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Reduced Adipose Glucocorticoid Reactivation and Increased Hepatic Glucocorticoid Clearance as an Early Adaptation to High-Fat Feeding in Wistar Rats

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Altered peripheral glucocorticoid metabolism may be important in the pathogenesis of obesity in humans and animal models. Genetically obese Zucker rats, Lep/ob mice, and obese humans exhibit increased regeneration of active glucocorticoids selectively in adipose tissue by 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD-1) and increased glucocorticoid clearance by hepatic A-ring reductases. We have examined whether dietary obesity in rats induces the same changes in glucocorticoid metabolism. Male Wistar rats were weaned onto high-fat (HF; 45% kcal from fat) or control (10% fat) diets. After 3 wk, HF rats showed no differences in weight but were glucose intolerant, had lower 11β-HSD-1 activity in liver (3.8 ± 0.2 *vs.* 4.9 ± 0.2 pmol product/min·mg protein; $P < 0.01$), sc fat (0.03 ± 0.01 *vs.* 0.09 ± 0.01 pmol product/min·mg protein; $P < 0.01$), and omental fat (0.02 ± 0.001 *vs.* 0.03 ± 0.003 pmol/ product/min·mg protein; $P < 0.05$) and higher hepatic 5β-reductase activity (0.26 ± 0.05 *vs.* 0.10 ± 0.007 pmol product/min·mg protein; $P < 0.05$). After 20 wk, HF rats were obese, hyperglycemic, and hyperinsulinemic, but differences in 11β-HSD-1 and 5β-reductase activities were no longer apparent. Mature male rats given HF diets for 24 or 72 h showed increased hepatic 5β-reductase activity and a trend for decreased sc adipose 11β-HSD-1 activity. Dietary obesity is not accompanied by the changes in 11β-HSD-1 and 5β-reductase expression and activity observed in genetically obese rodents. Acute exposure to HF diet alters glucocorticoid metabolism, predicting lower hepatic and adipose intracellular glucocorticoid concentrations, which may be a key mechanism protecting against the metabolic complications of obesity. (*Endocrinology* 146: 913–919, 2005)

OBESITY IN HUMANS is associated with a number of cardiovascular risk factors including hypertension, insulin resistance, type 2 diabetes, and hyperlipidemia. This cluster of features, termed the metabolic syndrome, is also found in Cushing's syndrome where it is caused by increased circulating levels of glucocorticoids. Additionally, increased cortisol concentrations in blood, saliva, and urine are associated with hypertension, insulin resistance, and hyperlipidemia (1).

Such observations have prompted a number of studies in animal models, which have sought to determine whether abnormalities in glucocorticoid secretion, action, or metabolism may underlie the development of visceral adiposity and the metabolic syndrome. One such model, the obese Zucker rat, which is homozygous for a mutation in the leptin receptor gene, exhibits insulin resistance and hypertension (2), which may reflect increased glucocorticoid action resulting from abnormalities at several levels of control. The hypothalamic-pituitary-adrenal (HPA) axis is activated in obese rats (3, 4), and tissue-specific changes in key glucocorticoid-metabolizing enzymes suggest that local changes in peripheral glucocorticoid metabolism and hence intracellular exposure may be important (2). Thus, increased glucocorticoid conversion by hepatic A-ring reductases may enhance glucocorticoid clearance and contribute to compensatory activation of the HPA axis (2). In addition, recent evidence suggests that the metabolites generated by 5α-reductase type 1 in liver may contribute to glucocorticoid receptor (GR) activation (5). Furthermore, tissue-specific changes in 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD-1), which regenerates active glucocorticoids from their inactive 11-keto metabolites (6), predict increased reactivation of glucocorticoids in adipose tissue but impaired reactivation in the liver in obese Zucker rats (2), findings that are reproduced in Lep/ob mice (7, 8). The alterations in 11β-HSD-1 and A-ring reductase activity in these rodent models parallel the changes observed in human obesity (9–12). The potential importance of this is further emphasized by the findings from two other rodent models; fat-selective transgenic overexpression of 11β-HSD-1 in mice is associated with the development of visceral obesity and the metabolic syndrome (8), whereas in contrast, mice homozygous for a targeted disruption of the 11β-HSD-1 gene exhibit attenuated gluconeogenic responses to stress (13), enhanced hepatic insulin sensitivity, and a cardioprotective lipid profile (14) and resist visceral obesity on exposure to a high-fat (HF) diet (15). Moreover, treatment with a selective inhibitor of 11β-HSD-1 increases insulin sensitivity in hyperinsulinemic, hyperglycemic mice (16).

