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The metabolic syndrome phenotypically resembles Cushing's, but plasma cortisol levels are normal. Intracellular cortisol regeneration by 11ß-hydroxysteroid dehydrogenase type 1 in adipose tissue might explain this paradox, but is this a realistic therapeutic target?

Inhibition of 11ß-hydroxysteroid dehydrogenase type 1 as a promising therapeutic target

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Chronically elevated glucocorticoid levels cause obesity, diabetes, heart disease, mood disorders and memory impairments. 11ß-hydroxysteroid dehydrogenase type 1 (11ß-HSD1) catalyses intracellular regeneration of active glucocorticoids (cortisol, corticosterone) from inert 11-keto forms in liver, adipose and brain, amplifying local action. Obese humans and rodents show increased 11ß-HSD1 in adipose tissue. Transgenic mice overexpressing 11ß-HSD1 selectively in adipose tissue faithfully recapitulate metabolic syndrome. Conversely, 11ß-HSD1 knockout mice have a 'cardioprotective' phenotype, whose effects are also seen with 11ß-HSD1 inhibitors in rodents. However, any major metabolic effects of 11ß-HSD1 inhibition in humans are, as yet, unreported. 11ß-HSD1 null mice also resist congitive decline with ageing, and this is seen in humans with a prototypic inhibitor. Thus 11ß-HSD1 inhibition is an emerging pleiotropic therapeutic target.

The prevalence of obesity and its metabolic complications has been increasing rapidly over the past two decades [1]. Obesity is associated with an increased risk of type 2 diabetes, metabolic syndrome, cardiovascular disease, stroke and certain cancers. The World Health Organisation has estimated that worldwide 1.6 billion adults are overweight with at least 400 million of them clinically obese [2]. These gloomy raw statistics include increasing numbers of children and adolescents, foreshadowing a worsening trend in the future. Given that medical science has always risen to epidemic challenges, the paralleled increase in our understanding of metabolic pathways underlying obesity and its metabolic consequences is not surprising. Whether or not obesity in the absence of its complicating disorders increases mortality remains contentious [3]. What is clear is that obesity is major risk factor for several disorders that are themselves associated with high morbidity and mortality. These include type 2 diabetes, dyslipidaemia, hypertension and cardiovascular disease (which, together with visceral/abdominal obesity, comprise the metabolic syndrome), as well as several cancers, respiratory disorders, gallstones, osteoarthritis, depression and anxiety. Whilst prevention of these complications of obesity is a major impetus for research into prevention and treatment, another important if more contentious driving force of the extensive research in the field of obesity is the growing public expectation of a pharmaceutical antidote to our 'obesogenic environment'. Many novel druggable molecular targets have recently been identified. Several have entered drug development and even clinical practice,

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including rimonabant, a selective endocannabinoid (CB1) receptor antagonist, and exenatide, a glucagon-like peptide-1 (GLP-1) mimetic.

Growing evidence indicates the cooperation between metabolic and inflammatory pathways is disrupted in the pathogenesis of the metabolic complications of obesity [4]. Identification and targeting of central molecules involved in the integration of metabolic and immune/inflammatory responses appear to have good prospects for a successful therapeutic approach in the metabolic syndrome. The promising reports announcing the benefits of thiazolidinediones, statins and salicylates to control inflammatory processes and to improve metabolic parameters support this notion.

Glucocorticoids, obesity and metabolic disease

Glucocorticoids are well-known ubiquitous hormones playing a key role in modulating immune and inflammatory responses, regulating energy metabolism and cardiovascular homeostasis and the body's responses to stress. Opposing the action of insulin, glucocorticoids stimulate production of glucose, switching the homeostatic balance towards catabolism. Thus, glucocorticoids promote gluconeogenesis but inhibit beta-cell insulin secretion and peripheral glucose uptake [5,6]. They also increase protein breakdown and lipolysis with consequent fatty acid mobilisation. Patients with endogenous or exogenous glucocorticoid excess (Cushing's syndrome) develop visceral obesity, insulin resistance, type 2 diabetes, dyslipidemia, hypertension and increased cardiovascular mortality.

The striking similarity of phenotype between rare Cushing's and the common Metabolic Syndrome/idiopathic obesity spectrum has spurred the search for a common underlying mechanism. Plasma cortisol levels are, however not notably elevated in simple obesity or the Metabolic Syndrome, at least in the absence of marked complications. It has been hypothesised that tissue-specific differences in glucocorticoid metabolism and hence increased local cellular corticosteroid exposure may explain this apparent paradox. Since most of the features of Cushing's syndrome are reversible by removal of glucocorticoid excess, manipulations reducing cortisol action at a local cellular or tissue level might provide a novel therapeutic strategy for the Metabolic Syndrome.

Glucocorticoids (cortisol in humans and most mammals, corticosterone in rats and mice) are produced by the adrenal cortex and regulated by ACTH under the control of hypothalamic-pituitary-adrenal (HPA) axis. As little as 5% of cortisol circulates free in the plasma, with the majority bound with high-affinity corticosteroid-binding globulin, which may act as a transporter of steroid to target cells, as well as lower affinity proteins such as albumin. The production of glucocorticoids is contingent upon the pronounced circadian rhythm (high during the active phase, low during quiescence/sleep) and episodic stressful events that stimulate the HPA axis, and considerable variations of free plasma cortisol occur. The dynamic range encompasses very low nanomolar levels at the nadir to low micromolar concentrations during severe stress [7]. Cortisol is believed to diffuse across cell membranes and then binds to cytoplasmic glucocorticoid receptors (GR) and in some tissues mineralocorticoid receptors (MR), which then translocate to the nucleus. GR and MR are ligand-gated transcription factors [8] that regulate a plethora of genes directly or through interactions with other transcription factors [9,10].

Until recently, it was axiomatic that the major determinant of corticosteroid action was the level of free cortisol in the plasma and the densities of GR and MR in target tissues. However, it has recently become apparent that tissue-specific metabolism of glucocorticoids, notably by 11ß-hydroxysteroid dehydrogenases (11ß-HSDs), alters tissue glucocorticoid levels and hence receptor access. 11ß-HSD catalyses the interconversion of non-receptor binding and therefore inert 11-ketosteroids, cortisone and 11-dehydrocorticosterone (11-DHC), and their receptor-binding active 11-hydroxy forms, cortisol and corticosterone (Figure 1). Inactive cortisone circulates unbound at around 100 nm in humans, and therefore its concentration is greater than active cortisol, notably during the diurnal nadir. In rats, 11-DHC levels are also around 50–100 nM, though its levels in mice are lower.

11ß-hydroxysteroid dehydrogenases (11ß-HSDs)

Two isoforms of 11ß-HSD are known, the products of distinct genes [7]. 11ß-HSD2, a high-affinity NAD-dependent dehydrogenase, is expressed mainly in mineralocorticoid target tissues (kidney, colon, salivary glands) [11]. This distribution reflects its role in protecting intrinsically non-selective MR from activation by cortisol and corticosterone, and thereby enabling selective aldosterone binding [12]. Additionally, 11ß-HSD2 is highly expressed in the placenta and the developing fetus, providing a potent barrier to maternal glucocorticoids [13].

In contrast, 11ß-HSD1 is a lower-affinity NADP(H)-dependent enzyme, which though bi-directional in purified preparations and tissue homogenates, acts as a predominant 11-ketoreductase in intact cells and organs [14,15]. 11ß-HSD1 is expressed primarily in tissues with high sensitivity to glucocorticoids (liver, adipose tissue, brain, lung) [16–18]. 11ß-HSD1 is active as a dimer and exhibits cooperative kinetics with cortisone and 11-DHC as substrates [19]. Thus, 11ß-HSD1 dynamically adapts to nanomolar as well as micromolar concentrations of 11-keto steroids. Both isozymes contain an N-terminal membrane-insertion sequence, thus enabling anchoring in the endoplasmic reticulum (ER) [20]. The catalytic moiety of 11ß-HSD2 faces the cytoplasm, while 11ß-HSD1 is directed into the ER lumen [15]. This has significant implications for cofactor availability (NAD+/NADPH ratio) and potential bi-directionality of 11ß-HSD1. The co-localisation of 11ß-HSD1 in the lumenal surface of the ER membrane with hexose-6-phosphate dehydrogenase (H6PDH), which catalyses the first two steps of the pentose-phosphate pathway generating NADPH, provides a supply of co-substrate to drive the predominant oxoreductase direction of 11ß-HSD1 in intact cells [21,22]. H6PDH knockout mice are unable to convert 11-dehydrocorticosterone to corticosterone, but efficiency of the opposite 'dehydrogenase' reaction is unaffected [23].

