

# Mechanisms of dysregulation of 11 $\beta$ -hydroxysteroid dehydrogenase type 1 in obese Zucker rats

D E W Livingstone, C J Kenyon and B R Walker

University of Edinburgh, Department of Medical Sciences, Western General Hospital, Edinburgh EH4 2XU, UK  
(Requests for offprints should be addressed to B R Walker; Email: B.Walker@ed.ac.uk)

## Abstract

Obesity has been associated with alterations in glucocorticoid metabolism in both man and rodents, but the underlying mechanisms remain undefined. We have previously reported tissue-specific alterations in 11 $\beta$ -hydroxysteroid dehydrogenase type 1 (11 $\beta$ -HSD1) in obese Zucker rats predicting that reactivation of corticosterone is decreased in liver but increased in omental fat. The mechanisms of dysregulation of 11 $\beta$ -HSD1 in obesity are not known, and in this study we have investigated the potential role of glucocorticoids and insulin. In one experiment lean and obese Zucker rats were adrenalectomised, and in a second experiment they were sensitised to insulin by treatment with either metformin or rosiglitazone.

Adrenalectomy (ADX) of obese animals attenuated weight gain, normalised hepatic 11 $\beta$ -HSD1 kinetics by an effect on  $V_{\max}$  ( $V_{\max}$  in sham-operated animals,  $6.6 \pm 1.1$  nmol/min per mg in lean vs  $3.4 \pm 0.6$  in obese,  $P < 0.01$ ; in ADX animals  $5.9 \pm 1.1$  in lean vs  $6.9 \pm 1.8$  in obese, NS), and reversed the difference in omental fat 11 $\beta$ -HSD1 activity ( $18.9 \pm 4.2\%$  in lean ADX vs  $8.2 \pm 2.3$  in obese ADX,  $P = 0.03$ ).

Both metformin and rosiglitazone improved insulin sensitivity in obese, but not lean animals, and had no effect on 11 $\beta$ -HSD1 activity in either liver or fat. However, both treatments normalised adrenal hypertrophy in obese animals ( $48 \pm 29$  mg in obese vehicle vs  $37 \pm 1.2$  in metformin and  $38 \pm 1.8$  in rosiglitazone treated, both  $P < 0.01$ ), and rosiglitazone tended to attenuate hypercorticonsteronaemia in obese rats. Neither treatment attenuated weight gain; in fact, weight gain was enhanced by rosiglitazone in obese rats.

In summary, altered 11 $\beta$ -HSD1 activity in obese Zucker rats is reversible following adrenalectomy, but the mechanism is unclear since adrenalectomy also normalises many other metabolic abnormalities. The current study suggests that hyperinsulinaemia is not responsible for tissue-specific dysregulation of 11 $\beta$ -HSD1. However, insulin sensitisation did reverse adrenal hypertrophy, suggesting that hyperinsulinaemia may be a key factor contributing to activation of the hypothalamic–pituitary–adrenal (HPA) axis in obesity independently of tissue-specific changes in 11 $\beta$ -HSD1.

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## Introduction

It has been proposed that glucocorticoids are important in primary obesity (Bjorntorp *et al.* 1999). We have recently described tissue-specific alterations of peripheral glucocorticoid metabolism in the leptin-resistant Zucker rat model of obesity (Livingstone *et al.* 2000). Reactivation of corticosterone by 11 $\beta$ -hydroxysteroid dehydrogenase type 1 (11 $\beta$ -HSD1) was lower in the liver but higher in adipose tissue when compared with lean Zucker rats. Obese humans also have impaired reactivation of cortisone to cortisol in liver (Stewart *et al.* 1999), and have similar alterations in urinary glucocorticoid metabolite profiles (Andrew *et al.* 1998, Stewart *et al.* 1999). These changes in glucocorticoid metabolism may influence both corticosteroid receptor activation in liver and fat as well as feedback regulation of the hypothalamic–pituitary–adrenal (HPA)

axis, and could contribute to increased glucocorticoid secretion in obesity.

