. By the standards of the age, Dryden's woman was not only believed to be improving her complexion but also to be healthy and resistant to episodic famine and contagion. Today, in contrast, obesity itself has reached epidemic proportions for the world's affluent nations and, increasingly, for developing countries too. Excess fat contributes to early morbidity and mortality, bringing a latter-day irony to Dryden's line. While obesity's primary cause is a chronic imbalance between calorie intake and energy expenditure, underlying vulnerabilities within individuals modulate the likeli-

hood of its development and complications. The metabolic syndrome (Reaven's syndrome X; the insulin resistance syndrome) describes a constellation of cardiovascular risk factors — specifically, insulin resistance, type 2 diabetes, dyslipidemia, and hypertension (Reaven, 1993,2002). The relative risk of morbidities in the metabolic syndrome is increased by the co-occurrence of obesity – notably, visceral (abdominal, android, central) obesity. Despite debate about its definition, the metabolic syndrome is increasing rapidly in prevalence and represents a major burden upon health-care delivery organisations worldwide. Understanding the pathogenesis and potential treatments for visceral obesity and its cardiometabolic associations is a high priority.

Endocrinologists have long puzzled over the strong morphological and metabolic similarities between the rare Cushing's syndrome of endogenous or exogenous glucocorticoid excess and the metabolic syndrome (Walker and Seckl, 2001). Excessive cortisol secretion in Cushing's syndrome is a classic cause of secondary obesity. Effects of excess cortisol on adipose tissue are complex, with an increase in central (i.e., visceral, abdominal, facial, and nape of neck) fat deposition, while peripheral fat is reduced. This may result from opposing effects of glucocorticoids that, on the one hand, increase lipolysis and downregulate lipoprotein lipase — thereby liberating free fatty acids (FFAs) from peripheral fat — but, on the other hand, stimulate preadipocyte differentiation and enhance substrate flux in favour of gluconeogenesis and triglyceride synthesis in central fat (Samra *et al.*, 1998; Andrews and Walker, 1999). Additionally important in glucocorticoid-induced obesity is central stimulation of appetite (Udden *et al.*, 2003), mediated by complex interactions between hypothalamic responses to glucocorticoids, their effects upon target appetite-controlling neurotransmitters such as neuropeptide Y (NPY), and both central and peripheral effects upon adipose-derived endocrine mediators such as leptin and gut hormones (Solano and Jacobson, 1999). Other central effects of increased glucocorticoid action include depression (Cohen, 1980), which intriguingly is also linked specifically to idiopathic visceral obesity (Ahlberg *et al.*, 2002).

Raised plasma cortisol levels cause phenotypic changes in Cushing's syndrome. Against this background, the importance of glucocorticoids in obesity has been investigated in animal models and in humans. Many studies have focused on glucocorticoid secretion and circulating blood levels of steroids. This research has shown that cortisol levels are modestly, if at all, elevated in patients with the metabolic syndrome and even may be reduced in simple obesity (i.e., obesity without diabetes or other disease states complicating increased fat mass) (Walker and Seckl, 2001). This conundrum recently was readdressed and some progress made in disentangling the mechanisms and importance of altered tissue sensitivity to glucocorticoids. This, then, is a story of cellular corticosteroid receptors, and particularly of prereceptor metabolism by enzymes, notably by the hitherto-obscure 11beta-hydroxysteroid dehydrogenases (11β-HSDs).

II. Blood Glucocorticoid Levels in Obesity

A. GLUCOCORTICOID CONTROL: THE HYPOTHALAMIC-PITUITARY-ADRENAL AXIS

Until recently, it was axiomatic that the major determinants of corticosteroid action were levels of steroid hormones in the blood, modified by binding to plasma proteins (notably, corticosteroid binding globulin (CBG)), and the densities of intracellular receptors in target organs. Plasma glucocorticoid levels are, of course, determined by the activity of the hypothalamic-pituitary-adrenal (HPA) axis, a classical short-term neuroendocrine feedback circuit. The HPA axis functions to restrict excursions of circulating glucocorticoid concentrations within fairly tight limits, with forward drive at the diurnal maximum or during stress rapidly attenuated by negative feedback of adrenal steroids upon pituitary corticotrophs, hypothalamic paraventricular nucleus (PVN) neurons that synthesise corticotropin-releasing hormone (CRH) and vasopressin (AVP), as well as a number of suprahypothalamic sites. Of the latter, the hippocampus, a part of the limbic system, has attracted much attention, as it contains a very high density of intracellular receptors for glucocorticoids and is a key locus for glucocorticoid effects upon memory and perhaps mood (de Kloet, 1991; McEwen, 1999). The hippocampus appears to act as a "brake" upon the HPA axis, since lesions interfere with glucocorticoid feedback control of HPA stress responses (Jacobson and Sapolsky, 1991). Increased HPA axis activity sufficient to cause raised blood cortisol levels would be a convenient mechanism to explain the Cushingoid aspects of the metabolic syndrome and simple visceral obesity. Data relating to glucocorticoid hypersecretion in the metabolic syndrome/visceral obesity continuum are described briefly in the following section.

B. THE HPA AXIS AND CORTISOL DYNAMICS IN OBESITY

A number of case-control studies have suggested that obesity is associated with increased urinary free cortisol (UFC) excretion (Strain *et al.*, 1980; Marin *et al.*, 1992; Pasquali *et al.*, 1993a). This association is particularly prominent in subjects with abdominal obesity, at least in women (Pasquali *et al.*, 1993b). A caveat is that UFC, though a useful marker of HPA axis activation, forms a very small fraction of total cortisol metabolite excretion. More convincingly, recent large studies confirm that total cortisol production rate is somewhat enhanced in obesity in men as well as women (Andrew *et al.*, 1998; Fraser *et al.*, 1999; Stewart *et al.*, 1999). This is further supported by evidence that HPA axis responsiveness to stimuli such as acute stress, CRH/AVP (Pasquali *et al.*, 1999), adrenergic manipulations (Pasquali *et al.*, 2000), hypoglycaemia, or a standard meal (Marin *et al.*, 1992; Hautanen and Adlercreutz, 1993) is enhanced in idiopathic obesity, particularly visceral obesity (Pasquali *et al.*, 2000). Moreover,

the cortisol response to particular types of food may differ with the locus of adiposity, as viscerally obese women show lower HPA axis responses to high-fat and protein but greater responses to high-carbohydrate meals, compared to peripherally obese and lean subjects (Vicennati *et al.*, 2002). However, in obesity, overall plasma cortisol levels are not consistently elevated. Indeed, peak plasma cortisol levels in the morning are typically *low* in the obese (Ljung *et al.*, 1996; Phillips *et al.*, 1998; Rosmond *et al.*, 1998; Walker *et al.*, 1999), though South Asians may be an exception (Ward *et al.*, 2003). While some data in humans suggest that plasma cortisol is higher during the evening nadir in obesity, flattening the diurnal variation (Ljung *et al.*, 1996; Rosmond *et al.*, 1998), evidence is lacking for marked elevations of basal plasma cortisol in obesity. The combination of increased secretion with low morning plasma levels suggests that the diurnal variation of cortisol secretion is disrupted and/or that peripheral metabolism of cortisol is enhanced.

As explanation for the increased glucocorticoid production and exaggerated cortisol responses in obesity, both increased forward drive to the HPA axis — perhaps reflecting some form of primary meal-related abnormality of cortisol release — moderate chronic stress (Rosmond *et al.*, 1998), and/or reduced sensitivity to HPA axis feedback have been suggested (Ljung *et al.*, 1996; Di Blasio *et al.*, 2003; Jessop *et al.*, 2003). Indeed, obesity has been associated with reduced sensitivity to glucocorticoid feedback (Ljung *et al.*, 1996; Jessop *et al.*, 2003), an effect thought to be mediated via altered sensitivity of glucocorticoid receptor (GR). However, increased feedback sensitivity may not be applicable to obese men (Pasquali *et al.*, 2002), although this sex is typified by a more-android adipose distribution! Several studies suggest that individuals with common GR polymorphisms demonstrate HPA axis abnormalities in addition to a peripheral phenotype of increased adiposity, insulin resistance, and hypertension (Bjorntorp *et al.*, 1999; Rosmond *et al.*, 2000; Ljung *et al.*, 2002; Di Blasio *et al.*, 2003). Whether this GR genotype is a primary cause of obesity or whether obesity reflects HPA activation remains unclear but is the topic of ongoing investigations. An additional complexity stems from recent data showing that, in the nucleus, GR homodimers are part of a macromolecular complex with specific coactivator and corepressor proteins that, in combination, determine the actions of glucocorticoids-GR upon a target gene. Variation in density or function of coactivators and corepressors may underlie aspects of interindividual variation in glucocorticoid sensitivity (Li *et al.*, 2003).

For the metabolic syndrome, case-control and cross-sectional studies have suggested that high blood pressure associates with somewhat elevated cortisol concentrations (in blood, saliva, or urine) (Watt *et al.*, 1992; Filipovsky *et al.*, 1996; Stolk *et al.*, 1996; Rosmond *et al.*, 1998; Walker *et al.*, 1998; Fraser *et al.*, 1999; Brunner *et al.*, 2002). Similarly, insulin resistance and glucose intolerance are associated with higher circulating cortisol levels (Phillips *et al.*, 1998;

Reynolds *et al.*, 2001). Higher morning plasma cortisol concentrations and increased responsiveness to corticotropin (ACTH) occur in adults having the additional cardiovascular risk factor of low birth weight (Phillips *et al.*, 1998,2000; Levitt *et al.*, 2000; Reynolds *et al.*, 2001). However, all these associations between cortisol activity and hypertension/insulin resistance are *independent* of obesity. In such studies, obesity again is associated with lower (not higher) plasma cortisol, no change in (renal) 11β-HSD2, and no difference in dermal sensitivity (vasoconstrictor responses) to glucocorticoids.

From these observational studies, it is not possible to dissect causality but they do not strongly favour a unifying hypothesis that ascribes the associations between obesity and other features of the metabolic syndrome to enhanced cortisol secretion. Rather, a primary or secondary increase in HPA axis activity may occur in subjects with hypertension and/or insulin resistance that do not directly predispose to obesity. Clearly, this syndrome cannot result from simple HPA activation or generalised alterations in tissue glucocorticoid sensitivity, yet the parallels with Cushing's remain persuasive.

Thinking this through, it is plausible that the observed combination of increased total cortisol secretion with lower plasma cortisol levels during peak secretion in obesity reflects increased peripheral cortisol clearance. More than 20 years ago, obesity was associated with increased cortisol clearance rates (Strain *et al.*, 1980; Lottenberg *et al.*, 1998). Increased glucocorticoid clearance could itself reduce plasma levels and lead to compensatory HPA axis activation. Any such activation would lead to adrenal hyperresponsivity to stress and other stimuli.

III. Corticosteroid Receptors and Obesity

Tissue sensitivity to glucocorticoids is determined by the density of receptors (and transcriptional cofactors and post-transcriptional mechanisms) in a particular cell type and, crucially, by tissue-specific metabolism by enzymes. In addition to evidence for altered sensitivity to glucocorticoids in HPA feedback sites, peripheral tissue sensitivity to glucocorticoids may be increased in the metabolic syndrome. For example, patients with essential hypertension or type 2 diabetes have enhanced tissue sensitivity to glucocorticoids, as measured by the intensity of dermal vasoconstriction following topical application of beclomethasone (Walker *et al.*, 1996,1998; Andrews *et al.*, 2002). In addressing tissue sensitivity to glucocorticoids, we will first review data on receptors and then concentrate upon enzymes.

Glucocorticoids act predominantly via nuclear receptors. There are two subtypes, GR and mineralocorticoid receptors (MR). These receptors are essential for life, since knockout (KO) of either by homologous recombination in mice is lethal at (for GR) or soon after (for MR) birth (Cole *et al.*,

1995; Berger *et al.*, 1998). GR are near ubiquitous, albeit their density varies considerably between cell types. MR are highly restricted to aldosterone target organs (i.e., distal nephron, distal colon, sweat and salivary glands) as well as some central nervous system (CNS) regions, notably, the hippocampus, and cardiovascular structures, including the heart. Metabolic effects associated with abdominal obesity are thought to originate in visceral adipose tissue and the liver, key sites determining hepatic glucose insulin and lipid homeostasis, as well as in skeletal muscle, the principal organ of glucose uptake. These tissues all highly express GR but have little or no MR. The importance of GR in obesity has been indicated by both its attenuation with adrenalectomy and GR antagonists in animal models and the effects on various mice bearing transgenic GR manipulations. However, the phenotypes are complex, since GR in the CNS also play a key role in HPA axis control such that a generalised transgenic reduction of GR causes obesity (Pepin *et al.*, 1989). This is probably because HPA axis overactivity and marked hypercorticosteronaemia override the reduced density of GR in peripheral tissues. Such outcomes are predicted by the relatively low affinity of GR, which is little occupied by basal levels of glucocorticoids but fully occupied under conditions of glucocorticoid excess (e.g., severe stress) (de Kloet, 1991).

GR density in key metabolic cells, at least in skeletal muscle and adipose tissue, shows considerable interindividual variation. Importantly, in humans, GR mRNA levels in skeletal muscle correlate with insulin sensitivity and blood pressure (Reynolds *et al.*, 2002; Whorwood *et al.*, 2002). Moreover, dermal (vascular) sensitivity to GR agonists correlates with component disorders of the metabolic syndrome (Walker *et al.*, 1996,1998; Andrews *et al.*, 2002) and is associated with a common bclI restriction fragment length polymorphism (RFLP) of the GR gene (Panarelli *et al.*, 1998), though any functional implications of this particular polymorphism are unclear. Thus, it has been hypothesised that genetic and environmental variations in GR density in specific metabolic tissues underlie the metabolic syndrome. Moreover, a mechanistic basis for such tissue-specific variation in GR expression has been provided through demonstration that the GR gene has a number of alternate untranslated exon1/promoter sequences in the 5′ region that, at least in lymphocytes and the brain, can drive tissue-specific expression under some circumstances (McCormick *et al.*, 2000). However, in obesity, GR mRNA is not consistently elevated in adipose tissue (Lindsay *et al.*, 2003; Wake *et al.*, 2003). This may reflect the fact that simple obesity and the metabolic syndrome can exist as discrete associated metabolic defects within a continuum of (visceral) obesity. Again, mechanisms pertaining to other features of the metabolic syndrome appear to differ from those that operate in obesity.