Although there has been extensive study of glucocorticoid metabolism in monogenic forms of obesity in rodents, little

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Abbreviations: GR, Glucocorticoid receptor; HF, high fat; HPA, hypothalamic-pituitary-adrenal; 11β-HSD-1, 11β-hydroxysteroid dehydrogenase 1; PPAR, peroxisome proliferator-activated receptor; TG, triglyceride.

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is known about the effects of dietary obesity on glucocorticoid signaling in rodent models and whether this is more relevant to human physiology. In mice, our recent data have shown that HF feeding induces a marked fall, rather than an increase, in adipose 11β-HSD-1, suggesting a novel physiological adaptation to HF feeding (17). Intriguingly, this decrease in 11β-HSD-1 was more marked in obesity-resistant strains, suggesting that this might be a protective mechanism (17). To explore further the effect of dietary obesity and to extend our previous studies on obesity-associated alterations in A-ring metabolism of glucocorticoids, we have used the Wistar rat, which has been shown to develop obesity and insulin resistance on a HF diet (18), to investigate whether dietary obesity is associated with the same changes in glucocorticoid metabolism as occur in monogenic rodent and human obesity. Additionally, we have investigated the time course of changes in these enzymes in response to the development of dietary obesity.

Materials and Methods

Animal maintenance

Eight nulliparous 200- to 250-g female Wistar rats (Charles River UK Ltd., Margate, UK) were maintained under controlled lighting (lights on 0700–1900 h) and temperature (22 C), with *ad libitum* access to standard rat chow (Special Diet Services, Witham, UK) and water. They were timed mated and delivered on d 21. At weaning (3 wk), male offspring were weighed, and four males were selected randomly from each litter. Two males from each litter were weaned on to a HF diet (diet D12451, Research Diets, New Brunswick, NJ) and two onto a control diet (diet D01072401, Research Diets) (Table 1). Diet D12451 has been shown to be effective in inducing obesity in rodents (19), and the control diet was developed to match as closely as possible the sucrose, protein, and micronutrient content of the HF diet. Animals were weighed weekly and handled daily for 2 wk before investigation. Eight rats on each diet were investigated after 3 wk on the diet (acute group) and the remainder after 20 wk on the diet (chronic group). Tail-nick blood samples were obtained at 0800 and 1900 h for diurnal variation in plasma corticosterone. To minimize any effect of stress on plasma corticosterone levels, animals were removed from their individual cages and samples taken as quickly as possible. Glucose tolerance tests were performed as described below. Animals were subsequently culled by decapitation between 0900 and 1100 h after an overnight fast, and trunk blood was collected. Tissues were dissected immediately, weighed, and snap-frozen on dry ice. All animal procedures were carried out under the terms of the UK Animals (Scientific Procedures) Act 1986.

TABLE 1. Diet constituents (Research Diets, New Brunswick, NJ)

	Control diet D01072401		HF diet D12451	
	g	kcal	g	kcal
Composition				
Protein	19.2	20	23.7	20
Carbohydrate	67	70	41	35
Fat	4.3	10	24	45
Total	91	100	89	100
kcal/g	3.85		4.73	
Ingredients				
Casein	200	800	200	800
L-Cystine	3	12	3	12
Corn starch	452	1809	73	291
Maltodextrin 10	75	300	100	400
Sucrose	173	691	173	691
Cellulose	50	0	50	0
Soybean oil	25	225	25	225
Lard	20	180	178	1598

Alterations in glucose and insulin homeostasis have been documented after 5-h exposure to iv triglyceride (TG) infusions (20) or 24-h exposure to a HF diet in rats (21), although the effect of such a short-term exposure on glucocorticoid-metabolizing enzymes is not known. Thus, to explore the acute effects of a HF diet on glucocorticoid-metabolizing enzymes, we elected to study the effects of a HF diet at 24 and 72 h. Therefore, a separate experiment was performed in which 12 6-wk-old male Wistar rats were commenced on the HF and 12 on the control diet. After 24 or 72 h on the diets, six control and six HF-fed animals were weighed and killed, and tissues were dissected as above.

