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Functional variability in corticosteroid receptors is a major component of strain differences in fat deposition and metabolic consequences of enriched diets in rat

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Abstract

We aimed to distinguish mineralocorticoid (MR) from glucocorticoid receptor (GR) actions in the nutritional differences between the Fischer 344 (F344) and LOU/C (LOU) rat strains. The decrease of urinary Na+/K+ ratio induced via MR activation by aldosterone and decrease of circulating lymphocyte counts exerted via GR activation by dexamethasone revealed a higher efficiency of corticosteroid receptor in LOU than in F344 rats. Afterward, we submitted F344 and LOU male rats to adrenalectomy and to substitution treatments with agonists of MR or GR under 3 successive diets—standard, free choice between chow and pork lard, and an imposed high-fat/high-sugar diet—to explore the involvement of the interactions between activation of corticosteroid receptors and diet on food intake, body composition, and metabolic blood parameters in these rats. Lastly, we measured energy expenditure and substrate oxidization in various experimental conditions in LOU and F344 rats by indirect calorimetry. In LOU rats, we showed greater basal and MR-induced energy expenditure, diet-induced thermogenesis, and lipid oxidization. We showed that the F344 rat strain constitutes a relevant model of the unfavorable effects exerted by glucocorticoids via GR on food preference for high-calorie diets, abdominal fat deposition, diabetes, and other deleterious consequences of visceral obesity. Contrary to F344 rats, the LOU rats did not exhibit the expected visceral fat deposition linked to GR activation. This strain is therefore a relevant model of resistance to diet-induced obesity and to the deleterious effects exerted by glucocorticoids on metabolism.
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1. Introduction

Prevalence of obesity is growing in industrialized countries, with many health and social consequences [1]. In both humans and rats, obesity appears to be inherited as a polygenic trait [2]. Interindividual differences in vulnerability to weight gain and fat deposition are exacerbated by environmental pressure, such as overconsumption of dietary fat and stressful lifestyle. Human abdominal obesity has been associated with perturbations in the hypothalamo-pituitary-adrenal (HPA) axis [3]. These alterations, among others, concern glucocorticoid production and metabolism [4]. Moreover, most obesity syndromes in animals depend on functional HPA axis [5]. Our aim is to explore the nutritional consequences of genetic variations in HPA axis activity. Glucocorticoid actions on target tissues, such as liver or fat, are dependent not only on circulating levels but also on hormone bioavailability (plasma transcortin and 11β-hydroxysteroid dehydrogenase [6,7]) and on the transduction efficiency at the level of their receptors [8]. Indeed both corticosteroid receptors, that is, mineralocorticoid (MR) and glucocorticoid (GR) receptors, are involved in metabolic regulations and in the development of fat stores and their pathologic consequences (such as the metabolic syndrome [9,10,11]). After adrenalectomy (ADX) in rat, the weight gain is increased to normal by MR activation with low doses of corticosterone or MR agonists via an up-regulation of food intake and efficiency [12] and adipogenesis [13,14]. It is decreased by GR activation with high doses of corticosterone or GR agonists via catabolic effects on fat and protein stores [15]. The proteolysis induced by GR activation is more marked than the lipolysis, altering the body distribution of lipid stores to increase abdominal fat

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mass at the expense of muscular mass [15]. Cushing syndrome in humans and animals illustrates the link between high glucocorticoids and accumulation of central fat [16]. Glucocorticoids stimulate also preadipocyte differentiation and drive adipose tissue distribution and function via both MR and GR [13,17]. The accumulation of abdominal fat they favor in rodents and humans is involved in the metabolic disturbances linked to obesity [18]. Indeed, in contrast to the subcutaneous fat, the abdominal fat mass exhibits high rates of basal and hormone-induced lipolysis (including at metabolically inappropriate times [19]). Glucocorticoids also induce hepatic gluconeogenesis and exert anti-insulin actions that worsen type 2 diabetes mellitus [4].

The present experiments aimed to explore the involvement of functional differences in MR and GR in nutritional differences between 2 inbred rat strains, Fischer 344 (F344) and LOU/C (LOU), especially in their vulnerability or resistance to fat deposition, respectively, and their sensitivity to enriched diets (gene × environment interaction). F344 rats have been shown to store excess triglycerides in liver and muscle and to exhibit abdominal fat accumulation, insulin resistance, and dyslipidemia under standard diet [20]. LOU rats are characterized by healthy aging and lower fat mass compared with other rat strains [21]. We have shown that, in contrast with F344 rats that develop visceral obesity in response to high-fat diet, LOU rats are resistant to diet-induced obesity [22]. Moreover, we have shown that LOU rats exhibit lower corticosterone levels across the circadian rhythm and during the recovery following a restraint stress as compared with F344 rats [23]. The first part of our study was devoted to compare the efficiency of both corticosteroid receptor types between F344 and LOU strains. To that end, we measured the decrease of urinary Na+/K+ ratio induced via MR activation by aldosterone [24] and the decrease of circulating lymphocyte counts exerted via GR activation by dexamethasone (DEX) in these strains [25]. To explore the metabolic consequences of the functional differences that we found in their corticosteroid receptors, physiologic (food intake, body weight gain) and biochemical (plasma corticosterone, insulin, leptin, glucose, and free fatty acids) measurements were made under standard diet, then under free choice between chow and pork lard, and lastly under an