The aim of this investigation was to gain insight into the possible mechanisms of dysregulation of 11 $\beta$ -HSD1 in obesity. Two of the major regulators of 11 $\beta$ -HSD1 are glucocorticoids, which are associated with up-regulation of 11 $\beta$ -HSD1 activity and expression (Low *et al.* 1994b), and insulin, which down-regulates 11 $\beta$ -HSD1 expression (Hammami & Siiteri 1991, Jamieson *et al.* 1995). Obese Zucker rats have increased plasma and urinary glucocorticoid levels when compared with lean animals (Cunningham *et al.* 1986, Fletcher *et al.* 1986, Guillaume-Gentil *et al.* 1990, Dryden *et al.* 1995, Livingstone *et al.* 2000), and are insulin resistant and hyperinsulinaemic (York *et al.* 1972). Furthermore, 11 $\beta$ -HSD activities are disrupted in some other rat models associated with insulin resistance, including Lyon, Bianchi–Milan and

spontaneously hypertensive rats (Stewart *et al.* 1993, Takeda *et al.* 1993, Lloyd-MacGilp *et al.* 1999). In Zucker obese rats, there is evidence that glucocorticoids contribute to the obesity, since weight gain is attenuated by adrenalectomy or treatment with glucocorticoid receptor antagonists (Yukimura *et al.* 1978, Freedman *et al.* 1986, Langley & York 1990). To investigate whether glucocorticoids and/or insulin have a role in the alterations of 11 $\beta$ -HSD1 in Zucker obese rats we carried out two experiments. In one study, Zucker rats were adrenalectomised; in a second study animals were treated with either the biguanide oral hypoglycaemic agent metformin, or the insulin-sensitising peroxisome proliferator-activated receptor- $\gamma$  (PPAR $\gamma$ ) agonist rosiglitazone (BRL 49653).

## Materials and Methods

### Animals

Groups of eight 5-week-old male obese and lean Zucker rats (Harlan Orlac, Bicester, UK) were characterised by phenotype, maintained under controlled conditions of light (on 0800 h to 2000 h) and temperature (21 °C), and allowed free access to standard rat chow (Special Diet Services, Witham, UK) and drinking water for 1 week before either surgery or treatment. Animal experiments were carried out under Home Office licence (PPL no. 60/01562).

Six-week-old rats were anaesthetised with 4% halothane and either bilaterally adrenalectomised (ADX) or sham-operated through dorsal incisions. All rats were then maintained on 0.9% saline drinking water and weighed every second day.

Alternatively, drug treatment was commenced when the animals were 6 weeks of age. Metformin (43 mg/kg body weight) or rosiglitazone (1 mg/kg body weight) were administered by gavage daily at 0900 h. Drugs were dissolved in water for administration, and a matched volume of water (1 ml/kg) was administered daily to the control groups. Animals were weighed regularly to allow accurate dosing with drugs, and to follow the progress of weight gain. Food intake for each cage of four animals was measured daily. After 2 weeks of treatment animals underwent an oral glucose tolerance test which consisted of an overnight fast followed by an oral glucose load of 2 g/kg body weight at 0900 h. Blood samples were taken by tail-nick at 0, 30 and 120 min after the glucose bolus and were analysed for glucose and insulin.

At 9 weeks of age, i.e. 3 weeks after surgery or the commencement of drug treatment, animals were decapitated at 0900–1100 h and trunk blood collected. Tissues were dissected and whole adrenals were blotted dry and weighed. Portions of other tissues were snap frozen on dry ice or mechanically homogenised in Krebs Ringer buffer (118 mM NaCl, 3.8 mM KCl, 1.19 mM KH<sub>2</sub>PO<sub>4</sub>,

2.54 mM CaCl<sub>2</sub>, 1.19 mM MgSO<sub>4</sub>, 25 mM NaHCO<sub>3</sub>, pH 7.4).

### Plasma assays

Plasma corticosterone levels were measured in terminal blood samples collected at 0900–1100 h using an in-house RIA (Al-Dujaili *et al.* 1981). The inter- and intra-assay coefficients of variation were <10%. Glucose concentrations were determined by the hexokinase method using a glucose assay kit (Sigma, Poole, UK), for which the inter- and intra-assay coefficients of variation were both <2%. Insulin was measured using a rat <sup>125</sup>I-insulin RIA kit (Amersham, Buckinghamshire, UK), for which the inter- and intra-assay coefficients of variation were <15 and <10% respectively.

