Strain differences in hypothalamic–pituitary–adrenocortical axis function and adipogenic effects of corticosterone in rats

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Abstract

Our aim was to explore the nutritional consequences of functional variations in the hypothalamic–pituitary–adrenocortical (HPA) axis in rats. We first aimed to compare the HPA axis activity and reactivity to stress between Fischer 344 (F344) and LOU/C (LOU) strains that differ in food behavior and metabolism. When compared with F344 rats, LOU rats showed lower corticosterone (Cort) levels across the circadian cycle and after restraint stress. Then, we compared the effects of adrenalectomized (ADX) and Cort substitution after ADX on food intake, body weight gain, body composition, and biochemical parameters related to metabolism and HPA axis function between 1) the F344 rat strain, a model of HPA axis hyperactivity and hyperreactivity to stress, and characterized by a large fat mass; 2) the LOU strain, shown to exhibit hypoactive/hyporeactive HPA axis, reduced fat mass, and resistance to diet-induced obesity; and 3) the Lewis (LEW) strain, a third condition of fat deposition (high) related to HPA axis function (low activity/reactivity). The F344 and LEW strains exhibited classical responses to ADX and Cort. On the contrary, LOU rats showed an apparent insensitivity to ADX. Despite the highest effects of Cort related to glucocorticoid receptor (on thymus weight, corticotropin-releasing factor, or corticosteroid-binding globulin), the LOU strain was insensitive to Cort effects on body weight, liver, and abdominal fat mass. These characteristics could be involved in the leanness, insensitivity to diet-induced obesity, and healthy aging in LOU. Our study shows the relevance of comparing the F344, LOU, and LEW strains to cover the complexity of interactions between metabolism and HPA axis function.

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Introduction

Prevalence of obesity in humans continues to increase in industrialized countries, mainly in children, which constitutes a pressing problem of public health in the industrialized world. Obesity results from an imbalance between caloric intake and energy expenditure, which induces body fat accumulation. This body fat excess leads to severe metabolic disturbances (e.g., hyperglycemia, hyperlipidemia, hyperinsulinemia) that are not only involved in the pathological consequences of obesity such as hypertension, type 2 diabetes, or cancer, but also in aggravating factors of adipogenesis (Girod & Brotman 2003). However, the response to environmental pressure (sedentary life, fat-, and sugar-rich foods) shows high interindividual variation, and the involvement of genetic factors in this variability has been demonstrated by family and twin studies in humans (Li *et al.* 2005, Loos & Rankinen 2005), the comparison of inbred strains (Schalling *et al.* 1999) and selection experiments in animals (Levin *et al.* 1997). A key process involved in this variability lies in the responsivity of the hypothalamic–pituitary–adrenocortical (HPA) axis. Indeed, corticosteroid hormones are implicated in nutritional regulations 1) by their action on central monoaminergic systems and hypothalamic peptides controlling food intake (Gibson 2006), and on the autonomous nervous system regulating thermogenesis and pancreatic secretions (Seematter *et al.* 2004) and 2) by a tissue-specific peripheral alteration of body reserves of carbohydrates (liver and muscles), proteins (muscular mass), and fats (white adipose tissue; Schalling *et al.* 1999).

Our aim was to explore the nutritional consequences of genetic variation in the HPA axis by the comparison of three inbred rat strains, Fischer 344 (F344), LOU/C (LOU), and Lewis (LEW). The F344 strain has been classically used as a model of HPA axis hyperactivity and hyperreactivity to stress, often in comparison with the LEW strain, of which the hypoactive and hyporeactive HPA axis has been associated with its vulnerability to autoimmune diseases (Dhabbar *et al.* 1995). When compared with other strains, the LOU rat strain exhibits healthy aging and lower fat mass (Alliot *et al.* 2002, Veyrat-Durebex *et al.* 2005). We have also

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shown that, in contrast to the diet-induced development of visceral obesity in F344 and LEW rats (Nave *et al*. 2003, Helies *et al*. 2005), LOU rats were resistant to diet-induced obesity (Helies *et al*. 2005). F344 rats have also been described as storing excess triglycerides in liver and muscle. They exhibit insulin and leptin resistance and dyslipidemia associated with abdominal fat accumulation (Levy *et al*. 2002). Considering the disturbances in the function of HPA axis playing a 'permissive' role in metabolic troubles linked to obesity (Ljung *et al*. 2002), we hypothesized that the nutritional differences observed between F344, LOU, and LEW rats could be related to the functional differences revealed in the HPA axis.

The first part of our study aimed to compare the HPA axis function between LOU and F344 rat strains. To that end, we measured corticosterone secretion during the circadian cycle and during recovery following restraint and metabolic stress (insulin-induced hypoglycemia) in both strains. Then, to unravel the network of interactions between the components of the HPA axis and to remove any strain differences in corticosterone availability, F344, LOU, and LEW male rats were adrenalectomized (ADX) and treated with increasing and fixed doses of corticosterone administered by s.c. pellets (0–100 mg). LEW rats were added to the study of F344 and LOU rats in order to introduce a third condition of fat deposition related to the HPA axis function. Therefore, in the second experiment, we compared the effects of ADX and corticosterone substitution on food intake, body composition, parameters related to metabolism (plasma glucose, insulin, free fatty acids, and leptin), and parameters related to the HPA axis function (corticotropin-releasing factor (CRF) expression in the hypothalamic paraventricular nucleus, plasma adrenocorticotropic hormone (ACTH), and corticosteroid-binding globulin (CBG)) between F344 (hyperactive HPA axis, large fat mass), LOU (hypoactive HPA axis, low fat mass), and LEW rats (hypoactive HPA axis, large fat mass).

Materials and Methods

Animals, general

Experiments were performed in accordance with the principles and guidelines of the French legislation on animal welfare: Journal Official number 87-848. All animals were born and raised in the laboratory from LOU (strain previously supplied by Harlan, Gannat, France), LEW, and F344 breeders (Iffa Credo, L'Arbresle, France). Rats were housed in standard collective cages, in a temperature-controlled room (23 ± 1 °C) with a 12 h light: 12 h darkness cycle (lights on at 0700 h). They were fed with SAFE-A03 chow (3·2 kcal/g metabolizable energy, Scientific Animal Food & Engineering, Villemoisson-sur-Orge, France) until weaning at 28 days of age, and subsequently with SAFE-A04 (2·9 kcal/g metabolizable energy). Water was available *ad libitum*. All rats were allowed to adapt to the animal room and placed two per cage for 10 days before the start of the experiments.

Experiment 1

Animals Our first experiment aimed to explore the HPA axis activity and reactivity to psychological and metabolic stress in LOU and F344 rats. It was conducted in 12-week-old males and females. The same rat cohort ($n=8$ per sex and strain) was randomly used for basal and restraint stress measurements. Another cohort ($n=8$ per sex and strain) was used for the insulin-induced hypoglycemic stress. Stress experiments were performed between 0900 and 1100 h.

Blood sampling under basal conditions Rats were removed from their home cage, and blood was taken from a nick of the tail, within 1 min for every 6 h at 0000, 0600, 1200, and 1800 h to measure basal plasma corticosterone levels throughout the circadian cycle.