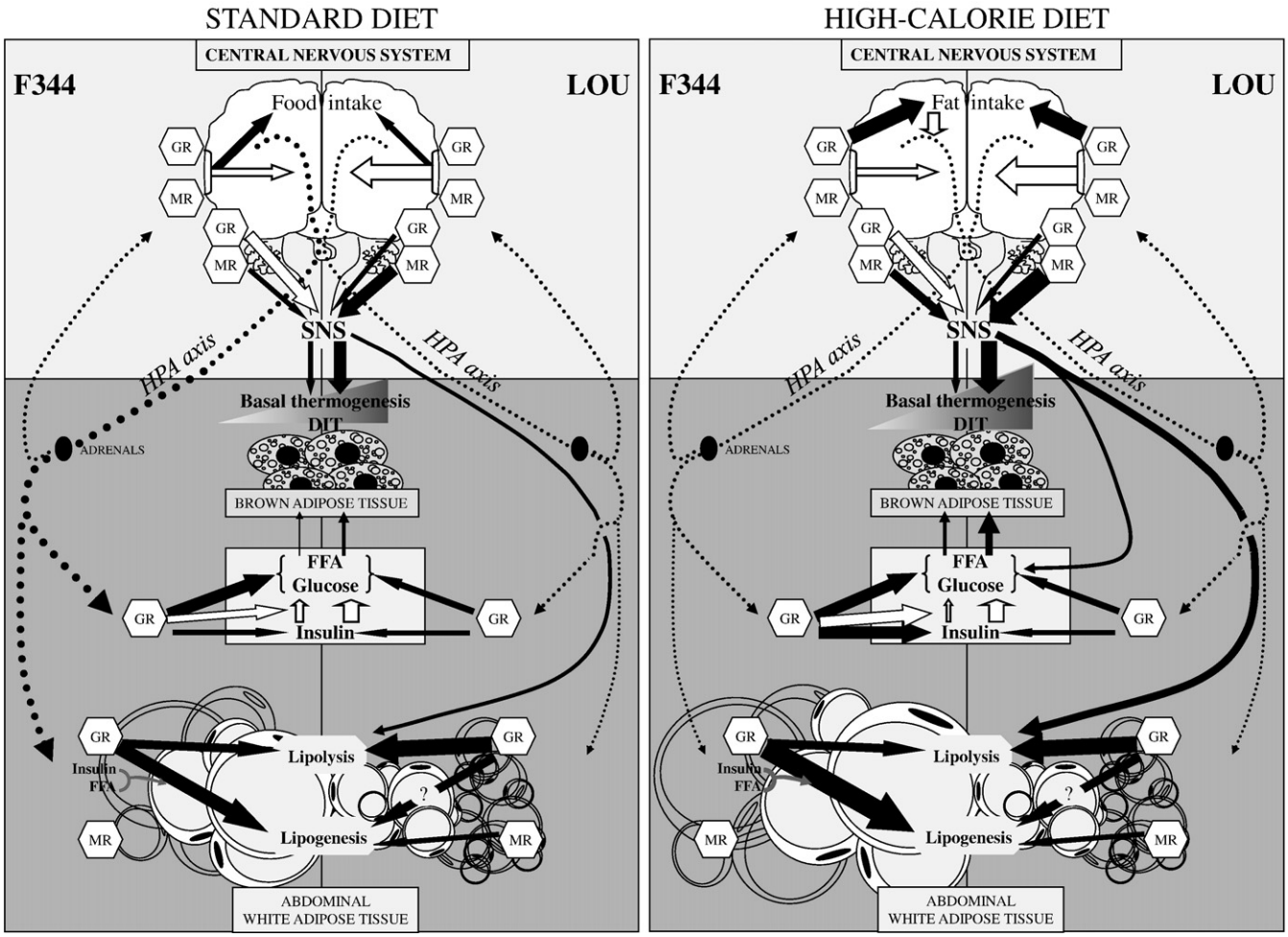

Fig. 1. MR and GR implications in the nutritional differences between F344 and LOU rat strains under standard and high-calorie diets. The black arrows represent the activations; and the white arrows, the inhibitions. The width of arrows illustrates the amplitude of the modifications.

imposed high-fat/high-sugar diet in F344 and LOU rats submitted to ADX and to substitution treatments with MR or GR agonists. Because the nutritional differences between F344 and LOU rats could also involve catabolic processes, the third part of our study was devoted to the measurements by indirect calorimetry of energy expenditure and substrate oxidization in control, fasting, and refeeding conditions. We also measured the effects of a norepinephrine acute treatment and of ADX ± MR/GR agonists under standard and high-fat diets on calorimetric data in both strains. This study demonstrates large differences in MR- and GR-related actions between F344 and LOU rats (Fig. 1, at the disposal of the reader from the start) and their role in vulnerability to fat deposition, metabolic disorders linked to visceral obesity, and energy expenditure.

2. Materials and methods

2.1. Animals and diets

Experiments were conducted in accordance with the principles and guidelines of the French legislation on animal welfare, Journal Officiel no. 87-848, and under veterinary supervision. All rats were born and raised in the laboratory from LOU (Harlan, Lyon, France) and F344 breeders (Iffa Credo, L'Abresle, France). They were housed in standard collective cages in a temperature-controlled room (23°C ± 1°C) with a 12:12-hour light-dark cycle (lights on at 7:00 AM). In standard conditions, they were fed with SAFE-A03 chow (3.2 kcal/g metabolizable energy [ME], Scientific Animal Food & Engineering, Villemoisson-sur-Orge, France) until weaning at 28 days of age and subsequently with SAFE-A04 (2.9 kcal/g ME). Water was available ad libitum.

For the study of the interaction between MR/GR function and diet composition, rats received successively 3 diets ad libitum for 10 days each: (1) the STANDARD diet composed of SAFE-A04 chow (2.9 kcal/g ME); (2) the LARD diet with free choice between pork subcutaneous fat (8.5 kcal/g ME) and SAFE-A04 chow; and (3) the OCCIDENTAL diet (SAFE Occidental Diet, 3.82 kcal/g ME) composed of 16% protein (11% from animal and 5% from vegetal origin), 16% lipids (3.6% palmitic, 0.4% palmitoleic, 2.0% stearic, 6.2% oleic, 2.5% linoleic, 0.2% linolenic, 0.2% gadoleic), 46% carbohydrates (29% starch, 11% saccharose, 0.7% glucose, 0.3% fructose, 2.4% cellulose), 1.5% minerals, and 1% vitamins. Animals, food, and saline were weighed daily.

2.2. Effect of MR and GR agonists on biological targets

For the comparison of MR efficiency between F344 and LOU rats, 8 male rats per strain (12 weeks old) were randomly used for the measurement of aldosterone and DEX effects. We aimed to explore the effect of aldosterone on urinary excretion of electrolytes as a measurement of MR efficiency. Rats were familiarized with individual metabolic cages and handling procedures 4 days before the experiment. They received a subcutaneous injection of aldosterone (100 μg/100 g of body weight [BW]). Controls received corresponding amounts of vehicle, and a water load (3 mL) was administered intraperitoneally to all rats. Urine was collected for 8 hours after injection as previously described [26], centrifuged, and 5-fold diluted. Na+ and K+ urinary concentrations were measured with a flame photometer (Model 410; Sherwood Scientific, Cambridge, United Kingdom). We also tested the effect of DEX on circulating lymphocyte count. Rats were placed 2 per cage and allowed to adapt to the animal room for 4 days before the start of this experiment. Blood samples were collected from a nick of the tail in chilled tubes coated with a 10% EDTA solution, before and 2 hours after a DEX subcutaneous injection (5 μg/100 g of BW [25]). Plasma lymphocyte numeration was performed on total blood with an MS4+ blood analyzer (Melet-Schloessing, Paris, France).

2.3. Involvement of MR and GR in the nutritional differences between F344 and LOU rats

This experiment aimed to compare the effects of standard and high-calorie diets between F344 and LOU rats and to investigate the role played by MR and GR in these effects. Bilateral ADX was performed under pentobarbital anesthesia (0.1 mL/100 g of BW) on 12-week-old LOU and F344 male rats (n = 24 per strain). Sham-operated rats (n = 8 per strain) were submitted to anesthesia and bilateral laparotomy. Incisions were closed with surgical gut and wound clips. At the time of surgery, sham animals and 8 ADX rats of each strain were given saline as drinking fluid (0.5% NaCl). The other ADX rats were distributed between 2 experimental groups: (1) the ADX + deoxycorticosterone (DOC) group received saline supplemented with DOC at 5 μg/mL of saline; (2) the ADX + DEX group received saline with DEX at 5 μg/mL of drinking fluid. The agonist concentrations were chosen according to our previous results on F344 and other rat strains [24]. At the end of STANDARD and LARD diets, rats were fasted overnight; and a blood sample was taken within 1 minute from a nick of the tail. On the 10th day of the OCCIDENTAL diet, rats were also fasted overnight and killed by decapitation. Blood samples were collected in chilled tubes coated with a 10% EDTA solution and centrifuged at 4000*g* for 15 minutes at 4°C. Plasma aliquots were stored at −80°C for subsequent measurements. Corticosterone concentrations were measured by radioimmunoassay with a commercial kit (MP Biomedicals, Orangeburg, NY). Plasma insulin and leptin were also measured with RIA kits (Insik 5; DiaSorin, Antony, France, and Linco Research, St Charles, MO, respectively). Plasma glucose and nonesterified fatty acids (FFA) were measured with colorimetric kits (Biolabo, Maizy, France, and Wako Chemicals, Neuss, Germany, respectively). Rats were dissected to evaluate their 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 (subcutaneous fat between the lower part of the rib cage and the thighs). Carcass, skin, and organs (brown adipose tissue, thymus, and liver) were also weighed.