11ß-HSD1 in obesity and the metabolic syndrome

To test the hypothesis that tissue-specific regulation of 11ß-HSD expression and activity contributes to obesity and its metabolic consequences, several animal studies have been performed. In leptin-resistant fatty Zucker rats, obesity associates with decreased 11ß-HSD1 activity and expression in the liver, but increased 11ß-HSD1 in the adipose tissue, notably in visceral fat [24]. Similar changes have been reported in leptin-deficient *ob*/*ob* mice [25]. It is noteworthy that basal 11ß-HSD1 levels are higher in peripheral

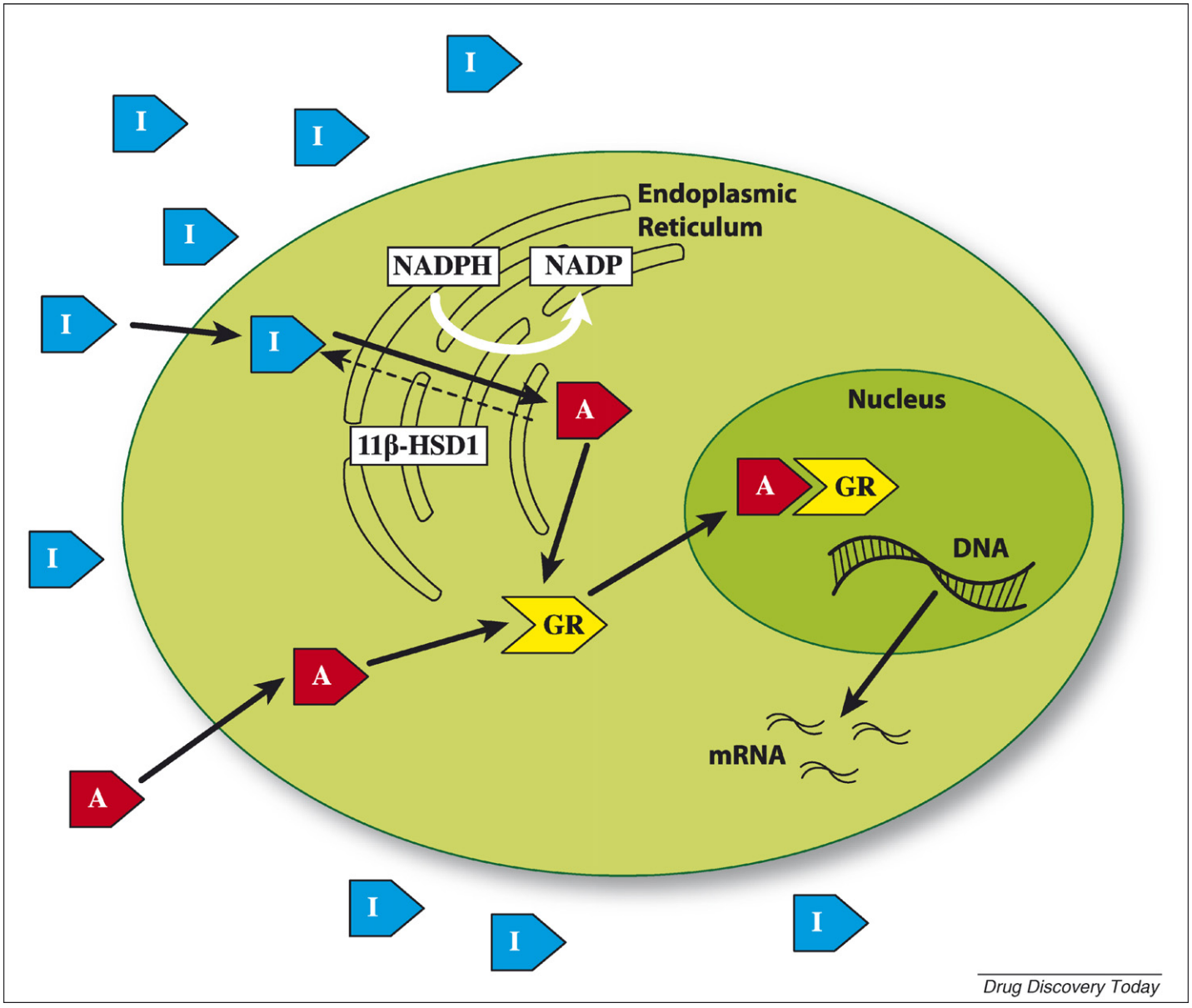

FIGURE 1

Schematic representation of the intracellular 11ß-HSD1-dependent regeneration of glucocorticoids. Glucocorticoids diffuse across cell membrane and bind to glucocorticoid receptors (GR; yellow) in the cytoplasm. When not activated by the ligand, GR are protected from the trafficking to the nucleus by the formation of chaperons. Once activated by the ligand, a complex is translocated rapidly into the nucleus where it binds to the promoter region of glucocorticoid-responsive genes and leads to an increase or repression of genes transcription. Since the production of glucocorticoids is contingent upon circadian rhythm, considerable variations of free plasma cortisol occur during diurnal changes. Inactive form of glucocorticoid (blue) circulates unbound, and therefore its concentration is usually greater than active form (red). 11ß-HSD1 (acting predominantly as a NADPH-dependent reductase *in vivo*) is located in the endoplasmic reticulum (ER). It interconverts active and inactive glucocorticoids, thus regulating glucocorticoid receptor activation intracellularly.

(subcutaneous) than in central (visceral/mesenteric) adipose tissue in such rodent models.

Initial studies of humans, which measured the ratio of cortisol to cortisone metabolites in urine as an indirect index of total body 11ß-HSD activity, produced inconsistent results, reporting increased [26,27], decreased [27,28] and unchanged [29] urinary '11ß-HSD index' values in obesity (Summary of representative human studies in Table 1). Such ratios, however, are inadequate as they may be influenced by other enzymes involved in the metabolism of cortisol (11ß-HSD2, 5α- and 5ß-reductases, 3α-HSDs, etc.). Additionally, opposing, tissue-specific changes in 11ß-HSD1 are difficult to dissect with such 'whole body' estimates. More recent studies have attempted various tissue-specific measures in humans to accommodate such concerns. Such work has generally, but not exclusively, suggested that obese humans, as monogenic obesity in rodents, show selective downregulation of 11ß-HSD1 in liver and upregulation in adipose tissue.

Downregulation of hepatic 11ß-HSD1 in obesity: a potential protective mechanism against diabetes

Hepatic 11ß-HSD1 activity (measured as the conversion of an oral dose of cortisone into cortisol in plasma after 'first pass' metabolism) has been consistently decreased in obesity [27,28,30]. This is not just driven by insulin resistance, since downregulation of

TABLE 1

Summary of the most representative human studies

Subjects	Changes in 11β-HSD1 expression/ activity in fat and liver	Correlation with obesity/metabolic syndrome	Method employed	Refs
16 patients undergoing abdominal surgery	↑ 11β-HSD1 activity in stromal cells from visceral, but not subcutaneous fat	Increased visceral 11β-HSD1 activity correlates with obesity	Visceral and sc fat biopsies, stromal cells culture	[16]
36 patients divided in three groups according to BMI	↓ 11β-HSD1 activity in obese subjects	11β-HSD1 inhibition correlates with central obesity	Urine cortisol metabolites	[28]
34 men with different BMI and insulin sensitivity	↑ 11β-HSD1 in sc fat and ↓ hepatic 11β-HSD1 in obesity	Increased adipose and decreased hepatic 11β-HSD1 correlate with obesity	Urine cortisol metabolites, sc fat biopsies, oral cortisone acetate test	[27]
12 lean and 18 obese patients	↑ 11β-HSD1 mRNA in sc fat in adipocytes and in visceral fat in both adipocytes and stroma from obese patients	Overexpression of 11β-HSD1 in fat correlates with metabolic disorders linked to obesity	*In situ* hybridisation	[37]
40 obese women	↑ 11β-HSD1 in fat and ↓ hepatic 11β-HSD1 in obesity ↑ cortisol clearance	Increased 11β-HSD1 in fat, decreased in liver and enhanced inactivation of cortisol by A ring reductases with compensatory activation of the HPA axis correlate with obesity	Urine cortisol metabolites, sc fat biopsies, oral cortisone acetate test	[30]
25 unmedicated lean men with glucose intolerance and 25 healthy men	Normal 11β-HSD1 activity in adipose tissue and ↓ hepatic 11β-HSD1 In subjects with impaired glucose intolerance	Enhanced *in vivo* peripheral tissue sensitivity to glucocortiocids, tissue-specific alterations in 11β-HSD1 activity and increased excretion of A-ring reduced metabolites of cortisol correlate with impaired glucose tolerance without obesity	Urine cortisol metabolites, Skin beclomethasone test	[160]
32 women undergoing elective abdominal surgery	No difference in adipose 11β-HSD1 mRNA between visceral and sc fat tissue, ↑ 11beta-HSD1 activity in visceral preadipocytes	No correlation of 11β-HSD1 mRNA with obesity. Enhanced preadipocyte proliferation within visceral adipose tissue contribute to increases in visceral fat in obese patients	Visceral and sc fat biopsies, preadipocytes culture	[38]
25 healthy men	↑ adipose 11β-HSD1 activity and increased urinary excretion of 5alpha- and 5beta-reduced cortisol metabolites in obesity. Fatty liver is associated with selective increase of 5beta-reduced cortisol metabolites	No correlation of visceral fat mass with changes in cortisol metabolism	Urine cortisol metabolites, sc fat biopsies	[73]
12 Caucasian 19 Pima Indian	↑Adipose 11β-HSD1 in obesity	Sc adipose 11β-HSD1 activity correlates with central adiposity and hyperinsulinemia. No difference in enzyme activity between Pima Indians and Caucasians	Sc fat biopsies	[35]
16 men and 16 women	↑ Adipose 11β-HSD1 mRNA and activity in obesity	Adipose 11β-HSD1 mRNA and activity correlate with obesity. Leptin but not angiotensinogen and GR were correlated with 11β-HSD1 and obesity	Sc fat biopsies	[36]
33 men with diabetes type 2 38 healthy subjects	No change in 11β-HSD1 activity in diabetic subjects	Impaired 11β-HSD1 activity correlates with obesity in healthy subjects but not in diabetics. Failure to downregulate11β-HSD1 activity with diabetes may potentate dyslipidemia, insulin resistance, and obesity	Urine cortisol metabolites	[31]
17 monozygotic twins	↑ Adipose 11β-HSD1 mRNA and protein in obesity	Increased 11β-HSD1 mRNA in sc fat correlates with obesity and insulin resistance	Sc fat biopsies	[34]
12 obese subjects on ten-week very low fat diet	↑ Adipose 11β-HSD1 activity and mRNA in isolated adipocytes	Weight loss increases 11β-HSD1 mRNA in adipose tissue. Decreased 11β-HSD1 activity and expression in obesity may act as a compensatory mechanism to enhance insulin sensitivity	Urine cortisol metabolites, sc fat biopsies, preadipocyte culture	[90]
11 healthy subjects		Splanchnic 11β-HSD1-dependent re-generation of cortisol contributes significantly to the whole body cortisol production. Thus, alterations in splanchnic cortisol production may contribute to visceral obesity	Hepatic venous and leg catheterisation and isotype infusion (*in vivo* splanchnic 11β-HSD1 activity)	[161]