Glucose tolerance tests

Animals were fasted overnight, and tests commenced at 0900 h the following morning. A glucose load of 2 g/kg was administered by gavage, and blood samples were collected by tail nicking at 0, 30, and 120 min. Plasma was stored at –20 C.

Plasma measurements

Glucose was determined by the enzymatic (hexokinase) method (Sigma, Poole, UK). Plasma insulin concentration was determined by ELISA (Crystal Chem Inc., Chicago, IL). TGs and nonesterified fatty acids were determined using kits (Roche Diagnostics Ltd., Lewes, UK, and Wako Pure Chemical Industries Ltd., Osaka, Japan). Plasma corticosterone was measured by RIA (22).

Measurement of enzyme activities

In vivo, 11β-HSD-1 is a reductase, converting inactive 11-dehydrocorticosterone to corticosterone. However, *in vitro*, the dehydrogenase direction is preferred, and reductase activity is less stable (23), so 11β-HSD-1 activity was quantified by conversion of corticosterone to 11-dehydrocorticosterone as previously described (2). When driven by excess NADP cofactor, this activity is proportional to total protein. All reactions described below were optimized to ensure they were in the linear part of the relationship between product formation, protein concentration, and time.

Samples of liver (0.2 mg/ml protein), sc fat (0.5 mg/ml protein), and omental fat (0.5 mg/ml protein) were incubated in duplicate at 37 C in buffer containing 25 nM $[^3H]_4$corticosterone (3 and 20 wk) or 100 nM $[^3H]_4$corticosterone (24 and 72 h) and NADP (2 mM). After incubation for 15 min (liver), 3 h (sc fat, acute group), 6 h (sc fat, chronic group), and 3 h (omental fat), steroids were extracted with ethyl acetate, separated by thin layer chromatography (mobile phase, 92% chloroform, 8% ethanol; TLC aluminum sheets, VWR, Lutterworth, Leicestershire, UK), and quantified after exposure to a phosphorimager tritium screen for 24 h (Fuji Photo Film Co. Ltd., Tokyo, Japan). 11β-HSD-1 activity was expressed as pmol product (tritiated 11-dehydrocorticosterone)/min·mg protein after correction for apparent conversion in reactions without homogenates.

For measurement of 5β-reductase activity, liver (100 mg wet weight) was homogenized in sucrose buffer (0.25 M, pH 7.5), HEPES (10 mM), and dithiothreitol (1 mM). Cytosolic and microsomal subfractions were separated by differential centrifugation (24). Briefly, homogenized liver samples were centrifuged (1000 × *g* at 4 C for 10 min) and the supernatant removed and centrifuged (34,000 × *g* at 4 C for 30 min). The resulting supernatant was further centrifuged (124,000 × *g* at 4 C for 60 min), yielding cytosolic supernatant for analysis. The protein concentration was determined colorimetrically. Cytosolic preparations (0.4 mg/ml protein) were incubated in duplicate at 37 C in potassium phosphate buffer (0.1 M, pH 7.5) containing glucose-6-phosphate (5 mM), glucose-6-phosphate dehydrogenase (0.1 U/ml), NADPH (2 mM), $[^3H]_4$corticosterone (50 nM), and unlabeled corticosterone (9.95 μM). After 2 h, steroids were extracted with ethyl acetate (2.5ml), the organic layer was reduced to dryness under nitrogen, and the residue was dissolved in the mobile phase (water/acetonitrile/methanol, 60/10/30). Steroids were separated by HPLC on a Water Symmetry RP8 column (15 cm, pore size 3.5 μm) at 35 C, and their concentrations were quantified by on-line scintillation counting. 5β-Reductase activity was expressed as pmol product ($[^3H]_4$5β-tetrahydrocorticosterone)/min·mg protein.

Radiolabeled steroids were obtained from Amersham Pharmacia Biotech (Aylesbury, UK). Solvents were HPLC glass-distilled grade from Rathburn Chemicals (Walkerburn, UK). Other chemicals were from Sigma.

mRNA isolation and quantification

RNA was isolated from tissues using TriZol (Invitrogen Life Technologies/Life Technologies, Inc., Paisley, UK) and quantified spectrophotometrically at OD_{260}. The integrity of total RNA was assessed using agarose gel electrophoresis. RNA was quantified by Northern blot for 5α-reductase type 1 and 5β-reductase in liver. Omental fat yielded small amounts of RNA, and we therefore used real-time PCR quantitative analysis of mRNAs for 11β-HSD-1, GR mRNA, and 5α-reductase type 1.