IV. Tissue Metabolism of Glucocorticoids

A. BASIC BIOCHEMISTRY

The enzymes directly metabolising cortisol include the steroid A-ring reductases (5α- and 5β-reductases), 6β-hydroxylase, 20-reductase, and 11β-HSDs. In rats and mice, which lack 17-hydroxylase in their adrenal cortex, the principal glucocorticoid is corticosterone, which is subject to analogous metabolism. This review will focus on the role of 11β-HSDs.

B. HISTORY OF 11β-HSD

50 years ago, Amelung and colleagues (1953) discovered an enzyme that catalysed the interconversion of 11-hydroxy glucocorticoids (cortisol, corticosterone) and 11-keto forms (cortisone, 11-dehydrocorticosterone): 11β-hydroxysteroid dehydrogenase (Figure 1). While 11-hydroxyglucocorticoids are active at receptors, 11-ketosteroids have very low affinity for GR and MR and appear to all intents and purposes inert. This 11β-HSD activity subsequently was described in a broad range of cells and tissues (Monder and White, 1993). In the 1980s, Monder and his coworkers in New York purified an 11β-HSD activity from rat liver (Lakshmi and Monder, 1988). Homogenates, microsomal preparations, and purified enzyme from rat liver catalysed both 11β-dehydrogenation of cortisol to inert cortisone and, typically to a lesser extent, the 11β-reduction of cortisone to active cortisol. Thus, 11β-HSD was thought to represent one of several arcane pathways for glucocorticoid clearance, so no specific function was ascribed to it.

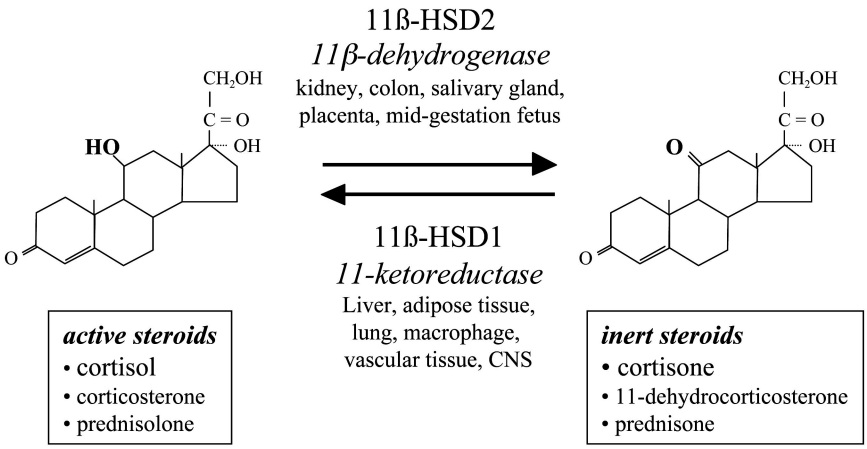

FIG. 1. 11beta-Hydroxysteroid dehydrogenase (11β-HSD). CNS, central nervous system.

Subsequently, two 11β-HSD isozymes have been characterised, isolated, and their cDNAs cloned, the products of distinct and only distantly related genes (reviewed in White *et al.*, 1997; Stewart and Krozowski, 1999; Seckl and Walker, 2001).

C. 11β-HSD TYPE 2

11β-HSD type 2 is a high-affinity (low nM Km for cortisol), nicotinamide adenine dinucleotide (NAD)-dependent dehydrogenase that rapidly converts active cortisol to inert cortisone (Albiston *et al.*, 1994; Brown *et al.*, 1996b). Although expressed in many tissues during fetal life, including the placenta (Brown *et al.*, 1996a), in adults, 11β-HSD2 is expressed principally in tissues where aldosterone induces its classical effects on sodium excretion, including distal nephron, sweat glands, salivary glands, and colonic mucosa (Smith *et al.*, 1996; Hirasawa *et al.*, 1997). There is some expression in other epithelial cells, such as in lung (Page *et al.*, 1994) and endothelium (Brem *et al.*, 1998; Hadoke *et al.*, 1998; Christy *et al.*, 2003). 11β-HSD2 functions predominantly to exclude glucocorticoids from intrinsically nonselective MR *in vivo* (Edwards *et al.*, 1988; Funder *et al.*, 1988). In an analogous action, 11β-HSD2 in placenta and fetal tissues appears to exclude glucocorticoids from GR *in utero* (Benediktsson *et al.*, 1997; Seckl *et al.*, 2000). Inhibition of this enzyme with liquorice (Stewart *et al.*, 1987) or disruption of the 11β-HSD2 gene in mice (Kotelevtsev *et al.*, 1999) or humans (Mune *et al.*, 1995; Dave-Sharma *et al.*, 1998) leads to the syndrome of apparent mineralocorticoid excess (AME). In AME, glucocorticoids illicitly occupy renal MR, causing sodium retention, hypertension, and hypokalemia. 11β-HSD2 inhibition also reduces fetal growth and alters tissue maturation (Lindsay *et al.*, 1996a,b).

D. 11β-HSD TYPE 1

1. Biochemistry and Distribution

In contrast to the well-defined functions of 11β-HSD2, although 11β-HSD1 was the first isoform to be identified and characterised, it remained until recently the Cinderella of this story. 11β-HSD1 has a much-lower affinity (i.e., low μM Km) for cortisol and corticosterone, is a nicotinamide adenine dinucleotide phosphate (NADP(H))-dependent enzyme, and is widely distributed. In cell homogenates or microsomal preparations, 11β-HSD1 readily converts cortisol to cortisone. Thus, for several years, it was thought to be responsible for inactivation of cortisol in the kidney. However, more-recent data in intact cells either transfected with 11β-HSD1 cDNA or in primary cells in culture show that in most, if not all, intact cells and organs, this enzyme is a predominant 11-ketoreductase, reactivating inert cortisone into cortisol (Low *et al.*, 1994a;

Hundertmark *et al.*, 1995; Jamieson *et al.*, 1995; Rajan *et al.*, 1996). The preponderant reductive direction *in vivo* appears to be due more to the intracellular redox context of 11β-HSD1 in the inner leaflet of the endoplasmic reticulum than to any structural feature, such as glycosylation (Agarwal *et al.*, 1995). The mechanisms determining reaction directionality have not been elucidated fully. Nonetheless, 11β-HSD1 co-precipitates with the NADPH-generating enzyme, hexose-6-phosphate dehydrogenase (Ozols, 1995), which may dictate the predominant 11-ketoreductase action of 11β-HSD1 *in vivo* (Draper *et al.*, 2003). Short-term, post-translational changes such as enzyme phosphorylation also may be important, particularly to explain the apparent instability of the 11-ketoreductase activity in homogenates despite plentiful NADPH, but remain to be clarified. Biochemical investigation of expressed 11β-HSD1 suggests that the reductase reaction has cooperative rather than Michaelis-Menten kinetics, unlike the dehydrogenase, ensuring cortisol generation across a wide range of substrate concentrations (Maser *et al.*, 2002), as observed *ex vivo* in perfused liver (Jamieson *et al.*, 2000).

In a few studies (e.g., in Leydig cells), 11β-dehydrogenase activity has been reported in apparently intact cell preparations (Gao *et al.*, 1997; Ge and Hardy, 2000). Others have found predominantly 11-ketoreduction in similar preparations (Leckie *et al.*, 1998). The discordance of reported reaction direction in intact cells, largely involving Leydig cells and preadipocytes (Bujalska *et al.*, 2002), remains unresolved but is an important issue, given the current enthusiasm to decrease/inhibit 11-ketoreductase as a therapeutic target. It has been suggested that some 11β-HSD1 may have been liberated from possible damaged cells in studies reporting 11β-dehydrogenase activity (Seckl and Walker, 2001), since this direction is more stable in homogenates and broken-cell preparations. More plausibly, adipose tissue dissociation and culture lead to early induction of adipocyte cytokine synthesis and release (Ruan *et al.*, 2003). Cytokines have potent effects upon 11β-HSD1 and other adipocyte products, which may explain some discordances in the human adipose 11β-HSD1 activities between freshly isolated tissue and cultured cells.

11β-HSD1 is expressed in kidneys of rats but negligible expression occurs in adult human kidney (Stewart *et al.*, 1994). However, 11β-HSD1 is widely expressed in other tissues in humans (Ricketts *et al.*, 1998) and rodents (Moisan *et al.*, 1990b), including liver (Agarwal *et al.*, 1989; Tannin *et al.*, 1990), adipose tissue (Bujalska *et al.*, 1997; Napolitano *et al.*, 1998), lung (Hundertmark *et al.*, 1993), skeletal muscle, cardiac and vascular smooth muscle (Walker *et al.*, 1991; Brem *et al.*, 1995; Christy *et al.*, 2003), anterior pituitary gland, brain (including hippocampus) (Moisan *et al.*, 1990a,b; Lakshmi *et al.*, 1991; Sakai *et al.*, 1992), and adrenal cortex (Shimojo *et al.*, 1996). Notably, these locations have high GR rather than MR expression, with the exception of hippocampus and heart, where MR act as high-affinity sites for physiological glucocorticoids rather than aldosterone *in vivo* (Whorwood *et al.*, 1991).

These observations in cells suggested a novel role for 11β-HSD1 involving reactivation rather than inactivation of glucocorticoid. This, indeed, occurs in intact organs, at least in the liver. Isolated perfused cat (Bush *et al.*, 1968) or rat (Jamieson *et al.*, 2000) liver models suggest that 11β-HSD1, which is the only isozyme expressed in liver, is a predominant 11β-reductase with a high capacity for reactivating 11-ketosteroid substrate over a broad range of substrate concentrations. These findings can be extrapolated to human liver *in vivo*, since historical work suggests that, on oral administration, cortisone (the first pharmacological glucocorticoid used in man) is rapidly activated to cortisol. Indeed, recent studies confirm that very little oral cortisone reaches the systemic circulation (A. Jamieson *et al.*, 1999) and that hepatic vein cortisol/cortisone ratios are very high (Walker *et al.*, 1992). 11-ketoreductase activity has been shown in other human tissues *in vivo*, including subcutaneous adipose tissue (Katz *et al.*, 1999).

2. Substrate Levels

For 11-ketoreductase to play a physiological role in regulating receptor exposure to active glucocorticoids (as opposed to a pharmacological role when cortisone is administered), there must be a substantial pool of substrate inert 11-ketosteroids in the circulation and tissues. *In vivo*, the main source of 11-ketosteroid is 11β-HSD2 dehydrogenation of cortisol and corticosterone, which predominantly occurs in the kidney (Whitworth *et al.*, 1989). In humans, cortisone circulates at levels approximating 50–100 nmol/l (Walker *et al.*, 1992). While this level is lower than diurnal peak cortisol of 400–600 nmol/l, cortisone is largely unbound, while $\approx$ 95% of cortisol is sequestered by binding to plasma proteins such as CBG (Dunn *et al.*, 1981). Moreover cortisone, unlike cortisol, shows no pronounced diurnal rhythm and so is constantly available for conversion to active glucocorticoid (Walker *et al.*, 1992). Estimates of "free" cortisol levels are rather imprecise but approximate 0.5–1 nmol/l at the diurnal nadir. In the rat, plasma concentrations of 11-dehydrocorticosterone are also $\approx$ 50 nmol/l, though in the mouse, levels are lower ($\approx$ 3–5 nmol/l) (Kotelevtsev *et al.*, 1997). Thus, for at least the quiescent part of the diurnal cycle, circulating cortisone levels equal or exceed free cortisol levels and similar ratios pertain in rodents.

E. FUNCTIONAL STUDIES OF 11β-HSD1 IN LIVER

Initial findings suggesting that 11β-HSD1 increases effective intracellular glucocorticoid action were obtained in liver. Here, glucocorticoids oppose the actions of insulin, for example, by upregulating expression of the key enzyme for gluconeogenesis, phosphoenolpyruvate carboxykinase (PEPCK). In male rats, estradiol potently downregulates 11β-HSD1 expression (Low *et al.*, 1993) and, only in the presence of glucocorticoids, downregulates PEPCK expression (P.M. Jamieson *et al.*, 1999). Such indirect studies, as well as the use of relatively

nonselective, liquorice-based inhibitors (Walker *et al.*, 1995; Andrews *et al.*, 2003), indicate that impaired activity of 11β-HSD1 in liver is associated with features of reduced glucocorticoid action and increased insulin sensitivity in hepatocytes. This has been supported by recent studies with selective 11β-HSD1 inhibitors (Alberts *et al.*, 2002,2003) (see below).