Restraint stress Rats were restrained for a period of 20 min in plastic bags with a breathing hole (Harvard Apparatus, Ealing, Courtaboeuf, France) between 0900 and 1100 h as described previously (Chaouloff 1994). In <1 min from placement in the restraint, blood samples were collected from a nick of the tail for the measurement of basal (T0), stress peak (T0 + 50 min), and recovery (T0 + 110 min) values of plasma corticosterone concentration (times were chosen following our previous results; Sarrieau & Mormede 1998, Sarrieau *et al*. 1998).

Insulin-induced hypoglycemic stress F344 and LOU rats were fasted overnight before the test. Rats were removed from their home cage, and blood was taken from a nick of the tail in order to measure basal plasma glucose and corticosterone concentrations (T0). Two minutes later, rats were injected i.p. with insulin (Actrapid Humarin, NOVONordisk, Mainz, Germany, 4 IU/kg). Then, blood samples were collected at T0 + 30 min and T0 + 90 min as described above, and in the literature (Karteszi *et al*. 1982, Osako *et al*. 1999).

Plasma measurements Samples were collected in chilled tubes coated with 10% EDTA solution and centrifuged at 4000 ***g*** for 15 min at 4 °C. Plasma was then stored at −80 °C for subsequent measurements of corticosterone and glucose. Plasma glucose levels were measured in duplicate by the glucose oxidase technique with a colorimetric kit (Biolabo, Maizy, France). Corticosterone concentrations were measured in duplicate by RIA with a commercial kit (MP Biomedicals, Orangeburg, NY, USA).

Data analysis Results were expressed as means ± S.E.M. Data were analyzed by a three-way ANOVA with strain (F344, LOU) and sexes as two between-subject factors, and time as within-repeated factor. *Post hoc* Newman–Keuls tests were performed when ANOVA was significant ($P<0·05$).

Experiment 2

To test the assumption of an association between differences in the HPA axis function and nutritional differences in rats, we compared the effects of ADX and corticosterone substitution after ADX on food intake, body weight gain, body composition, and biochemical parameters related to metabolism and the HPA axis function between the F344, LOU, and LEW rat strains. We restricted this study to a male rat population in order to lighten the results discussed in this publication, and to allow us to refer to previous works concerning metabolism or HPA axis function, which were mostly carried out in males.

Animals Bilateral ADX (40 rats per strain, in two successive replications) was performed under pentobarbital anesthesia (0·1 ml/100 g of body weight) on 12-week-old LOU, F344, and LEW males. Sham-operated animals (eight rats per strain, in two successive replications) were subjected to anesthesia and bilateral laparotomy. Incisions were closed with surgical gut and wound clips. At the time of surgery, a pellet was implanted subcutaneously in the interscapular region of each rat. Sham-operated animals and eight ADX rats of each strain received paraffin pellets weighing about 100 mg (Sham and ADX+0 groups respectively). The other ADX rats were given 100 mg fused pellets composed of various percentages of corticosterone and cholesterol, and distributed among four experimental groups, ADX+12·5, ADX+25, ADX+50, and ADX+100, treated with pellets containing 12·5, 25, 50, and 100% of corticosterone respectively. The pellets remained *in situ* until killing. The percentages of corticosterone used in pellets were chosen in order to cover mineralocorticoid receptor (MR; from 12·5 to 25%) and then glucocorticoid receptor (GR; from 25%) activation, and according to previous studies investigating physiological regulations by corticosterone in rats (Levin *et al.* 1987, Duclos *et al.* 2004). All rats were given saline (0·5% NaCl) after surgery. Animals, food, and drinking fluid were weighed every 2 days for 2 weeks. Then, rats were fasted overnight, weighed, and killed by decapitation (from 0900 to 1100 h).

Body composition Four depots of adipose tissue were carefully removed and weighed: epididymal (around testis and ductus deferens), retroperitoneal (along the posterior wall, from the kidney to the hip region), mesenteric (along the mesentery, starting from the lesser curvature of the stomach and ending at the sigmoid colon), and inguinal (s.c. fat between the lower part of the rib cage and the thighs). Liver and thymus were also excised and weighed.

CRF expression in the paraventricular nucleus Brains were dissected out, embedded in Tissue Tek (Sakura Finetek Europe BV, Zoeterwoude, The Netherlands), frozen in isopentane cooled at −50 °C, and stored at −80 °C. Coronal sections were cut at 12 μm in a cryostat, collected onto gelatinized slides, and stored at −80 °C until use. Hybridization procedure was conducted as described previously (Ribot *et al.* 2003) with a radioactive antisense cRNA CRF probe. The hybridization signal was analyzed by macro-autoradiography over the paraventricular nucleus area in three adjacent series of eight sections spaced 72 μm.

Hormonal and biochemical parameters ACTH, CBG, corticosterone, glucose, nonesterified free fatty acids (FFA), insulin, and leptin concentrations were measured in plasma. Trunk blood was collected into chilled tubes coated with 10% EDTA solution and centrifuged (4000 ***g***, 15 min, 4 °C) in order to extract plasma, which was stored at −80 °C.

HPA axis Plasma ACTH was measured in duplicate with an RIA kit (ACTH Radioisotopic Assay, Nichols Institute Diagnostics, San Juan Capistrano, CA, USA).

CBG concentration was measured by competitive protein binding between cold corticosterone and ^{3}H-corticosterone in a 50 μl plasma volume (Johnson *et al.* 2005). Endogenous steroids were first removed with dextran-coated charcoal (DCC). After centrifugation to remove DCC (9000 ***g***, 15 min, 4 °C), the supernatants were diluted 100 times in a Tris–HCl buffer (pH 7·4) and incubated with 1·50–200 nM ^{3}H-corticosterone in the presence (non-specific binding) or absence (specific binding) of 0·8–100 μM unlabeled steroid for 20 min at 40 °C, and for 2 h at 4 °C. Separation of CBG-bound steroid was achieved by incubation with DCC (10 min at 4 °C), followed by centrifugation (2000 ***g***, 15 min at 4 °C). The supernatants (200 μl) were added to 3·5 ml scintillating liquid and subjected to β-counting. Saturation curves were analyzed by Scatchard analysis with Prism software (GraphPad, San Diego, CA, USA).

Corticosterone concentrations were determined as described in experiment 1.

Metabolism Plasma glucose and FFA were measured in duplicate with colorimetric kits (Biolabo and Wako Chemicals, Neuss, Deutschland respectively). Plasma insulin and leptin were measured in triplicate with RIA kits (Insik 5, DiaSorin SA, Antony, France, and Linco Research, St Charles, MO, USA respectively).

Data analysis Results were expressed as means ± S.E.M. Data were analyzed by a three-way ANOVA with strain (F344, LOU and LEW) and treatments (effect of corticosterone substitution treatments versus Sham or ADX+0 values) as two between-subject factors, and time as within-repeated factor when necessary (food and fluid intake, body weight gain). *Post hoc* Newman–Keuls tests were performed when ANOVA was significant ($P<0·05$).

Results

Experiment 1

The HPA axis activity and reactivity to psychological and metabolic stress in LOU and F344 rats were explored in this experiment.