For Northern blots, 10 μg RNA was blotted onto a Bio-Rad Laboratories, Inc. (Richmond, CA) ζ-Probe nylon membrane, and 5α-reductase type 1 and 5β-reductase mRNAs were identified as previously described (25). Membranes were hybridized overnight at 55 C with rat 5α- or 5β-reductase cDNA labeled with [^{32}P]deoxy-CTP using a random primed DNA labeling kit (Roche Diagnostics). Hybridized probe was quantified using a Fuji Photo Film Co., Ltd. (Tokyo, Japan) FLA2000 fluorescent image analyzer. Membranes were rehybridized with a U1 probe using the same method to control for differences in mRNA loading and transfer.

For real-time PCR for 11β-HSD-1, GR, and 5α-reductase type 1 mRNA levels, cDNA was synthesized from 0.5-μg RNA samples using the Promega reverse transcription system (Promega, Southampton, UK). PCR amplification using glyceraldehyde 3-phosphate dehydrogenase primers on a proportion of the cDNA samples confirmed successful RT. Real-time PCR was performed using the TaqMan ABI Prism 7900 sequence detector with cycling parameters of 50 C for 2 min, 95 C for 10 min, 40 cycles of 95 C for 15 sec, and 60 C for 1 min. Data acquisition was processed with Sequence Detector 1.6.3 software. mRNA expression was determined from standard curves generated for each primer-probe set using serial dilutions of cDNA from the tissue studied. Cyclophilin was used as the endogenous reference to normalize the transcript levels. Oligonucleotide primers and TaqMan probes for 11β-HSD-1, GR, 5α-reductase type 1, and cyclophilin are detailed below.

11β-HSD-1. Primer sequences were as follows: forward, 5′-TCATAGACACAGAAACAGCTTTGAAA-3′; reverse, 5′-CTCCAGGGCGCATTCCT-3′. The probe was 5′-6-FAM-CTGGGATAATCTTGAGTCAAGCTGCTCCC-TAMRA-3′.

GR. Primer sequences were as follows: forward, 5′-GGGTACTCAAGCCCTGGAATG-3′; reverse, 5′-CCCGTAATGACATCCTGAAGCT-3′. The probe was 5′-6-FAM-CCACGGGACCACCTCCCAAGC –TAMRA-3′.

5α-reductase type 1. Primer sequences were as follows: forward, 5′-CTGTTTCCTGACAGGCTTTGC-3′; reverse, 5′-GCCTCCCCTGGGTATCTTGT-3′. The probe was 5′-6-FAM-CAGACCACATCCTGAGGAATCTGAGAAAACC-TAMRA-3′.

Cyclophilin. Primer sequences were as follows: forward, 5′-CCCACCGTGTTCTTCGACAT-3′; reverse, 5′-GAAAGTTTTCTGCTGTCTTTGGAACT-3′. The probe was 5′-VIC-CAAGGGCTCGCCATCAGCCGTTAMRA-3′.

Statistics

Data are expressed as mean ± SEM unless otherwise stated and were analyzed using unpaired *t* tests and two-way or repeated-measures ANOVA (glucose tolerance tests and longitudinal body weight).

Results

Effects of HF diet on body composition and insulin sensitivity

After 24 h, 72 h, and 3 wk, HF rats did not show discernible differences in body weight or in organ weight. From 60 d, HF rats had higher body weights ($P < 0.05$) (Fig. 1). At culling, HF rats had increased retroperitoneal fat weight (21.7 ± 1.9 *vs.* 12.9 ± 0.9 g; $P < 0.005$) but no differences in the weight of other organs measured.

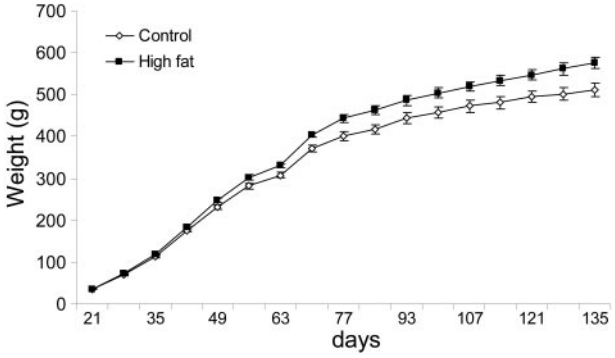

FIG. 1. Weight gain of animals on HF or control diets. Mean body weight from weaning until d 135 (n = 8 HF and 7 control) is shown. Data are mean ± SEM. By repeated-measures ANOVA, $P = 0.03$ for effect of diet.