To explore this further, 11β-HSD1 KO mice have been generated (Kotelevtsev *et al.*, 1997). These mice appear to develop normally and are viable, fertile, and apparently normotensive. This model shows that 11β-HSD1 is the sole major 11β-reductase, at least in mice, since adrenalectomised 11β-HSD1 KO mice cannot convert administered 11-dehydrocorticosterone to active corticosterone. Plasma corticosterone levels are modestly elevated at the diurnal nadir, presumably due to somewhat deficient feedback upon the HPA axis (11β-HSD1 is expressed in hippocampus, PVN, and anterior pituitary) (Harris *et al.*, 2001). However, despite slightly elevated basal plasma corticosterone levels, 11β-HSD1$^{-/-}$ mice have a phenotype compatible with impaired intracellular glucocorticoid regeneration and reduced antagonism of insulin action. They show impaired induction of the key glucocorticoid-inducible hepatic enzymes PEPCK and glucose-6-phosphatase on fasting and an attenuated hyperglycemic response to novel environment stress or chronic high-fat feeding (Kotelevtsev *et al.*, 1997). Importantly, the mice have more-than-adequate, stress-induced HPA axis responses (Harris *et al.*, 2001) and do not exhibit hypoglycaemia with prolonged fasting (Kotelevtsev *et al.*, 1997). Further aspects of the 11β-HSD1 null phenotype affect hepatic metabolic control and cardiovascular risk factors (Morton *et al.*, 2001). *Ad lib*-fed 11β-HSD1$^{-/-}$ mice have lower plasma triglyceride and elevated "cardioprotective" high-density lipoprotein (HDL) cholesterol levels (Morton *et al.*, 2001). This appears to be due to increased hepatic β oxidation of lipids, rather than to altered lipogenesis. Indeed, hepatic expression of peroxisome proliferator-activated receptor alpha (PPARα), a key glucocorticoid-sensitive transcription factor driving lipid metabolism, is elevated in fed 11β-HSD1$^{-/-}$ mice. The major murine HDL carrier, apolipoprotein AI, is increased in the null mice, whereas serum apolipoprotein CIII, which increases plasma triglycerides by inhibiting hepatic lipolysis and interfering with transfer of triglycerides to the liver, is reduced. Additionally, preliminary data suggest favourable effects upon haemostasis, as in liver, glucocorticoid-inducible Aα-fibrinogen transcript levels are reduced (Morton *et al.*, 2001).

F. 11β-HSD1 IN ADIPOSE TISSUE

1. Isozymes and Directionality

Glucocorticoids play a key role both in the regulation of adipose tissue metabolism and in the differentiation of preadipocytes into adipocytes (Gaillard

et al., 1991). 11β-HSD expression in adipose tissue was noted in the 1980s and thought to be a dehydrogenase (Monder and White, 1993). Studies in adipose cells derived from the mammary gland confirmed the presence of 11β-dehydrogenase activity *in vitro* (Quirk *et al.*, 1991). More recently, 11β-HSD in adipose tissue has been re-examined in light of the 11-ketoreductase predominance of this isozyme in intact hepatocytes and whole liver.

In terms of isozymes, 11β-HSD1, but not 11β-HSD2, mRNA is expressed in rat white (epididymal) adipose tissue (WAT) (Napolitano *et al.*, 1998) and in human adipose tissue (Bujalska *et al.*, 1997; Sun *et al.*, 1997; Paulmyer-Lacroix *et al.*, 2002). The enzyme appears to be expressed similarly in adipocytes and the stromal vascular fraction (enriched with preadipocytes and vascular cells) in the rat, though perhaps is more highly concentrated in adipose stromal cells in humans (Bujalska *et al.*, 1997). 11β-HSD also is expressed in murine fibroblast-adipocyte 3T3-F442A and 3T3-L1 cell lines, which closely reproduce preadipocyte differentiation *in vitro* when cultured in the presence of inducing agents, including dexamethasone. These respond to glucocorticoids in a similar manner to adipose tissue *in vivo* (MacDougald *et al.*, 1994; MacDougald and Lane, 1995). 3T3 cells exclusively express the 11β-HSD1 isozyme and, in intact cells, the reaction direction is solely 11-ketoreduction (Napolitano *et al.*, 1998). 11β-HSD1 mRNA and activity in 3T3 cells increases markedly with differentiation of cells from their preadipocyte to mature adipocyte state. 11β-HSD1 appears to be a late-differentiation gene in 3T3 cells. 3T3 cell *in vitro* differentiation is promoted by insulin and glucocorticoids. However, the increase in 11β-HSD1 with differentiation does not appear to be mediated directly by these hormones, since, at least in differentiated 3T3 cells, insulin and glucocorticoids reduce 11β-HSD1 expression. By contrast, intact human adipose stromal cells cultured in the presence of insulin and cortisol have been reported to show bi-directional 11β-HSD1 activity, although 11-ketoreductase activity predominates in cells derived from omental fat, with further induction by glucocorticoids in mature cells (Bujalska *et al.*, 1997).

Some recent findings suggest that 11β-HSD1 reaction direction may alter in adipose tissue with differentiation state (Bujalska *et al.*, 2002), although this was not apparent in a clonal 3T3 cell model (Napolitano *et al.*, 1998). Thus, primary cultures of human stromal and mature adipocytes taken from patients undergoing elective abdominal surgery showed predominant 11β-dehydrogenase activity in freshly isolated stromal cells from the omental compartment, whereas intact mature adipocytes had predominantly 11-ketoreductase activity. Following culture for 2 weeks, 11-ketoreductase activity predominated, despite no significant changes in 11β-HSD1 mRNA. The authors speculated that since glucocorticoids inhibit cell proliferation, dehydrogenase in immature preadipocytes facilitates proliferation. However, once

differentiation is initiated, 11-ketoreductase amplifies glucocorticoid levels, promoting adipogenesis. While these findings might be explained by cell breakage leading to liberation of enzyme into a homogenate-like state in the early phases of cell culture and/or by cytokine-inductive effects of adipose tissue dissociation and early culture *in vitro*, an intriguing biochemical mechanism for shifts in 11β-HSD1 directionality has been advanced. This advocates varying developmental expression of hexose-6-phosphate dehydrogenase, which co-precipitates with 11β-HSD1 and acts to generate NADPH inside the lumen of the endoplasmic reticulum in adipose tissue (Stegeman and Klotz, 1979). Intriguingly, while polymorphisms in the 11β-HSD1 gene (HSD11B1) correlate rather poorly (Draper *et al.*, 2002; Gelernter-Yaniv *et al.*, 2003), if at all (Caramelli *et al.*, 2001), with adipose distribution in humans, changes in the hexose-6-phosphate dehydrogenase gene may be key. Thus, in three patients with rare cortisone reductase deficiency (Phillipov *et al.*, 1996), null mutations in the HSD11B1 gene have not been found. Recent data suggest that such individuals have more-subtle mutations in both HSD11B1 and in hexose-6-phosphate dehydrogenase, resulting in both low 11β-HSD1 expression and low endoplasmic reticulum NADPH generation, with consequent loss of 11β-HSD1 reductase activity (Draper *et al.*, 2003). It remains to be established whether or not this mechanism applies to more-common cases of 11β-HSD1 dysregulation in adipose tissue with obesity and associated insulin-resistance states, such as the polycystic ovary syndrome, which phenotypically most closely resembles cortisone reductase deficiency.

2. Depot Differences

Fat depots may differ in 11β-HSD1 activity, although no clear consensus exists. Discrepancies may reflect species differences or assay conditions employed. Thus, in humans, cultures of omental stromal cells have more 11β-HSD1 than subcutaneous stromal cells (Bujalska *et al.*, 1999). In contrast, in various nonobese strains of mice, 11β-HSD1 mRNA expression and activity are higher in freshly isolated peripheral than visceral fat depots (Morton *et al.*, 2003). This is consistent with the idea that increased glucocorticoid reactivation correlates with increased differentiation potential of this depot. These interspecies differences may reflect the conditions of assay; *ex vivo* cultures in high glucocorticoid concentrations employed in humans perhaps favour increased activity in visceral fat because this depot has more GR (Masuzaki *et al.*, 2001) and/or has greater capacity for glucocorticoid-induced differentiation of immature to mature (i.e., higher 11β-HSD1-expressing) adipocytes.

3. Molecular Regulatory Mechanisms

Several studies have examined regulation of 11β-HSD1 expression in adipose tissue. Insulin appears to reduce 11β-HSD1 gene expression in fat cells. Although glucocorticoids reduce adipose 11β-HSD1 expression in some systems, in others, they stimulate its activity, with effects reflecting the assay conditions and perhaps species. Proinflammatory cytokines such as tumor necrosis factor alpha (TNFα) and interleukin-1 (IL1) increase and anti-inflammatory cytokines reduce 11β-HSD1 in adipose cells *in vitro* (Handoko *et al.*, 2000; Friedberg *et al.*, 2003). As detailed earlier, both glucocorticoids and insulin exert effects, albeit *in vitro*. However, regulation by other nuclear receptors remains less certain. Thus, while PPARγ ligands such as thiazolidinediones downregulate 11β-HSD1 in 3T3 cells *in vitro* and in epididymal fat in mice *in vivo* (Berger *et al.*, 2001), the same was not observed in lean or obese Zucker rats (Livingstone *et al.*, 2000b). Liver X receptor (LXR) agonists also partially downregulate 11β-HSD1 *in vitro* and *in vivo* (Stulnig *et al.*, 2002). The mechanism may be indirect, which may explain the discrepancies observed in such studies. In terms of more-direct effects, CCAAT/enhancer binding protein alpha (C/EBPα) binds to several sites on the 11β-HSD1 promoter and, when transfected into human hepatoma hepG2 cells, increases 11β-HSD1 promoter activity (Williams *et al.*, 2000). In contrast, C/EBPβ acts as a dominant-negative repressor of 11β-HSD1 transcription when added to C/EBPα, although alone, it is a weak inducer. Electrophoretic mobility shift assays (EMSA) using nuclear extracts from undifferentiated and differentiated 3T3-F442A cells suggest that C/EBPα also may regulate 11β-HSD1 expression in adipocytes (Williams *et al.*, 1993). These data suggest the possibility that 11β-HSD1 regulation in the adipocyte by insulin and glucocorticoids may be mediated indirectly through changes in C/EBP-related proteins. Since under many circumstances, 11β-HSD1 regulation in liver and adipose tissue is discordant, as with other well-characterised C/EBP-regulated genes such as PEPCK, the fine details of control are likely to be tissue specific. 11β-HSD1 mRNA regulation in 3T3-F442A cells parallels glycerol-3-phosphate dehydrogenase, a key enzyme in triglyceride synthesis and a well-characterised marker of adipocyte differentiation (Moustaid *et al.*, 1990).

G. AP2–11β-HSD1 ADIPOSE OVEREXPRESSING TRANSGENIC MICE: A MODEL OF THE METABOLIC SYNDROME

The preponderance of data suggest that 11-ketoreductase activity is increased in adipose tissue in obese rodent models and humans (see below). The key question is whether increased adipose 11β-HSD1 is a cause or a consequence of obesity and any associated metabolic syndrome. To dissect this, mice over-

expressing 11β-HSD1 selectively in adipose tissue have been generated, exploiting the adipocyte fatty acid binding protein (aP2) promoter (Masuzaki *et al.*, 2001). Two lines have been selectively developed to exhibit 2- to 3-fold overexpression of 11β-HSD1 in adipose tissue, which represents the degree of enzyme increase found in obese humans and animals. These aP2–11β-HSD1 mice are viable and appear developmentally normal. The transgene is expressed in all adipose depots examined and in brown adipose tissue (BAT) but is absent from other 11β-HSD targets such as the brain, liver, skeletal muscle, and kidney. The effect of the transgene is to approximately double corticosterone levels within adipose tissue, while circulating glucocorticoid levels are unaltered, at least under basal conditions. As a consequence of local intra-adipose glucocorticoid excess, aP2–11β-HSD1 mice are modestly obese. Strikingly, the obesity is predominantly intra-abdominal, with a $>$ 3-fold increase in the mesenteric fat depot, whereas peripheral fat depots are significantly, but much less spectacularly, increased. The visceral adipose expansion in these animals (which overexpress 11β-HSD1 similarly in all adipose beds) may relate to much-greater levels of GRα in visceral adipose than peripheral depots in mice (Masuzaki *et al.*, 2001). Indeed, the transgenics show increased expression of the glucocorticoid target gene lipoprotein lipase, particularly in mesenteric adipose tissue that could drive lipid accumulation in this adipose depot. It is interesting that GRα is not downregulated in the transgenic animals. Glucocorticoid autoregulation of GR is highly tissue specific and is only seen acutely in many tissues.

In association with this localised change in glucocorticoid exposure and the consequent increased visceral fat mass, aP2–11β-HSD1 mice develop all major features of the metabolic syndrome. The animals are markedly glucose intolerant and insulin resistant, features exacerbated by high-fat feeding. The animals do not show obvious fasting hyperglycemia but this becomes markedly manifest after a glucose load, suggesting the deficit is in peripheral glucose uptake rather than a predominant increase in hepatic glucose production. They also show dyslipidemia with elevated FFA and triglyceride levels (Masuzaki *et al.*, 2001). Glucocorticoids are potent secretagogues of leptin from adipose tissue. Serum leptin levels are elevated in the transgenic animals disproportionately to their degree of obesity, suggesting leptin resistance, as observed in human obesity.

aP2–11β-HSD1 transgenic mice show adipocyte hypertrophy but not hyperplasia (Masuzaki *et al.*, 2001). This is perhaps not surprising, as aP2 is a late differentiation gene in adipocytes, so expression and effects in preadipocytes would not be expected. Within adipocytes themselves, aP2–11β-HSD1 mice show changes concordant with decreased sensitivity to insulin and/or increased corticosterone levels. Thus, transgenic adipose tissue has decreased expression of the insulin-sensitising factor adiponectin (acrp30, adipoQ) (Yamauchi *et al.*, 2002) and increased expression of TNFα, an adipose cytokine that causes insulin

resistance (Hotamisligil *et al.*, 1993). Both are commensurate with the effects of glucocorticoid excess (Fasshauer *et al.*, 2002; Viengchareun *et al.*, 2002). Serum TNFα levels also are elevated. In contrast, adipose mRNA-encoding resistin, which may lead to insulin resistance (Steppan *et al.*, 2001), is reduced in transgenic adipose tissue, perhaps as a consequence of glucocorticoid excess (Viengchareun *et al.*, 2002). Intriguingly, angiotensinogen mRNA, which normally is expressed at low levels in adipose tissue, is strikingly elevated in aP2–11β-HSD1 transgenic mouse fat. This glucocorticoid-regulated transcript may underpin the marked hypertension seen in these animals. The hypertension is associated with renin-angiotensin-aldosterone system (RAAS) activation and is strikingly sensitive to low doses of an angiotensin II receptor antagonist (Masuzaki *et al.*, 2003). Finally, the animals show intriguing hyperphagia, indicating the potential for a novel pathway, other than leptin deficiency (though leptin resistance is not excluded), of signalling between the adipocyte and CNS appetite centres. The aP2–11β-HSD1 transgenic mouse faithfully models the major features of the metabolic syndrome.