Circadian cycle of corticosterone secretion In both sexes, F344 rats exhibited a higher secretion of corticosterone than LOU rats during the darkness period (strain effect: $F_{1,119}=27{\cdot}82$, $P<0{\cdot}001$; strain×time interaction: $F_{3,117}=4{\cdot}88$, $P<0{\cdot}01$; Fig. 1a). The circadian cycle in female rats was more pronounced than in males (sex×time interaction: $F_{3,117}=5{\cdot}17$, $P<0{\cdot}01$).

Response to a restraint stress The restraint stress increased plasma corticosterone in both sexes and both strains (time effect: $F_{2,85}=66{\cdot}50$, $P<0{\cdot}001$; Fig. 1b), but to a greater extent in females than in males (sex×time interaction: $F_{2,85}=7{\cdot}53$, $P<0{\cdot}001$), and more in F344 than in LOU rats (strain×time interaction: $F_{2,85}=4{\cdot}45$, $P<0{\cdot}01$).

Response to insulin-induced hypoglycemia *Plasma glucose levels*. Insulin decreased glucose levels 30 and 90 min after injection in both sexes and both strains (time effect: $F_{2,88}=154{\cdot}39$, $P<0{\cdot}001$; Fig. 2a), but significantly more in LOU than in F344 rats (strain×time interaction: $F_{2,88}=25{\cdot}08$, $P<0{\cdot}001$).

Plasma corticosterone levels. F344 rats exhibited higher plasma corticosterone concentrations than LOU rats (strain effect: $F_{1,89}=6{\cdot}90$, $P<0{\cdot}05$; Fig. 2b). Plasma corticosterone was increased by insulin injection in both sexes (time effect: $F_{2,88}=113{\cdot}37$, $P<0{\cdot}001$), but to a greater extent in females than in males (sex×time interaction: $F_{2,88}=17{\cdot}24$, $P<0{\cdot}001$).

Experiment 2

This experiment allowed us to compare the effects of ADX and corticosterone substitution after ADX on food intake, body weight gain, body composition, and biochemical parameters related to metabolism and the HPA axis function between the F344, LOU, and LEW rat strains.

***In vivo* measurements** *Saline and food intake*. ADX increased strongly the saline intake of the three strains (treatment effect: $F_{5,67}=5{\cdot}24$, $P<0{\cdot}001$; Table 1), and particularly in LEW rats (strain×treatment interaction: $F_{10,134}=3{\cdot}21$, $P<0{\cdot}05$). Saline intake was not altered by corticosterone treatment in any strain. Food intake was not significantly different between groups or between treatments.

Body weight gain. Initial body weight did not differ across experimental groups in each strain. The percentages of weight gain or loss are presented in Table 1. During the first day of experimental procedures, F344 and LEW rats showed higher body weights than LOU rats (strain effect: $F_{2,142}=264{\cdot}35$, $P<0{\cdot}001$). ADX induced weight loss in F344 and LEW rats

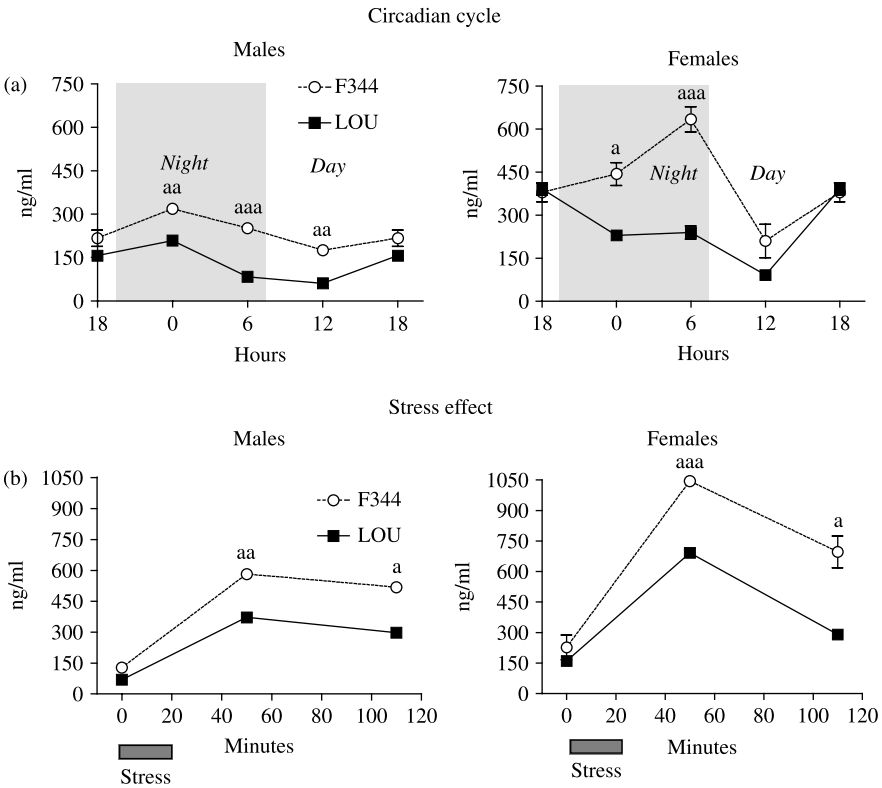

Figure 1 Plasma corticosterone (a) during the circadian cycle and (b) after a restraint stress. Strain differences: $^{a}P<0{\cdot}05$, $^{aa}P<0{\cdot}01$, $^{aaa}P<0{\cdot}001$.