### Measurement of 11 $\beta$ -HSD1 activity and kinetics

*In vivo* 11 $\beta$ -HSD1 is a reductase, converting inactive 11-dehydrocorticosterone to corticosterone. However, in tissue preparations dehydrogenase activity predominates so we quantified 11 $\beta$ -HSD1 activity by conversion of corticosterone to 11-dehydrocorticosterone. 11 $\beta$ -HSD1 activity was measured in homogenates of tissues by incubating in duplicate at 37 °C in Krebs Ringer buffer containing 0.2% glucose, NADP (2 mM) and [<sup>3</sup>H]corticosterone (100 nM). Conditions were optimised for each tissue to ensure first-order kinetics and the protein concentrations of homogenates used were 10  $\mu$ g/ml for liver and 1 mg/ml for omental fat. After 60 min, steroids were extracted with ethyl acetate, the organic phase evaporated under nitrogen and extracts re-suspended in mobile phase (20% methanol, 30% acetonitrile and 50% water). Steroids were separated by HPLC using a reverse phase  $\mu$ -Bondapak C18 column and quantified by on-line liquid scintillation counting. In addition, in samples from the adrenalectomy experiment, hepatic 11 $\beta$ -HSD1 kinetics were determined in microsomes prepared from tissue homogenates as previously described (Livingstone *et al.* 2000).

### Statistics

All data are expressed as mean  $\pm$  standard error. Data were analysed using Student's *t*-test for paired data, or ANOVA followed by *post hoc* tests.

## Results

### Effects of adrenalectomy

As expected, obese animals gained more weight in the 21-day study period than the lean animals (Table 1) and adrenalectomy attenuated this weight gain. Plasma

**Table 1** Body and organ weights of sham-operated and adrenalectomised lean and obese Zucker rats

	Sham-operated		Adrenalectomised	
	Lean	Obese	Lean	Obese
Weight gain, 21 days (g)	78.0 ± 4.4	104.1 ± 4.0*	38.8 ± 3.8	50.5 ± 6.2
Adrenal weight (mg)	38.4 ± 2.1	54.3 ± 6.8*	n.d.	n.d.
Adrenal weight/ body weight (mg/g)	0.17 ± 0.01	0.20 ± 0.03	–	–
Plasma corticosterone (nM)	78 ± 48	446 ± 91*	n.d.	n.d.

Data are means ± s.e.m.s; \* $P < 0.05$  compared with lean sham-operated group. n.d., not detectable; limit of detection for corticosterone RIA was 6.25 nM.  $n = 8$  in all groups, except lean adrenalectomised where  $n = 7$ .

corticosterone levels were significantly elevated in sham-operated obese Zucker rats, and corticosterone was not detected in the plasma of any of the adrenalectomised animals.

Hepatic 11 $\beta$ -HSD1 kinetics were analysed using the Lineweaver–Burke linear transformation of the Michaelis–Menten equation (Lineweaver & Burke 1934). The Michaelis constant ( $K_m$ ) was not different between lean and obese animals and was unaffected by adrenalectomy. Maximum velocity ( $V_{max}$ ) values for obese animals were approximately half those for lean animals (Fig. 1). Adrenalectomy normalised  $V_{max}$  in obese animals but had no effect in lean animals.

11 $\beta$ -HSD1 activity in omental fat tended to be higher in obese than lean rats ( $P = 0.13$ ; Fig. 2). Adrenalectomy tended to decrease omental fat 11 $\beta$ -HSD1 activity in obese rats, but increase it in lean rats, so that after adrenalectomy enzyme activity was lower rather than higher in obese animals ( $P = 0.03$ ).

#### Effects of insulin-sensitising drugs

By contrast with adrenalectomy, neither of the anti-diabetic treatments attenuated weight gain; in fact rosiglitazone caused an increase in weight gain and food intake (Table 2).

Results from glucose tolerance tests are shown in Fig. 3. Obese animals exhibited hyperglycaemia in the fasting state and 120 min after the glucose bolus. Insulin levels were markedly higher in obese animals at all time points in the glucose tolerance test. Neither metformin nor rosiglitazone affected plasma glucose or insulin in lean rats. In obese rats effects on plasma insulin were more striking than effects on plasma glucose: metformin decreased both fasting and 30 min plasma glucose levels, and decreased 30 min and 120 min insulin levels; rosiglitazone decreased only the 30 min glucose level, but reduced plasma insulin levels at all time points.