TABLE 1 (*Continued*)

Subjects	Changes in 11β-HSD1 expression/ activity in fat and liver	Correlation with obesity/metabolic syndrome	Method employed	Refs
Six lean and six obese men	↑ ***In vivo*** 11β-HSD1 activity in sc fat and unchanged total splanchnic 11β-HSD1 activity in obese men	Increased 11β-HSD1 activity selectively in adipose tissue correlates with obesity. Obese men are less susceptible than lean men to the insulin-sensitizing effects of carbenoxolone	Isotype infusion (*in vivo* splanchnic 11β-HSD1 activity), microdialysis of sc fat	[41]
Nine healthy men	↑ 11β-HSD1 activity in fat after meal	Hyperinsulinaemia and increased FFA induce acute increases in 11β-HSD1 activity in adipose tissue	Isotype infusion (*in vivo* splanchnic 11β-HSD1 activity), microdialysis of sc fat	[91]
18 healthy subjects	↑ 11β-HSD1 activity following mixed meal	Increased total body cortisol production after an ingestion of a mixed meal does not originate from alter splanchnic cortisol production	Isotype infusion (*in vivo* splanchnic 11β-HSD1 activity)	[92]
70 postmenopausal women	↑ 11β-HSD1 and ↓11β-HSD2 mRNA in sc fat in obese women	Increased 11β-HSD1 mRNA positively correlates with the waist circumference and is associated with decreased insulin sensitivity. No effect of weight loss on the expression of 11β-HSD1 and 11β-HSD2	Sc fat biopsies	[162]

The table presents an overview of the most significant human studies testing the hypothesis that suggests that changes in 11ß-HSD1 expression/activity contribute to obesity and its metabolic complications. It highlights the relationship between the method employed in the study and the corresponding outcomes.

hepatic 11ß-HSD1 activity is not seen in lean subjects with type 2 diabetes [31]. The absence of hepatic 11ß-HSD1 downregulation in obese diabetics emphasises its possible role in pathogenesis. This raises the intriguing hypothesis that 11ß-HSD1 inhibition in obese people who develop impaired glucose tolerance may protect from progression to the type 2 diabetes. In obese rats, the downregulation of hepatic 11ß-HSD1 activity apparently requires elevated circulating glucocorticoid levels and/or weight gain, but not insulin resistance alone, since neither thiazolidinediones nor metformin reversed the finding in Zucker rats [32]. The fundamental mechanism involved thus remains obscure.

Increased glucocorticoid regeneration in adipose tissue: a possible cause of metabolic syndrome

Increased fat mass in obese subjects is suggested to be associated with increased pre-adipocyte proliferation, differentiation and accumulation of lipid droplets, yet the underpinning mechanism remains unclear. Hence 11ß-HSD1 is highly expressed in fat and its mRNA and activity increases significantly with differentiation of pre-adipocytes to mature adipocytes [33], a new concept of its role as an autocrine regulator of fat mass emerged. Most human studies show increased 11ß-HSD1 in subcutaneous fat tissue in obesity [27,30,34–37] (Table 1). Tomlinson *et al.* [38], however found no correlation of 11ß-HSD1 in human fat tissue with obesity and hypothesised that this may have important implications for the enhancement of pre-adipocytes proliferation. It cannot be excluded, however, that even if there is no increase in the enzyme activity per gram of visceral adipose tissue, an increase in the volume of abdominal fat in obese subjects may account for the high cortisol levels delivered by the portal vein to the liver.

It has been suggested that visceral fat is more sensitive to glucocorticoids (higher GR and 11ß-HSD1 expression) and therefore it was hypothesised that a high ratio of cortisol/cortisone reactivation in visceral adipose tissue may be responsible for the 'Cushing's disease of the omentum' [16]. Introduction of a novel method by Andrew *et al.* [39] using deuterated cortisol tracer infusion enabled measurement *in vivo* of 11ß-HSD1 activity in the human splanchnic bed [40] and, separately, in liver to allow an estimate of visceral adipose tissue activity [41]. Such work showed that the splanchnic bed produces approximately one quarter of the amount of cortisol produced by the adrenal cortex [40]. Furthermore, one third of the splanchnic contribution to cortisol production appears to be from liver, with the rest assumed to be largely from visceral fat and other mesenteric sources, at least in healthy subjects [42]. Using this technique, Sandeep *et al.* [41] compared 11ß-HSD1 activity in lean and obese subjects. Obese men had no difference in whole-body regeneration cortisol from cortisone, but exhibited greater conversion of [^{3}H]cortisone to [^{3}H]cortisol in abdominal subcutaneous adipose tissue, as measured directly by microdialysis, suggesting again the downregulation of 11ß-HSD1 in liver in obesity. Thus, in obesity, whole-body 11ß-HSD1 activity is not reliably altered because increased activity of cortisol regeneration in adipose tissue is balanced by the parallel decrease of hepatic activity. The data support the notion that 11ß-HSD1 regeneration of glucocorticoids in visceral fat contributes substantially to the concentration of cortisol in the portal vein and therefore is an important determinant of the development of insulin resistance associated with abdominal obesity.

The role of 11ß-HSD1 in the pancreas and muscle

11ß-HSD1 is expressed in islets of Langerhans isolated from *ob/ob* mice and also from human pancreas [43]. 11ß-HSD1 is increased in islets of diabetic but not pre-diabetic Zucker rats [44]. Higher levels of 11ß-HSD1 mRNA and enzyme activity have been correlated with the appearance of diabetes and are increased further with disease progression [44]. Moreover, in Zucker rats, troglitazone-induced improvement in metabolic abnormalities correlated with a 40% decline in 11ß-HSD1 mRNA in the islets [44]. Incubation of islets with 11-dehydrocorticosterone resulted in dose-dependent inhibition of insulin secretion; the effect was reversed by carbenoxolone [43]. Selective inhibition of 11ß-HSD1, or GR antagonist treatment, in *ob/ob* mice attenuated 11-dehydrocorticosterone-induced

enhancement of 11ß-HSD1 activity [45]. Any fundamental mechanistic importance of these interesting observations remains unclear.

Alongside liver and fat, skeletal muscles are a major target for insulin-mediated glucose uptake. In obese patients presenting with features of metabolic syndrome, it has been demonstrated that decreased levels of non-oxidative glucose disposal are determined by impaired insulin action predominantly in skeletal muscle [46]. In human skeletal myoblasts, 11ß-HSD1 correlates with insulin sensitivity and blood pressure [47]. Little else is known about the importance of the low levels of 11ß-HSD1 in skeletal muscle. In contrast, 11ß-HSD1 is more clearly expressed in vascular smooth muscle [48]. 11ß-HSD2 is also present in the endothelium, so 11ß-HSD1 effects are likely to be very cell-specific. Whilst 11ß-HSD1 has no effect on vasoconstrictor/dilator function in healthy vessels [49], a role in angiogenesis, typically inhibited by glucocorticoids, has recently been reported [50]. 11ß-HSD1 maintains an anti-angiogenic tone *in vivo*. Perhaps in consequence, 11ß-HSD1−/− mice have markedly improved myocardial function (ejection fraction) 1 week after experimental infarction despite identical infarct size. Selective 11ß-HSD1 inhibition also reduces atheroma formation [51], an effect that may include effects in vascular smooth muscle.