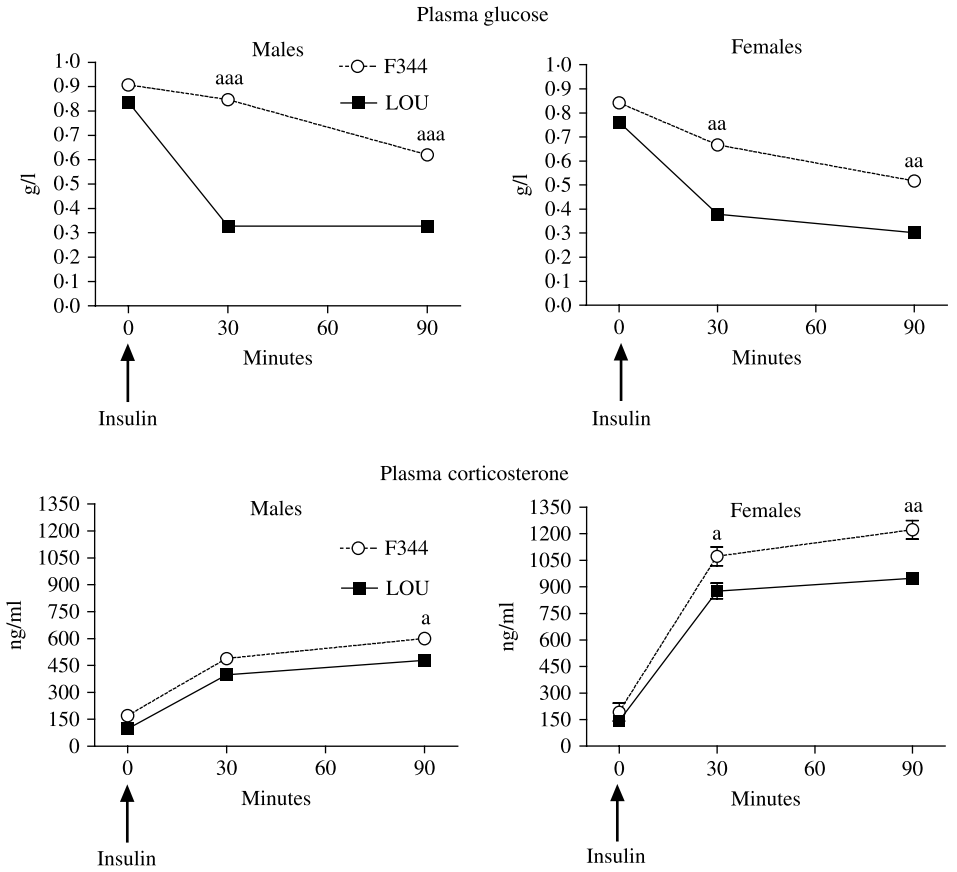

Figure 2 Plasma glucose and corticosterone concentrations in response to the hypoglycemic stress induced by insulin. F344 and LOU rats were fasted overnight before the test. Rats were removed from their home cage and blood was taken from a nick of the tail before (T0) and after insulin injection at 4 IU/kg (T0+30 min, T0+90 min). Strain differences: $^{a}P<0{\cdot}05$, $^{aa}P<0{\cdot}01$, $^{aaa}P<0{\cdot}001$.

Table 1 Total saline intake, food intake, and body weight (BW) gain during the 2 weeks after adrenalectomy (ADX) in Fischer 344 (F344), LOU, and Lewis (LEW) rats

		Treatment					
	Strain	ADX+0	ADX+12·5	ADX+25	ADX+50	ADX+100	Sham
Data							
Saline intake	F344	$57{\cdot}4\pm12{\cdot}4^{a}$	$70{\cdot}8\pm12{\cdot}5^{a}$	$72{\cdot}1\pm8{\cdot}6^{a}$	$51{\cdot}4\pm5{\cdot}5^{a}$	$55{\cdot}0\pm1{\cdot}7^{a}$	$47{\cdot}8\pm2{\cdot}5^{a}$
(ml/100 g BW/14 days)	LOU	$58{\cdot}9\pm8{\cdot}9^{a}$	$77{\cdot}3\pm13{\cdot}7^{a}$	$63{\cdot}3\pm3{\cdot}9^{a}$	$58{\cdot}6\pm1{\cdot}9^{a}$	$65{\cdot}1\pm7{\cdot}4$	$62{\cdot}0\pm7{\cdot}9$
	LEW	$88{\cdot}0\pm5{\cdot}0^{b}$	$106{\cdot}7\pm13{\cdot}0^{b}$	$107{\cdot}9\pm12{\cdot}0^{b}$	$86{\cdot}4\pm2{\cdot}2^{b}$	$80{\cdot}4\pm4{\cdot}9$	$51{\cdot}0\pm1{\cdot}9^{\dagger}$
Food intake	F344	$37{\cdot}4\pm3{\cdot}4$	$52{\cdot}1\pm5{\cdot}5$	$54{\cdot}0\pm7{\cdot}7$	$47{\cdot}9\pm6{\cdot}4$	$55{\cdot}3\pm8{\cdot}9$	$69{\cdot}5\pm15{\cdot}6$
(g/100 g BW/14 days)	LOU	$47{\cdot}6\pm12{\cdot}0$	$61{\cdot}2\pm12{\cdot}6$	$58{\cdot}1\pm15{\cdot}1$	$58{\cdot}6\pm13{\cdot}6$	$60{\cdot}4\pm12{\cdot}2$	$62{\cdot}4\pm13{\cdot}7$
	LEW	$39{\cdot}7\pm8{\cdot}2$	$53{\cdot}6\pm9{\cdot}0$	$57{\cdot}0\pm9{\cdot}8$	$62{\cdot}0\pm12{\cdot}2$	$65{\cdot}1\pm14{\cdot}1$	$64{\cdot}8\pm12{\cdot}7$
BW gain	F344	$-11{\cdot}0\pm5{\cdot}0^{a}$	$-2{\cdot}3\pm2{\cdot}6^{*}$	$-3{\cdot}5\pm2{\cdot}4^{*}$	$-6{\cdot}6\pm2{\cdot}6^{a}$	$-1{\cdot}9\pm1{\cdot}4^{a,*}$	$1{\cdot}5\pm1{\cdot}6^{\dagger}$
(g/100 g BW/14 days)	LOU	$-0{\cdot}5\pm2{\cdot}1^{b}$	$-2{\cdot}7\pm4{\cdot}4$	$0{\cdot}4\pm1{\cdot}3$	$-3{\cdot}0\pm3{\cdot}3^{a}$	$-10{\cdot}8\pm2{\cdot}2^{b,\dagger}$	$-0{\cdot}8\pm1{\cdot}4$
	LEW	$-8{\cdot}0\pm3{\cdot}1^{a}$	$1{\cdot}6\pm1{\cdot}8^{\dagger}$	$0{\cdot}1\pm1{\cdot}4^{*}$	$4{\cdot}9\pm2{\cdot}1^{b,\ddagger}$	$0{\cdot}1\pm3{\cdot}1^{a,*}$	$2{\cdot}9\pm2{\cdot}7^{\dagger}$

Sham-operated and ADX rats of each strain received paraffin pellets weighing about 100 mg (Sham and ADX+0 groups respectively). The other ADX rats were given 100 mg fused pellets composed of various percentages of corticosterone and cholesterol, and distributed among four experimental groups, ADX+12·5, ADX+25, ADX+50, and ADX+100, treated with pellets containing 12·5, 25, 50, and 100% of corticosterone respectively for 2 weeks. For the same treatment, strains with different letters differ significantly ($P<0{\cdot}05$). Differences from the ADX group: $^{*}P<0{\cdot}05$, $^{\dagger}P<0{\cdot}01$, $^{\ddagger}P<0{\cdot}001$.

only (strain×treatment interaction: $F_{10,134}=2{\cdot}56$, $P<0{\cdot}01$). Corticosterone treatment restored dose-dependently the weight gain to sham values in these strains. On the contrary, neither ADX nor low doses of corticosterone induced any effect on the weight gain of LOU rats. The highest corticosterone dose (ADX+100) induced weight loss in this strain (treatment effect in LOU rats: $F_{5,43}=12{\cdot}84$, $P<0{\cdot}01$). Body weight curves showing the repeated data of each experimental group are presented in a supplementary figure available online at http://joe.endocrinology-journals.org/content/vol195/issue3/.

Body composition *Body weight at killing*. LEW sham rats showed a greater body weight than F344 (strain effect: $F_{2,142}=215{\cdot}33$, $P<0{\cdot}001$) or LOU rats ($P<0{\cdot}001$ versus F344 and LEW; Fig. 3a). ADX induced a strong weight loss in F344 and LEW rats only (strain×treatment interaction: $F_{10,134}=1{\cdot}99$, $P<0{\cdot}05$), whereas the substitution treatment by corticosterone restored the body weight of ADX groups to sham values. On the contrary, in *post hoc* analyses, the treatment effect did not reach significance in the LOU strain.