After 3 wk, HF rats were glucose intolerant (Fig. 2A) but had no difference in plasma insulin levels. By 20 wk, although there was an age-related increase in basal and 120-min glucose and insulin values, HF rats were both hyperglycemic and hyperinsulinemic after a glucose load when compared with controls at this time point (Fig. 2B). HF rats showed a trend toward lower plasma TGs after 3 wk and a significant decrease after 20 wk. There were no differences in serum nonesterified fatty acids at either time point (Table 2).

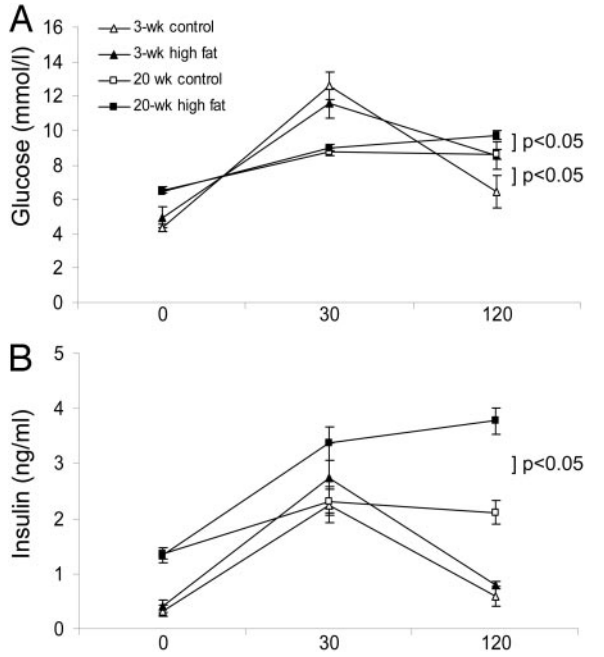

FIG. 2. Glucose tolerance tests. Effect of HF diet for 3 wk (n = 8 HF and 8 control) and 20 wk (n = 8 HF and 7 control) on plasma concentrations of glucose and insulin during glucose tolerance tests is shown. Data are mean ± SEM. *P* values on the graphs refer to *post hoc* tests comparing two groups by ANOVA. A, Glucose (mM). At 3 and 20 wk, plasma glucose was elevated by HF diet. By repeated-measures ANOVA, $P < 0.05$. B, Insulin (ng/ml). HF diet had no effect at 3 wk but induced marked hyperinsulinemia at 20 wk in the HF group. By repeated-measures ANOVA, $P < 0.005$.

TABLE 2. Body weight, plasma parameters, GR mRNA, 5α- and 5β-reductases, and 11β-HSD-1 mRNA from animals at 3 and 20 wk

	3 wk		20 wk	
	Control (n = 8)	HF (n = 8)	Control (n = 7)	HF (n = 8)
Weight (g)	188 ± 10	194 ± 9	541 ± 20	618 ± 17[a]
Serum (mM)				
Fatty acids	0.49 ± 0.06	0.43 ± 0.02	0.50 ± 0.03	0.49 ± 0.04
TGs	1.30 ± 0.20	0.85 ± 0.09	2.03 ± 0.4	1.30 ± 0.07[a]
GR mRNA				
Liver	1.04 ± 0.03	0.99 ± 0.02	0.98 ± 0.01	1.01 ± 0.03
Omental fat	1.02 ± 0.08	1.00 ± 0.06	0.98 ± 0.05	0.95 ± 0.10
11β-HSD-1 mRNA				
Liver	0.92 ± 0.09	0.95 ± 0.04	1.06 ± 0.04	0.97 ± 0.02
Omental fat	0.93 ± 0.04	1.05 ± 0.07	0.93 ± 0.14	0.99 ± 0.06
5α-Reductase type 1 mRNA				
Liver	1.80 ± 0.06	1.95 ± 0.10	1.67 ± 0.12	1.77 ± 0.10
Omental fat	1.10 ± 0.04	1.16 ± 0.10	0.86 ± 0.07	0.93 ± 0.04
5β-Reductase mRNA				
Liver	1.69 ± 0.09	1.76 ± 0.08	2.62 ± 0.12	2.24 ± 0.17

Data are the mean ± SEM. The 5α- and 5β-reductase mRNAs are expressed normalized to U1 in liver. GR and 11β-HSD-1 mRNAs in liver and omental fat and 5α-reductase mRNA in omental fat are expressed normalized to cyclophilin.