The aP2–11β-HSD1 transgenic mouse permits speculation about the subtle but important phenotypic differences between Cushing's syndrome and the metabolic syndrome. If the metabolic syndrome is, indeed, generated by local glucocorticoid excess inside adipose tissue and/or liver but with normal circulating cortisol levels, then tissue without the adipose-hepatic axis will be unaffected. We would not necessarily expect Cushingoid skin thinning, vascular fragility, delayed wound healing, myopathy, or CNS effects, which may well reflect the actions of *systemic* glucocorticoid excess upon skin, blood vessels, skeletal muscle, and brain without the compass of *local* adipose and hepatic glucocorticoid excess seen in the 11β-HSD1 transgenic models.

H. LIVER GLUCOCORTICOID EXCESS: APOE–11β-HSD1 TRANSGENIC MICE

Blood from visceral adipose tissue drains via the hepatic portal vein to the liver. Unsurprisingly, therefore, aP2–11β-HSD1 transgenic mice have elevated levels of corticosterone and FFA in the portal plasma, implying that the liver is exposed to excess glucocorticoids. To address this and to examine whether excess 11β-HSD1 in the liver *per se* can cause the metabolic syndrome, transgenic mice overexpressing 11β-HSD1 in the liver have been generated under the ApoE promoter. These mice are viable and appear normal. As adults, the mice show modest insulin resistance and hypertriglyceridemia, along with substantially increased fat deposits in the liver. However, ApoE–11β-HSD1 transgenic mice have normal weight with normal distribution and mass of fat depots and show normal glucose tolerance. Intriguingly, the animals are hypertensive, with activation of the RAAS, in this case due, apparently, to overex-

pression of angiotensinogen in the liver but not adipose tissue. Overexpression of 11β-HSD1 in liver produces an attenuated metabolic syndrome phenotype *without* visceral obesity. This might be of pathogenic relevance in cases such as patients with the insulin resistance of myotonic dystrophy (Johansson *et al.*, 2001), in which liver 11β-HSD1 activity is raised.

I. DEFICIENCY OF 11β-HSD1 IN ADIPOSE TISSUE: LESSONS FROM THE KO MOUSE

11β-HSD1 expression in adipose tissue produces a striking metabolic syndrome phenotype. But what about enzyme deficiency (Figure 2)? Inhibitors have provided some conflicting data that may relate to difficulties in access of carbenoxolone to adipose tissue *in vivo*, at least in the doses administered to rodent models (Livingstone and Walker, 2003) and perhaps humans (Andrews *et al.*, 2003). Adiposity in 11β-HSD1 KO animals has been examined on both intrinsically obesity-resistant and obesity-prone genetic backgrounds. Intra-adipose corticosterone levels are lowered substantially in the 11β-HSD1$^{-/-}$ animal

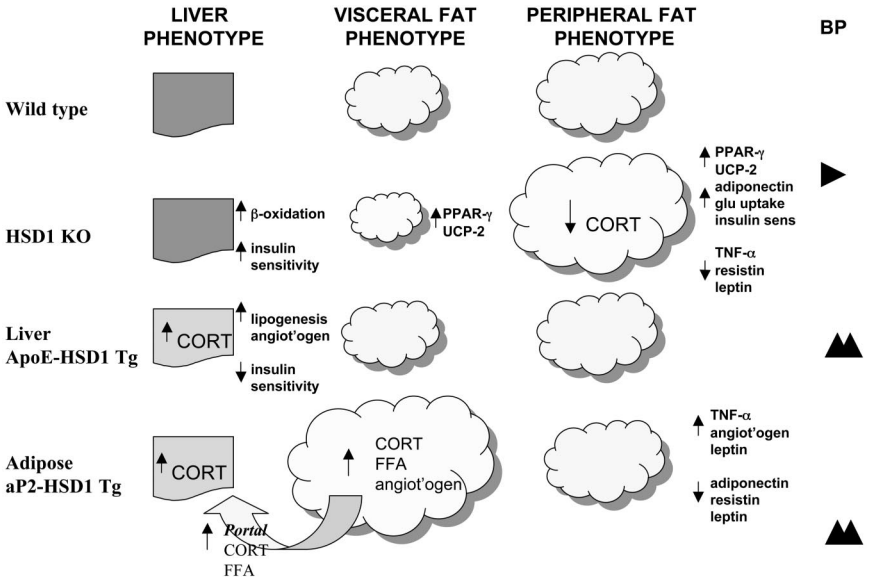

FIG. 2. Schematic of the phenotypes of mice lacking or overexpressing 11β-HSD1 in metabolic tissues. The size of the representations of fat depots reflects the mass observed. Paler liver colours represent fat accumulation. Abbreviations: BP, blood pressure; KO, knockout; Tg, transgenic; PPARγ, peroxisome proliferator-activated receptor gamma; UCP-2, uncoupling protein 2; TNFα = tumor necrosis factor alpha; HSD, hydroxysteroid dehydrogenase; FFA, free fatty acid; CORT, corticosterone.

in the face of modestly elevated plasma corticosterone concentrations (Morton *et al.*, 2003). On the obesity-prone C57Bl/6J genetic background, high-fat diet-fed 11β-HSD1 KO mice gain significantly less weight than controls, despite relative hyperphagia. This appears due to an enhanced metabolic rate, as judged by increased core body temperature (Morton *et al.*, 2002). With high-fat diet on either genetic background, 11β-HSD1 KO mice preferentially gain adipose tissue in the "metabolically safer" peripheral depots rather than in the "metabolically disadvantageous" visceral sites. While the explanation of fat redistribution in 11β-HSD1$^{-/-}$ animals is uncertain, these mice show higher expression of the thiazolidinedione target adipogenic transcription factor PPARγ receptor in all adipose tissue beds. Furthermore, 11β-HSD1$^{-/-}$ mice show a greater increase of adipose PPARγ receptors with high-fat feeding than wild-type (WT) mice. PPARγ ligands cause fat redistribution to the periphery, which may underpin the favourable morphology seen (Kelly *et al.*, 1999; Sewter *et al.*, 2002). Additionally, 11β-HSD1 null animals show greater induction of uncoupling protein-2 (UCP-2) in mesenteric adipose tissue than WT mice (Morton *et al.*, 2002), which may allow local calorie wastage rather than storage as fat (Digby *et al.*, 2000). UCP-2 is downregulated by glucocorticoids (Udden *et al.*, 2001) and upregulated by PPARγ activation (Kelly *et al.*, 1998), so its induction in the 11β-HSD1 null mouse adipose tissue is not unexpected.

In terms of adipose endocrine changes, adipose leptin mRNA and plasma leptin levels are reduced in 11β-HSD$^{-/-}$ mice, particularly in peripheral adipose. On the obesity-prone C57Bl/6J genetic background, 11β-HSD1 null animals are clearly insulin sensitised and resist hyperglycaemia that occurs with high-fat feeding in WT mice. This occurs at least partly at the adipocyte level, since isolated primary adipocytes show increased basal and insulin-stimulated glucose uptake (Morton *et al.*, 2003). Adipocyte resistin and TNFα mRNAs are reduced, whereas adiponectin is increased, again compatible with an adipose-mediated, insulin-sensitised phenotype. Thus, overall, the mouse shows improved glucose tolerance, increased insulin sensitivity, and reduced intratissue glucocorticoid levels in the face of modest hypercorticosteronaemia (Harris *et al.*, 2001). These beneficial effects of deletion in 11β-HSD1 in adipose tissue are accompanied by changes in hepatic gene expression consistent with increased β oxidation of lipids in the liver (Morton *et al.*, 2001).

Although the relative importance of liver and adipose tissues in contributing to this beneficial phenotype remains to be determined, it is clear that loss of 11β-HSD1 has few, if any, deleterious metabolic effects, at least as assessed in this mouse model. Indeed, the lifespan of this model is apparently normal and the animals resist the normal glucocorticoid-associated decline of memory and other cognitive processes with ageing (Yau *et al.*, 2001). Thus, loss of this enzyme appears to be beneficial.

1. Why Might 11β-HSD1 Exist?

However, the phenotype of the 11β-HSD1$^{-/-}$ mouse and the various transgenic and pharmacological manipulations detailed here raise the evolutionary question of the overall biological purpose of such an apparently virtueless enzyme. It cannot exist merely as a convenient target for therapy. We can only speculate. Given that our vertebrate biological antecedents had to suffer considerably more starvation than plenty, perhaps the boost of glucocorticoid from local tissue reactivation, particularly during the diurnal nadir, provided an extra lift to metabolic processes underpinning calorie generation. This might well have assisted survival during periods of starvation. Even during relative nutritional plenty, the sustenance of some key metabolic organs, while others, including the HPA system, were quiescent (i.e., during sleep), may have had an advantage. However, in modern westernised existence with an excess of calorifically rich foods available at every street corner, perhaps such activity becomes redundant, particularly as obesity and its metabolic consequences become the problem. It is an intriguing notion that an enzyme putatively required to help survive starvation becomes a metabolic "appendix" in the presence of nutritional plenty and a target for therapy of the consequences of calorific excess.

V. 11β-HSD1 and Glucocorticoid Metabolism in Obesity

The combination of increased total cortisol secretion with lower plasma cortisol levels during peak secretion suggests that peripheral cortisol clearance may be increased in obesity. Any such activation would lead to adrenal hyperresponsivity to stress and other stimuli, as observed in obesity. In the 2 decades since excess cortisol production and clearance rates were first noted in obesity (Strain *et al.*, 1980), the observation has been repeated many times (Andrew *et al.*, 1998,2002; Lottenberg *et al.*, 1998). Increased glucocorticoid clearance could reduce plasma levels and lead to compensatory HPA axis activation. The enzymes responsible for increased glucocorticoid clearance in obesity have been identified through analysis of cortisol metabolites in urine. Increased relative excretion of A-ring reduced metabolites of cortisol has been reported that is attributed to activation of the hepatic A-ring reductases (Andrew *et al.*, 1998; Fraser *et al.*, 1999). A-ring reductases (5α- and -β) are activated in both animal (Livingstone *et al.*, 2000a) and human obesity (Andrew *et al.*, 1998,2002). The underlying cause of increased enzyme activity is unknown but these enzymes may be regulated by insulin, lipids, and substrate availability. However, here, we concentrate upon another pathway, the 11β-HSDs.

A. 11β-HSD1 IN OBESITY

Measurement of 11β-HSD1 *in vivo* in humans is not straightforward (Walker, 2000). The original assessment relied upon measuring ratio of the most-abundant urinary metabolites of cortisol and cortisone (i.e., 5α- and 5β-tetrahydrocortisols/5β-tetrahydrocortisone). However, this ratio also is influenced by activities of other enzymes, including 11β-HSD2, 5α-reductase, 5β-reductase, and 3α-HSDs. For example, carbenoxolone, which inhibits both renal 11β-HSD2 and hepatic 11β-HSD1, does not alter the ratio of cortisol/cortisone metabolites (Stewart *et al.*, 1990). In obesity, the urinary cortisol/cortisone metabolite ratio has been reported to be increased (Andrew *et al.*, 1998; Rask *et al.*, 2002; Tiosano *et al.*, 2003), unchanged (Fraser *et al.*, 1999; Reynolds *et al.*, 2001), or decreased (Stewart *et al.*, 1999; Rask *et al.*, 2001). This inconsistency may depend in part upon variations — for example, with sex — in 5α- and 5β-reductase activity. Recent findings suggest that increased fat content of the liver, which constitutes a risk factor for insulin resistance over and above the effect of obesity, is associated with increased urinary excretion of 5β-reduced cortisol metabolites. Liver fat content was shown to be a more-powerful determinant of cortisol/cortisone metabolite ratios than the degree of obesity (Westerbacka *et al.*, 2003). However, this important confounder has not been accounted for in most studies in which liver fat content was not measured. Another source of inconsistency of cortisol/cortisone metabolite ratio in obesity is the possible influence of tissue-specific disruption of 11β-HSD1 activity. It is this last crucial point that turns out to be the key issue in obesity.

Studies in leptin-resistant obese Zucker rats revealed that obesity was associated with decreased 11β-HSD1 expression and activity in liver but increased 11β-HSD1 in omental adipose tissue (Livingstone *et al.*, 2000a). This led to dissection of potential tissue-specific differences in obese humans. In humans, conversion of cortisone after oral administration to cortisol in peripheral plasma, reflecting first-pass metabolism by hepatic 11β-HSD1, is impaired in obesity (Stewart *et al.*, 1999; Rask *et al.*, 2001,2002). In contrast, in subcutaneous abdominal adipose tissue, 11β-HSD1 activity is increased both *in vivo* and *in vitro* (Rask *et al.*, 2001,2002; Lindsay *et al.*, 2003; Wake *et al.*, 2003). Further studies have confirmed that the increased 11β-HSD1 activity in biopsies is accompanied by increased 11β-HSD1 mRNA (Paulmyer-Lacroix *et al.*, 2002; Lindsay *et al.*, 2003; Wake *et al.*, 2003; Westerbacka *et al.*, 2003). By analogy with the transgenic mouse having 3-fold overexpression of 11β-HSD1 in adipose tissue (Masuzaki *et al.*, 2001,2003), one would expect a Cushingoid phenotype to result from the similar magnitude of increase in adipose 11β-HSD1 documented in human obesity. Interestingly, while increased subcutaneous adipose 11β-HSD1 is associated with insulin resistance in obesity, it is not linked specifically with visceral adiposity or hypertension. In these respects, mice and

men may differ but it remains to be determined whether 11β-HSD1 mRNA or activity is increased in intact *omental* adipose tissue in obese subjects (Tomlinson *et al.*, 2002).