2.4. Energy expenditure and substrate utilization in F344 and LOU rats

We used an OXYLET indirect calorimeter (Bioseb, Chaville, France) to examine energy expenditure and substrate utilization in F344 and LOU rats. Measurements were conducted in individual Plexiglas cages (20 × 21 cm; Rubbermaid, Winchester, VA) at 23°C ± 1°C. The small size of these cages reduces the influence of strain differences in locomotor activity. Outdoor air is sucked through the cages (1.5 l/min) by a pump and an expansion unit (Model MM-100; CWE, Ardmore, PA). Cage air is then directed to an air dryer filled with anhydrous CaCl2 (WA Hammond Drierite, Xenia, OH) and a gas analyzer (Metabolic Monitor Model MMX-8; CWE, Ardmore, PA). Food and saline ± DOC or DEX were available ad libitum and changed daily. Downstream gases were successively analyzed in 8 different cages for 30 seconds (the system was rinsed for 90 seconds between each cage [27]). After a period of adaptation of 24 hours, O_2 and CO_2 concentrations were measured continuously for the entire 12-hour dark phase and for 10 hours of the light phase (2 hours required to calibrate the system, clean the cages, and change and weigh rats, food, and water; the time of handling was changed each day). Energy expenditure was calculated according to the Weir method [28] and followed exactly the same profile as the amount of O_2 consumed (VO_2, which we chose for illustrations). Respiratory quotient was calculated by dividing the amount of CO_2 produced by the amount of O_2 consumed. Protocols classically used to investigate calorimetric data in rats were also applied to F344 and LOU rats [29,30].

2.4.1. Control, fast, and refeeding

F344 and LOU rats (n = 8 male rats per strain, 12 weeks old) were submitted to indirect calorimetry in control condition, then during a 24-hour fast, followed by a 24-hour refeeding period. During fast, rats had ad libitum access to drink. On the following day, ad libitum access to food was allowed to rats from 9:00 AM onward.

2.4.2. Norepinephrine acute treatment

Another group of rats (n = 8 male rats per strain, 12 weeks old) was submitted to an intraperitoneal injection of norepinephrine (200 μg/kg at 2:00 PM [31]) in basal condition and after a 24-hour fast.

2.4.3. ADX, DOC, and DEX treatments; standard and high-fat diets

We compared the effects of ADX, DOC, and DEX treatments on VO_2 and respiratory quotient under STANDARD and LARD diets in F344 and LOU rats (12 weeks old, n = 4 male rats per strain and per treatment). In both strains, each experimental group received successively the 10-day STANDARD and LARD diets. In consideration of the similitude between the results we obtained with LARD and OCCIDENTAL diets in experiment 2, we limited the calorimetry experiment to the effects of LARD diet.

2.5. Data analysis

Results were expressed as means ± standard errors of mean. Data were analyzed by 2-way analysis of variance (ANOVA) with strain (F344, LOU) and treatment (aldosterone, DEX, ADX, DOC, and sham/control conditions) as 2 between-subject factors. Subsequently, 2 other ANOVAs were realized: one considering DOC effects vs ADX and sham conditions, and the other testing DEX effects vs ADX and sham conditions, to avoid the underestimation of effects due to opposing actions of both substitution treatments. The strain specificity of treatments was discussed when the strain × treatment was significant ($P < .05$). Calorimetric data were averaged per hour for the effect of norepinephrine and per day vs per night for the other measurements. A third factor with repeated data was added to ANOVA when necessary (time). Post hoc Newman-Keuls tests were performed when ANOVAs were significant ($P < .05$).

3. Results

3.1. Experiment 1: comparison of MR and GR efficiency between F344 and LOU rats

3.1.1. Effect of aldosterone on excretion of electrolytes

F344 and LOU rats showed the same urinary Na+/K+ ratio in control condition (Fig. 2A), but aldosterone decreased this ratio in LOU rats only ($P < .01$).

3.1.2. Effect of DEX on circulating lymphocytes

In control condition, lymphocyte count was higher in LOU than in F344 rats ($P < .001$) (Fig. 2B). DEX reduced lymphocyte count in both strains, but to a greater extent in LOU than in F344 rats (−80.30% ± 0.30% vs −73.3% ± 0.30%, $P < .05$).

3.2. Experiment 2: involvement of MR and GR in the nutritional differences between F344 and LOU rats

3.2.1. STANDARD diet

3.2.1.1. Food intake. In sham condition, caloric intake did not differ between the F344 and LOU strains. In other conditions (ADX, or MR or GR agonists), caloric intake was greater in LOU than in F344 rats ($P < .05$) (Fig. 3A).

3.2.1.2. Body weight gain. In sham condition, food efficiency was lower in LOU than in F344 rats: 36.30 ± 5.40 vs 47.01 ± 4.00 mg of weight gain per kilocalorie ingested, respectively ($P < .05$) (Fig. 3A). In both strains,

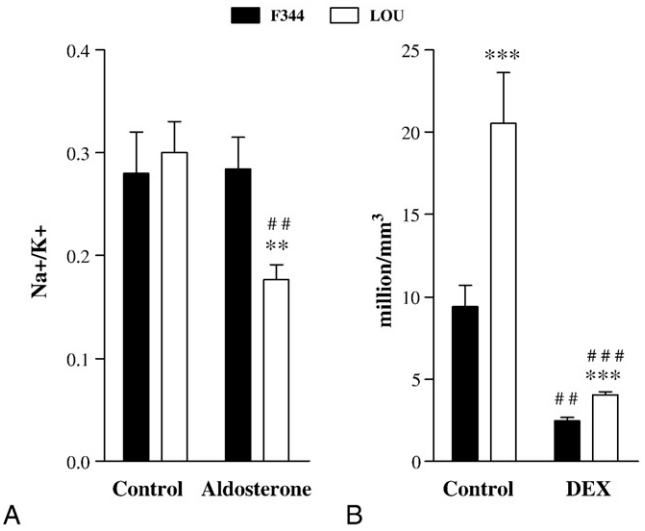

Fig. 2. Effects of aldosterone (A) and DEX (B) on urinary Na+/K+ ratio and plasma lymphocyte counts, respectively. Differences between strains: $^{**}P < .01$ and $^{***}P < .001$. Differences from the control condition: # #$P < .01$ and # # #$P < .001$.

DOC substitution treatment increased slightly the weight gain of ADX rats ($P < .05$), whereas DEX induced a strong weight loss ($P < .001$).

3.2.1.3. Plasma hormones and metabolites. ADX rats showed plasma corticosterone concentrations below assay detection limits. In sham condition, plasma corticosterone was higher in F344 than in LOU rats ($P < .01$, Fig. 4). As shown in Supplemental Table S1, plasma glucose was higher in F344 than in LOU rats in sham condition ($P < .01$). It was decreased by ADX in both strains ($P < .05$) and restored by DEX to sham value in F344 rat. DEX increased it beyond sham value in LOU strain ($P < .001$). DEX increased strongly FFA concentrations in both strains, but to a greater extent in F344 rats ($P < .001$ vs ADX and sham) than in LOU rats ($P < .01$ vs ADX and sham, $P < .001$ vs F344 DEX rats). In LOU rats, ADX reduced plasma insulin ($P < .001$ vs sham); and DEX restored it to the sham value. In F344 rats, ADX did not induce significant effect on plasma insulin and leptin, whereas DEX increased plasma insulin and leptin up the sham and ADX values ($P < .001$).

3.2.2. LARD diet

3.2.2.1. Food intake. All experimental groups approximately doubled their caloric intake under LARD diet ($P < .001$, Fig. 3B). In both strains, sham rats ate about 70% of their total intake as lard. Although the total calorie intake was not changed by ADX and MR/GR ligands, the preference for lard (vs chow) was decreased to 50% by ADX ($P < .01$ in F344, $P < .001$ in LOU rats) and restored to sham value by DEX only.

3.2.2.2. Body weight gain. LOU rats exhibited globally a lower body weight gain than F344 rats ($P < .001$) (Fig. 3B). In sham condition, food efficiency was much higher in F344 than in LOU rats (60.69 ± 8.55 vs 29.67 ± 3.98 mg per kilocalorie, respectively; $P < .001$). When considering ADX + DOC groups per se, food efficiency was decreased by LARD to a greater extent in LOU than in F344 rats (−66.12% in comparison with the STANDARD diet, ie, 23.48 ± 5.90 mg/kcal in LOU, vs −35.42% to 51.51 ± 11.89 mg/kcal in F344 rats, $P < .001$). DEX induced a strong weight loss in both strains ($P < .001$ vs ADX and sham), but to a greater extent in LOU than in F344 rats ($P < .001$).