[a] $P < 0.05$ for HF *vs.* control at 20 wk.

HPA axis

The HF diet had no effect on nadir 0800 h plasma corticosterone levels after 3 or 20 wk. However, the HF diet lowered peak 1900 h plasma corticosterone at 20 wk (Fig. 3). There were no differences in mRNA for hepatic or omental fat GR at 3 or 20 wk (Table 2).

11β-HSD-1

After 24 and 72 h of HF feeding, there were no differences in hepatic or omental fat 11β-HSD-1 activity, but sc fat 11β-HSD-1 activity tended to decrease (Fig. 4; $P = 0.06$). After 3 wk of HF diet, 11β-HSD-1 activity decreased in liver and sc and omental fat (Fig. 4), although no changes were found in mRNA levels (Table 2). By 20 wk, however, the difference in 11β-HSD-1 activity between HF and control-fed animals was no longer apparent.

A-ring reductases

The HF diet increased hepatic 5β-reductase activity (Fig. 5) but not mRNA (Table 2). This was detectable after 24 h on the HF diet and was maintained at 3 wk. However, after 20 wk of HF feeding, the difference in 5β-reductase activity was no longer apparent. There were no differences at 3 or 20 wk in mRNA levels for hepatic and omental fat 5α-reductase type 1 (Table 2).

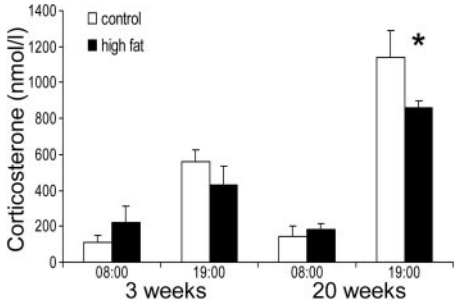

FIG. 3. HPA axis. Effect of HF diet for 3 wk (n = 8 HF and 8 control) and 20 wk (n = 8 HF and 7 control) on plasma concentrations of corticosterone at 0800 and 1900 h is shown. Data are mean ± SEM. Peak corticosterone was reduced in HF animals at 20 wk; *, $P = 0.04$.

Discussion

These results demonstrate that short-term HF feeding in rats is associated with acute changes in glucocorticoid-metabolizing enzymes, with down-regulation of liver and adipose 11β-HSD-1 activity and increased hepatic 5β-reductase activity. In contrast, chronic HF feeding is associated with the development of obesity, insulin resistance, and altered HPA axis activity, in the absence of changes in peripheral glucocorticoid metabolism.

Evidence from animal models of genetic obesity suggest that changes in peripheral glucocorticoid metabolism may be important in the pathogenesis of obesity and/or its metabolic sequelae (2, 7, 8, 26). In peripheral tissues, the metabolism of glucocorticoids by the isozymes of 11β-HSD is important in modulating the access of glucocorticoids to the corticosteroid receptors GR and mineralocorticoid receptor, thereby modulating local glucocorticoid effects (23), and in some rodent models tissue-specific alterations in the activity of 11β-HSD-1 may be important in the pathogenesis of obesity (2, 7, 8). Additionally, in Zucker rats, obesity and insulin resistance are associated with increased glucocorticoid inactivation by hepatic A-ring reductases. This may increase glucocorticoid clearance and lead to a compensatory activation of the HPA axis (2).