Plasma corticosterone levels were rather variable in this experiment, probably reflecting poorly controlled stress at the time of decapitation (this experiment was performed in

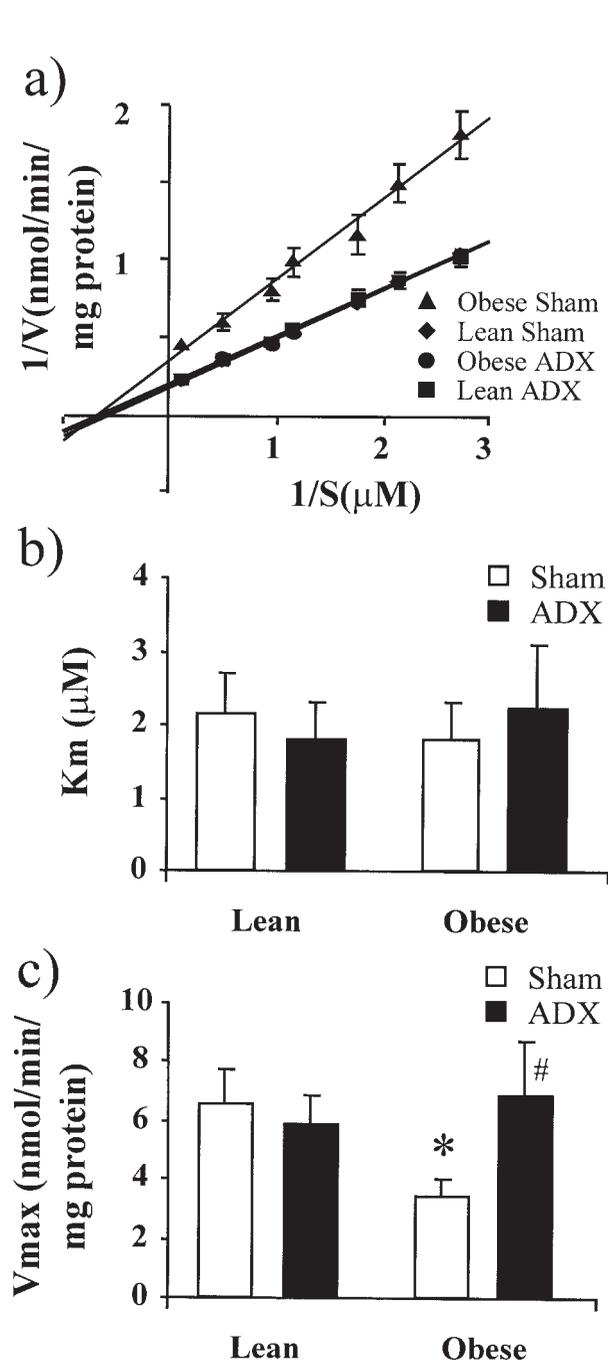
a different animal unit from the adrenalectomy experiment), so that trends rather than statistically significant differences were observed (Table 2). In lean animals, neither plasma corticosterone nor adrenal weight was affected by metformin or rosiglitazone. However, in obese animals, where plasma corticosterone and adrenal weight were higher, both metformin and rosiglitazone reduced adrenal weight, but only rosiglitazone reduced plasma corticosterone (Table 2).

In vehicle treated animals, hepatic 11 $\beta$ -HSD1 activity (Fig. 4a) was lower, and omental fat 11 $\beta$ -HSD1 activity (Fig. 4b) was higher ( $P = 0.02$ ), in obese than in lean rats. Neither metformin nor rosiglitazone altered hepatic or omental fat 11 $\beta$ -HSD1 activity.

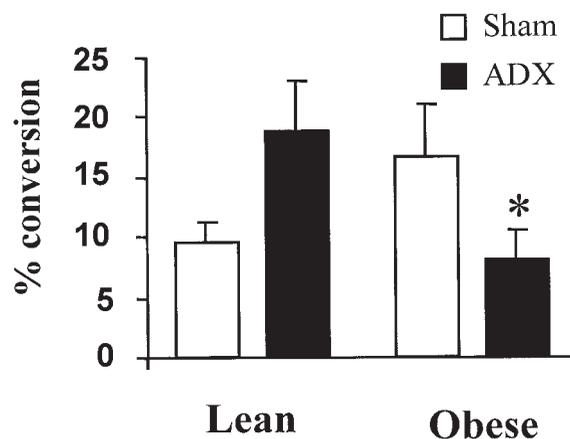
## Discussion

These data confirm previous reports that obese Zucker rats show activation of the HPA axis (Livingstone *et al.* 2000), impaired glucose tolerance and insulin resistance (York *et al.* 1972), and tissue-specific alterations in peripheral corticosterone metabolism (Livingstone *et al.* 2000). In addition, we found that adrenalectomy had tissue-specific effects to normalise 11 $\beta$ -HSD1 activity: impaired 11 $\beta$ -HSD1 activity in liver of obese rats was restored; enhanced 11 $\beta$ -HSD1 activity in omental fat of obese rats was reduced. In contrast, insulin sensitisation with metformin or rosiglitazone had no effect on aberrant 11 $\beta$ -HSD1 activity in either tissue, but normalised the adrenal hypertrophy in obese rats.