Polymorphisms in the gene encoding 11ß-HSD1

HSD11B1 is located on chromosome 1 in humans and mice, whereas *HSD11B2* is located on chromosome 16 in humans and chromosome 8 in mice. *HSD11B1* spans 30 kb, mostly due to a large intron 4 [52]. Several polymorphisms in the *HSD11B1* locus have been described to date (31 SNPs are within intron 4, one in the 3′-untranslated region, three in 5′-regions and four in 3′-gene regions [53]). Detailed descriptions can be found in the recent review by Tomlinson *et al*. [53]. Attempts to link polymorphisms of *HSD11B1* with obesity have been negative in some populations [52,54], but associations are reported in others [55,56] and in porcine obesity [57]. Specific polymorphisms of *HSD11B1* associate with insulin sensitivity/diabetes [58], with hypertension [59], with PCOS [60] and with apolipoprotein levels [56]. Thus *HSD11B1* is a reasonable candidate gene for obesity/metabolic syndrome spectrum disorders, but any major causal role of genetic variation remains unproven. Importantly, studies in identical twins support environmental causes for the association between increased adipose 11ß-HSD1 expression and obesity [34].

There are 11 reported cases of deficiency of 11ß-HSD1, at least as reported under the term 'cortisone reductase deficiency' (CRD) [61]. Most of the cases affect women. The clinical presentation resembles polycystic ovarian syndrome (PCOS) with acne, hirsutism, oligo-amenorrhoea and infertility. Obesity has been reported for a few cases [62]. The phenotype is thought to be due to ACTH-mediated 'adrenal androgen' excess, secondary to the failure to regenerate cortisol, which results in feedback activation of the HPA axis. Although no gross deletions or re-arrangements in *HSD11B1* have been found in most CRD cases [63–65], two polymorphisms in complete linkage disequilibrium within intron 3 of *HSD11B1* were described in three cases associated with exon 5 polymorphisms of *H6PD* gene (encoding H6PDH) [66]. Thus, a concept emerged that CRD is caused by a combination of mutations in *HSD11B1* and *H6PD*. White [67], however found the co-occurrence of these polymorphisms in normal subjects. San Millan *et al*. also showed that the triallelic genotypes *HSD11B1* 83,557insA and *H6PD* R453Q found in CRD do not always cause CRD but that in their group of patients with PCOS, the *H6PD* gene variant was associated with increased cortisol and 17-hydroxyprogesterone levels [68]. Gambineri *et al*. reported that *HSD11B1* 83,557insA contributed to increased cortisol clearance and compensatory adrenal hyperandrogenism in lean women with PCOS but might potentially play a protective role against obesity and dyslipidemia [60].

Does elevated adipose 11ß-HSD1 cause metabolic disorders?

Transgenic overexpression of 11ß-HSD1 models the metabolic syndrome

To dissect the pathogenic implications of elevated adipose 11ß-HSD1 in obesity, transgenic mice with 2–3-fold overexpression of 11ß-HSD1 in fat were generated, exploiting the adipocyte fatty acid binding protein (aP2) promoter [69] (Table 2). These aP2-HSD1 transgenic mice have elevated corticosterone levels in adipose tissue but unaltered plasma concentrations. The mice develop many features of the metabolic syndrome: glucose intolerance and insulin resistance (exacerbated further by high-fat feeding), dyslipidemia, apparent leptin resistance [69] and hypertension associated with renin-angiotensin-aldosterone system activation [70]. Adipokines associated with insulin resistance (resistin, TNFα, leptin) are elevated and insulin-sensitising adiponectin is reduced. aP2-HSD1 mice are hyperphagic and obese, predominantly in the visceral fat depot. Expression of the GRα receptor was higher in visceral compared to subcutaneous fat, while the expression of the transgene *HSD11B1* was similar in all fat depots [69]. The greater effects in visceral adipose may reflect the higher GR and/or higher lipoprotein lipase (LPL) in mesenteric fat depot.

aP2-HSD1 transgenic mice have elevated corticosterone and free fatty acids levels in the hepatic portal vein that drains blood from visceral fat to the liver. To examine the impact of elevated liver, glucocorticoids mice overexpressing 11ß-HSD1 selectively in the liver under the control of the ApoE promoter have been generated (Table 2). ApoE-HSD1 transgenic mice develop mild insulin resistance, fatty liver and dyslipidemia without impairment of glucose tolerance, obesity or changes in adipose distribution [71]. Interestingly, ApoE-HSD1 transgenic mice have higher hepatic expression of LXR and PPARα suggesting increased lipid synthesis as well as clearance, and CYP7a, indicating enhanced bile acid synthesis. The mice are also hypertensive, perhaps driven by increased angiotensinogen synthesis in the liver. ApoE-HSD1 mice may model the attenuated metabolic syndrome without obesity, and indeed increased 11ß-HSD1 activity in liver is seen in patients with the insulin resistance of myotonic dystrophy [72]. In idiopathic steatohepatitis, liver 11ß-HSD1 levels are not downregulated [73], but any role of the enzyme in this prevalent problem are speculative.

Is 11ß-HSD1 a therapeutic target? 11ß-HSD1$^{-/-}$ mice resist the metabolic syndrome

Elevated 11ß-HSD1 levels in adipose tissue in human obesity and the phentoype of two murine models overexpressing 11ß-HSD1 in

TABLE 2

Transgenic mice models with genetic manipulation of 11ß-HSDs

Transgenic mouse model	Effect on enzyme activity	Phenotype	Refs
aP2-HSD1 transgenic	↑11β-HSD1 in adipose tissue	Visceral obesity Insulin resistance Type 2 diabetes Hyperphagia Hypertension Dyslipidemia	[69,70]
apoE-HSD1 transgenic	↑11β-HSD1 in liver	Modest insulin resistance Fatty liver Dyslipidemia Hypertension	[71]
11β-HSD1 knockout	No 11β-HSD1 activity	Resistance to diet-induced obesity Peripheral fat redistribution Hyperphagia Absence of hyperglycemia upon starvation and stress challenges ↑ Insulin sensitivity HPA axis hyperactivity to stress Adrenal hyperplasia ↑ HDL, ↓ LDL, ↓ TG ↓ Fibrinogen Protection from age-related cognitive impairment	[75–78,138]
aP2-HSD2 transgenic	↑11β-HSD2 predominantly in subcutaneous adipose tissue	Resistance to diet-induced obesity Hypophagia Increased energy expenditure ↑ Insulin sensitivity	[79]

To identify the tissue-specific role of 11ß-HSD1, several transgenic mice models were generated. Overexpression of 11ß-HSD1 selectively in adipose tissue was associated with manifestation of phenotypic features resembling full metabolic syndrome. In contrast, 11ß-HSD1−/− mice or mice with overexpression of 11ß-HSD2 in adipose tissue presented 'cardioprotective' phenotype.

adipose tissue and liver provided evidence that inhibition of glucocorticoid regeneration, especially in fat, might be a therapeutic target for metabolic syndrome. Since carbenoxolone does not inhibit 11ß-HSD1 in adipose tissue *in vivo* [41,74], 11ß-HSD1−/− mice have offered a unique possibility to model the potential effects of this possible therapeutic strategy. 11ß-HSD1−/− mice are viable and healthy [75] but are unable to convert inert 11-dehydrocorticosterone to corticosterone. To compensate for their decreased production of active glucocorticoids, 11ß-HSD1−/− mice have adrenal hyperplasia and hyper-responsiveness to exogenous ACTH *in vitro* and *in vivo* [76]. 11ß-HSD1−/− mice have improved glucose tolerance, plausibly due to decreased activation of key enzymes involved in gluconeogenesis in the liver (PEPCK and glucose-6-phosphatase), but do not show hypoglycemia when fasted [75]. 11ß-HSD1−/− mice have reduced triglyceride and NEFA levels, lower hepatic fibrinogen synthesis (reduced hypercoagulability) and raised HDL cholesterol (increased apolipoprotein AI, reduced apolipoprotein CIII), factors associated with a cardioprotective phenotype. These changes are driven by increased lipid beta-oxidation (mCPT-I, UCP-2, ACO) and increased PPARα [77].

Moreover, 11ß-HSD1−/− mice are insulin-sensitised, notably in adipocytes *in vitro* [78] and adipose tissue *ex vivo* (decreased resistin and TNFα, but increased adiponectin and PPARγ) [77]. Interestingly, on a high-cholesterol diet, wild-type mice showed a switch in lipoprotein profile from HDL to LDL, whereas 11ß-HSD1−/− mice have lower plasma cholesterol level and a higher HDL to total cholesterol ratio. On the obesity- prone C57Bl/6J genetic background, high-fat diet-fed 11ß-HSD1−/− mice gain significantly less weight than controls, despite relative hyperphagia. With high-fat diet 11ß-HSD1−/− mice preferentially gain weight in peripheral, rather than in visceral fat depot. Increased expression of PPARγ and UCP-2 in 11ß-HSD1−/− visceral adipose tissue could explain those changes in accumulation of fat [77,78].