The mechanisms underlying increased adipose 11β-HSD1 in obesity are uncertain. 11β-HSD1 transcription is highly regulated, including by many factors that are altered in obesity (e.g., cytokines, sex steroids, growth hormone (GH), insulin, PPARα and -γ agonists). In obese Zucker rats and ob/ob mice, downregulation of hepatic 11β-HSD1 is reversible with other manipulations that induce weight loss (Livingstone *et al.*, 2000b; Liu *et al.*, 2003). Attempts to link the 11β-HSD1 genotype with obesity have not been successful (Caramelli *et al.*, 2001; Draper *et al.*, 2002), although arguably the intermediate phenotypes (i.e., anthropometric measurements or urinary cortisol/cortisone metabolite ratios) employed are too insensitive to allow clear inferences about the influence of known polymorphisms in the 11β-HSD1 gene.

B. 11β-HSD1 AND GH

In addition to dysregulation in patients with idiopathic obesity, the central adiposity of GH deficiency and hypothalamic obesity may be associated with changes in cortisol metabolism and 11β-HSD1 activation. 11β-HSD1 is inhibited by GH and insulin-like growth factor-1 (IGF-1) *in vitro* (Napolitano *et al.*, 1998; Tomlinson *et al.*, 2001). In humans with hypopituitarism and acromegaly, this effect may be important (Moore *et al.*, 1999; Trainer *et al.*, 2001; Tiosano *et al.*, 2003). Indeed, GH therapy reduces cortisol:cortisone metabolite ratios in patients with hypopituitarism (Weaver *et al.*, 1994) and in idiopathic obesity (Tomlinson *et al.*, 2003). As in studies of idiopathic obesity, the difficulty is in the interpretation of urinary cortisol:cortisone metabolite ratios, which do not reflect any tissue-specific changes. *In vivo* animal studies have shown that GH regulates liver 11β-HSD1, with the direction of change dependent upon sex-specific patterns of pulsatile GH release (Low *et al.*, 1994b). These findings may not explain changes in adiposity but may give a mechanism for improvements in insulin sensitivity in liver alone when GH is administered at the constant levels typical of female patterns that downregulate hepatic 11β-HSD1. Whether such effects pertain to humans is not fully known.

C. 11β-HSD IN THE BRAIN IN OBESITY

While not the main object of this review (for details of 11β-HSDs in the CNS, see Seckl, 1997; Yau and Seckl, 2001), specific CNS changes in 11β-HSDs in obese rodents have been reported that add to the complexity of tissue-specific dysregulation. In particular, obese Zucker rats have reduced 11β-HSD1 mRNA in the hippocampus (Mattsson *et al.*, 2003). The enzyme functions as a reductase in hippocampal neurons (Rajan *et al.*, 1996). The reduced expression of 11β-

HSD1 may, in part, underlie the reduced sensitivity to glucocorticoid feedback inhibition of the HPA axis and consequent hypercorticosteronaemia of Zucker rats. Moreover, the feedback defect appears linked more to MR than GR, both in obese rodents (Mattsson *et al.*, 2003) and humans (Jessop *et al.*, 2003). Therefore, studies with GR-selective agonists like dexamethasone may be misleading (Weyer *et al.*, 1997). Whether such effects occur in obese humans is unknown (and is probably unknowable) but 11β-HSD1 is expressed in the human hippocampus (Sandeep *et al.*, 2002) and may be targeted by 11β-HSD inhibitors (Jellinck *et al.*, 1993).

VI. 11β-HSD1 as a Drug Target for Therapy in the Metabolic Syndrome

While the various mouse models described here represent a major series of advances in understanding the role of tissue (intracrine) glucocorticoid levels in generating visceral obesity and aspects of the metabolic syndrome, several discordances in human pathophysiology remain to be adequately accounted for.

First, studies showing increased 11β-HSD1 activity in adipose tissue in humans mainly have investigated abdominal subcutaneous fat. While this is not necessarily as distinct from visceral adipose as other subcutaneous depots — and subcutaneous fat is perhaps the major source of adipose hormones and cytokines — this depot is not the same as visceral fat and probably contributes little, if anything, to the portal venous influx to the liver.

Second, mice are not humans in key aspects of their biochemistry. Thus, murine HDL cholesterol represents the majority of total cholesterol, while in humans, the preponderance is reversed, with most cholesterol transported in low-density lipoprotein (LDL) particles. Mice have corticosterone as their sole glucocorticoid, whereas in humans, cortisol predominates. Corticosterone has a higher affinity for GR and MR. Mice do not spontaneously get atheroma without a specific mutation (e.g., ApoE$^{-/-}$), whereas humans do.

Third, while human subcutaneous adipose 11β-HSD1 correlates with obesity and increased insulin resistance, it does not correlate closely with the *distribution* of body fat, notably in the visceral compartment (Westerbacka *et al.*, 2003).

Fourth, it remains unclear whether 11β-HSD1 activity is also increased in omental adipose tissue in obese humans and drives a Cushingoid phenotype of central obesity (Bujalska *et al.*, 1997). One study has reported that 11β-HSD1 in omental adipose obtained during surgery is not correlated with obesity. Indeed, when adipocytes were cultured, there was an inverse correlation between 11β-HSD1 and obesity (Tomlinson *et al.*, 2002), although the effects of anaesthesia and/or stress on the subsequent pattern of gene expression and 11β-HSD1 activity and direction are difficult to anticipate. Attempts to demonstrate a close association between increased 11β-HSD1 and intra-adipose cortisol levels or

glucocorticoid-dependent gene transcription have been inconclusive (Lindsay *et al.*, 2003; Wake *et al.*, 2003). It has been proposed that the level of 11β-HSD1 mRNA expression and total protein may not be the most-important determinant of net cortisol generation from cortisone, since the balance between reductase and dehydrogenase activities may be crucially dependent upon cofactor generation by hexose-6-phosphate dehydrogenase (Draper *et al.*, 2003). While this hypothesis has not been tested definitively, it is notable that subjects with combined 11β-HSD1 and hexose-6-phosphate dehydrogenase mutations putatively conferring impaired conversion of cortisone to cortisol are not universally lean (Phillipou *et al.*, 1996; A. Jamieson *et al.*, 1999; Draper *et al.*, 2003).

Fifth, understanding the molecular mechanisms regulating 11β-HSD1 is key. While insulin and other hormones exert indirect effects in rodents, the mechanisms in humans are poorly understood. However, recent data suggest that patients with glucose intolerance have paradoxically normal cortisol secretion, which is perhaps inappropriately high, given enhanced central and peripheral tissue sensitivity to glucocorticoids (Andrews *et al.*, 2002). In these subjects, "normal" 11β-HSD1 activity in adipose tissue with reduced hepatic activity suggests that tissue-specific changes in 11β-HSD1 in hyperglycemia differ from those in primary obesity (Andrews *et al.*, 2002). Thus, increased adipose 11β-HSD1 appears to relate specifically to obesity, rather than to hyperglycaemia or insulin resistance (Westerbacka *et al.*, 2003) *per se*.

Despite these caveats, 11β-HSD1 inhibition is a tempting target for treatment of the metabolic syndrome and its complications. In this line, at least one selective inhibitor has been formulated and doubtless others will be developed.

A. INHIBITOR STUDIES: OLD AND NEW AGENTS

Carbenoxolone, the hemisuccinate of glycyrrhetinic acid, which is the main active derivative of liquorice, has been a prototype, though nonselective, 11β-HSD1 inhibitor (Stewart *et al.*, 1990). Carbenoxolone enhances insulin sensitivity, as measured by a euglycaemic hyperinsulinaemic clamp (Walker *et al.*, 1995), an effect attributed to enhanced hepatic insulin sensitivity. Stable isotope glucose tracers have been used to demonstrate that, in lean patients with type 2 diabetes, carbenoxolone inhibits hepatic glucose production (Andrews *et al.*, 2003). However, in contrast with enhanced adipose insulin sensitivity and glucose disposal in 11β-HSD1 KO mice, carbenoxolone has no effect on glucose disposal, perhaps because carbenoxolone poorly inhibits adipose 11β-HSD1 (Livingstone and Walker, 2003). Moreover, liver 11β-HSD1 is downregulated in obesity, so that the incremental effect of inhibition may be smaller in the obese (Stewart *et al.*, 1999; Rask *et al.*, 2001,2002). Whether inhibition in liver alone, and resulting enhancement of hepatic insulin sensitivity, is sufficient to confer clinically useful metabolic benefits is uncertain.

Recently, a new, potent, nonsteroidal class of selective inhibitors of 11β-HSD1 has been described, the arylsulfonamidothiazoles (Barf *et al.*, 2002). These agents inhibit 11β-HSD1 and enhance insulin action in liver, decreasing PEPCK and glucose-6-phosphatase expression and lowering blood glucose concentrations in KKA^Y diabetic and ob/ob obese mice (Alberts *et al.*, 2002,2003). However, euglycaemic hyperinsulinaemic clamps in mice suggested that the selective 11β-HSD1 inhibitor did not increase peripheral glucose uptake (Alberts *et al.*, 2003). What remains to be established for 11β-HSD1 inhibitors is whether their effects extend beyond the liver and include benefits in adipose tissue, where the most-beneficial target is likely to be.

VII. Summary

Close phenotypic similarities exist between rare Cushing's syndrome, caused by excess circulating glucocorticoids, and the much more-common visceral obesity (with or without the metabolic syndrome), although plasma cortisol levels in the latter typically are normal. This chapter reviews recent data suggesting that this paradox may be explained by increased local tissue activity of glucocorticoids in simple obesity syndromes. In particular, we concentrate on the emerging role of 11β-HSD1, an enzyme that catalyses the regeneration of active glucocorticoids in adipose tissue and liver as both a cause and a therapeutic target in this increasingly prevalent group of disorders.

ACKNOWLEDGMENTS

We are grateful to the Wellcome Trust and British Heart Foundation for generous research support.

REFERENCES

Agarwal AK, Monder C, Eckstein B, White PC 1989 Cloning and expression of rat cDNA encoding corticosteroid 11dehydrogenase. J Biol Chem 264:18939–18943

Agarwal AK, Mune T, Monder C, White PC 1995 Mutations in putative glycosylation sites of rat 11beta- hydroxysteroid dehydrogenase affect enzymatic activity. Biochem Biophys Acta 1248:70–74

Ahlberg AC, Ljung T, Rosmond R, McEwen B, Holm G, Akesson HO, Bjorntorp P 2002 Depression and anxiety symptoms in relation to anthropometry and metabolism in men. Psychiat Res 112(2):101–110

Alberts P, Engblom L, Edling N, Forsgren M, Klingstrom G, Larsson C, Ronquist-Nii Y, Ohman B, Abrahmsen L 2002 Selective inhibition of 11 beta-hydroxysteroid dehydrogenase type 1 decreases blood glucose concentrations in hyperglycaemic mice. Diabetologia 45(11): 1528–1532

Alberts P, Nilsson C, Selen G, Engblom L, Edling N, Norling S, Forsgren M, Klingstrom G, Larsson C, *et al.* 2003 Selective inhibition of 11β-hydroxysteroid dehydrogenase type 1

improves hepatic insulin sensitivity in hyperglycaemic mice strain. Endocrinology 144:4755–4762

Albiston AL, Obeyesekere VR, Smith RE, Krozowski ZS 1994 Cloning and tissue distribution of the human 11β-hydroxysteroid dehydrogenase type 2 enzyme. Mol Cell Endocrinol 105(2): R11–R17

Amelung D, Huebener HJ, Roka L, Meyerheim G 1953 Conversion of cortisone to compound F. J Clin Endocrinol Metab 13:1125

Andrew R, Phillips DIW, Walker BR 1998 Obesity and gender influence cortisol secretion and metabolism in man. J Clin Endocrinol Metab 83:1806–1809

Andrew R, Gale CR, Walker BR, Seckl JR, Martyn CN 2002 Glucocorticoid metabolism and the metabolic syndrome: associations in an elderly cohort. Exp Clin Endocrinol Diab 110(6): 284–290

Andrews R, Walker B 1999 Glucocorticoids and insulin resistance: old hormones, new targets. Clin Sci 96:513–523

Andrews RC, Herlihy O, Livingstone DEW, Andrew R, Walker BR 2002 Abnormal cortisol metabolism and tissue sensitivity to cortisol in patients with glucose intolerance. J Clin Endocrinol Metab 87(12):5587–5593

Andrews RC, Rooyackers O, Walker BR 2003 Effects of the 11 beta-hydroxysteroid dehydrogenase inhibitor carbenoxolone on insulin sensitivity in men with type 2 diabetes. J Clin Endocrinol Metab 88(1):285–291

Barf T, Vallgarda J, Emond R, Haggstrom C, Kurz G, Nygren A, Larwood V, Mosialou E, Axelsson K, *et al.* 2002 Arylsulfonamidothiazoles as a new class of potential antidiabetic drugs. Discovery of potent and selective inhibitors of the 11 beta-hydroxysteroid dehydrogenase type 1. J Med Chem 45(18):3813–3815

Benediktsson R, Calder AA, Edwards CRW, Seckl JR 1997 Placental 11β-hydroxysteroid dehydrogenase type 2 is the placental barrier to maternal glucocorticoids: *ex vivo* studies. Clin Endocrinol 46:161–166

Berger J, Tanen M, Elbrecht A, Hermanowski-Vosatka A, Moller DE, Wright SD, Thieringer R 2001 Peroxisome proliferator-activated receptor-γ ligands inhibit adipocyte 11β-hydroxysteroid dehydrogenase type 1 expression and activity. J Biol Chem 276:12629–12635