Normalisation of weight gain in obese rats by adrenalectomy has been reported previously (Yukimura *et al.* 1978), and is associated with normalisation of many of their metabolic abnormalities, including hyperinsulinaemia (Castonguay *et al.* 1986). The current data show that reversal of this metabolic syndrome by adrenalectomy is associated with normalisation of hepatic 11 $\beta$ -HSD1 kinetics, i.e. an increase in  $V_{max}$  without altering  $K_m$ . The kinetic data suggest that the abnormality in hepatic 11 $\beta$ -HSD1 in the obese Zucker rat is due to down-regulation



**Figure 1** Hepatic 11 $\beta$ -HSD1 kinetics from adrenalectomy experiment. Data are means  $\pm$  S.E.M.s. \* $P < 0.05$  comparing lean and obese sham-operated animals; # $P < 0.05$  compared with sham-operated animals of the same phenotype; Sham denotes sham-operated animals; and ADX denotes adrenalectomised animals. (a) Lineweaver–Burke plots for summarised data.  $K_m$  (b) and  $V_{max}$  (c) were calculated from Lineweaver–Burke plots for each individual animal.  $n = 8$  in all groups except lean adrenalectomised where  $n = 7$ .



**Figure 2** 11 $\beta$ -HSD1 activity in omental fat from adrenalectomy experiment. Data are means  $\pm$  S.E.M.s; Sham denotes sham-operated animals and ADX denotes adrenalectomised animals. 11 $\beta$ -HSD1 activity was assessed as percentage conversion of corticosterone to 11-dehydrocorticosterone. \* $P < 0.05$  comparing lean and obese animals receiving the same treatment.  $n = 8$  in all groups except lean adrenalectomised where  $n = 7$ .

of protein, and that normalisation of enzyme activity is due to an increase in protein, rather than removal of a competitive inhibitor. One explanation for this is that hypercorticonaemia is responsible for dysregulation of 11 $\beta$ -HSD1 activity in obesity. However, it is difficult to address this directly because any manipulation which restores circulating glucocorticoid levels in adrenalectomised obese rats also restores additional metabolic disturbances such as hyperinsulinaemia (Freedman *et al.* 1986).

The alterations in 11 $\beta$ -HSD1 in omental fat in obese Zucker rats are qualitatively different from those in liver. The current results confirm increased 11 $\beta$ -HSD1 activity in omental fat from obese animals (Livingstone *et al.* 2000), albeit that this was statistically significant in the insulin-sensitising experiment but a non-significant trend in the sham-adrenalectomised animals. After adrenalectomy, this difference was reversed, so that omental fat 11 $\beta$ -HSD1 activity was lower in obese animals. Relatively little is known about regulation of 11 $\beta$ -HSD1 expression in rat adipose tissue, but experiments in human cultured cells suggest that both glucocorticoids and insulin may up-regulate activity (Bujalska *et al.* 1997). However, our observation that adrenalectomy up-regulates 11 $\beta$ -HSD1 activity in omental fat of lean animals, by contrast with the lack of effect in liver of lean rats, reinforces previous observations that regulation of this enzyme is tissue specific (Low *et al.* 1994b), and suggests that it is unlikely that chronic hypercorticonaemia is responsible for up-regulation of adipose 11 $\beta$ -HSD1 in obese Zucker rats.