To determine whether loss of glucocorticoids specifically in the adipose tissue might protect from the development of metabolic syndrome, mice ectopically overexpressing 11ß-HSD2, the reverse 11ß-dehydrogenase enzyme that potently inactivates corticosterone, under the control of the murine aP2 promotor (ap2-HSD2) have been generated [79] (Table 2). Surprisingly, expression and activity of the transgene was higher in subcutaneous than other fat depots. On high-fat diet, aP2-HSD2 mice show decreased food intake and increased energy expenditure [79]. They resist weight gain and have improved glucose tolerance and insulin sensitivity [79]. On high-fat diet these mice exhibit increased expression of PPARγ, UCP-2, PEPCK and adiponectin, and reduced expression of leptin, TNFα and resistin in adipose tissues [79]. Thus, apart from food intake (see below), aP2-11ß-HSD2 mice are phenotypically similar to 11ß-HSD1−/− mice emphasizing the importance of adipose tissue as a target for enzyme inhibition.

Although 11ß-HSD1−/− mice resist the metabolic consequences of a high-fat diet, they show hyperphagia, suggesting that 11ß-HSD1 plays a role in the central control of food intake and that this predominates over fat-derived signals [80]. Normal C57Bl/6 mice fed a high-fat diet show a rapid increase in 11ß-HSD1 and downregulation of agouti-related peptide (AgRP) specifically in the arcuate nucleus but show no increase in food intake to this palatable diet. In the absence of 11ß-HSD1 (knockout mice), high-

fat diet paradoxically upregulates the orexigenic AgRP mRNA associated with hyperphagia. Thus, it has been proposed that induction of 11ß-HSD1 in the arcuate serves to constrain intake of palatable energy dense foods by reducing AgRP expression with which the enzyme is co-localised. Pharmacological glucocorticoid manipulations, however increase AgRP suggesting an indirect effect. This might be an opioid pathway since treatment of high-fat-fed mice with the opioid antagonist naloxone induces a rise in arcuate AgRP and blocks the rise in 11ß-HSD1 [80]. The precise anatomy of the circuitry involved remains undetermined.

How is 11ß-HSD1 regulated?

Expression of 11ß-HSD1 is regulated by many factors including glucocorticoids, insulin, growth hormone, thyroid and sex hormones, cytokines, PPARα, PPARγ and perhaps other nuclear receptors (Figure 2). *In vivo*, in rat liver and hippocampus, glucocorticoids have been reported to regulate temporally 11ß-HSD1

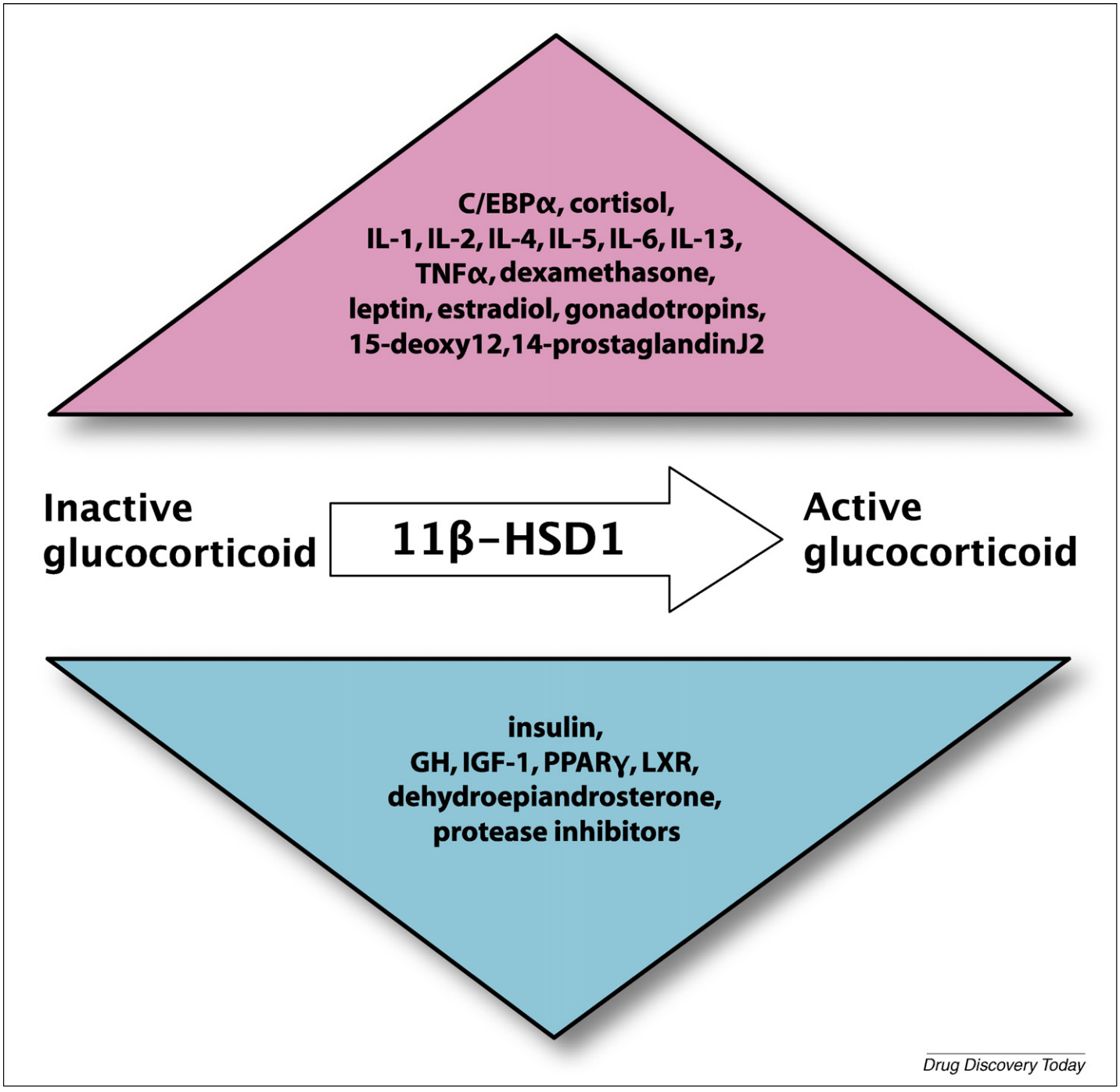

FIGURE 2

Schematic overview of the regulators of 11ß-HSD1 activity. The figure represents an overview of studies that analysed influence of various factors on the upregulation (pink triangle) and downregulation (blue triangle) of 11ß-HSD1 activity. Note: the effect may be tissue- and species-specific.

expression and activity [14]. In human skeletal muscle, 11ß-HSD1 expression is upregulated by physiological concentrations of cortisol in a dose-dependent manner [47]. 11ß-HSD1 expression and activity are increased with differentiation from pre-adipocytes to mature adipocytes and with differentiation to the adipocyte phenotype in 3T3 L1 cells [33]. Pro-inflammatory cytokines such as IL-1ß and TNFα have been reported to upregulate 11ß-HSD1 in adipocytes [81] and aortic smooth muscle cells [82]. Growth hormone (GH) inhibits 11ß-HSD1, in part through insulin-like growth factor-I (IGF-I) [83]. Patients with acromegaly treated with a growth hormone (GH) receptor antagonist show changes in cortisol metabolism [84]. Furthermore, it has been reported that ligands activating two nuclear receptors at the heart of lipid metabolism, LXR and PPARγ, downregulate 11ß-HSD1 expression and activity *in vitro* and *in vivo* in adipose cells [85,86]. Addition of cycloheximide revealed that ongoing protein synthesis is necessary for the effects of either LXR or PPARγ agonists on 11ß-HSD1 mRNA, indicating indirect molecular mechanism. In terms of more direct effects, CCAAT/enhancer binding protein alpha (C/EBPα) binds to several sides of 11ß-HSD1 promoter and positively regulates its activity in hepatocytes both *in vitro* and *in vivo* [87]. C/EBPβ also binds and weakly activates 11ß-HSD1 transcription, but its dominant effect may be to antagonise induction of the promoter by C/EBPα, at least in liver [87]. Finally, it should be pointed out that the regulation of 11ß-HSD1 transcription and activity is tissue-specific. Whilst some cells in the lung exploit a distinct promoter [88], this is not the case for adipose and most other tissues. The mechanisms underpinning the strikingly discordant changes in 11ß-HSD1 transcription in adipose, liver and other metabolic tissues remains unknown.