We demonstrate that alterations in hepatic and adipose 11β-HSD-1 and hepatic A-ring reductase activity occur as a very early response to HF feeding in Wistar rats and are present before the development of obesity. Hepatic 5β-reductase activity was increased after 24 h on the HF diet, and this persisted at 3 wk. No significant changes in 11β-HSD-1 activity were found at 24 or 72 h; however, activity was decreased in both liver and fat after 3 wk of exposure to a HF diet. Intriguingly, these changes in hepatic A-ring reductase activity and 11β-HSD-1 activity were not maintained after 20 wk of HF feeding, despite the development of obesity and marked insulin resistance, in contrast to the alterations in

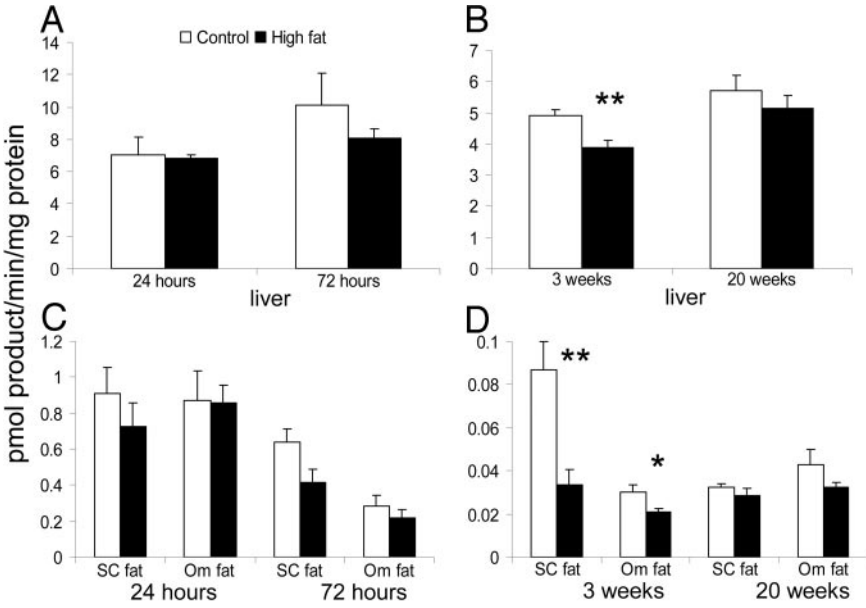

FIG. 4. 11β-HSD-1 activity. Effect of HF diet on 11β-HSD-1 activity after 24 and 72 h and 3 and 20 wk, expressed as pmol product (11-dehydrocorticosterone)/min·mg protein, is shown. Data are mean ± SEM. 11β-HSD-1 activity was measured in tissue homogenates in liver at 24 and 72 h (A) and at 3 and 20 wk (B) and in sc and omental (Om) fat at 24 and 72 h (C) ($P = 0.06$ for sc fat HF *vs.* control at 72 h) and at 3 and 20 wk (D); n = 6 HF and 6 control at 24 and 72 h, 8 HF and 8 control at 3 wk, and 8 HF and 7 control at 20 wk. *, $P < 0.05$; **, $P < 0.005$ for HF *vs.* control.

peripheral glucocorticoid metabolism in animal models of genetic obesity (2, 7). Thus, HF diet exposure is associated with rapid alterations in glucocorticoid metabolism; however, persistent changes in the activities of 11β-HSD-1 and A-ring reductase enzymes are not an invariable consequence of HF-diet-induced obesity. Additionally, the development of obesity can occur independently of altered 11β-HSD-1. Furthermore, it suggests that specific mechanisms for increased 11β-HSD-1 may operate only in some forms of obesity. Conceivably, such heterogeneity may underlie the discrepancies that have been observed between the majority of studies that suggest that idiopathic obesity in humans is associated with increased 11β-HSD-1 mRNA and activity in sc adipose (9–12, 27–29) and other studies that did not find this (30). This is a key issue, particularly if pharmacological inhibitors of 11β-HSD-1 are to be as effective in humans as they appear to be in rodent obesity (16, 31, 32).

Changes in GR density are reported to vary between obesity models (33–35), and we found no changes in peripheral GR mRNA levels with HF feeding. However, there were alterations in HPA axis activity. Thus, after 20 wk on the HF diet, nadir plasma corticosterone levels were unchanged, but peak levels were lower, in agreement with previous studies in rodents (36, 37) and humans (38, 39). This dissociation of altered HPA axis activity from altered peripheral glucocorticoid metabolism in dietary obesity suggests that HF feeding may be associated with alterations in central HPA axis control. In support of this, recent studies have shown that obesity in Zucker rats is associated with site-specific alterations in glucocorticoid-metabolizing enzymes and mineralocorticoid receptors in central feedback sites (40).