Thymus. ADX increased the thymus weight of the three strains (treatment effect: $F_{5,139}=20{\cdot}79$, $P<0{\cdot}001$; Fig. 3b), but to a greater extent in LOU (strain×treatment interaction: $F_{10,134}=2{\cdot}17$, $P<0{\cdot}05$; treatment effect in LOU: $F_{5,43}=17{\cdot}02$, $P<0{\cdot}001$) and LEW rats ($F_{5,43}=7{\cdot}73$, $P<0{\cdot}001$) than in F344 rats ($F_{5,43}=4{\cdot}03$, $P<0{\cdot}05$). Corticosterone treatment restored thymus weight to the sham value in LEW rats, whereas it induced a thymolysis beyond control values in LOU and F344 ADX+100 groups (Fig. 3b).

Liver. Liver was heavier in F344 than LEW rats (strain effect: $F_{2,16}=5{\cdot}51$, $P<0{\cdot}01$; Fig. 3c). ADX decreased liver weights (treatment effect: $F_{5,139}=3{\cdot}33$, $P<0{\cdot}01$), and the substitution corticosterone treatment dose dependently restored them to sham values, in F344 and LEW rats only (strain×treatment interaction: $F_{10,134}=3{\cdot}34$, $P<0{\cdot}001$). The *post hoc* treatment effect did not reach significance in LOU rats.

Fat mass. Abdominal fat depots. Retroperitoneal, epididymal, and mesenteric fat depots were weighed and expressed in percentages of body weight, as shown in Fig. 4a–c. Irrespective of the experimental condition, LOU rats exhibited lower retroperitoneal fat mass than F344 and LEW rats (strain effect: $F_{2,142}=129{\cdot}17$, $P<0{\cdot}001$). At moderate-to-high corticosterone concentrations, LOU rats also showed lower epididymal and mesenteric fat depots than F344 and LEW rats (strain effects: $F_{2,142}=239{\cdot}19$ and $114{\cdot}97$ respectively, $P<0{\cdot}001$). For all abdominal fat depots, the lipolytic effect of ADX and the adipogenic effect of corticosterone were measured in F344 and LEW rats only (strain×treatment interactions $F_{10,134}=4{\cdot}70$, $5{\cdot}10$, $4{\cdot}64$ in retroperitoneal, epididymal, and mesenteric fat respectively, $P<0{\cdot}001$).

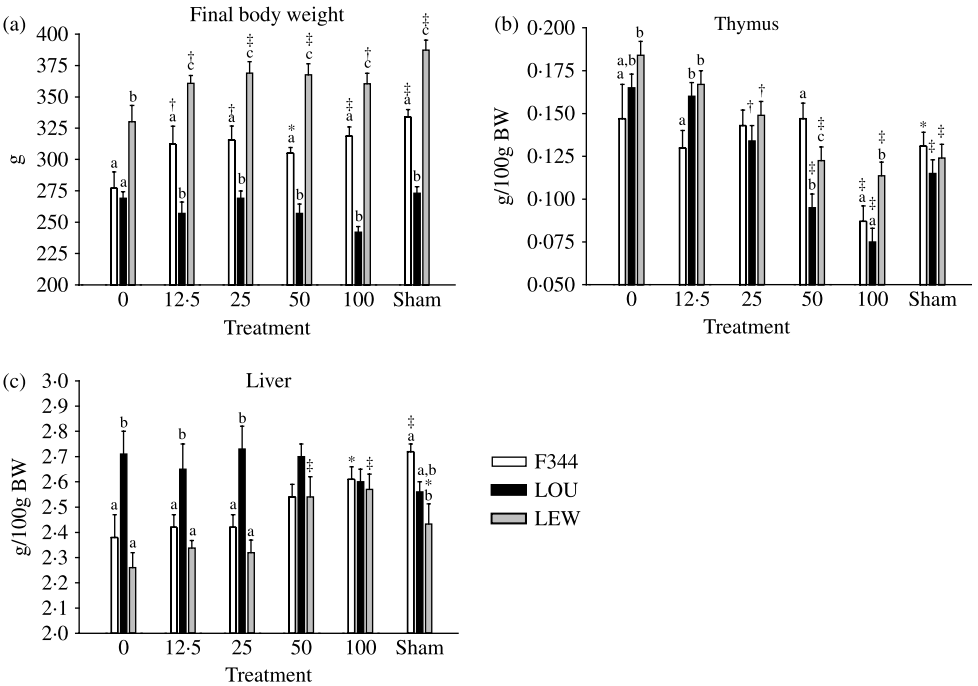

Figure 3 (a) Final body weight, (b) thymus, and (c) liver weights in F344, LOU, and LEW rats. ADX groups are represented on the abscissa by the percentage of corticosterone in their s.c. pellets (0, 12·5, 25, 50, and 100% respectively). Means were expressed according to the body weight (g/100 g BW). For the same treatment, strains with different letters differ significantly ($P<0{\cdot}05$). Differences from the ADX group: $^{*}P<0{\cdot}05$, $^{\dagger}P<0{\cdot}01$, $^{\ddagger}P<0{\cdot}001$.

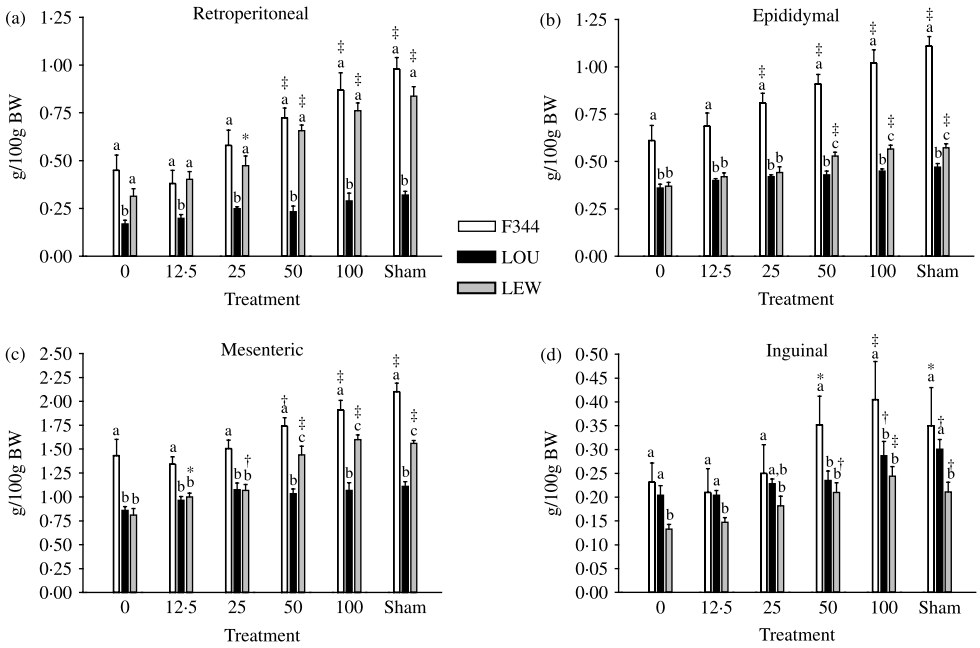

Figure 4 Percentages of abdominal fat depots (a–c) and inguinal fat depot (d, representative of the s.c. fat) in F344, LOU, and LEW rats. ADX groups are represented on the abscissa by the percentage of corticosterone in their s.c. pellets (0, 12·5, 25, 50, and 100% respectively). Means were expressed according to the body weight (g/100 g BW). For the same treatment, strains with different letters differ significantly ($P<0·05$). Differences from the ADX group: $^{*}P<0·05$, $^{\dagger}P<0·01$, $^{\ddagger}P<0·001$.