3.2.2.3. Plasma hormones and metabolites. Plasma corticosterone was not significantly different between strains (Fig. 4). The LARD diet reduced plasma corticosterone in F344 rats only ($P < .05$ vs STANDARD). In both strains, glucose levels were globally increased by the LARD compared with the STANDARD diet ($P < .05$, Fig. 5). ADX decreased plasma glucose in LOU rats only ($P < .001$). Glucose concentration was restored to sham value by both agonist treatments in these rats. In F344 rats, DEX induced a strong hyperglycemia ($P < .001$); and it increased plasma insulin to a greater extent in F344 ($P < .001$ vs ADX and sham) than in LOU rats ($P < .05$ vs ADX, $P < .001$ vs F344 rats). DEX also increased strongly FFA concentration in F344 rats only ($P < .001$, Fig. 5). Plasma leptin was decreased by ADX ($P < .001$) and strongly increased by DEX ($P < .001$) in F344 rats only (Fig. 5). In LOU rats, plasma leptin was increased by DOC treatment up the ADX value ($P > .01$), whereas it was strongly decreased by DEX ($P < .01$).

3.2.3. OCCIDENTAL diet

3.2.3.1. Food intake. In both strains, caloric intake was only slightly increased by the OCCIDENTAL diet compared with the STANDARD diet ($P < .05$, Fig. 3C). DEX decreased strongly food intake in both strains ($P < .001$ vs ADX), but to a greater extent in LOU than in F344 rats ($P < .01$).

3.2.3.2. Body weight gain. Whatever experimental condition, LOU rats gained less weight than F344 rats ($P < .001$, Fig. 3C). In sham condition, food efficiency was higher in F344 than in LOU rats (55.70 ± 8.78 vs −6.72 ± 2.89 mg/kcal, $P < .001$). When considering ADX + LOU groups, the food efficiency of the LOU strain was strongly decreased by the OCCIDENTAL diet (0.90 ± 7.16 under OCCIDENTAL vs 23.48 ± 5.90 under LARD, $P < .01$). DEX induced the same strong weight loss in both strains ($P < .001$ vs ADX and sham).

3.2.3.3. Plasma hormones and metabolites. The OCCIDENTAL diet increased plasma corticosterone in both strains ($P < .05$ vs STANDARD diets in F344 rats, $P < .001$ vs STANDARD and LARD diets in LOU rats, Fig. 4). In both strains, except in the ADX + DEX LOU group, plasma

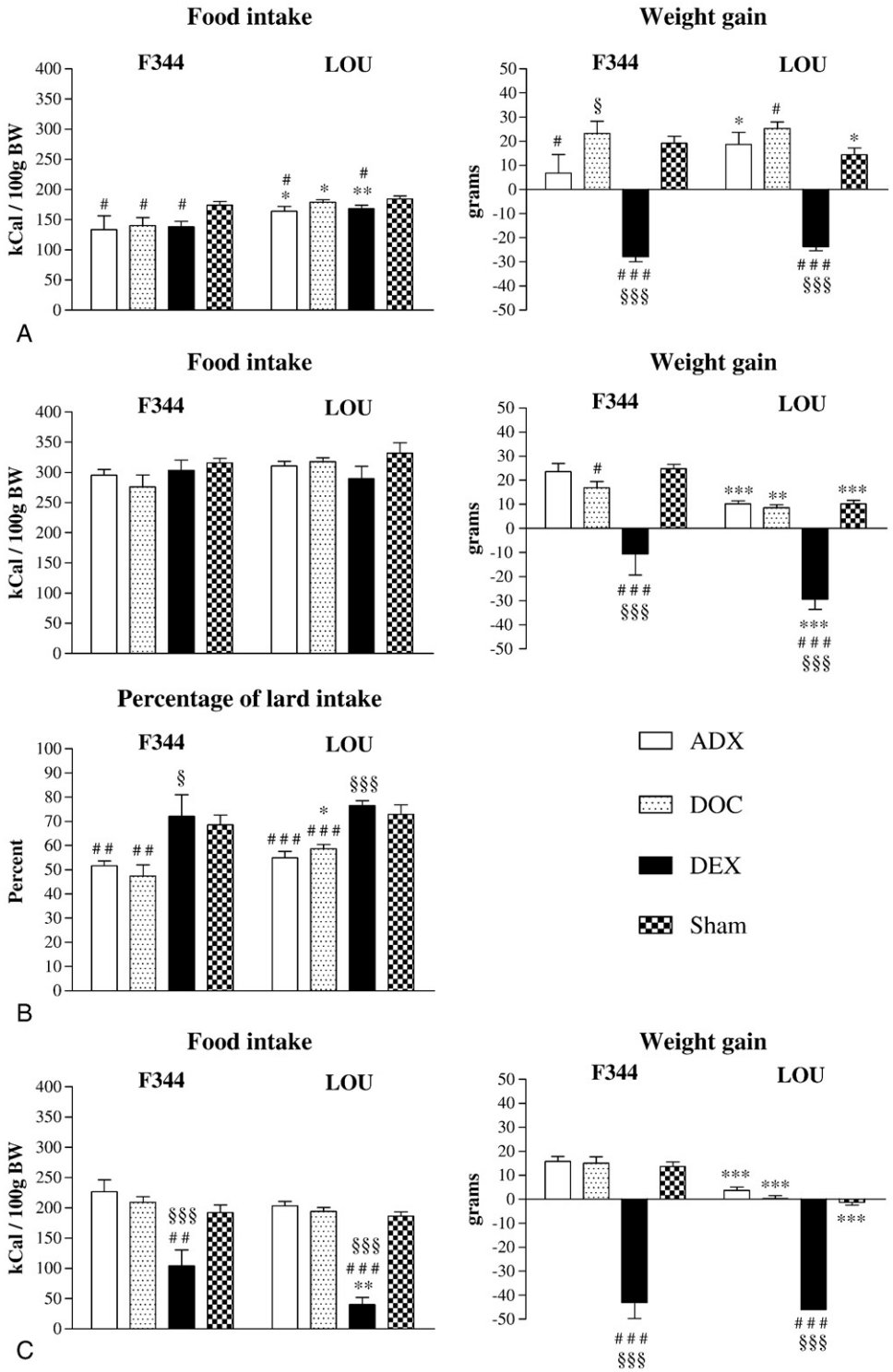

Fig. 3. Food intake and body weight gain under STANDARD (A), LARD (B), and OCCIDENTAL (C) diets in LOU and F344 rats. Differences between strains: *$P < .05$, **$P < .01$, and ***$P < .001$. Differences from ADX: §$P < .05$ and §§§$P < .001$. Differences from sham: #$P < .05$, # #$P < .01$, and # # #$P < .001$.

glucose increased under OCCIDENTAL diet compared with the STANDARD diet ($P < .001$, Supplemental Table S1). As found with LARD diet, DEX increased strongly plasma glucose in F344 strain ($P < .001$ vs ADX and sham). In this strain, plasma insulin was decreased by ADX ($P < .01$ vs sham) and increased strongly by DEX ($P < .001$ vs ADX and sham) to a greater extent than in LOU rats. The MR and GR agonists exerted inverse effects on plasma leptin between

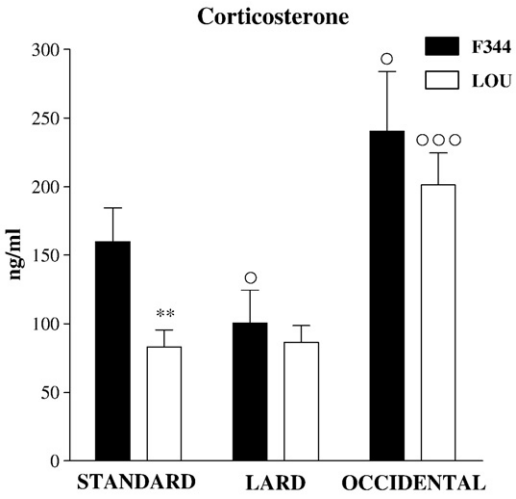

Fig. 4. Plasma corticosterone concentrations. Differences between strains: $^{**}P < .01$. Differences from the STANDARD diet: $^{\circ}P < .05$ and $^{\circ\circ\circ}P < .001$.

strains; that is, DOC decreased vs increased and DEX increased vs decreased leptin concentrations in F344 vs LOU, respectively (Supplemental Table S1).