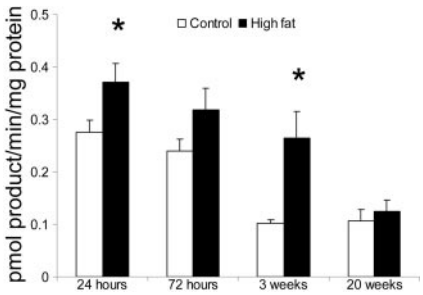

FIG. 5. 5β-Reductase activity. Effect of HF diet on hepatic 5β-reductase activity, expressed as pmol product (5β-tetrahydrocorticosterone)/min·mg protein, at 24 and 72 h and 3 and 20 wk is shown. Data are mean ± SEM. 5β-Reductase activity was measured in liver homogenates at 24 h (n = 6 HF and 6 control), 72 h (n = 6 HF and 6 control), 3 wk (n = 8 HF and 8 control), and 20 wk (n = 8 HF and 7 control). *, $P < 0.05$ for HF *vs.* control.

The mechanisms by which these enzymes are regulated remain unclear. Little is known about the regulation of metabolism of glucocorticoids by A-ring reductase enzymes, although recent evidence suggests that altered 5β-reductase activity in human obesity may be influenced more by nutritional status than by the degree of obesity (41). Transcriptional regulators of 11β-HSD-1 include glucocorticoids, insulin, TNF-α, thyroid hormones, sex steroids, GH, IGF-1, and cytokines (23). Chronic stress or elevated glucocorticoid levels are associated with reduced 11β-HSD-1 activity (42), and peroxisome proliferator-activated receptor (PPAR)-α, PPAR-γ, and liver X receptor agonists attenuate 11β-HSD-1 expression and activity (43–45). Despite the marked alterations in enzyme activity, no changes in 11β-HSD-1 or 5β-reductase mRNA were noted, suggesting that posttranscriptional regulation of enzyme activities may be occurring. Although by 3 wk HF animals were insulin resistant, the observed changes in 11β-HSD-1 and 5β-reductase activities appear independent of insulin resistance, because they are not maintained in the long term despite a further marked decline in insulin sensitivity. Indeed, these findings are in agreement with studies that show that insulin sensitization alone (using metformin or low-dose rosiglitazone) is insufficient to normalize the changes in 11β-HSD-1 enzyme activity in the obese Zucker rat (26), although high-dose PPAR-γ agonists can attenuate adipose tissue 11β-HSD-1 activity in *db/db* mice (43).

Thus, in Wistar rats, short-term HF feeding is associated

with acute changes in glucocorticoid metabolism in the absence of obesity or hyperinsulinemia. This increase in hepatic A-ring reductase activity, together with decreased 11β-HSD-1 activity, predicts reduced local glucocorticoid concentrations and may represent a short-term adaptive mechanism to limit the well established adverse metabolic consequences of HF feeding (2, 17). This notion is supported by the finding that adipose tissue overexpression of 11β-HSD-1 is associated with the development of obesity and features of the metabolic syndrome (8) and that adipose and liver 11β-HSD-1 deficiency (14, 15) and selective pharmacological inhibition of 11β-HSD-1 (16, 31, 32) have beneficial tissue-specific metabolic effects in genetic and dietary models of obesity. Indeed, we have also shown that a marked down-regulation of 11β-HSD-1 activity occurs with HF feeding in mice. This down-regulation is maintained with both short and chronic HF diet exposure (17) and is more pronounced in metabolic disease-resistant strains (17). However, unlike in mice, with chronic HF feeding, Wistar rats exhibit a relative loss of the potentially protective HF-mediated down-regulation of adipose 11β-HSD-1 and no longer exhibit higher 5β-reductase activity in liver. The reason for this difference remains unclear, although the failure to maintain reduced adipose and/or hepatic 11β-HSD-1 activity may be important in the pathogenesis of the metabolic sequelae associated with obesity and, indeed, may mean that Wistar rats are more susceptible than mice to the metabolic consequences of dietary obesity. These data support the hypothesis that variation in susceptibility to obesity and its metabolic consequences may, in part, be caused by interindividual differences in susceptibility to the dysregulation of 11β-HSD-1 (17). Furthermore, they suggest a novel and complementary hypothesis that a similar interindividual variation in hepatic clearance/metabolism of glucocorticoids by 5β-reductase may also contribute to metabolic disease susceptibility.

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