An alternative explanation for the effect of adrenalectomy is that reversal of insulin resistance is important, as

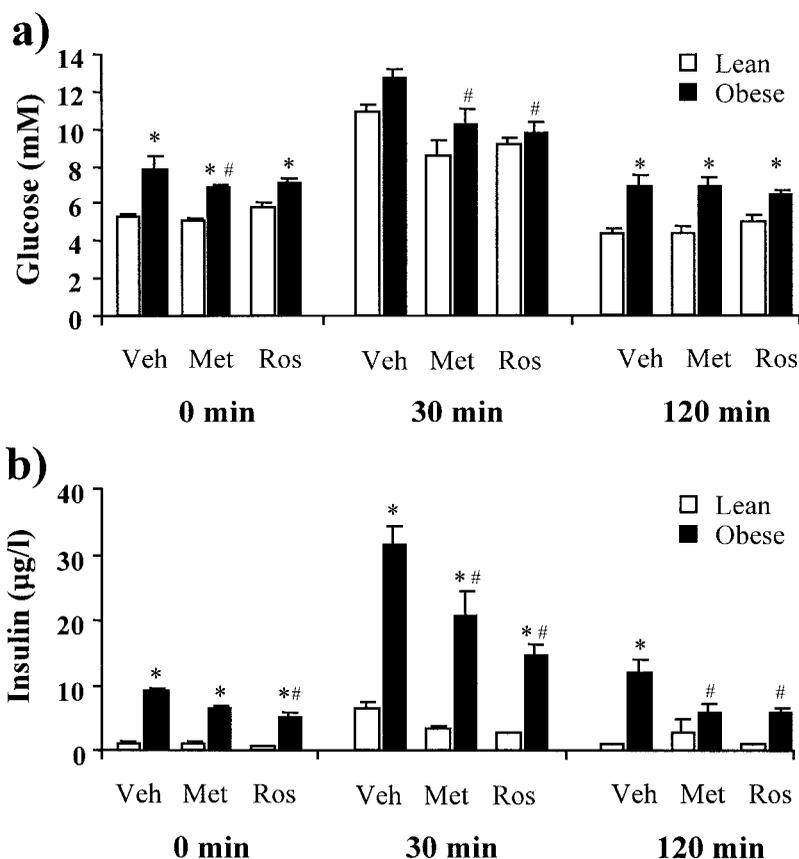
**Table 2** Body and organ weights of lean and obese Zucker rats treated with vehicle, metformin or rosiglitazone

	Vehicle		Metformin		Rosiglitazone	
	Lean	Obese	Lean	Obese	Lean	Obese
Weight gain, 21 days (g)	93 ± 5.5	137 ± 3.8*	90 ± 4.8	133 ± 1.8*	89 ± 5.7	152 ± 3.5*#
Food intake per day (g)	19.4 ± 0.4	28.3 ± 0.5*	19.8 ± 0.3	28.9 ± 0.5*	20.1 ± 0.4	31.9 ± 0.8*#
Adrenal weight (mg)	37.4 ± 3.5	48.4 ± 2.9*	32.9 ± 2.7	36.9 ± 1.2#	36.2 ± 1.9	37.8 ± 1.8#
Adrenal weight/body weight (mg/kg)	158 ± 15	153 ± 10	140 ± 13	112 ± 4.6*#	153 ± 9.5	107 ± 3.8*#
Plasma corticosterone (nM)	163 ± 74	302 ± 86	175 ± 48	340 ± 113	178 ± 46	160 ± 56

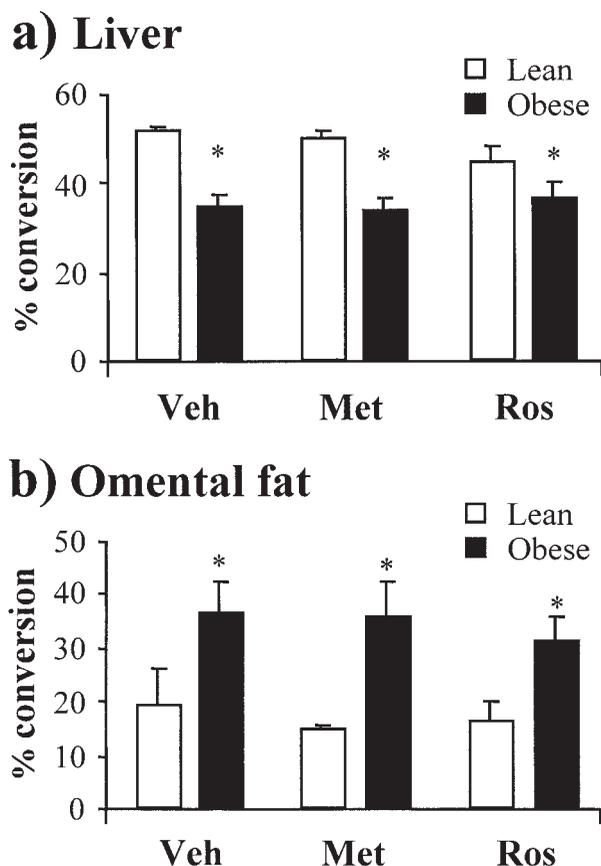
Data are means ± S.E.M.s; \**P*<0.05 comparing lean and obese animals receiving the same treatment; #*P*<0.05 compared with animals of the same phenotype receiving vehicle. *n*=8 in all groups.