3.2.4. Body composition

Dissections were conducted at the end of the OCCIDENTAL diet. Fat masses and organs were expressed in percentages of body weight. Whatever experimental condition, F344 rats showed a greater body weight than LOU rats ($P < .001$, Supplemental Table S2). DEX induced weight loss in both strains ($P < .001$ vs ADX and sham), but to a greater extent in the LOU strain ($P < .001$). In both strains, thymus weight was increased by ADX ($P < .05$) and decreased by DEX ($P < .001$ vs ADX and sham). The weight of brown adipose tissue was increased by DEX in F344 rats only ($P < .001$ vs ADX and sham, $P < .001$ vs LOU rats, Supplemental Table S2).

3.2.4.1. Carcass (lean mass). Sham, ADX, and ADX + DOC LOU rats presented a higher percentage of carcass than F344 rats ($P < .001$, Supplemental Table S2).

3.2.4.2. White adipose tissue. Whatever experimental condition, all abdominal fat depots were lower in LOU than in F344 rats ($P < .001$, Fig. 6). ADX reduced all the fat depots of F344 rats, whereas it induced no (mesenteric and epididymal) or a slight effect only (decrease of the retroperitoneal fat, $P < .05$, restored to sham by DOC) in LOU rats. When expressed in percentage of variation relative to ADX mean value, the DOC effect on abdominal fat depot was exerted in opposite directions between F344 and LOU rats (eg, for the visceral fat mass: −21.3% ± 5.9% in F344 vs +11.7% ± 5.5% in LOU rats, $P < .01$). The DEX treatment increased mesenteric and epididymal fat depots in F344 rats ($P < .001$ vs ADX), whereas it decreased all abdominal fat depots in LOU rats. Inguinal fat did not differ across strains. It was reduced by DEX in both strains ($P < .05$ vs sham in F344, $P < .001$ vs ADX and sham in LOU rats).

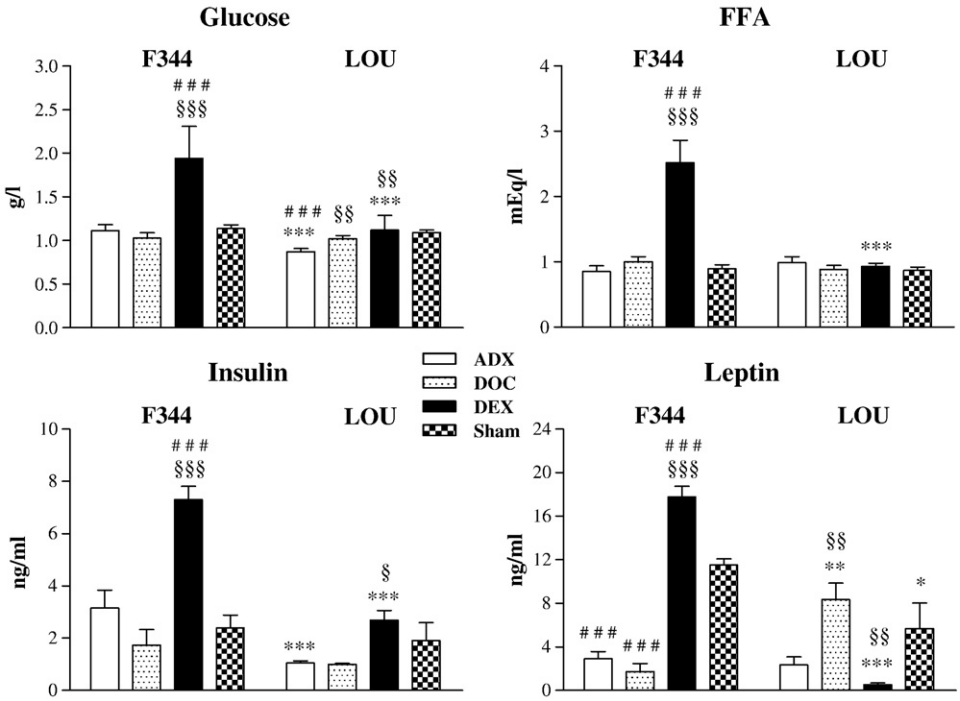

Fig. 5. Plasma glucose, FFA, insulin, and leptin concentrations after LARD diet. Data on STANDARD and OCCIDENTAL diets are shown in Supplemental Table S1. Differences between strains: $^{*}P < .05$, $^{**}P < .01$, and $^{***}P < .001$. Differences from ADX: §$P < .05$, §§$P < .01$, and §§§$P < .001$. Differences from sham: # # #$P < .001$.

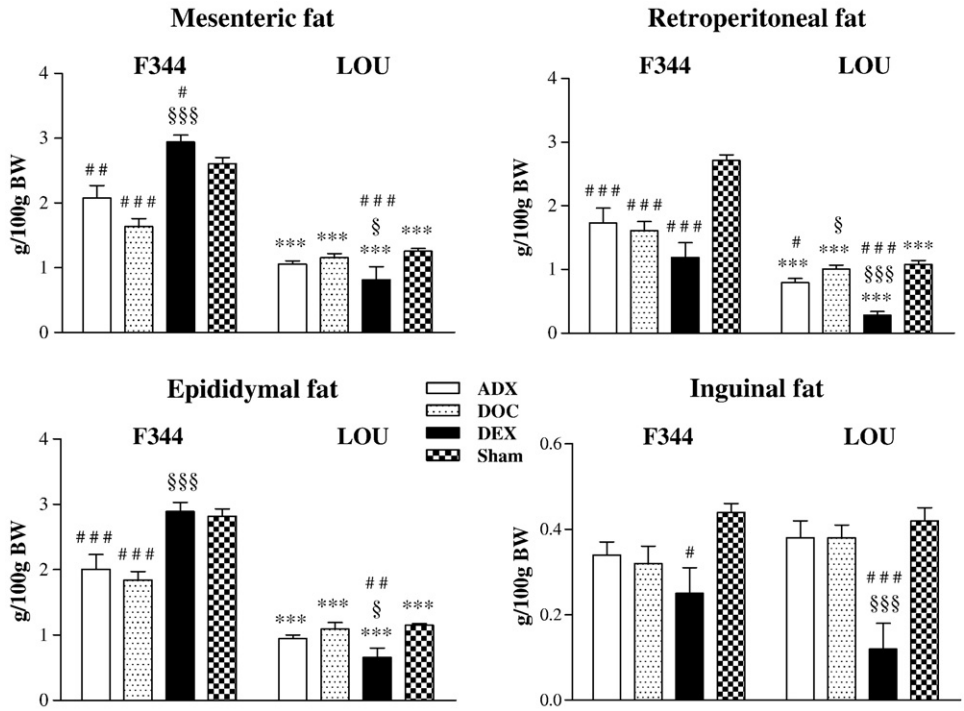

Fig. 6. Abdominal and inguinal fat masses of F344 and LOU rats at the end of the OCCIDENTAL diet. Body and organ weights are shown in Supplemental Table S2. Differences between strains: ***$P < .001$. Differences from ADX: §$P < .05$ and §§§$P < .001$. Differences from sham: #$P < .05$, # #$P < .01$, and # # #$P < .001$.

