Postnatal Diet-Induced Obesity in Rats Upregulates Systemic and Adipose Tissue Glucocorticoid Metabolism During Development and in Adulthood

Its Relationship With the Metabolic Syndrome

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In humans, a hyperactivity of glucocorticoid metabolism was postulated to be involved in the intrauterine programming of the metabolic syndrome in adulthood. We studied in rats the effects of overfeeding, obtained by reducing the size of the litter in the immediate postnatal period, a time crucial for neuroendocrine maturation such as late gestation in humans. Overfeeding induced early-onset obesity and accelerated the maturation of the hypothalamo-pituitary-adrenal (HPA) axis together with an upregulation of adipose tissue glucocorticoid receptor (GR) mRNA. In adulthood, neonatally overfed rats presented with moderate increases in basal and stress-induced corticosterone secretion and striking changes in visceral adipose tissue glucocorticoid signaling, that is, enhanced GR and 11β-hydroxysteroid dehydrogenase type 1 mRNA levels. The above-mentioned alterations in the endocrine status of overfed rats were accompanied by a moderate overweight status and significant metabolic disturbances comparable to those described in the metabolic syndrome. Our data demonstrate for the first time that postnatal overfeeding accelerates the maturation of the HPA axis and leads to permanent upregulation of the HPA axis and increased adipose tissue glucocorticoid sensitivity. Thus, the experimental paradigm of postnatal overfeeding is a powerful tool to understand the pathophysiology of glucocorticoid-induced programming of metabolic axes.
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Numerous clinical and biological findings indicate that glucocorticoids are involved in the pathophysiology of abdominal obesity and its accompanying complications. Indeed, an excess of glucocorticoids, when associated with hyperinsulinism, favors an increase of lipogenesis and a decrease of lipolysis, together with a stimulation of hepatic neoglucogenesis and an inhibition of peripheral glucose utilization (1). Alterations in the hypothalamo-pituitary-adrenal (HPA) axis have been described in human obesity and in rodent models of obesity. They could involve a hyperactivity of the central command of ACTH secretion, secondary to an increased exposure or sensitization to stress (2) or decreased negative glucocorticoid feedback (3). In addition, changes in peripheral glucocorticoid signaling with increased visceral adipose tissue glucocorticoid receptor (GR) concentrations and local reactivation of circulating inert cortisone (11-dehydrocorticosterone in rodents) to cortisol (corticosterone) driven by 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD-1) could play a pivotal role (4). However, the origins of the above-mentioned dysregulations have not been established.

Clinical and experimental evidence shows that the environment during the perinatal period plays an important role in the regulation of both metabolic and hormonal axes in adulthood. In humans, hyperglycemia and hyperinsulinemia in macrosomic fetuses of diabetic mothers were shown to favor later development of overweight (5). Conversely, it has been demonstrated that intrauterine growth retardation, with its subsequent catch-up growth that is associated with enhanced adiposity (6), is followed by increased prevalence of the metabolic syndrome in adults (7). The mechanisms underlying these abnormalities are not yet understood. In rodents, nutritional or pharmacological manipulations during the perinatal period have been shown to affect metabolic and/or endocrine regulations in adulthood. Nyirenda et al. (8) have demonstrated that chronic dexamethasone injection during the third week of gestation in rats induces glucose intolerance together with upregulation of hepatic PEPCK and GR mRNA in adult offspring. We have demonstrated that late gestation maternal undernutrition leads to a decrease of HPA axis activity at birth (9). However, it should be

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11β-HSD-1, 11β-hydroxysteroid dehydrogenase type 1; AT, adipose tissue; EAT, epididymal adipose tissue; FFA, free fatty acid; GR, glucocorticoid receptor; HPA, hypothalamo-pituitary-adrenal; MAT, mesenteric adipose tissue; NF, normo-feeding; OF, overfeeding; PVN, paraventricular nucleus.