Berger S, Bleich M, Schmid W, Cole TJ, Peters J, Watanabe H, Kriz W, Warth R, Greger R, *et al.* 1998 Mineralocorticoid receptor knockout mice: pathophysiology of Na+ metabolism. Proc Natl Acad Sci USA 95(16):9424–9429

Bjorntorp P, Holm G, Rosmond R 1999 Hypothalamic arousal, insulin resistance and type 2 diabetes mellitus. Diab Med 16:373–381

Brem AS, Bina RB, King T, Morris DJ 1995 Bidirectional activity of 11beta-hydroxysteroid dehydrogenase in vascular smooth muscle cells. Steroids 60:406–410

Brem AS, Bina RB, King TC, Morris DJ 1998 Localization of 2 11 beta-OH steroid dehydrogenase isoforms in aortic endothelial cells. Hypertension 31(1):459–462

Brown RW, Diaz R, Robson AC, Kotelevtsev Y, Mullins JJ, Kaufman MH, Seckl JR 1996a The ontogeny of 11β-hydroxysteroid dehydrogenase type 2 and mineralocorticoid receptor gene expression reveal intricate control of glucocorticoid action in development. Endocrinology 137:794–797

Brown RW, Kotolevtsev Y, Leckie C, Lindsay RS, Lyons V, Murad P, Mullins JJ, Chapman KE, Edwards CRW, *et al.* 1996b Isolation and cloning of human placental 11β-hydroxysteroid dehydrogenase-2 cDNA. Biochem J 313:1007–1017

Brunner EJ, Hemingway H, Walker BR, Page M, Clarke P, Juneja M, Shipley MJ, Kumari M, Andrew R, *et al.* 2002 Adrenocortical, autonomic, and inflammatory causes of the metabolic syndrome — nested case-control study. Circulation 106(21):2659–2665

Bujalska I, Kumar S, Stewart PM 1997 Central obesity: "Cushing's disease of the omentum." Lancet 349:1210–1213

Bujalska IJ, Kumar S, Hewison M, Stewart PM 1999 Differentiation of adipose stromal cells: the roles of glucocorticoids and 11 beta-hydroxysteroid dehydrogenase. Endocrinology 140(7): 3188–3196

Bujalska IJ, Walker EA, Hewison M, Stewart PM 2002 A switch in dehydrogenase to reductase activity of 11,6- hydroxysteroid dehydrogenase type 1 upon differentiation of human omental adipose stromal cells. J Clin Endocrinol Metab 87(3):1205–1210

Bush IE, Hunter SA, Meigs RA 1968 Metabolism of 11-oxygenated steroids. Metabolism *in vitro* by preparation of liver. Biochem J 107:239–258

Caramelli E, Strippoli P, Di Giacomi T, Tietz C, Carinci P, Pasquali R 2001 Lack of mutations of type 1 11 beta-hydroxysteroid dehydrogenase gene in patients with abdominal obesity. Endocr Res 27(1–2):47–61

Christy C, Hadoke P, Paterson J, Mullins J, Seckl J, Walker B 2003 11beta-hydroxysteroid dehydrogenase type 2 in mouse aorta. Localization and influence on response to glucocorticoids. Hypertension 42:580–587

Cohen S 1980 Cushing's syndrome: a psychiatric study of 29 patients. Br J Psychiat 136:120–124

Cole TJ, Blendy JA, Monaghan AP, Krieglstein K, Schmid W, Aguzzi A, Fantuzzi G, Hummler E, Unsicker K, *et al.* 1995 Targeted disruption of the glucocorticoid receptor gene blocks adrenergic chromaffin cell-development and severely retards lung maturation. Genes Dev 9(13):1608–1621

Dave-Sharma S, Wilson RC, Harbison MD, Newfield R, Azar M, Krozowski ZS, Funder JW, Shackleton CHL, Bradlow HL, *et al.* 1998 Extensive personal experience — examination of genotype and phenotype relationships in 14 patients with apparent mineralocorticoid excess. J Clin Endocrinol Metab 83:2244–2254

de Kloet ER 1991 Brain corticosteroid receptor balance and homeostatic control. Front Neuroendocrinol 12:95–164

Di Blasio A, van Rossum E, Maestrini S, Berselli M, Tagliaferri M, Podestà F, Koper J, Liuzzi A, Lamberts S 2003 The relation between two polymorphisms in the glucocorticoid receptor gene and body mass index, blood pressure and cholesterol in obese patients. Clin Endocrinol 59:68–74

Digby JE, Crowley VEF, Sewter CP, Whitehead JP, Prins JB, O'Rahilly S 2000 Depot-related and thiazolidinedione-responsive expression of uncoupling protein 2 (UCP2) in human adipocytes. Intl J Obes 24(5):585–592

Draper N, Echwald SM, Lavery GG, Walker EA, Fraser R, Davies E, Sorensen TIA, Astrup A, Adamski J, *et al.* 2002 Association studies between microsatellite markers within the gene encoding human 11 beta-hydroxysteroid dehydrogenase type 1 and body mass index, waist to hip ratio, and glucocorticoid metabolism. J Clin Endocrinol Metab 87(11):4984–4990

Draper N, Walker EA, Bujalska IJ, Tomlinson JW, Chalder SM, Arlt W, Lavery GG, Bedendo O, Ray DW, *et al.* 2003 Mutations in the genes encoding 11 beta-hydroxysteroid dehydrogenase type 1 and hexose-6-phosphate dehydrogenase interact to cause cortisone reductase deficiency. Nature Genet 34(4):434–439

Dunn JF, Nisula BC, Rodbard D 1981 Transport of steroid hormones — binding of 21 endogenous steroids to both testosterone-binding globulin and corticosteroid-binding globulin in human plasma. J Clin Endocrinol Metab 53:58–68

Edwards CRW, Stewart PM, Burt D, Brett L, McIntyre MA, Sutanto WS, de Kloet ER, Monder C 1988 Localisation of 11β-hydroxysteroid dehydrogenase-tissue specific protector of the mineralocorticoid receptor. Lancet ii:986–989

Fasshauer M, Klein J, Neumann S, Eszlinger M, Paschke R 2002 Hormonal regulation of adiponectin gene expression in 3T3-L1 adipocytes. Biochem Biophys Res Commun 290(3): 1084–1089

Filipovsky J, Ducimetiere P, Eschwege E, Richard J, Rosselin G, Claude J 1996 The relationship of blood pressure with glucose, insulin, heart rate, free fatty acids and plasma cortisol levels according to degree of obesity in middle-aged men. J Hypertens 14:229–235

Fraser R, Ingram MC, Anderson NH, Morrison C, Davies E, Connell JMC 1999 Cortisol effects on body mass, blood pressure, and cholesterol in the general population. Hypertension 33(6):1364–1368

Friedberg M, Zoumakis E, Hiroi N, Bader T, Chrousos GP, Hochberg Z 2003 Modulation of 11beta-hydroxysteroid dehydrogenase type 1 in mature human subcutaneous adipocytes by hypothalamic messengers. J Clin Endocrinol Metab 88(1):385–393

Funder JW, Pearce PT, Smith R, Smith AI 1988 Mineralocorticoid action: target tissue specificity is enzyme, not receptor, mediated. Science 242:583–585

Gaillard D, Wabitsch M, Pipy B, Negrel R 1991 Control of terminal differentiation of adipose precursor cells by glucocorticoids. J Lipid Res 32:569–579

Gao HB, Ge RS, Lakshmi V, Marandici A, Hardy MP 1997 Hormonal regulation of oxidative and reductive activities of 11beta-hydroxysteroid dehydrogenase in rat Leydig cells. Endocrinology 138:156–161

Ge RS, Hardy MP 2000 Initial predominance of the oxidative activity of type I 11 beta-hydroxysteroid dehydrogenase in primary rat Leydig cells and transfected cell lines. J Androl 21(2):303–310

Gelernter-Yaniv L, Feng N, Sebring NG, Hochberg Z, Yanovski JA 2003 Associations between a polymorphism in the 11 beta hydroxysteroid dehydrogenase type I gene and body composition. Intl J Obes 27(8):983–986

Hadoke PWF, Kotelevtsev Y, Williams BC, Seckl JR, Mullins JJ, Walker BR 1998 Understanding glucocorticoid-induced hypertension: evidence from transgenic mice that vasoconstrictor and dilator pathways are modulated by 11β-hydroxysteroid dehydrogenase type 2. J Hum Hypertens 12:1

Handoko K, Yang KP, Strutt B, Khalil W, Killinger D 2000 Insulin attenuates the stimulatory effects of tumor necrosis factor alpha on 11 beta-hydroxysteroid dehydrogenase 1 in human adipose stromal cells. J Steroid Biochem Mol Biol 72(3–4):163–168

Harris HJ, Kotelevtsev Y, Mullins JJ, Seckl JR, Holmes MC 2001 11β-hydroxysteroid dehydrogenase type 1 null mice have altered hypothalamic-pituitary-adrenal axis activity: a novel control of glucocorticoid feedback. Endocrinology 142:114–120

Hautanen A, Adlercreutz H 1993 Altered adrenocorticotropin and cortisol secretion in abdominal obesity: implications for the insulin resistance syndrome. J Intern Med 234:461–469

Hirasawa G, Sasano H, Takahashi K-I, Fukushima K, Suzuki T, Hiwatashi T, Toyota T, Krozowski Z, Nagura H 1997 Colocalization of 11beta-hydroxysteroid dehydrogenase type II and mineralocorticoid receptor in human epithelia. J Clin Endocrinol Metab 82:3859–3863

Hotamisligil G, Shargill N, Spiegelman B 1993 Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science 259:87–91

Hundertmark S, Ragosch V, Schein B, Buhler H, Fromm M, Lorenz U, Weitzel H 1993 11Beta-hydroxysteroid dehydrogenase of rat lung: enzyme kinetic, oxidase- reductase ratio, electrolyte and trace element dependence. Enzyme Prot 47:83–91

Hundertmark S, Buhler H, Ragosch V, Dinkelborg L, Arabin B, Weitzel HK 1995 Correlation of surfactant phosphatidylcholine synthesis and 11beta-hydroxysteroid dehydrogenase in the fetal lung. Endocrinology 136:2573–2578

Jacobson L, Sapolsky R 1991 The role of the hippocampus in feedback regulation of the hypothalamic-pituitary-adrenal axis. Endocr Rev 12:118–134

Jamieson A, Wallace AM, Walker BR, Andrew R, Fraser R, White PC 1999 Apparent cortisone reductase deficiency: a functional defect in 11β-hydroxysteroid dehydrogenase type 1. J Clin Endocrinol Metab 84:3570–3574

Jamieson PM, Chapman KE, Edwards CRW, Seckl JR 1995 11β-hydroxysteroid dehydrogenase is an exclusive 11β-reductase in primary cultured rat hepatocytes: effect of physicochemical and hormonal manipulations. Endocrinology 136:4754–4761

Jamieson PM, Nyirenda MJ, Walker BR, Chapman KE, Seckl JR 1999 Interactions between oestradiol and glucocorticoid regulatory effects on liver-specific glucocorticoid-inducible genes: possible evidence for a role of hepatic 11β-hydroxysteroid dehydrogenase type 1. J Endocrinol 160:103–109

Jamieson PM, Chapman KE, Walker BR, Seckl JR 2000 11β-hydroxysteroid dehydrogenase type 1 is a predominant 11β-reductase in the intact perfused rat liver. J Endocrinol 165:685–692

Jellinck PH, Monder C, McEwen BS, Sakai RR 1993 Differential inhibition of 11β-hydroxysteroid dehydrogenase by carbenoxolone in rat brain regions and peripheral tissues. J Steroid Biochem Molec Biol 46:209–213

Jessop D, Dallman M, Fleming D, Lightman S 2003 Resistance to glucocorticoid feedback in obesity. J Clin Endocrinol Metab 86:4109–4114

Johansson A, Andrew R, Forsberg H, Cederquist K, Walker BR, Olsson T 2001 Glucocorticoid metabolism and adrenocortical reactivity to ACTH in myotonic dystrophy. J Clin Endocrinol Metab 86:4276–4283

Katz J, Mohammed-Ali V, Wood P, Yudkin J, Coppack S 1999 An *in vivo* study of the cortisol-cortisone shuttle in subcutaneous abdominal adipose tissue. Clin Endocrinol 50: 63–68

Kelly IE, Han TS, Walsh K, Lean MEJ 1999 Effects of a thiazolidinedione compound on body fat and fat distribution of patients with type 2 diabetes. Diab Care 22(2):288–293

Kelly L, Vicario P, Thompson G, Candelore M, Doebber T, Ventre J, Wu M, Meurer R, Forrest M, *et al.* 1998 Peroxisome proliferator-activated receptors gamma and alpha mediate *in vivo* regulation of uncoupling protein (UCP-1, UCP-2, UCP-3) gene expression. Endocrinology 139:4920–4927

Kotelevtsev Y, Holmes MC, Burchell A, Houston PM, Schmoll D, Jamieson PM, Best R, Brown R, Edwards CRW, *et al.* 1997 11β-hydroxysteroid dehydrogenase type 1 knockout mice show attenuated glucocorticoid inducible responses and resist hyperglycaemia on obesity or stress. Proc Natl Acad Sci USA 94:14924–14929

Kotelevtsev Y, Brown RW, Fleming S, Kenyon CJ, Edwards CRW, Seckl JR, Mullins JJ 1999 Hypertension in mice lacking 11β-hydroxysteroid dehydrogenase type 2. J Clin Invest 103:683–689

Lakshmi V, Monder C 1988 Purification and characterisation of the corticosteroid 11β-dehydrogenase component of the rat liver 11β-hydroxysteroid dehydrogenase complex. Endocrinology 123:2390–2398

Lakshmi V, Sakai RR, McEwen BS, Monder C 1991 Regional distribution of 11β-hydroxysteroid dehydrogenase in rat brain. Endocrinology 128:1741–1748

Leckie CM, Welberg L, Seckl JR 1998 11β-hydroxysteroid dehydrogenase type 1 is a reductase in intact rat Leydig cells. J Endocrinol 159:233–238