3.3. Experiment 3: energy expenditure and substrate utilization in F344 and LOU rats

3.3.1. Calorimetric data in control, fasting, and refeeding conditions

In control condition, VO_2 was higher in LOU than in F344 rats during both the light ($P < .05$) and the dark ($P < .01$) phases (Fig. 7). Respiratory quotient was also higher in LOU rats during the light phase ($P < .05$). In both strains, VO_2 and respiratory quotient were decreased by fast ($P < .001$ in F344, $P < .01$ in LOU rats) and increased by refeeding ($P < .05$ and $P < .001$, respectively).

3.3.2. Acute treatment with norepinephrine

In control condition, norepinephrine increased VO_2 ($P < .001$) and decreased respiratory quotient ($P < .001$) in F344 rats only (Fig. 8). This effect did not reach significance during the fasting period.

3.3.3. ADX, DOC, and DEX treatment effects on calorimetric data

3.3.3.1. STANDARD diet. We found again a greater nocturnal VO_2 in LOU than in F344 rats ($P < .05$, Supplemental Table S3). During the light period, ADX decreased VO_2 in F344 rats only ($P < .05$). In LOU rats, DEX increased 24-hour VO_2 without changing respiratory quotient ($P < .01$ vs ADX). In F344 rats, DOC and DEX treatments decreased respiratory quotient during the light ($P < .01$ and $P < .05$ vs ADX, respectively) and dark ($P < .05$ vs ADX for both) periods.

3.3.3.2. LARD diet. During the day and compared with the STANDARD diet, the LARD diet increased VO_2 ($P < .01$) and inversely decreased respiratory quotient ($P < .01$) in LOU sham rats (Fig. 9). On the contrary, the LARD diet decreased the diurnal VO_2 ($P < .05$) of sham F344 rats. Whatever experimental condition, LOU rats exhibited a greater VO_2 ($P < .001$ on 24 h) and a lower respiratory quotient ($P < .001$ during the day [Fig. 9], $P < .05$ at night [Supplemental Table S3]) than F344 rats. In F344 rats, ADX increased VO_2 during the light period ($P < .01$, Fig. 9). During the day and compared with ADX, DOC exerted opposite effects on VO_2 between LOU (increase, $P < .05$) and F344 rats (decrease, $P < .05$). As with the STANDARD diet, DEX treatment increased the diurnal VO_2 of LOU rats ($P < .001$ vs ADX and sham, Fig. 9).

4. Discussion

We showed previously that the modulation by corticosterone of food intake, body composition, and biochemical parameters related to energy metabolism differs between F344 and LOU strains [32]. The aim of the present study was to distinguish MR from GR actions in these nutritional differences. The first part was devoted to compare MR and GR efficiency between F344 and LOU strains. To that end,

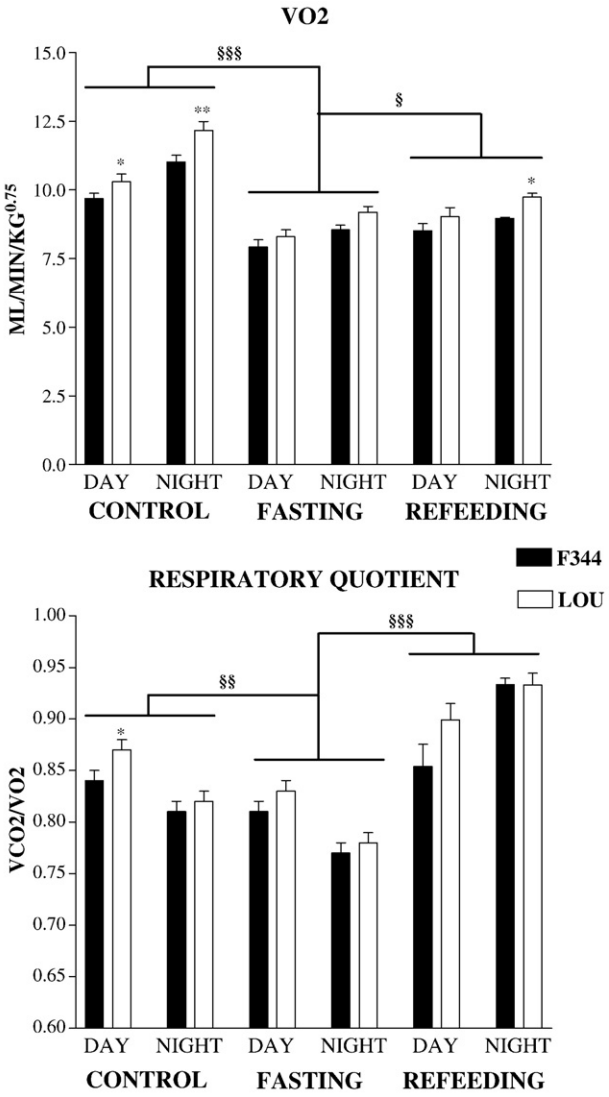

Fig. 7. Oxygen consumption and respiratory quotient determined by indirect calorimetry in F344 and LOU rats during the control condition, a 24-hour fasting period, and refeeding. Differences between strains: *$P < .05$ and **$P < .01$. Differences between periods: §$P < .05$, §§$P < .01$, and §§§$P < .001$.

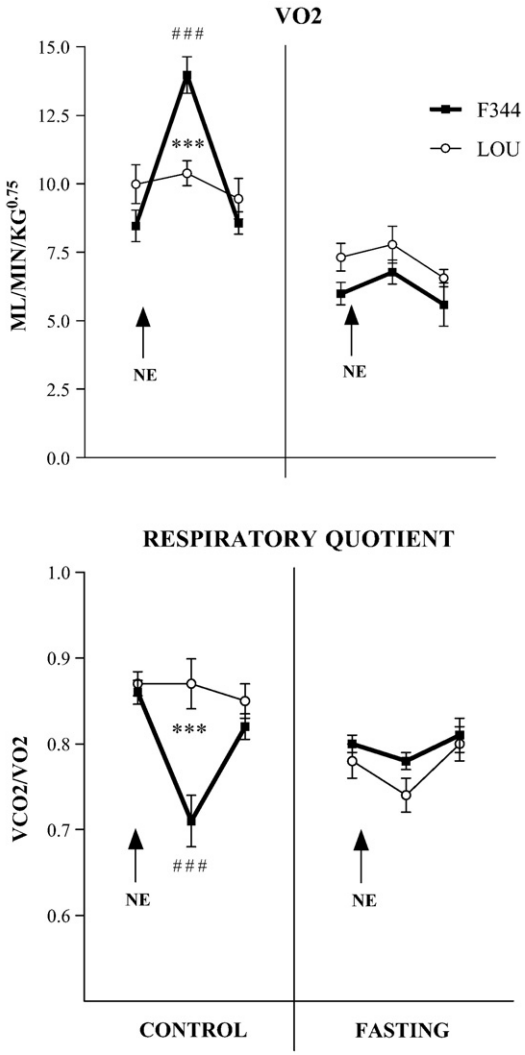

Fig. 8. Oxygen consumption and respiratory quotient in F344 and LOU rats in response to an intraperitoneal injection of norepinephrine (NE) during control and fasting conditions. Each point of the curves corresponds to the average of the 5 data collected by indirect calorimetry per rat for each hour before, just after, and 1 hour after injection. Differences between strains: ***$P < .001$. Significant effects of norepinephrine: # # #$P < .001$.

we measured the decrease of urinary Na+/K+ ratio induced via MR activation by aldosterone and the decrease of circulating lymphocyte counts exerted via GR activation by DEX in these strains (Fig. 2). Both receptors showed a greater efficiency in LOU than in F344 rats. In LOU rat strain, these functional characteristics could contribute to the low circulating levels of corticosterone measured in this strain by a stronger feedback exerted via corticosteroids through central MR and GR [32]. We have previously observed the same characteristics in the Brown Norway rat, another lean strain [26]. However, at first sight and according to what is classically described in the literature [15,16,33], high GR efficiency does not fit with the low abdominal fat mass characterizing Brown Norway and LOU strains. Further investigations were therefore required to find the possible common denominator between these functional characteristics in corticosteroid receptors and the leanness of these rat models. To that end, we submitted F344 and LOU male rats to ADX and to substitution treatments with agonists of MR (DOC) or GR (DEX) under 3 successive diets—STANDARD (chow), free choice between chow and pork lard (LARD diet), and an imposed high-fat/high-sugar diet (OCCIDENTAL)—to explore the involvement of the interactions between receptor activation and diet on nutritional parameters.