emphasized that, in rodents, neuroendocrine maturation occurs mainly during the postnatal period, as opposed to primates, in which it takes place during the third trimester of gestation (10). As a consequence, hormonal studies in rodents are more appropriate in the postnatal rather than in the prenatal period. It is established that, in rats, during the immediate postnatal period, environmental or pharmacological manipulations permanently program the HPA axis. Indeed, adult basal and/or stress-induced corticosterone secretion is increased after postnatal maternal separation (11), modified maternal behavior (11), or endotoxin injection (12) and is decreased after handling (11) and parenteral (13) or maternal (14) glucocorticoid treatment. Interestingly, it is known that postnatal overfeeding leads to overweight, metabolic disturbances, and hypertension in adulthood (15). We hypothesized that postnatal overfeeding may impair the development of central and/or peripheral glucocorticoid regulation that could affect the metabolic regulations.

RESEARCH DESIGN AND METHODS

All experimental procedures were approved by the Local Animal Care and Use Committee. Animals were housed under standard conditions of light (12-h light/dark cycle; lights on at 0600) and temperature (22–24°C), with free access to tap water and standard pellet diet. Virgin female Wistar rats (Janvier, Le Genest St Isle, France) were time-mated. At postnatal day 3 (P3), male pups were randomly distributed among the mothers. The litter size was adjusted to 10 newborns to induce normo-feeding (NF) or to three pups to induce overfeeding (OF). Animals were subsequently left undisturbed, except for weighing and cage cleaning at P8 and P14. Rats were weaned at P21, and body weight was recorded throughout life. At the adult age, six randomly selected animals per group were housed in metabolic cages to measure daily mean food intake and to collect urine.

Basal study. Ten NF and nine OF P8, P14, P21, or adult (4 months) rats were killed by stressless decapitation between 1400 and 1600. Trunk blood was collected in tubes with or without a 5% EDTA solution. Blood was centrifuged at 4,000 rpm for 20 min at 4°C, and the resulting plasma or serum was stored at −70°C until assay. Brain, adrenals, and liver were removed and frozen on dry ice. White fat pads were dissected, weighed, and frozen or paraffin-embedded. Fat pads were not dissected at P8 for methodological reasons. Tissues were stored at −70°C until further processing.

Intraperitoneal glucose tolerance test. Rats (n = 8/group) were fasted overnight and then injected intraperitoneally with 1.5 mg/kg D-glucose (30% stock solution in saline). Blood samples were taken under light Forene anesthesia by tail venesection before injection and 30 and 120 min after the glucose load.

Hormonal response to stress. Five days before the experiment, adult rats (n = 7/group) were subjected to intracarotid canulation under deep equitesine anesthesia (0.25 ml/100 g body wt). Canulae were flushed every day with a heparin-saline solution. Rats were placed into cages attached to a shaking platform for 10 min at 0800. Blood samples were taken from the canula before and 15, 30, 45, 60, 90, and 120 min after the onset of stress.

Assays. Corticosterone was assayed in plasma and urine (after ethanol or dichloromethane extraction, respectively) using a radioimmunoassay previously described (16). Adrenals were dissected free of fat, weighed to the nearest milligram, and homogenized in a solution of 0.9% NaCl. Adrenal corticosterone content was measured using the above-mentioned radioimmunoassay. Plasma glucose was measured using an enzymatic method (Bio-Mérieux, Marcy l'Etoile, France). Plasma insulin and leptin were assayed using radioimmunoassays (Linco Research, St. Charles, MO). Plasma lipids were assayed using automatized enzymatic assays (Vitros, Ortho-Clinical Diagnostics, Rochester, NY). Free fatty acids (FFAs) were assayed using an enzymatic method (Roche, Penzberg, Germany).