Levitt NS, Lambert EV, Woods D, Hales CN, Andrew R, Seckl JR 2000 Impaired glucose tolerance and elevated blood pressure in low birth weight, non-obese young South African adults: early programming of the cortisol axis. J Clin Endocrinol Metab 85:4611–4618

Li X, Wong J, Tsai S, Tsai M, O'Malley BW 2003 Progesterone and glucocorticoid receptors recruit distinct coactivator complexes and promote distinct patterns of local chromatin modification. Mol Cell Biol 23:3763–3773

Lindsay RS, Lindsay RM, Edwards CRW, Seckl JR 1996a Inhibition of 11β-hydroxysteroid dehydrogenase in pregnant rats and the programming of blood pressure in the offspring. Hypertension 27:1200–1204

Lindsay RS, Lindsay RM, Waddell B, Seckl JR 1996b Programming of glucose tolerance in the rat: role of placental 11β-hydroxysteroid dehydrogenase. Diabetologia 39:1299–1305

Lindsay RS, Wake DJ, Nair S, Bunt J, Livingstone DEW, Permana PA, Tataranni PA, Walker BR 2003 Subcutaneous adipose 11 beta-hydroxysteroid dehydrogenase type 1 activity and messenger ribonucleic acid levels are associated with adiposity and insulinemia in Pima Indians and Caucasians. J Clin Endocrinol Metab 88(6):2738–2744

Liu YJ, Nakagawa Y, Wang Y, Li RS, Li XJ, Ohzeki T, Friedman TC 2003 Leptin activation of corticosterone production in hepatocytes may contribute to the reversal of obesity and hyperglycemia in leptin-deficient ob/ob mice. Diabetes 52(6):1409–1416

Livingstone DEW, Walker BR 2003 Is 11 beta-hydroxysteroid dehydrogenase type 1 a therapeutic target? Effects of carbenoxolone in lean and obese Zucker rats. J Pharmacol Exp Therapeut 305(1):167–172

Livingstone DEW, Jones G, Smith K, Jamieson PM, Andrew R, Kenyon CJ, Walker BR 2000a Understanding the role of glucocorticoids in obesity: tissue-specific alterations of corticosterone metabolism in obese Zucker rats. Endocrinology 141:560–563

Livingstone DEW, Kenyon CJ, Walker BR 2000b Mechanisms of dysregulation of 11 beta-hydroxysteroid dehydrogenase type 1 in obese Zucker rats. J Endocrinol 167(3):533–539

Ljung T, Andersson B, Bengtsson B, Bjorntorp P, Marin P 1996 Inhibition of cortisol secretion by dexamethasone in relation to body fat distribution: a dose-response study. Obes Res 4:277–282

Ljung T, Ottosson M, Ahlberg AC, Eden S, Oden B, Okret S, Bronnegard M, Stierna P, Bjorntorp P 2002 Central and peripheral glucocorticoid receptor function in abdominal obesity. J Endocrinol Invest 25(3):229–235

Lottenberg SA, Giannella-Neto D, Derendorf H, Rocha M, Bosco A, Carvalho SV, Moretti AE, Lerario AC, Wajchenberg BL 1998 Effect of fat distribution on the pharmacokinetics of cortisol in obesity. Intl J Clin Pharmacol Therapeut 36(9):501–505

Low SC, Assaad SN, Rajan V, Chapman KE, Edwards CRW, Seckl JR 1993 Regulation of 11β-hydroxysteroid dehydrogenase by sex steroids *in vivo*: further evidence for the existence of a second dehydrogenase in rat kidney. J Endocrinol 139:27–35

Low SC, Chapman KE, Edwards CRW, Seckl JR 1994a Liver-type 11β-hydroxysteroid dehydrogenase cDNA encodes reductase not dehydrogenase activity in intact mammalian COS-7 cells. J Molec Endocrinol 13:167–174

Low SC, Chapman KE, Edwards CRW, Wells T, Robinson ICAF, Seckl JR 1994b Female pattern growth hormone secretion mediates the oestrogen-related decrease in hepatic 11β-hydroxysteroid dehydrogenase expression in the rat. J Endocrinol 143:541–548

MacDougald O, Lane M 1995 Transcriptional regulation of gene expression during adipocyte differentiation. Annu Rev Biochem 64:345–373

MacDougald O, Cornelius P, Lin F-T, Chen S, Lane M 1994 Glucocorticoids reciprocally regulate expression of the CCAAT/enhancer-binding protein and genes in 3T3-L1 adipocytes and white adipose tissue. J Biol Chem 269:19041–19047

Marin P, Darin M, Amemiya T, Andersson B, Jern S, Bjorntorp P 1992 Cortisol secretion in relation to body fat distribution in obese premenopausal women. Metab Clin Exper 41:882–888

Maser E, Volker B, Friebertshauser J 2002 11beta-hydroxysteroid dehydrogenase type 1 from human liver: dimerization and enzyme cooperativity support its postulated role as glucocorticoid reductase. Biochemistry 41:2459–2465

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

Masuzaki H, Yamamoto H, Kenyon CJ, Elmquist JK, Morton NM, Paterson JM, Shinyama H, Sharp MGF, Fleming S, *et al.* 2003 Transgenic amplification of glucocorticoid action in adipose tissue causes high blood pressure in mice. J Clin Invest 112(1):83–90

Mattsson C, Lai M, Noble J, McKinney E, Yau J, Seckl J, Walker B 2003 Reduced mineralocorticoid receptor and 11b-hydroxysteroid dehydrogenase type 1 expression in hippocampus of Zucker fat rats; mechanisms for altered feedback control of the hypothalamic-pituitary-adrenal axis in obesity. Endocrinology 144:2997–3003

McCormick J, Lyons V, Jacobson M, Diorio J, Nyirenda M, Weaver S, Yau JLW, Ester W, Meaney MJ, *et al.* 2000 5′-heterogeneity of glucocorticoid receptor mRNA is tissue-specific; differential regulation of variant promoters by early life events. Molec Endocrinol 14:506–517

McEwen BS 1999 Stress and hippocampal plasticity. Annu Rev Neurosci 22:105–122

Moisan M-P, Seckl JR, Brett LP, Monder C, Agarwal AK, White PC, Edwards CRW 1990a 11β-hydroxysteroid dehydrogenase messenger ribonucleic acid expression, bioactivity and immunoreactivity in rat cerebellum. J Neuroendocrinol 2:853–858

Moisan M-P, Seckl JR, Edwards CRW 1990b 11β-hydroxysteroid dehydrogenase bioactivity and messenger RNA expression in rat forebrain: localization in hypothalamus, hippocampus and cortex. Endocrinology 127:1450–1455

Monder C, White PC 1993 11β-hydroxysteroid dehydrogenase. Vitam Horm 47:187–271

Moore JS, Monson JP, Kaltsas G, Putignano P, Wood PJ, Sheppard MC, Besser GM, Taylor NF, Stewart PM 1999 Modulation of 11 beta-hydroxysteroid dehydrogenase isozymes by growth hormone and insulin-like growth factor: *in vivo* and *in vitro* studies. J Clin Endocrinol Metab 84(11):4172–4177

Morton N, Paterson J, Holmes MC, Walker B, Mullins J, Seckl J 2002 11β-Hydroxysteroid dehydrogenase type 1 null mice resist dietary obesity and its metabolic consequences. In: Program & Abstracts of the 84th Annual Meeting of The Endocrine Society, June 19–22, San Francisco, abstract OR17–3, p 87

Morton N, Paterson J, Masuzaki H, Holmes MC, Staels B, Fievet C, Walker B, Flier J, Mullins J, Seckl J 2003 Reduced 11β-hydroxysteroid dehydrogenase type 1-mediated intra-adipose glucocorticoid regeneration: a novel protective adaptation to and treatment for the metabolic syndrome. In: Program & Abstracts of the 85th Annual Meeting of The Endocrine Society, June 19–22, Philadelphia, abstract OR39–5, p 114

Morton NM, Holmes MC, Fievet C, Staels B, Mullins JJ, Seckl JR 2001 Improved lipid and lipoprotein profile, hepatic insulin sensitivity and glucose tolerance in 11beta-hydroxysteroid dehydrogenase type 1 null mice. J Biol Chem 276:41293–41300

Moustaid N, Lasnier F, Hainque B, Quignard-Boulange Q, Pairault J 1990 Analysis of gene expression during adipogenesis in 3T3-F442A preadipocytes: insulin and dexamethasone control. J Cell Biochem 42:243–254

Mune T, Rogerson FM, Nikkilä H, Agarwal AK, White PC 1995 Human hypertension caused by mutations in the kidney isozyme of 11β-hydroxysteroid dehydrogenase. Nature Genet 10:394–399

Napolitano A, Voice M, Edwards CRW, Seckl JR, Chapman KE 1998 11β-hydroxysteroid dehydrogenase type 1 in adipocytes: expression is differentiation-dependent and hormonally-regulated. J Steroid Biochem Molec Biol 64:251–260

Ozols J 1995 Lumenal orientation and post-translational modification of the liver microsomal 11β-hydroxysteroid dehydrogenase. J Biol Chem 270:2305–2312

Page N, Warriar N, Govindan M 1994 11-beta-hydroxysteroid dehydrogenase activity in human lung cells and transcription regulation for glucocorticoids. Am J Physiol — Lung Cell Molec Physiol 267:L464–L474

Panarelli M, Holloway CD, Fraser R, Connell JMC, Ingram MC, Anderson NH, Kenyon CJ 1998 Glucocorticoid receptor polymorphism, skin vasoconstriction, and other metabolic intermediate phenotypes in normal human subjects. J Clin Endocrinol Metab 83(6):1846–1852

Pasquali R, Cantobelli S, Casimirri F, Capelli M, Bortoluzzi L, Flamia R, Labate A, Barabara L 1993 The hypothalamic-pituitary-adrenal axis in obese women with different patterns of body fat distribution. J Clin Endocrinol Metab 77:341–346

Pasquali R, Gagliardi L, Vicennati V, Gambineri A, Colitta D, Ceroni L, Casimirri F 1999 ACTH and cortisol response to combined corticotropin releasing hormone-arginine vasopressin stimulation in obese males and its relationship to body weight, fat distribution and parameters of the metabolic syndrome. Intl J Obes 23(4):419–424

Pasquali R, Vicennati V, Calzoni F, Gnudi U, Gambineri A, Ceroni L, Cortelli P, Menozzi R, Sinisi R, *et al.* 2000 Alpha 2-adrenoceptor regulation of the hypothalamic-pituitary-adrenocortical axis in obesity. Clin Endocrinol 52(4):413–421

Pasquali R, Ambrosi B, Armanini D, Cavagnini F, Uberti ED, del Rio G, de Pergola G, Maccario M, Mantero F, *et al.* 2002 Cortisol and ACTH response to oral dexamethasone in obesity and effects of sex, body fat distribution, and dexamethasone concentrations: a dose-response study. J Clin Endocrinol Metab 87(1):166–175

Paulmyer-Lacroix O, Boullu S, Oliver C, Alessi MC, Grino M 2002 Expression of the mRNA coding for 11 beta-hydroxysteroid dehydrogenase type 1 in adipose tissue from obese patients: an *in situ* hybridization study. J Clin Endocrinol Metab 87(6):2701–2705

Pepin M-C, Beaulieu S, Barden N 1989 Antidepressants regulate glucocorticoid receptor messenger RNA concentrations in primary neuronal cultures. Mol Brain Res 6:77–83

Phillipou G, Palermo M, Shackleton C 1996 Apparent cortisone reductase deficiency; a unique form of hypercortisolism. J Clin Endocrinol Metab 81:3855–3860

Phillips DI, Fall CHD, Whorwood CB, Seckl JR, Wood PJ, Barker DJP, Walker BR 1998 Elevated plasma cortisol concentrations: an explanation for the relationship between low birthweight and adult cardiovascular risk factors. J Clin Endocrinol Metab 83:757–760

Phillips DIW, Walker BR, Reynolds RM, Flanagan DEH, Wood PJ, Osmond C, Barker DJP, Whorwood CB 2000 Low birth weight predicts elevated plasma cortisol concentrations in adults from 3 populations. Hypertension 35(6):1301–1306

Quirk SJ, Slattery JA, Funder JW 1991 Epithelial and adipose cells isolated from mammary glands of pregnant and lactating rats differ in 11β-hydroxysteroid dehydrogenase activity. J Steroid Biochem Molec Biol 37:529–534

Rajan V, Edwards CRW, Seckl JR 1996 11β-hydroxysteroid dehydrogenase in cultured hippocampal cells reactivates inert 11-dehydrocorticosterone, potentiating neurotoxicity. J Neurosci 16:65–70

Rask E, Olsson T, Soderberg S, Andrew R, Livingstone DEW, Johnson O, Walker BR 2001 Tissue-specific dysregulation of cortisol metabolism in human obesity. J Clin Endocrinol Metab 86:1418–1421

Rask E, Walker BR, Soderberg S, Livingstone DEW, Eliasson M, Johnson O, Andrew R, Olsson T 2002 Tissue-specific changes in peripheral cortisol metabolism in obese women: increased adipose 11 beta-hydroxysteroid dehydrogenase type 1 activity. J Clin Endocrinol Metab 87(7):3330–3336

Reaven G 2002 Metabolic syndrome — pathophysiology and implications for management of cardiovascular disease. Circulation 106(3):286–288

Reaven GM 1993 Role of insulin resistance in human-disease (syndrome-x) — an expanded definition. Annu Rev Med 44:121–131

Reynolds RM, Walker BR, Syddall HE, Andrew R, Wood PJ, Whorwood CB, Phillips DIW 2001 Altered control of cortisol secretion in adult men with low birth weight and cardiovascular risk factors. J Clin Endocrinol Metab 86:245–250