In control condition (sham ADX), F344 rats exhibited higher food efficiency than LOU rats under both STANDARD (as shown previously [23]) and high-calorie diets. In both strains, the weight gain was not proportional to the caloric intake. Therefore, fat ingestion might have activated

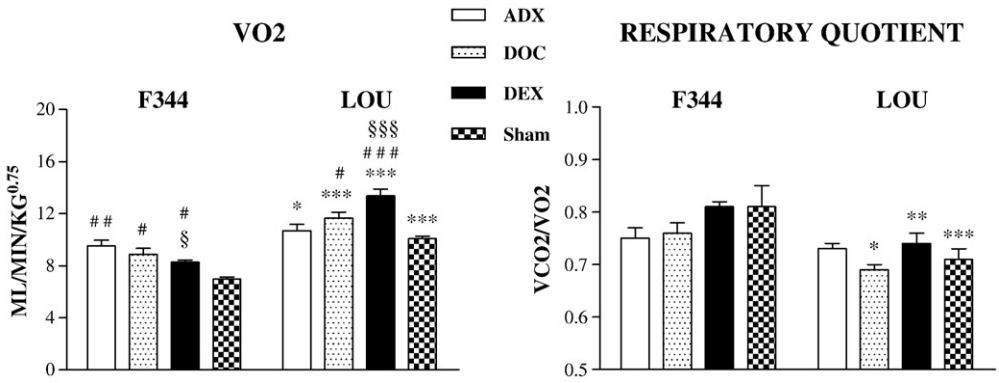

Fig. 9. Diurnal VO_2 and respiratory quotient measured by indirect calorimetry during LARD diet in F344 and LOU rats. Other data on VO_2 and respiratory quotient during STANDARD and LARD diets are shown in Supplemental Table S3. Differences between strains: $*P < .05$, $**P < .01$, and $***P < .001$. Differences from ADX: $§P < .05$ and $§§§P < .001$. Differences from sham: $\#P < .05$, $\# \#P < .01$, and $\# \# \#P < .001$.

defense catabolic mechanisms to limit the weight gain of both strains, such as diet-induced thermogenesis (DIT) via sympathetic nervous system (SNS) activation [34], but to a much greater extent in LOU than in F344 rats. Our measurements of energy expenditure and respiratory quotient by indirect calorimetry have investigated this assumption. Indeed, LOU rats exhibited higher VO_2 than F344 rats in sham condition and under standard diet (Fig. 7). The decrease in energy expenditure and respiratory quotient induced by fast [29] did not differ across strains. Refeeding-induced thermogenesis was exhibited by both strains, but also to a greater extent in LOU than in F344 rats at night. Moreover, lard ingestion increased energy expenditure in LOU rats (expected DIT [35]), whereas it decreased it in F344 rats (Fig. 9). The LARD diet reduced respiratory quotient in LOU rats only, suggesting a preferential use of fatty acids for oxidations in response to high-fat diet in LOU rats. On the contrary, F344 rats did not exhibit this compensatory metabolic response. These calorimetric data suggest that sympathetic activity is high in LOU rats. This could result from SNS hyperactivation via MR brain action (as shown recently in Sprague-Dawley rats by Zhang et al [36]), which we hypothesize to be higher in LOU than in F344 rats (Fig. 1). Perrin et al [37] showed previously an enhanced sympathetic activity in white and brown adipose tissues in LOU rats, but paradoxical results concerning brainstem and hypothalamic areas. Further investigations are needed to test our hypothesis.

Plasma corticosterone, glucose, insulin, FFA, and leptin were measured after an overnight fast at the end of each diet. F344 sham rats exhibited a higher corticosterone concentration than LOU rats in response to fast (Fig. 4), as we described previously after another metabolic stress (hypoglycemia induced by an insulin acute treatment [23]). Eating lard reduced the reactivity of HPA axis in F344 rats only. Such a "comforting" effect has been previously discussed for very palatable foods by Dallman and collaborators [3] and Gibson [38] in rodents and humans. La Fleur et al [39] have also shown that a free choice between lard and chow diminished corticotropin and corticosterone responses to a restraint stress in Sprague-Dawley rats. On the other hand, the OCCIDENTAL diet increased strongly plasma corticosterone in our strains. Interestingly, La Fleur et al [39] also showed that, when imposed, the high-fat diet increased HPA reactivity to stress in Sprague-Dawley rats. In sham condition, plasma glucose was higher in F344 than in LOU rats despite equivalent insulin levels, in agreement with our previous data [23] and with the insulin resistance described in older F344 rats by Levy et al [20]. At the end of the experiment, we measured the body composition of rats. Abdominal fat mass was twice higher in F344 than in LOU rats, contrary to the subcutaneous fat that did not differ between strains (Fig. 6). The greater abdominal fat depot measured in F344 strain fits with its resistance to insulin as widely described in the literature [40].

As expected [12,23], ADX decreased food intake, weight gain, and VO_2 in F344 rats. On the contrary, as previously shown in Brown Norway rats [26], weight gain was insensitive to ADX in LOU rats, suggesting the involvement of compensatory mechanisms for the lack of MR activation in these rats. In line with this result, ADX altered neither VO_2 nor respiratory quotient in LOU rats. On the other hand, insulin and glucose levels were sensitive to ADX in LOU rats and were decreased as expected [41]. In F344 rats, plasma insulin was not altered by ADX; but plasma glucose was restored by ADX to the classic value described in standard rats [20]. These results suggest the involvement of anti-insulin effects of corticosterone in the high plasma glucose of F344 rats [42], even under STANDARD diet (Fig. 1). In both strains, lard ingestion that accounted for 70% of the caloric intake of sham rats was reduced to 50% by ADX (Fig. 3), in accordance with previous studies showing that food preference for high-fat meals and other "comfort foods" depends on glucocorticoid action in rodents and humans [38,43]. Our previous study [23] showed that abdominal fat depot was decreased by ADX and dose-

dependently increased by corticosterone in F344 rats submitted to a standard diet, as in other rat strains [12,13]. On the contrary, we had not found any effect of ADX or corticosterone on abdominal fat in LOU rats. Here we globally confirm these results under high-calorie diets. ADX reduced all fat depots in F344 rats, whereas it induced no or a slight effect only in LOU rats. Under LARD diet, ADX increased energy expenditure and decreased respiratory quotient in F344 rats (Fig. 9), which allowed these rats to give the expected thermogenic response to the high-calorie diets [30] to limit fat deposition, as shown in other obesity models [44]. Nevertheless, ADX thermogenic effects were not sufficient to restrain weight gain or to improve completely resistance to insulin in F344 rats. In the same way, ADX did not counteract resistance to obesity in LOU rats. Balanced influence of both corticosteroid receptors is released by ADX, and the lack of effect of ADX does not necessarily mean a lack of implication of corticosteroids. Afterward, our aim was to distinguish MR from GR specific implication in F344 and LOU phenotypes by treating ADX rats with DOC or DEX, respectively.