In situ hybridization. Coronal 12-μm sections were cut in a cryostat microtome at −20°C through the hypothalamic paraventricular nucleus (PVN). Sections (12 or 20 μm) were cut in liver or epididymal adipose tissue (EAT) and mesenteric adipose tissue (MAT). Sections were thaw-mounted onto gelatin-coated slides, dried on a slide warmer, and kept at −70°C. In situ hybridization was performed as previously described (17). Corticotropin-releasing hormone (CRH), GR, PEPCK, and 11β-HSD-1 antisense probes were generated by in vitro transcription in the presence of ^{35}S-uridine triphosphate (Perkin Elmer, Paris, France) from cDNA inserted into pPCR script and corresponding to bases 261-1020, 1617-2150, 174-531, and 18-271 of their respective mRNA. Slides were exposed to X-ray films (BIOMAX MR; Kodak, Le Pontet, France) together with ^{14}C standards. Hybridization with the sense probes showed no signal, demonstrating the specificity of the probes (not shown). Hybridization signals were quantified on the film autoradiograms using the Image software and converted to nanocuries per gram using the ^{14}C standards. Because GR mRNA expression was very high in blood vessels, adult AT GR hybridizations were quantified on nuclear emulsion–dipped (K5, Ilford, Saint-Priest, France; diluted 1:1 in water) slides exposed for 1 month and counterstained with eosin. Brightfield images were captured with a charge-couple device camera and digitized. Semiquantitative analysis of GR mRNA was performed by measuring the surface of the hybridized areas using the Image software. Ten randomly chosen fields (0.5 mm^2) per section were analyzed, and the resulting average values were used for statistical analysis. Adipocyte surface was measured on counterstained paraffin AT sections.

Statistical analysis. Data are presented means ± SE. Statistical analysis was performed using the Statview analysis program. For body, hormonal basal measurements, and PVN in situ hybridizations, statistical analysis was performed using the Student's t test or two-way ANOVA followed by the Fisher's test, when appropriate. Circulating parameters during stress exposure or intraperitoneal glucose loading were analyzed using repeated-measures ANOVA followed by the Fisher's test. For AT in situ hybridization, we used the Mann-Whitney U test to compare mRNA expression between NF and OF rats and the paired Wilcoxon's test to compare mRNA expression between EAT and MAT. Adipocytes surfaces were compared with the Kolmogorov-Smirnov test. $P < 0.05$ was considered significant.

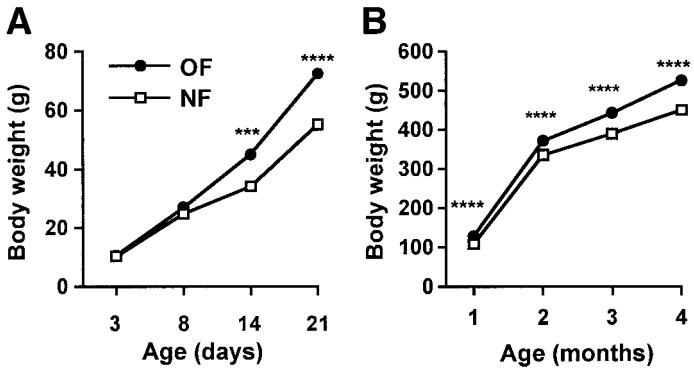

FIG. 1. Evolution of body weight (mean ± SE) during the suckling period (*A*) and after weaning (*B*) in NF (n = 10) or OF (n = 9) rats. ***P < 0.001, ****P < 0.0001 vs. NF animals.

RESULTS

Postnatal overfeeding induces early-onset obesity and overweight in adulthood. Body weight was not statistically different between groups at P3 and P8. Significant overweight was found in P14 and P21 and adult OF compared with NF rats (Fig. 1). An increase in all fat pad weight was found in P21 and adult OF rats and in adipocyte surface in adult animals (Table 1).