Reynolds RM, Chapman KE, Seckl JR, Walker BR, McKeigue PM, Lithell HO 2002 Skeletal muscle glucocorticoid receptor density and insulin resistance. JAMA 287(19):2505–2506

Ricketts ML, Verhaeg JM, Bujalska I, Howie AJ, Rainey WE, Stewart PM 1998 Immunohistochemical localization of type 1 11 beta-hydroxysteroid dehydrogenase in human tissues. J Clin Endocrinol Metab 83(4):1325–1335

Rosmond R, Dallman M, Bjorntorp P 1998 Stress-related cortisol secretion in men: relationships with abdominal obesity and endocrine, metabolic and haemodynamic abnormalities. J Clin Endocrinol Metab 83:1853–1859

Rosmond P, Chagnon YC, Chagnon M, Perusse L, Bouchard C, Bjorntorp P 2000 A polymorphism of the 5′-flanking region of the glucocorticoid receptor gene focus is associated with basal cortisol secretion in men. Metab Clin Exper 49(9):1197–1199

Ruan H, Zarnowski MJ, Cushman SW, Lodish HF 2003 Standard isolation of primary adipose cells from mouse epididymal fat pads induces inflammatory mediators and down-regulates adipocyte genes. J Biol Chem 278:47585–47593

Sakai RR, Lakshmi V, Monder C, McEwen BS 1992 Immunocytochemical localisation of 11beta-hydroxysteroid dehydrogenase in hippocampus and other brain regions of the rat. J Neuroendocrinol 4:101–106

Samra J, Clark M, Humphreys S, MacDonald I, Bannister P, Frayn K 1998 Effects of physiological hypercortisolemia on the regulation of lipolysis in subcutaneous adipose tissue. J Clin Endocrinol Metab 83:626–631

Seckl JR 1997 11β-hydroxysteroid dehydrogenase in the brain: a novel regulator of glucocorticoid action? Front Neuroendocrinol 18:49–99

Seckl JR, Walker BR 2001 11β-hydroxysteroid dehydrogenase type 1: a tissue-specific amplifier of glucocorticoid action. Endocrinology 142:1371–1376

Seckl JR, Cleasby M, Nyrenda MJ 2000 Glucocorticoids, 11β-hydroxysteroid dehydrogenase and fetal programming. Kidney Intl 57:1412–1417

Seckl JR, Sandeep T, Walker BR, Mullins JJ, Yau JL 2002 Glucocorticoids and cognitive deficits with aging: new approaches of potential clinical relevance. In: Program & Abstracts of the 84th Annual Meeting of The Endocrine Society, June 19–22, San Francisco, abstract S14–3, p 30

Sewter CP, Blows F, Vidal-Puig A, O'Rahilly S 2002 Regional differences in the response of human pre-adipocytes to PPAR gamma and RXR alpha agonists. Diabetes 51(3):718–723

Shimojo M, Whorwood C, Stewart P 1996 11beta-Hydroxysteroid dehydrogenase in the rat adrenal. J Mol Endocrinol 17**:**121–130

Smith R, Maguire J, Stein-Oakley A, Sasano H, Takahashi K, Fukushima K, Krozowski Z 1996 Localization of 11beta-hydroxysteroid dehydrogenase type II in human epithelial tissues. J Clin Endocrinol Metab 81:3244–3248

Solano JM, Jacobson L 1999 Glucocorticoids reverse leptin effects on food intake and body fat in mice without increasing NPY mRNA. Am J Physiol Endocrinol Metab 277(4):E708–E716

Stegeman J, Klotz A 1979 A possible role for microsomal hexose-6-phosphate dehydrogenase in microsomal electron transport and mixed-function oxidase activity. Biochem Biophys Res Commun 87:410–415

Steppan C, Bailey S, Bhat S, Brown E, Banerjee R, Wright C, Patel H, Ahima R, Lazar M 2001 The hormone resistin links obesity to diabetes. Nature 409:307–312

Stewart P, Murry B, Mason J 1994 Human kidney 11-hydroxysteroid dehydrogenase is a high affinity nicotinamide adenine dinucleotide-dependent enzyme and differs from the cloned type 1 isoform. J Clin Endocrinol Metab 79:480–484

Stewart PM, Krozowski ZS 1999 11 beta-hydroxysteroid dehydrogenase. Vitam Horm 57:249–324

Stewart PM, Valentino R, Wallace AM, Burt D, Shackleton CHL, Edwards CRW 1987 Mineralocorticoid activity of liquorice: 11β-hydroxysteroid dehydrogenase deficiency comes of age. Lancet ii:821–824

Stewart PM, Wallace AM, Atherden SM, Shearing CH, Edwards CRW 1990 Mineralocorticoid activity of carbenoxolone: contrasting effects of carbenoxolone and liquorice on 11β-hydroxysteroid dehydrogenase activity in man. Clin Sci 78:49–54

Stewart PM, Boulton A, Kumar S, Clark PMS, Shackleton CHL 1999 Cortisol metabolism in human obesity: impaired cortisone $\rightarrow$ cortisol conversion in subjects with central adiposity. J Clin Endocrinol Metab 84(3):1022–1027

Stolk R, Lamberts S, de Jong F, Pols H, Grobbee D 1996 Gender differences in the associations between cortisol and insulin sensitivity in healthy subjects. J Endocrinol 149:313–318

Strain G, Zumoff B, Strain J 1980 Cortisol production in obesity. Metab Clin Exper 29:980–985

Stulnig TM, Oppermann U, Steffensen KR, Schuster GU, Gustafsson JA 2002 Liver X receptors downregulate 11 beta-hydroxysteroid dehydrogenase type 1 expression and activity. Diabetes 51(8):2426–2433

Sun K, Yang K, Challis JRG 1997 Differential expression of 11beta-hydroxysteroid dehydrogenase types 1 and 2 in human placenta and fetal membranes. J Clin Endocrinol Metab 82:300–305

Tannin GM, Agarwal AK, Monder C, New MI, White PC 1991 The human gene for 11-beta-hydroxysteroid dehydrogenase – structure, tissue distribution, and chromosomal localization. J Biol Chem 266:16653–16658

Tiosano D, Eisentein I, Militianu D, Chrousos G, Hochberg Z 2003 11beta-Hydroxysteroid dehydrogenase activity in hypothalamic obesity. J Clin Endocrinol Metab 88:379–384

Tomlinson J, Crabtree N, Holder G, Toogood A, Shackelton C, Stewart P 2003 Low-dose growth hormone inhibits 11 hydroxysteroid dehydrogenase type 1 but has no effect upon fat mass in patients with simple obesity. J Clin Endocrinol Metab 88:2113–2118

Tomlinson JW, Moore J, Cooper MS, Bujalska I, Shahmanesh M, Burt C, Strain A, Hewison M, Stewart PM 2001 Regulation of expression of 11beta-hydroxysteroid dehydrogenase type 1 in adipose tissue: tissue-specific induction by cytokines. Endocrinology 142(5):1982–1989

Tomlinson JW, Sinha B, Bujalska I, Hewison M, Stewart PM 2002 Expression of 11 beta-hydroxysteroid dehydrogenase type 1 in adipose tissue is not increased in human obesity. J Clin Endocrinol Metab 87(12):5630–5635

Trainer P, Drake W, Perry L, Taylor N, Besser G, Monson J 2001 Modulation of cortisol metabolism by the growth hormone receptor antagonist pegvisomant in patients with acromegaly. J Clin Endocrinol Metab 86:2989–2992

Udden J, Folkesson R, Hoffstedt J 2001 Downregulation of uncoupling protein 2 mRNA in women treated with glucocorticoids. Intl J Obes 25(11):1615–1618

Udden J, Bjorntorp P, Arner P, Barkeling B, Meurling L, Rossner S 2003 Effects of glucocorticoids on leptin levels and eating behaviour in women. J Intern Med 253(2):225–231

Vicennati V, Ceroni L, Gagliardi L, Gambineri A, Pasquali R 2002 Response of the hypothalamic-pituitary-adrenocortical axis to high-protein/fat and high-carbohydrate meals in women with different obesity phenotypes. J Clin Endocrinol Metab 87(8):3984–3988

Viengchareun S, Zennaro MC, Pascual-Le Tallec L, Lombes M 2002 Brown adipocytes are novel sites of expression and regulation of adiponectin and resistin. FEBS Lett 532(3):345–350

Wake DJ, Rask E, Livingstone DEW, Soderberg S, Olsson T, Walker BR 2003 Local and systemic impact of transcriptional up-regulation of 11 beta-hydroxysteroid dehydrogenase type 1 in adipose tissue in human obesity. J Clin Endocrinol Metab 88(8):3983–3988

Walker B 2000 How will we know if 11-hydroxysteroid dehydrogenases are important in common diseases? Clin Endocrinol 52:401–402

Walker B, Seckl J 2001 Cortisol metabolism. In: Björntorp P, ed. International Textbook of Obesity. Chichester, UK: John Wiley and Sons; 241–268

Walker BR, Soderberg S, Lindahl B, Olsson T 2000 Independent effects of obesity and cortisol in predicting cardiovascular risk factors in men and women. J Intern Med 247:198–204

Walker BR, Yau JLW, Burt D, Brett L, Seckl JR, Williams BC, Monder C, Edwards CRW 1991 Distribution of 11β-hydroxysteroid dehydrogenase bioactivity in rat vasculature: localization of messenger RNA expression and immunoreactivity. Endocrinology 124:3305–3312

Walker BR, Campbell JC, Fraser R, Stewart PM, Edwards CRW 1992 Mineralocorticoid excess and inhibition of 11β-hydroxysteroid dehydrogenase in patients with ectopic ACTH syndrome. Clin Endocrinol 37:483–492

Walker BR, Connacher AA, Lindsay RM, Webb DJ, Edwards CRW 1995 Carbenoxolone increases hepatic insulin sensitivity in man: a novel role for 11-oxosteroid reductase in enhancing glucocorticoid receptor activation. J Clin Endocrinol Metab 80:3155–3159

Walker BR, Best R, Shackleton CHL, Padfield PL, Edwards CRW 1996 Increased vasoconstrictor sensitivity to glucocorticoids in essential hypertension. Hypertension 27:190–196

Walker BR, Phillips DIW, Noon JP, Panarelli M, Andrew R, Edwards HV, Holton DW, Seckl JR, Webb DJ, *et al.* 1998 Increased glucocorticoid activity in men with cardiovascular risk factors. Hypertension 31:891–895

Ward AMV, Fall CHD, Stein CE, Kumaran K, Veena SR, Wood PJ, Syddall HE, Phillips DIW 2003 Cortisol and the metabolic syndrome in South Asians. Clin Endocrinol 58(4):500–505

Watt G, Harrap S, Foy C, Holton D, Edwards H, Davidson H, Connor J, Lever A, Fraser R 1992 Abnormalities of glucocorticoid metabolism and the renin-angiotensin system: a four corners approach to the identification of genetic determinants of blood pressure. J Hypertens 10:473–482

Weaver J, Thaventhiran L, Noonan K, Burrin J, Taylor N, Norman M, Monson J 1994 The effect of growth hormone replacement on cortisol metabolism and glucocorticoid sensitivity in hypopituitary adults. Clin Endocrinol 41:639–648

Westerbacka J, Yki-Järvinen H, Vehkavaara S, Häkkinen A, Andrew R, Wake D, Seckl J, Walker B 2003 Body fat distribution and cortisol metabolism in healthy men: enhanced 5-reductase and lower cortisol/cortisone metabolite ratios in men with fatty liver. J Clin Endocrinol Metab 88:4924–4931

Weyer C, Gudat U, Heise T, Kimmerle R, Santen R, Schlaghecke R, Spraul M, Berger M 1997 Integrity of the hypothalamo-pituitary-adrenal axis in morbid human obesity. Endocrinol Metab 4(6):397–401

White PC, Mune T, Agarwal AK 1997 11beta-Hydroxysteroid dehydrogenase and the syndrome of apparent mineralocorticoid excess. Endocr Rev 18:135–156

Whitworth JA, Stewart PM, Burt D, Atherden SM, Edwards CRW 1989 The kidney is the major site of cortisone production in man. Clin Endocrinol 31:255–361

Whorwood CB, Donovan SJ, Flanagan D, Phillips DIW, Byrne CD 2002 Increased glucocorticoid receptor expression in human skeletal muscle cells may contribute to the pathogenesis of the metabolic syndrome. Diabetes 51(4):1066–1075

Whorwood CB, Franklyn JA, Sheppard MC, Stewart PM 1991 Tissue localization of 11B-hydroxysteroid dehydrogenase and its relationship to the glucocorticoid receptor. J Steroid Biochem Molec Biol 41:21–28

Williams L, Napolitano A, Rajan V, Edwards CRW, Seckl JR, Chapman KE 1993 C/EBP — a putative transcriptional regulator of 11β-OHSD. J Endocrinol 139(suppl):P7

Williams LJS, Lyons V, MacLeod I, Rajan V, Darlington GJ, Poli V, Seckl JR, Chapman KE 2000 C/EBPα regulates hepatic transcription of 11β-hydroxysteroid dehydrogenase type 1; a

novel mechanisms for cross-talk between the C/EBP and glucocorticoid signalling pathways. J Biol Chem 275:30232–30239

Yamauchi T, Kamon J, Minokoshi Y, Ito Y, Waki H, Uchida S, Yamashita S, Noda M, Kita S, *et al.* 2002 Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. Nature Med 8:1288–1295

Yau JLW, Seckl JR 2001 11 beta-hydroxysteroid dehydrogenase type I in the brain; thickening the glucocorticoid soup. Mol Psychiat 6(6):611–614

Yau JLW, Noble J, Kenyon CJ, Hibberd C, Kotelevtsev Y, Mullins JJ, Seckl JR 2001 Lack of tissue glucocorticoid reactivation in 11β-hydroxysteroid dehydrogenase type 1 knockout mice ameliorates age-related learning impairments. Proc Natl Acad Sci USA 98:4716–4721