High-fat diet induced downregulation of 11ß-HSD1 activity

To gain an insight into the molecular mechanism that drives diet-induced obesity in mice, Morton *et al.* [89] studied the expression of 11ß-HSD1 in both obesity and metabolic disease-prone (C57Bl/6J) and resistant (A/J) strains. With chronic high-fat diet C57Bl/6J mice increased weight and became profoundly hyperinsulinemic and glucose intolerant. A/J mice in contrast resisted these metabolic changes. Intriguingly, high-fat diet decreases 11ß-HSD1 in all fat depots, whereas in liver the enzyme is unaffected. 11ß-HSD1 activity was downregulated with high-fat diet in all adipose depots in both strains. A/J mice, however have lower basal expression and greater downregulation of 11ß-HSD1 in adipose tissues. It was therefore hypothesised that downregulation of adipose tissue 11ß-HSD1 with a high-fat diet is a protective mechanism against metabolically detrimental consequences. These data suggest that individual susceptibility for the development of metabolic disease as a consequence of long-term high-fat diet exposure is related *inter alia* to the degree of downregulation of 11ß-HSD1 in adipose tissue; it perhaps relates to the genetics of murine strain differences. Given the largely positive correlation between adipose 11ß-HSD1 and obesity-induced insulin resistance in humans, it might be hypothesised that HF-mediated downregulation of adipose 11ß-HSD1 is a beneficial adaptation that is perhaps diminished in humans prone to metabolic disease. The molecular explanation for the downregulation of 11ß-HSD1 upon high-fat diet and its variation with strain is unknown and may relate to polymorphisms of *HSD11B1,* as mooted in humans [56,58,60,68]. Alternatively, differences in free fatty acids and lipid products could activate nuclear receptors including LXR and PPARs and consequently variably downregulate 11ß-HSD1.

Regulation of human 11ß-HSD1 activity by feeding

Although insulin and other hormones exert direct effects on 11ß-HSD1 in rodents, the regulatory mechanism in humans is poorly understood. Weight loss has been reported to increase 11ß-HSD1 mRNA in human fat [90]. Recently it has been hypothesised that in humans 11ß-HSD1 may be also involved in acute metabolic response to feeding [91,92]. Sandeep *et al.* [41] provided evidence that insulin induced a rapid downregulation of adipose 11ß-HSD1 in lean, but not in obese, patients. Postprandially, when insulin is secreted, cortisol availability in adipose tissue appears to be controlled independently from the alterations of the HPA axis [91]. Failure to reduce adipose tissue cortisol generation by insulin in obese subjects may be a decisive factor determining susceptibility to further obesity.

The role of 11ß-HSD1 in resolution of inflammation

The anti-inflammatory properties of glucocorticoids are well known, and these steroids are commonly used in clinical treatment of inflammatory disorders. Notwithstanding this, it has been suggested that although glucocorticoids have immunosuppressive effects in pharmacological doses, at physiological levels they provide a more immunomodulatory influence [6]. Chronic inflammation in such conditions as rheumatoid arthritis and asthma is believed to result from disturbance of the equilibrium between the type-1 (pro-inflammatory) and type 2 (anti-inflammatory) immune responses [93]. It has recently been proposed that 11ß-HSD1 may be involved in the resolution of inflammation [94]. Reduced 11ß-HSD1 has been reported in the synovium in rheumatoid arthritis, suggesting deficient glucocorticoid regeneration may be implicated in pathogenesis [95]. 11ß-HSD1 but not 11ß-HSD2 is expressed in differentiated macrophages, denritic cells, T and B cells and acts as a reductase [96–98]. Moreover, macrophages deficient in 11ß-HSD1 are less able to ingest and therefore remove apoptotic neutrophils *in vitro* and *in vivo*, suggesting that deficiency of the enzyme may indeed contribute to the persistence of inflammation, at least in the peritoneum [99]. Cytokines upregulate 11ß-HSD1 activity in many key cells involved in the inflammatory response including glomerular mesangial cells [100,101], macrophages [98,99], T, B and dendritic cells [96,97], human fibroblasts derived from synovium, bone marrow and skin [102] and adipocytes [81]. Perhaps this engenders a local feedback mechanism where pro-inflammatory stimuli enhance re-generation of active glucocorticoids and therefore minimise any tendency to prolong inflammation.

Regulation of 11ß-HSD1-mediated re-generation of active glucocorticoids in immune-competent cells also appears to act as an autocrine mechanism regulating function. Dendritic cells stimulated by innate immunity signals increase cortisone reductase activity [97], whereas when activated by adaptive immune response (CD40 ligation) their capacity to generate active cortisol decreases. Intracellular reactivation of glucocorticoids by 11ß-HSD1 has been also demonstrated to provide CD4+, CD8+ and B220+ lympocytes with an intracrine mechanism controlling

their viability [96]. Thus, it has been suggested that 11ß-HSD1-dependent control of glucocortiocoid bio-availability inside immune cells provides a checkpoint attenuating unwanted immune activity. Thus, modulation of 11ß-HSD1 activity selectively in immune-inflammatory cells may present as a new therapeutic strategy for chronic inflammatory disease.

Inhibition of 11ß-HSD1 as a therapeutic target

It has been hypothesised that decreasing glucocorticoid activity in adipose tissue and liver might protect against the detrimental metabolic consequences of obesity. Two principal therapeutic strategies to diminish the exaggerated activation of a receptor may be identified: antagonism of the receptor and/or its signalling pathway or reducing ligand availability, either systemically or locally. Administration of GR antagonist RU38486 in Cushing's syndrome patients and in *db/db* mice decreases plasma glucose levels [103,104]. This approach is, however, limited by difficulties of achieving any tissue specificity and the adverse consequences of general glucocorticoid deficiency (e.g. Addison's disease). Moreover, long-term therapy with GR antagonists leads to activation of the HPA axis and reversal of the competitive blockade, as well as adrenal hyperplasia [105]. Thus, targeting pre-receptor glucocorticoid activation by 11ß-HSD1 has some attractions if any compensatory HPA axis activation is incomplete and adrenal stress responses are maintained.

Natural 11ß-HSD1 inhibitors

Derivatives of the licorice root (*Glycyrrhiza glabra*), including glycyrrhetinic acid and its synthetic hemisuccinyl ester, carbenoxolone, are potent (IC_{50} nM *in vitro*) but non-specific 11ß-HSD inhibitors [106]. A variety of endogenous steroids and their metabolites [107], as well as bile acids such as chenodeoxycholic acid [108], have 11β-HSD inhibitory properties. Various environmental chemicals and food ingredients also interfere with glucocorticoid metabolism by 11ß-HSDs [109], notably flavanone, 2′-hydroxyflavanone-extracts from fruits and vegetables and coffee extracts [110]. 11ß-HSD1 has also recently been reported to catalyse interconversion of additional substrates including 7-oxy-dehydroepiandrosterone metabolites and 7-oxysterols [111,112]. Thus, 11ß-HSD1-dependent glucocorticoid conversion may perhaps be attenuated by competition for these alternative substrates.

Studies with 'natural' 11ß-HSD inhibitors

Studies using the prototypic drug, carbenoxolone, showed hepatic insulin sensitisation in lean healthy subjects [113] and patients with type 2 diabetes [114] but not obese men [41] (Table 3). The insulin sensitisation appears due to reduced hepatic glucose production and glycogenolysis, rather than any effect on peripheral glucose uptake, perhaps because carbenoxolone fails to inhibit 11ß-HSD1 in adipose tissue in rats or humans [41,74]. This raises the notion that a successful inhibitor for the treatment of obese patients with diabetes type 2/metabolic syndrome should be effective in adipose tissue. Interestingly, a recent study on healthy women with normal BMI reported significant changes in circumference and thickness of the superficial thigh fat layer after one month treatment with topical glycyrrhetinic acid [115]. Such non-selective licorice-based compounds also potently inhibit 11ß-HSD2 causing renal sodium retention, hypertension and hypokalaemia [48,116,117]. They also have effects on other short-chain dehydrogenases, such as 15-prostaglandin dehydrogenase [117] and on gap junctions [118], though these effects occur at higher concentrations than 11ß-HSD inhibition.

Novel compounds selectively inhibiting 11ß-HSD

Biovitrum first reported selective 11ß-HSD1 inhibitors (Table 3). Their arylsulphonamidothiazole compounds efficiently inhibit

TABLE 3

Prototypic 11ß-HSD1 inhibitors

Inhibitor	Selectivity	Animal studies	Human studies	Carbohydrate metabolism	Lipid metabolism	CNS effect	Proposed application	Disadvantages
Carbenoxolone	Non-selective	[74]	[41,113,114, 139,145,163]	↑ Insulin sensitivity ↓ Hepatic gluconeogenesis	↓ Cholesterol ↓ Lipolysis	Protection from age-related cognitive impairment.	• Obesity • Type 2 diabetes • Cognitive impairment • Glaucoma	• No penetration to fat tissue • Requires addition of amiloride
Compound 544 Merck	Selective	[51]	No	↑ Insulin sensitivity ↓ Fasting glucose ↑Glucose tolerance test ↓ Food intake	↓ Triglyceride ↓ Cholesterol ↓ Free fatty acids ↓ Adipose tissue mass	Not tested	• Atherosclerosis • Type 2 diabetes • Obesity	• No human studies
BVT.2733 Biovitrum	Selective	[119–121, 164]	No	↑ Insulin sensitivity ↓ Hepatic gluconeogenesis ↓ Circulating glucose ↓ Circulating insulin	↓ Triglyceride ↓ Cholesterol ↓ Free fatty acids	Not tested	• Type 2 diabetes	• No human studies • No changes in body weight • Only small decrease of food intake

The table summarises results of the early pharmaceutical programs targeting 11ß-HSD1. It presents a short description of the efficacy of non-selective and prototypic selective 11ß-HSD1 inhibitors with the particular focus on their effects on the carbohydrate and lipid metabolism.