Inguinal fat depot (representative of the s.c. fat). LEW sham rats had lower inguinal fat depots than F344 or LOU rats (strain effect: $F_{2,16}=6·15$, $P<0·01$). The three strains presented the same regulation profile of their inguinal fat mass by corticosterone (Fig. 4d): a decrease induced by ADX and a dose-dependent restoration to control values by substitution treatment (treatment effect: $F_{5,139}=12·16$, $P<0·001$; no strain×treatment interaction).

CRF expression in the paraventricular nucleus CRF expression in the hypothalamic paraventricular nucleus was higher in LOU than that in F344 and LEW sham rats (strain effect: $F_{2,16}=9·14$, $P<0·001$). It was increased by ADX (treatment effect: $F_{5,120}=12·99$, $P<0·001$; $P<0·01$ versus sham value in F344, $P<0·001$ in LEW, NS in LOU; no strain×treatment interaction), and was inhibited by the 50 and 100 mg corticosterone pellets in the same proportions in the three strains (Fig. 5a).

Hormonal and biochemical parameters *HPA axis.* ACTH. ADX increased ACTH concentration (treatment effect: $F_{5,139}=13·47$, $P<0·001$), but to a greater extent in the F344 strain than in the others (strain×treatment interaction: $F_{10,134}=2·62$, $P<0·01$). The corticosterone replacement treatment decreased ACTH concentration when compared with ADX value from the lowest dose without any restoration to the sham value in F344 rats, with restoration to the sham level from the lowest dose in LOU rats, and from the 50 mg dose in LEW rats (Fig. 5b).

CBG. F344 and LEW rats presented higher CBG concentrations than LOU rats (strain effect $F_{2,142}=21·96$, $P<0·001$). Plasma CBG concentrations were increased by ADX in the three strains (global treatment effect: $F_{5,139}=9·86$, $P<0·001$; ADX effect: $F_{1,47}=29·96$, $P<0·001$), but to a greater extent in F344 and LOU than in LEW rats (strain×treatment interaction in ADX effect: $F_{2,46}=3·59$, $P<0·05$; Fig. 5c).

Corticosterone. All ADX rats showed plasma corticosterone concentrations below assay detection limits (Fig. 5d). Substitution treatments induced the same dose-dependent increase profile of plasma corticosterone concentration in the three strains (from $34·1\pm4·5$ to $188·3\pm17·2$ ng/ml, treatment effect: $F_{5,139}=48·80$, $P<0·001$; no strain×treatment interaction). In sham condition, LOU rats showed lower corticosterone concentrations than F344 and LEW rats (strain effect: $F_{2,16}=9·15$, $P<0·001$).

Metabolism. Glucose. Glucose levels were lower in LEW than those in F344 and LOU rats (strain effect: $F_{2,142}=15·51$, $P<0·001$; Table 2). The global treatment effect was significant ($F_{5,139}=3·03$, $P<0·05$), but *post hoc* analyses revealed a significant treatment effect in LOU only ($F_{5,43}=2·50$, $P<0·05$). Glucose levels were reduced by ADX ($F_{5,43}=2·98$, $P<0·05$) and restored to sham levels from the 50 mg dose of corticosterone ($P<0·05$) in LOU.

Insulin. Neither treatment nor strain affected plasma insulin concentration.

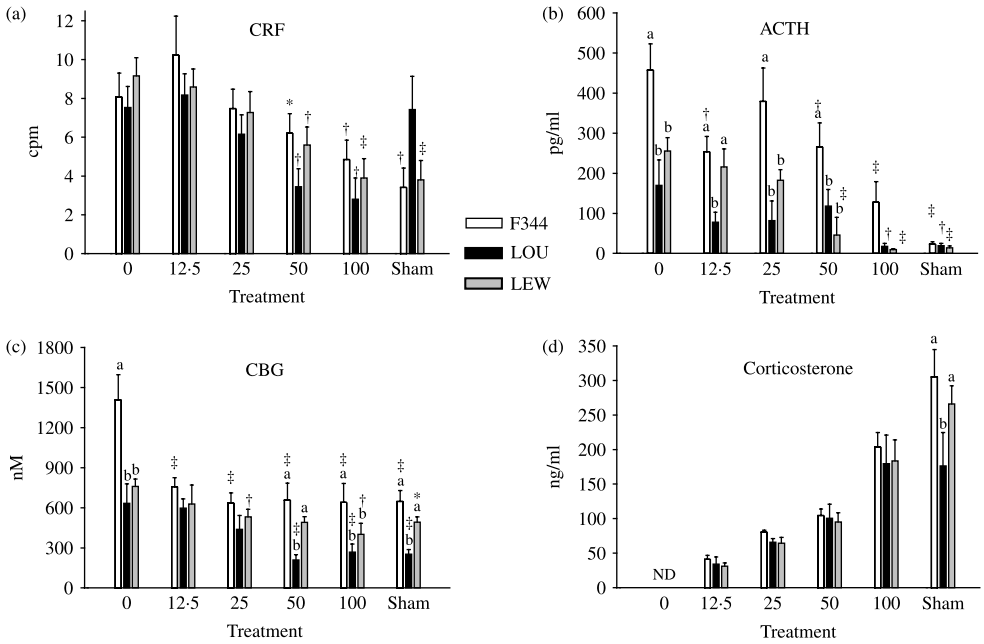

Figure 5 HPA axis parameters: CRF expression in the paraventricular nucleus (a) and plasma concentrations of ACTH (b), CBG (c) and corticosterone (d) in F344, LOU, and LEW rats. ADX groups are represented on the abscissa by the percentage of corticosterone in their s.c. pellets (0, 12·5, 25, 50, and 100% respectively). For the same treatment, strains with different letters differ significantly ($P<0·05$). Differences from the ADX group: $^{*}P<0·05$, $^{\dagger}P<0·01$, $^{\ddagger}P<0·001$. ND, not detectable.

Free fatty acids. Globally, the LOU strain presented lower plasma FFA concentration than the other strains (strain effect: $F_{2,138}=4·70$, $P<0·05$; Table 2).

Leptin. ADX decreased and high corticosterone increased plasma leptin concentrations in F344 and LEW rats only (strain×treatment interaction: $F_{10,131}=6·67$, $P<0·001$; treatment effects: $F_{5,40}=17·14$, $P<0·001$ and $F_{5,43}=2·97$, $P<0·05$ respectively; Table 2).