insulin down-regulates 11β-HSD1 activity in liver (Hammami & Siiteri 1991) and may up-regulate it in fat (Bujalska *et al.* 1997). Both metformin and rosiglitazone enhanced insulin sensitivity in obese, but not lean, Zucker rats. The mechanism of their action is probably different, with metformin lowering fasting glucose and acting

principally on hepatic gluconeogenesis, while rosiglitazone may have more important effects in adipose tissue. While metformin treatment had no effect on food intake or body weight, rosiglitazone treatment caused an increase in both food intake and weight gain in obese animals, as previously reported (Wang *et al.* 1997). However, neither



**Figure 3** Glucose tolerance tests from insulin-sensitising experiment. Data are means ± S.E.M.s; \**P*<0.05 comparing lean and obese animals receiving the same treatment; #*P*<0.05 compared with animals of the same phenotype receiving vehicle. Veh denotes vehicle-treated animals; Met, metformin treatment; and Ros, rosiglitazone treated. (a) Glucose levels and (b) insulin levels at 0, 30 and 120 min after a bolus of 2 g/kg glucose. *n*=8 in all groups.



**Figure 4** 11 $\beta$ -HSD1 activities from insulin-sensitising experiments. Data are means  $\pm$  S.E.M.s; \* $P < 0.05$  comparing lean and obese animals receiving the same treatment; # $P < 0.05$  compared with animals of the same phenotype receiving vehicle. Veh denotes vehicle-treated animals; Met, metformin treatment; and Ros, rosiglitazone treated. 11 $\beta$ -HSD1 activity was assessed as percentage conversion of corticosterone to 11-dehydrocorticosterone.  $n = 8$  in all groups.

insulin-sensitising agent affected 11 $\beta$ -HSD1 activity in liver or adipose tissue.

Mechanisms for activation of the HPA axis in obesity are poorly understood. Enhanced peripheral glucocorticoid clearance, resulting from increased 5 $\alpha$ -reductase and impaired hepatic 11 $\beta$ -HSD1 activities (Livingstone *et al.* 2000), may be an important stimulus (Strain *et al.* 1982). However, although neither metformin nor rosiglitazone affected 11 $\beta$ -HSD1 activity in peripheral tissues, both treatments normalised the adrenal hypertrophy in obese Zucker rats. Moreover, there was a trend for normalisation of plasma corticosterone by rosiglitazone, albeit contrary to a previous report (Wang *et al.* 1997). Effects on adrenal size without changes in plasma glucocorticoid levels may reflect actions of insulin-sensitising agents on other glucocorticoid-metabolising enzymes, such as 5 $\alpha$ -reductase. Alternatively, insulin increases activity and

expression of the steroidogenic enzyme 3 $\beta$ -hydroxysteroid dehydrogenase in the adrenal cortex (Kristiansen *et al.* 1997), and impaired steroidogenesis in insulin resistance could play a role in adrenal hypertrophy in the obese Zucker rat. Effects on adrenal size accompanied by a reduction in plasma corticosterone are more consistent with effects of insulin resistance or hyperinsulinaemia on central control of the HPA axis. Further studies of the effects of insulin on the HPA axis in insulin-resistant models are required to differentiate these possibilities.

In conclusion, in obesity down-regulation of 11 $\beta$ -HSD1 may attenuate glucocorticoid action in liver and may enhance metabolic clearance of glucocorticoids. By contrast, enhanced 11 $\beta$ -HSD1 in omental fat may maintain local glucocorticoid concentrations and promote lipogenesis. Both of these phenomena are reversible following adrenalectomy, but the mechanism is unclear and does not appear to relate to hyperinsulinaemia. Alternative mediators of tissue-specific dysregulation of 11 $\beta$ -HSD1, including growth hormone (Low *et al.* 1994a) and gonadal steroids (Low *et al.* 1993), may be more important in obesity. However, hyperinsulinaemia may be a key factor contributing to activation of the HPA axis in obesity independently of tissue-specific changes in 11 $\beta$ -HSD1.

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