The effect of DOC on abdominal fat deposition diverged between F344 and LOU rats. DOC was ineffective in F344 rats. On the contrary, in LOU rats, DOC increased abdominal fat, in accordance with other in vivo [13,45] and in vitro studies on MR action on adipogenesis [14,46]. The greater MR sensitivity in LOU was also measured on plasma leptin, increased by DOC in LOU rats only, in parallel to the visceral fat as usually described in rats [47]. Under high-calorie diets, DOC increased also plasma glucose in LOU rats only, without any change in their insulin level, which could result from a preferential use of lipids for thermogenesis and/or from a hyperglycemic effect of SNS activation by central MR [36,48]. Calorimetric studies permitted to test these assumptions. We submitted F344 and LOU rats to a norepinephrine acute treatment in control and fasting conditions. Control F344 rats exhibited the expected increase of VO_2 and decrease of respiratory quotient induced by norepinephrine [35]. On the contrary, LOU rats were insensitive to these effects, as measured during fast in both strains (Fig. 8). Knowing that fast classically reduces body sensitivity to norepinephrine by activation of SNS in rats [49], the insensitivity to norepinephrine in LOU rats could support our assumption of a hyperactivity of their SNS. The diurnal thermogenesis of LOU rats was increased by DOC, so that it became much higher than that of F344 rats (Fig. 9). In LOU rats, the low respiratory quotient (0.7) suggests an exclusive use of lipids for oxidization, which could contribute to the high plasma glucose induced by DOC (nonuse of carbohydrates) in these rats (Fig. 5). Our results are consistent with the lack of increase of food efficiency by DOC observed under LARD diet and with our hypothesis of thermogenesis exacerbated by SNS activation via MR in LOU rats [36,50]. On the contrary, DOC treatment decreased the diurnal VO_2 of F344 rats. The functional peculiarity of the MR of LOU strain allows a better adaptation to high-fat unbalanced diets by easily using visceral fat as a buffer toward the flow of lipids and by prioritizing fatty acid oxidization (Fig. 1). Interestingly, the greater sensitivity of MR that we showed in LOU rats by a classic urinary test with aldosterone was also found in DOC action on metabolic processes.

DEX caused a strong weight loss under all diets and in both strains by a strong mobilization of body reserves of carbohydrates, proteins, and fat [51]. Under STANDARD diet, DEX increased energy expenditure in LOU rats only. DEX caused a strong lipolysis in F344 rats, as suggested by the high plasma FFA measured in ADX + DEX F344 group. The respiratory quotient was decreased by DEX in F344 rats, but insufficiently to counteract the very high inflow of FFA in their plasma. If not oxidized, FFA might be recaptured by adipose tissues, but also inappropriately stored in muscles, liver, and pancreatic β-cells [52]. According to Levy et al [20], the second process appears to be predominant in F344 rats. By their insulinotropic effect on pancreatic β-cells coupled to their role as substrate in the gluconeogenesis induced by glucocorticoids [52], high FFA levels might have contributed to the high levels and poor effects of insulin in F344 rats, as described in obese humans [53]. Plasma FFA levels were increased neither by high-fat diet nor by DEX in LOU rats, despite equivalent lard ingestion as F344 rats. Once again, this could involve a very quick and efficient use of their plasma FFA for oxidization, as suggested by a respiratory quotient near to 0.7 during the day in LOU rats only (Fig. 9).

DEX restored the preference for lard of ADX rats to 70% in both strains. Such an increase of the motivational value of palatable food via GR has been described in Sprague-Dawley rats by La Fleur et al [33] and in humans submitted to chronic stress [38]. The weight loss induced by DEX was attenuated by lard ingestion in F344 rats, suggesting that preference for high-fat diets could be exacerbated by GR activation to limit the weight loss and to provide the energetic fuel to cope with a stressful situation [41]. This was not the case under OCCIDENTAL diet, as the anorexia induced by DEX in both strains also contributed to the high weight loss observed in ADX + DEX groups.

As above for the MR, GR activation by DEX induced opposite effects on body composition between F344 and LOU rats (Figs. 1 and 6). As described in other strains [12], DEX increased the percentage of abdominal fat in F344 rats. On the contrary, DEX exerted the same lipolytic action on abdominal as on peripheral fats in LOU rats. These contrasting effects of DEX on adipose tissues between F344 and LOU rats fit the opposite regulation exerted by DEX on their plasma leptin. The summation of the MR- and GR-mediated opposite effects on fat mass may have rendered corticosterone effects nonobservable in LOU rats in our previous study [23]. In LOU rats, the paradoxical effect of DEX on visceral fat deposition could originate in a defective synergy between insulin and glucocorticoids to induce adipogenesis [54] or in an excessive activation of

hormone-sensitive lipase via GR and/or β-adrenergic receptors [19]. It is surprising that despite a greater efficiency of their GR, some phenotypes are modified to a lower extent in LOU than in F344 rats by DEX treatment and, in F344 rats, always toward an aggravation of the metabolic syndrome. For instance, plasma insulin and glucose were increased by DEX in both strains under LARD as under STANDARD diet, but to a greater extent in F344 than in LOU rats. This confirms the relevance of using the F344 rat strain as a model of the deleterious effects of corticotropic hyperactivity or chronic stressful conditions associated to high-fat diets, such as metabolic syndrome and insulin resistance [22,23], and as described in obese humans [55]. DEX decreased the diurnal VO_2 of F344 rats in accordance with Strack et al [56], showing an inhibition of thermogenesis by corticosterone in rats. Interestingly, the effect of corticosterone described by Strack and collaborators was significant only in rats rendered diabetic. Our data are also in line with a central inhibition of SNS by GR in the F344 strain (Fig. 1), as described in other rat strains [57], and with an exacerbation of the metabolic effects of high-fat diets by glucocorticoids. On the contrary, the LOU rat strain appears insensitive to the central inhibition of SNS by glucocorticoids because their VO_2 was preserved (at night) or increased (in the day) by DEX. In LOU, such a resistance of the SNS to GR-induced regulation could explain the unexpected lower GR effects on glucose and insulin that we underscored in comparison to F344 rats [58]. Keeping in mind that MR targets are insensitive to ADX in LOU rats, the fact that GR-induced actions are sometimes higher and other times lower in LOU than in F344 rats could depend on the nature of the dimerization during transduction (homo- vs hetero-dimerization with other nuclear factors including MR [59]).

5. Conclusion

This study confirms the involvement of genetic factors in individual vulnerability to the unfavorable impact of stressful environment, that is, of high glucocorticoid levels, on the consequences of inadequate food choices and overfeeding [42,52,59]. The F344 rat strain constitutes a relevant model of the involvement of GR activation in food preference for high-calorie diets, abdominal fat deposition, type 2 diabetes mellitus, and other deleterious consequences of visceral obesity [18,19]. The LOU rats exhibit a high sensitivity to MR-induced thermogenesis probably via a central SNS activation, associated to a low sensitivity to the GR-induced SNS inhibition, which could both contribute to their leanness and resistance to diet-induced obesity. By oxidizing preferentially FFA, the LOU rat lives in a constant state of caloric restriction, which could be involved in its long and healthy aging [60,61]. Our study also supports the recent assumption of a neuroprotective role of MR [62]. The LOU rat strain constitutes a relevant model of resistance to diet-induced obesity and to the deleterious effects exerted by glucocorticoids on metabolism and fat deposition. Our study highlights the implication of functional variability in HPA axis and, particularly in both corticosteroid receptor types, in the complex interactions between the physiopathology of nutrition and genetics.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.metabol.2010.07.005.

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