Discussion

The first part of our study aimed to compare the HPA axis activity and reactivity to stress between F344 and LOU rats, previously shown to differ in food behavior and metabolism (Couturier *et al.* 2002, Helies *et al.* 2005). When compared with F344 rats, animals from the LOU strain showed lower corticosterone levels across the circadian cycle and during recovery following restraint

Table 2 Plasma levels of metabolic parameters

		Treatment					
	Strain	ADX+0	ADX+12·5	ADX+25	ADX+50	ADX+100	Sham
Data							
Glucose (g/l)	F344	1·08±0·17	0·92±0·07^{a}	0·89±0·11^{a}	1·11±0·04^{a}	1·07±0·02^{a,b}	1·33±0·05^{a}
	LOU	0·87±0·07	1·04±0·11^{a}	1·06±0·23^{a}	1·19±0·14^{a,*}	1·31±0·11^{a,†}	1·22±0·16^{a,*}
	LEW	0·88±0·16	0·70±0·07^{b}	0·61±0·10^{b}	0·81±0·09^{b}	0·84±0·08^{b}	0·89±0·09^{b}
Insulin (ng/ml)	F344	1·98±0·71	2·39±0·61	2·74±0·64	2·05±0·52	2·93±0·82	4·00±0·89
	LOU	1·76±0·53	2·97±1·22	2·32±1·08	2·45±0·73	2·25±0·36	3·31±0·79
	LEW	2·52±0·70	2·94±0·68	3·71±0·97	3·02±0·79	4·25±0·71	4·17±0·51
FFA (mEq/l)	F344	0·59±0·04	0·72±0·06	0·69±0·02^{a,b}	0·75±0·04^{a}	0·72±0·08^{a}	0·64±0·10
	LOU	0·68±0·15	0·78±0·17	0·60±0·15^{b}	0·58±0·08^{b}	0·50±0·06^{b}	0·55±0·06
	LEW	0·78±0·05	0·72±0·04	0·83±0·08^{a}	0·76±0·07^{a}	0·82±0·10^{a}	0·67±0·07
Leptin (ng/ml)	F344	0·47±0·25	0·24±0·27	0·39±0·23	0·76±0·25	1·39±0·23^{a,†}	3·05±0·21^{a,‡}
	LOU	0·23±0·11	0·25±0·13	0·56±0·15	0·72±0·39	0·53±0·12^{b}	0·37±0·13^{b}
	LEW	0·53±0·15	0·52±0·06	0·28±0·10	0·68±0·28	1·14±0·26^{a,*}	1·15±0·43^{c,*}

Sham and ADX rats of each strain received paraffin pellets. ADX+12·5, ADX+25, ADX+50, and ADX+100 groups were treated with pellets containing 12·5, 25, 50, and 100% of corticosterone respectively for 2 weeks. For the same treatment, strains with different letters differ significantly ($P<0·05$). Differences from the ADX group: $^{*}P<0·05$, $^{\dagger}P<0·01$, $^{\ddagger}P<0·001$.

stress. In response to insulin treatment, glucose levels decreased in both sexes and strains, but to a greater extent in LOU than in F344 rats, in accordance with the insulin resistance described by Levy *et al.* (2002) in old F344 rats. Plasma corticosterone concentration was increased by insulin-induced hypoglycemia in both strains, but stayed higher in F344 than in LOU rats. Considering the disturbances in the HPA axis function playing a 'permissive' role in metabolic disturbances associated with obesity (Ljung *et al.* 2002), the nutritional differences observed between F344 and LOU rats could be associated with the functional differences in their HPA axis. To test this assumption, we compared the effects of ADX and corticosterone substitution after ADX on food intake, body weight gain, body composition, and biochemical parameters related to metabolism and HPA axis function between F344, LOU, and LEW males. LEW rats were added to the study of F344 and LOU rats in order to introduce a third condition of fat deposition related to HPA axis function. Indeed, the LEW strain has been described classically to exhibit a low HPA axis activity and reactivity to stress (similar to LOU; Dhabhar *et al.* 1995) and a high percentage of body fat mass (similar to F344; Nave *et al.* 2003).

According to Devenport *et al.* (1989), the effects of corticosterone on body weight follow a bell-shaped curve with increasing concentrations of the hormone as a result of different components of corticosterone actions on consummatory behaviors and metabolism (Tempel & Leibowitz 1994, Santana *et al.* 1995). Optimal growth is reached at physiological levels. Body weight gain is reduced by ADX, restored to normal by low doses of corticosterone or MR agonists (Devenport *et al.* 1991), and decreased by high doses of corticosterone or GR agonists via catabolic effects on fat and protein stores (Santana *et al.* 1995). As we described previously (Helies *et al.* 2005), the body weight of F344 and LEW sham rats was markedly greater than that of LOU sham rats, in spite of an equal nose–tail length. F344 and LEW rats exhibited the classical weight loss after ADX, partly related to a decrease in their food intake. On the contrary, as described previously in Brown Norway rats (Marissal-Arvy *et al.* 2004), LOU rats showed an apparent insensitivity to ADX, suggesting the involvement of compensatory mechanisms for the lack of MR activation in these rats. The lowest doses of corticosterone (MR effect) restored the weight gain of F344 and LEW rats to sham values. A strong decrease in body weight gain was induced by the highest dose of corticosterone in LOU rats only, associated with a slight decrease in their food intake, suggesting that this strain is more sensitive to the catabolic effects exerted by corticosterone via GR. Thymus weight has been described classically as an index of GR activation in rodents (Levin *et al.* 1987, Cador *et al.* 1993, Young *et al.* 1995, Duclos *et al.* 2004). At equal corticosterone dose, thymolysis reflects GR sensitivity and/or efficiency. It reached significance from the 25 mg corticosterone treatment in LOU and LEW rats, whereas it was significant in F344 rats with the 100 mg dose only. This marked GR effect of corticosterone in the LOU and LEW strains could involve a higher bioavailability of corticosterone (rise in the free active fraction of the steroid) and/or a greater efficiency of GR transduction processes (Miller *et al.* 1997).

The body fat content and distribution are mainly controlled by the sympathetic nervous system (SNS, lipolytic; Bartness & Bamshad 1998, Fliers *et al.* 2003) and the HPA axis (Bjorntorp 1991). Low doses of corticosterone or aldosterone via MR have been described to exert lipogenic effects in rats (Devenport *et al.* 1991). Glucocorticoids are described classically to induce lipolysis and proteolysis to provide energetic fuels for the stress response (Smith & Vale 2006). They are also known to increase preadipocyte differentiation (Joyner *et al.* 2000) and adipogenesis via GR synergistically with insulin in the abdominal area (Fried *et al.* 1993, Ramsay 1996). The proteolysis induced by GR activation is more marked than the lipolysis, altering the body distribution of lipid stores to increase abdominal fat mass at the expense of muscular mass (Sjögren *et al.* 1994, Abate & Garg 1995, Wajchenberg 2000). A recent study conducted by Zhang *et al.* (2006) showed that specific activation of the hypothalamic MR stimulates the SNS. On the contrary, glucocorticoids are described classically to decrease norepinephrine synthesis, release, and reuptake via GR in the central nervous system (Kvetnansky *et al.* 1995). The HPA axis and SNS also interact continuously in periphery as, according to biological conditions, i.e. the presence of insulin or not, glucocorticoids can inhibit or potentiate catecholamine lipolytic action respectively (Ottosson *et al.* 2000, Girod & Brotman 2003). In sham condition, LOU rats showed a lower abdominal fat mass than F344 and LEW rats. The resistance to diet-induced obesity and the leanness of LOU rats could originate in the HPA axis hypoactivity observed in this study, and/or sympathetic hyperactivity (as suggested recently by Perrin *et al.* 2003). ADX decreased the percentage of body fat mass of F344 and LEW rats. On the contrary, in LOU rats, we did not measure any effect of ADX on abdominal fat depot. This characteristic might partly contribute to the lack of weight loss after ADX in this strain. As expected according to the involvement of glucocorticoids in the regulation of fat depots summed up as above, and as described classically in other rat strains (Devenport *et al.* 1991, Tempel & Leibowitz 1994), the body fat proportion was dose dependently increased by the substitution corticosterone treatment in the F344 and LEW strains. This effect was induced by different corticosterone doses according to the strain and the fat pad considered: 12·5 mg pellet in LEW versus 50 mg in F344 rats for the mesenteric fat depot, 25 mg versus 50 mg respectively for the retroperitoneal fat, 50 mg versus 25 mg for the epididymal fat, and 50 mg in both strains for the inguinal (s.c.) fat. Therefore, F344/LEW and fat mass differences could involve various MR/GR actions on a background of different nervous (SNS) and/or hormonal (sexual steroids, insulin) regulations (McCarty 2001). On the contrary, LOU rats were insensitive to the adipogenic effect exerted by corticosterone on abdominal fat. This model is all the more interesting since the visceral fat mass is involved in the metabolic disturbances linked to obesity (McCarty 2001, Pitombo *et al.* 2006), which