11ß-HSD1 both *in vitro* and *in vivo* and showed impressive isoform-specificy (>200-fold selectivity over human and murine 11ß-HSD2) [119]. BVT.2733 has an IC_{50} of 96 nM for mouse 11ß-HSD1 [120]. *In vivo* BVT.2733 lowers plasma glucose and insulin in various hyperglycemic mice (ob/ob, db/db and KKAy) reduces hepatic expression of PEPCK and G-6-P mRNAs and therefore decreases hepatic glucose production [121]. In accordance to observations in humans, inhibition of 11ß-HSD1 achieves beneficial glucose lowering only in type 2 diabetes mice models but does not alter plasma glucose levels in controls [121]. Moreover, BVT.2733 decreased cholesterol, free fatty acids and triglyceride levels, replicating the phenotype of 11ß-HSD1−/− mice.

Merck's compound 544 (Table 3) also shows selective inhibition of 11ß-HSD1 activity [51]. The drug lowers body weight and appetite in diet-induced obesity in mice. In parallel to previous findings, compound 544 also lowers fasting glucose, improves insulin resistance, glucose tolerance and serum lipids in a mouse model of type 2 diabetes. Intriguingly, it has a pronounced atheroprotective effect in vulnerable $ApoE^{-/-}$ mice [51]. Furthermore, RU 486 showed very similar effects to the 11ß-HSD1 inhibitor, suggesting that reducing the intracellular glucocorticoid signal accounted for the efficacy of compound 544.

11ß-HSD1 inhibition and atherosclerosis

Treatment of ApoE−/− mice, which are susceptible to 'western' diet induced atheroma, with 11ß-HSD1 inhibitors protects against the development of atherosclerosis [51]. Since a decrease in atherosclerosis is more pronounced than might be expected by the serum lipid-lowering effect, it raises the possibility that the additional factors may play a role. The inflammatory component of atherogenesis involves monocyte accumulation, differentiation into macrophages and foam cell formation. As atherosclerotic lesions advance, macrophages increasingly take up oxidised lipoproteins and vascular wall cells secrete inflammatory cytokines, growth factors and adhesion molecules that together contribute to the formation of advanced lesions. 11ß-HSD1 is expressed in both vascular smooth muscle cells, perhaps endothelial cells [82,122], differentiated macrophages [98,99], and is upregulated by inflammatory cytokines providing a local mechanism to control inflammation [82]. However, by analogy with the peritoneum, deficiency of 11ß-HSD1 should exacerbate inflammation, potentially worsening disease. Alternatively, 11ß-HSD1 in inflammatory cells may impact upon their infiltration, proliferation and differentiation, which may be enhanced by glucocorticoids in the physiological range [123]. In this case, inhibition of the enzyme might be advantageous in the developing atherosclerotic lesion.

11ß-HSD1 also catalyses the conversion of highly atherogenic 7-ketocholesterol to more soluble 7ß-hydroxycholesterol [112]. 7-oxygenated forms of cholesterol are present in the plasma [124–126], with much higher concentrations in foam cells and atherosclerotic plaques [127]. 11ß-HSD1 plays an apparent role in the clearance of 7-ketocholesterol [112]. Whether or not loss of this reaction contributes to the beneficial effects of 11ß-HSD1 inhibition in atherosclerotic plaque formation remains unclear.

Another important possible indication for 11ß-HSD1 inhibitors is associated with the ability of glucocorticoids to enhance angiogenesis [50]. *In vitro* and *in vivo* studies demonstrated that 11ß-HSD1, by locally amplifying glucocorticoid action in the vessel wall, tonically represses angiogenic responses. Moreover, seven days after coronary artery ligation, 11ß-HSD1−/− mice show increased vascularisation in the infarcted myocardium, associated with partial protection against myocardial dysfunction [50]. Effects of 11ß-HSD1 inhibition on pathological neovascularisation, for instance, in the diabetic eye and in cancers and metastasis remain to be determined, but are potential toxicities.

A viable drug target for cognitive impairment?

Glucocorticoids play myriad important functions in the central nervous system. They are involved in neurotransmission, cellular metabolism, neuronal division and survival [128–130]. Chronic elevation of glucocorticoids, as in Cushing's syndrome, is associated with affective, cognitive and even psychotic disorders [129]. Accumulating evidence suggest that cognitive impairments with aging associate with elevated glucocorticoid levels in rodents and humans [131]. Indeed, maintenance of low glucocorticoid level throughout life, either *via* neonatal 'programming' of tighter HPA axis control or by adrenalectomy with low-dose glucocorticoid replacement in mid-life, prevent the emergence of cognitive deficits with age [132,133].

11ß-HSD1 is widely expressed in brain [134], notably in hippocampus, cerebellum and neocortex, suggesting its potential involvement in such processes as memory and learning. The enzyme is also expressed, albeit at lower levels, in the hypothalamus and anterior pituitary, indicating a role in neuroendocrine control [135]. 11ß-HSD1 is maintained in primary cultures of hippocampal cells, where it is a reductase and inhibited by carbenoxolone. The enzyme acts *in vivo* to amplify the known neuro-endangering actions of glucocorticoids, potentiating excitatory aminoacid-induced neurotoxity [136,137]. This appears to be important *in vivo*, as aged $11ß\text{-}HSD1^{-/-}$ mice resist the usual cognitive impairments seen in aged wild-type mice [138]. *In situ* hybridisation studies in post-mortem human brain confirmed the expression of 11ß-HSD1 but not 11ß-HSD2 in hippocampus, prefrontal cortex and cerebellum [139]. In two small, randomised, double-blind, placebo-controlled, crossover studies, carbenoxolone improved verbal fluency in healthy elderly men and verbal memory in patients with type 2 diabetes [139]. Cognitive improvements with 11ß-HSD inhibition have also been reported in rodents [140].

Finally, a potential link between cognition and metabolism should be highlighted. Since 11ß-HSD1−/− mice are insulin sensitised and have an atheroprotective lipid profile, it might be anticipated that the neuroprotective effect of the enzyme inhibitors could be secondary to metabolic and vascular effects. Chronic hyperglycemia in type 2 diabetes indeed associates with mild cognitive impairments [141]. Polymorphisms in *HSD11B1* gene have been linked to type 2 diabetes and hypertension, at least in Native Americans, and a rare polymorphism (rs846911-C/A) has been correlated with an increased risk of Alzheimer's disease [142]. In a study of 194 participants of the Scottish Mental Survey, the common variants, however did not associate with cognitive impairment with ageing and the rare polymorphism was not detected [143]. Although carbenoxolone enhances insulin sensitivity in healthy young volunteers [113] and

patients with type 2 diabetes [114], in the elderly cognition studies, however there were no effects on indices of glycaemic control or serum lipids. The potentially synergistic effects of 11ß-HSD1 inhibition on the brain and metabolism appear propitious, but the locus of action of selective 11ß-HSD1 inhibitors needs to be defined.

Topical 11ß-HSD1 inhibitors for the treatment of glaucoma

Raised intraocular pressure is a well-recognised feature of Cushing's syndrome. Topical or systemic glucocorticoid administration results in rise in intraocular pressure and subsequently may cause iatrogenic glaucoma. Intraocular pressure is kept under control by the balance between the production and outflow of aqueous humour. Mammalian ocular tissues involved in the regulation of aqueous humour express 11ß-HSD1 [144]. In the support of the hypothesis that 11ß-HSD1 enhanced activity may be important for the etiology of glaucoma systemic administration of carbenoxolone to healthy volunteers resulted in a reduction of intraocular pressure [145]. Selective 11ß-HSD1 inhibitors administered topically are a potential novel therapy for glaucoma.

New perspective for the treatment of glucocorticoid-induced osteoporosis

Glucocorticoids play an important role in human osteoblast differentiation, proliferation and matrix mineralisation [146–148]. Clinical use of glucocorticoids is associated with the development of osteoporosis. Although young 11ß-HSD1−/− mice have normal bones [149], this may not be pertinent to elderly humans. 11ß-HSD1 is expressed in human bone and cultured osteoblasts and is regulated by pro-inflammatory cytokines and glucocorticoids [150,151]. Moreover, 11ß-HSD1 is regulated in differentiation-dependent manner in osteoblasts [147]: non-differentiated pre-osteoblasts have low alkaline phosphatase activity but increased 11ß-HSD1 activity; the opposite features are found in differentiated osteoblasts. Interestingly, in primary cultures of human osteoblasts, 11ß-HSD1 activity increases with age of the patient, suggesting a link with the age-related incidence of osteoporosis [151]. The level of 11ß-HSD1 activity in human bone correlates with susceptibility of glucocorticoid (prednisone)-induced changes on bone turnover, although this is perhaps expected since inert prednisone is converted by 11ß-HSD1 to active prednisolone [152]. Inhibition of 11ß-HSD1 by carbenoxolone suppresses bone resorption markers [150]. The role of 11ß-HSD1 inhibitors upon human bones *in vivo* requires further study.