are moreover aggravated by glucocorticoids. At last, this process might contribute to the greater weight loss induced by the highest dose of corticosterone in LOU, by a loss of muscular mass not compensated by an increasing abdominal fat depot. On the contrary, corticosterone induced the expected increase of s.c. fat in LOU. These differential effects of corticosterone on fats illustrate regional specificity (visceral versus s.c.) of the regulations exerted by the HPA axis and SNS. For instance, they could involve the greater norepinephrinergic tone (i.e., lipolysis) suggested in abdominal versus peripheral fat by Bartness & Bamshad (1998), associated or not with a high central MR activation stimulating SNS tone in LOU rats (Zhang *et al.* 2006). Previously, we performed a genetic study on a Brown Norway×F344 F2 population that revealed a gain of function mutation in the Brown Norway MR, which was involved in the insensitivity of Brown Norway rats to ADX (Marissal-Arvy *et al.* 2004). An MR harboring a gain of function could potentiate GR-related catabolic and feedback effects, notably by heterodimerization (Derfoul *et al.* 2000). It could also induce a high central stimulation of the SNS (Zhang *et al.* 2006) in lean rat strains such as the Brown Norway and LOU strains. Further investigations will be conducted to find the possible common denominator between functional differences in corticosteroid receptors, specific involvement of MR versus GR in the central regulation of SNS by corticosterone, and the nutritional characteristics of our rat models.

As described classically in the literature (Kumar & Leibowitz 1988), the liver weight was decreased by ADX and returned to control by high doses of corticosterone via GR in F344 and LEW rats. On the contrary in LOU, as for body weight and fat, the liver weight was modified neither by ADX nor by corticosterone substitution treatment.

To complete the comparison of the regulation exerted by corticosterone on body composition between F344, LOU, and LEW rats, we investigated corticosterone action on parameters related to the HPA axis (hypothalamic CRF expression, plasma ACTH, and CBG) and metabolism (plasma glucose, insulin, FFA, and leptin). The corticosterone effect on HPA axis is the reflection of MR/GR activation and the negative feedback exerted by this steroid on its own secretion. In sham condition, CRF expression in the hypothalamic paraventricular nucleus did not differ across the three strains, confirming the previous results obtained by Rivest and Rivier in F344 and LEW males (Rivest & Rivier 1994). ADX increased CRF expression in F344 and LEW rats only. CRF hypothalamic expression was decreased from the 50 mg corticosterone treatment in the three strains probably via classical GR activation (Tanimura & Watts 1998). Control ACTH concentrations did not differ across strains. ADX increased ACTH to a greater extent in F344 than in LOU and LEW rats. ACTH was brought back to sham values by corticosterone in a dose-dependent manner and in the same proportion in the three strains. This effect reached significance from the lowest corticosterone dose in F344 rats, implicating MR-related regulation of plasma ACTH in this strain, as already described in Sprague–Dawley rats by Levin *et al.* (1987). In sham groups and at the 50 and 100 mg corticosterone doses, LOU rats showed lower plasma CBG concentrations than F344 and LEW rats. Therefore, in LOU rats, the greater free fraction of corticosterone might contribute to the higher effects induced by corticosterone on their carcass, thymus weight, or CRF.

Corticosterone exerts classically a GR-related hyperglycemic effect via the mobilization of carbohydrate body stores (in liver and muscles), gluconeogenesis (Goldstein *et al.* 1993, Opherk *et al.* 2004), and anti-insulin effects on glucose uptake and utilization by tissues (Andrews & Walker 1999). Whenever corticosterone was present in plasma (control or pellet treatment), glucose levels of LEW rats were lower than those of LOU or F344 rats and than those already published (Levy *et al.* 2002). To our knowledge, this peculiarity of LEW rats has not been explored yet. Glucose levels were decreased by ADX in LOU rats only, and then restored classically to sham levels by the highest corticosterone doses. Insulin concentrations were the same in the three rat strains in control and were not altered by ADX. Plasma insulin tended to be increased by the highest doses of corticosterone, but this effect did not reach significance in our study. ADX and corticosterone effects on insulin have been described more clearly by other authors, but in other rat strains and after different duration of ADX (Dallman *et al.* 1993, La Fleur *et al.* 2004). In sham condition, plasma FFA concentrations did not differ across strains. We observed much lower FFA concentration in LOU than that in F344 and LEW rats at moderate-to-high doses of corticosterone. Nevertheless, the high SNS tone (Perrin *et al.* 2003) associated with the greater corticosterone effects in LOU would be expected to induce a high basal lipolysis. In this case, FFA would be instantly used for gluconeogenesis or oxidized in this strain. This assumption will be explored by measurements with indirect colorimetry. In sham condition, the strain differences in plasma leptin levels reflected exactly the differences observed in fat mass depot, i.e., F344>LEW>LOU rats. As described classically in the literature (Mora & Pessin 2002, Zhang *et al.* 2005), plasma leptin was decreased by ADX in F344 and LEW rats. Leptin secretion followed the fat mass increase but was not restored to the sham level by corticosterone supplementation in these rats, suggesting the involvement of other adrenal factor(s) in the regulation of leptin production. As for abdominal fat mass, we observed neither ADX nor corticosterone effect on leptin concentration in LOU rats.

In conclusion, we showed here that despite the greatest effects of high doses of corticosterone on thymus, carcass weight, hypothalamic CRF, or plasma CBG in the LOU strain when compared with the F344 and LEW strains, this strain was completely insensitive to the anabolic effects of corticosterone, i.e., on body weight, liver, and abdominal fat depots. The knowledge of the mechanisms involved could bring critical information on important features of this strain, its leanness and insensitivity to diet-induced obesity, as well as its longevity with healthy aging (Veyrat-Durebex *et al.* 2005). The LOU rat strain should be useful as a model for the resistance to the central adipogenic effects exerted by glucocorticoids. Molecular mechanisms implicated in such a resistance in abdominal adipose

tissues should be compared with other models of glucocorticoid resistance (Schaaf & Cidlowski 2002) to identify fat specificity and potential therapeutic targets to prevent the metabolic syndrome.

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