Key issues in drug development

Isozyme selectivity

Animal models described above, and experiments using 11ß-HSD1 inhibitors, have highlighted effects upon hepatic gluconeogenesis [75,120], regulation of adipocyte differentiation, lipid metabolism [69,153], lipoprotein profiles [77], insulin secretion and sensitivity [43], and blood pressure [70]. Several natural product derivatives, including carbenoxolone, glycyrrhetinic acid, metyrapone and ketoconazole have been shown to exhibit inhibitory activity against 11ß-HSD. However, none is fully selective for 11ß-HSD1. Inhibition of 11ß-HSD2 produces apparent mineralocorticoid excess, an undesirable effect. Pharmacophore modelling and a variety of screening approaches have revealed many small molecules that selectively inhibit 11ß-HSD1 but not 2 [117], perhaps reflecting the minor sequence identity between *HSD11B1* and *HSD11B2* [154]. It will be however beneficial to test other enzymes belonging to short-chain dehydrogenase/reductase (SDR) family that share higher homology with 11ß-HSD1 to avoid potential adverse effects by cross-inhibition of, for instance, 17ß-HSDs, key to sex steroid metabolism [117].

Tissue specificity

As outlined above, probably the key metabolic tissue to target is adipose tissue. Whether or not inhibition in liver is additionally beneficial remains to be determined. 11ß-HSD1−/− mice show elevated plasma corticosterone levels at the diurnal nadir, adrenal hypertrophy and exaggerated responses to stress [75,76] implying impaired glucocorticoid feedback. Whilst limited penetration through blood–brain barrier could be potentially beneficial for stabilisation of such 'compensatory' HPA axis activation [76], recent data in 11ß-HSD1−/− mice with complementation (rescue) of the enzyme solely in the liver [155] suggest that this may not be effective. The lesson from the 11ß-HSD1−/− mouse is that despite modest activation of the HPA axis, levels of glucocorticoids *inside* key metabolic and CNS cells are reduced. Crucially, the HPA axis forward drive apparently remains intact at times of stress, preventing glucocorticoid insufficiency. Whether or not the increase in ACTH with 11ß-HSD1 deficiency [76] triggers increased adrenal, androgen production can only be satisfactorily answered in humans, since the rodent adrenal cortex does not synthesise such steroids. Potentially, however, by analogy to CRD, hirsutism may be an adverse effect of clinical 11ß-HSD1 inhibition. Finally, since circulating cortisol production undergoes a pronounced circadian rhythm, when free cortisol levels exceed the levels required for substantial GR activation in a particular tissue, 11ß-HSD1 inhibition may have only minimal effects on GR responsive gene targets. It is therefore possible that a specific dosing strategy may be necessary to overcome this and that inhibition in humans may have less impact than seen in rodents with their differing physiology and energy economy.

Numerous structural classes of selective 11ß-HSD1 inhibitors have been recently proposed including sulfonamidooxazoles and beta-ketosulfones from Wyeth [156], perhydroquinolylbenzamides from Novartis [157], benzothiazoles from the University of Bath and Imperial College London [158], adamantyl amide 11ß-HSD1 inhibitors from our laboratory [159]. The current goal is to identify potent and selective 11ß-HSD1 inhibitors that possess desirable pharmacokinetic properties. Improving the potency and pharmaceutical properties of those prototypic compounds will be crucial for successful discovery of effective human therapeutic agents. Human 11ß-HSD1 inhibitors are now entering clinical trials, starting with BVT-3498 (Amgen Inc-Biovitrum). Given a high spectrum of biological pathways under control of glucocorticoids, it is not surprising that proposed applications of 11ß-HSD1 inhibitors span the gamut from insulin resistance, type 2 diabetes, obesity and related metabolic disturbances to cognitive impairments, glaucoma, muscle atrophy and osteoporosis (Figure 3). Thus, it may be predicted that for different therapeutic applications, distinct classes of inhibitors with various pharmacokinetic parameters and

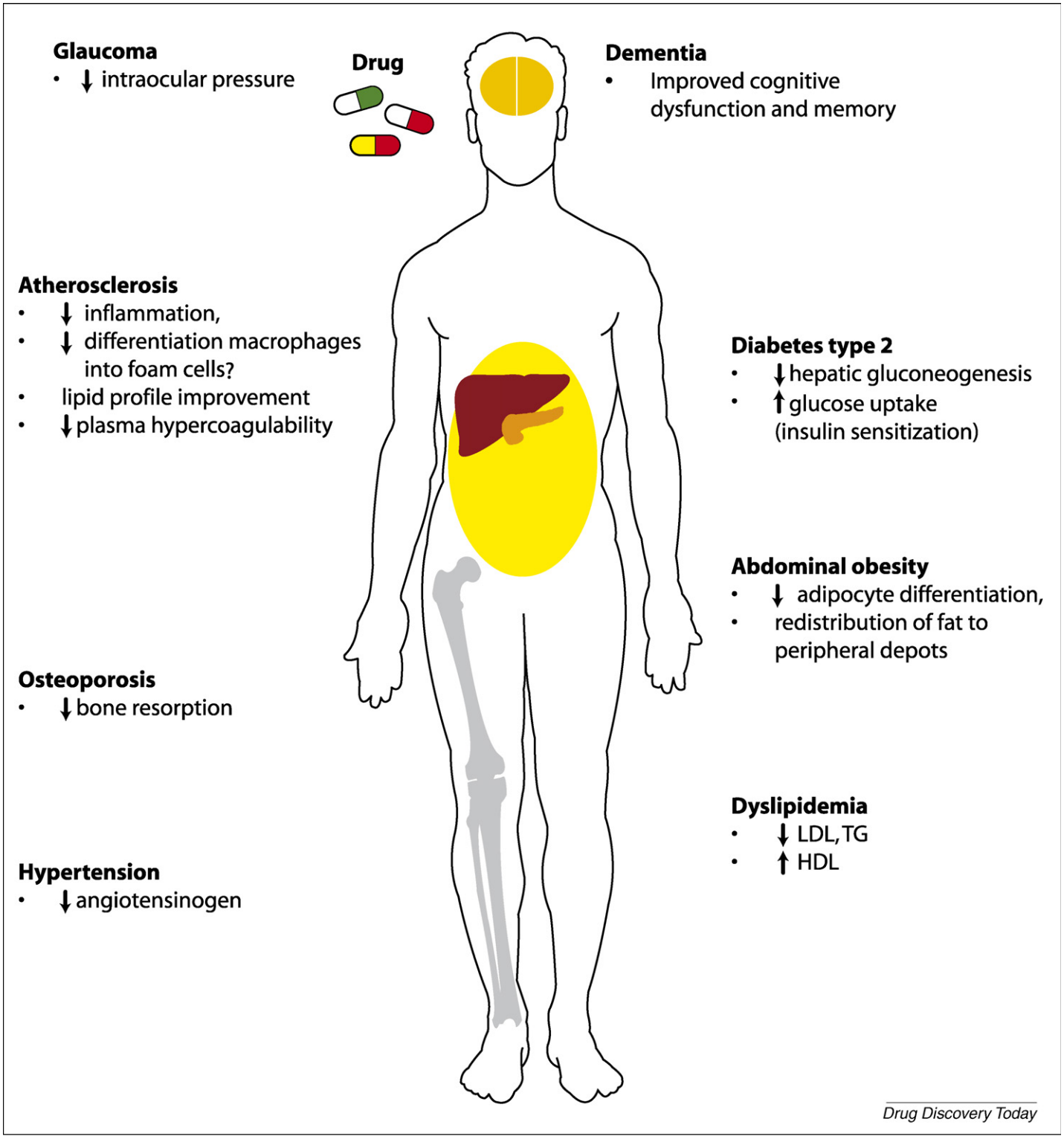

FIGURE 3

Potential clinical indications of the 11ß-HSD1 inhibitors. Early results of studies with 11ß-HSD1 inhibitors suggest their good prospects in the therapy of the obesity-induced metabolic syndrome. Given a high range of biological pathways controlled by glucocorticoids, the manipulation of their levels, however might be considered in the treatment of many other medical disorders in which a decreased intracellular concentration of active glucocorticoids is desirable. The figure presents a list of proposed indications of the new class of drugs with a brief description of plausible mechanisms of action.

tissue-penetration/specificity might be required. Interestingly, many patent applications suggest the combination of 11ß-HSD1 inhibitors with antihypertensive agents, cannabinoid receptor type 1 antagonists and other metabolic drugs. This represents a sensible trend to multifactorial and pathogenically complex disorders, recognizing the unlikelihood of any universal 'magic bullet' and pragmatically endorsing the less euphonically named 'magic shotgun' approach.

Conclusion

Here we present a review of animal and human studies on 11ß-HSD1 and give a rationale for the development of a new class of drugs by the pharmaceutical industry. The broad range of reported functions of 11ß-HSD1 point to the possibility that its inhibition would be successful in the treatment, prevention and prophylaxis of a range of medical disorders in which a decreased intracellular concentration of active glucocorticoids is desirable. Inevitably, for any potent therapy, there will be adverse effects, but the normal lifespan of 11ß-HSD1−/− mice reassures that these are unlikely to be ubiquitous or even commonly severe.

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