Developing OF rats have glucose intolerance and increased circulating leptin and FFA levels. In the NF group, plasma glucose did not differ with age, whereas insulin and insulin-to-glucose ratio were significantly decreased at P14. In OF animals, none of the above-mentioned parameters was affected at P8, whereas glycemia was increased at P14 but not at P21, and insulin and insulin-to-glucose ratio were enhanced at P14 and P21. Plasma FFA levels decreased with age and were not different between groups. Circulating leptin decreased after P8 in both groups, whereas it was elevated in OF rats compared with NF animals (Table 2). The nutritional manipulation did not change the concentrations of the mRNAs coding for GR, 11β-HSD-1, and PEPCK in the liver of P21 animals (299 ± 10 vs. 301 ± 11, 333 ± 41 vs. 323 ±

TABLE 1
Fat pad weight and adipocyte surface in P14, P21, and adult NF ($n = 10$) or OF ($n = 9$) rats

	NF	OF
Age P14		
Epididymal (mg)	87.1 ± 4.8	128.8 ± 14.0*
Retroperitoneal (mg)	100.0 ± 9.0	161.0 ± 11.5*
Perirenal (mg)	49.2 ± 6.6	59.0 ± 5.1*
Mesenteric (mg)	177.1 ± 15.7	210.4 ± 17.7*
Age P21		
Epididymal (mg)	186.2 ± 10.5	363.7 ± 30.4†
Retroperitoneal (mg)	180.4 ± 8.3	434.2 ± 43.2†
Perirenal (mg)	114.3 ± 10.7	165.4 ± 21.1‡
Mesenteric (mg)	329.1 ± 28.3	621.9 ± 51.8†
Age 4 months		
Epididymal (g)	6.33 ± 0.39	10.65 ± 0.87§
Retroperitoneal (g)	7.00 ± 0.58	9.34 ± 0.83‡
Perirenal (g)	1.91 ± 0.17	2.79 ± 0.21‡
Mesenteric (g)	6.16 ± 0.30	8.37 ± 0.64§
Adipocytes surface		
Epididymal (μm^2)	3,578 ± 64	3,986 ± 112†
Mesenteric (μm^2)	2,593 ± 111	3,042 ± 70†

Data are means ± SE. The experiment was repeated once with the same consistent results. *NS; †$P < 0.0001$, ‡$P < 0.05$, §$P < 0.01$ vs. NF rats.

40, and 216 ± 22 vs. 253 ± 21 nCi/g in NF or OF rats, respectively; $P > 0.05$).

Adult animals neonatally overfed show hyperphagia, glucose intolerance, and plasma lipid and leptin disturbances. OF rats had higher total and nocturnal food intake (24.1 ± 0.5 vs. NF 20.6 ± 0.4 g/day, $P < 0.001$, and 18.2 ± 1.1 vs. NF 13.0 ± 1.3 g/12 h, $P < 0.05$, respectively). Fed OF rats showed increases in circulating insulin, glucose, and FFAs compared with controls. Circulating total cholesterol, HDL cholesterol, and triglycerides did not change between groups (Table 2). Compared with normofeeding, overfeeding induced an increase in fasting plasma insulin, insulin-to-glucose ratio, and FFAs (93 ± 18 vs. NF 27 ± 3 pmol/l, $P < 0.05$; 20.0 ± 4.3 vs. NF 4.9 ± 0.6, $P < 0.05$; and 0.23 ± 0.03 vs. NF 0.12 ± 0.02 g/l, $P < 0.01$, respectively), whereas fasting glycemia was not changed (5.2 ± 0.2 vs. NF 5.4 ± 0.1 mmol/l, $P > 0.05$). After an intraperitoneal glucose load (Fig. 2), OF rats had higher circulating levels of glucose and insulin compared with controls. Adult OF rats presented with increased plasma leptin levels (Table 2), which were clearly disproportionately elevated because circulating leptin concentrations were still significantly increased after adjustment for body weight (11.82 ± 1.32 ng/ml, $P < 0.05$ vs. NF). The nutritional manipulation did not change the concentrations of the mRNA coding for GR, 11β-HSD-1, and PEPCK in the liver of adult animals (251 ± 10 vs. 264 ± 13, 396 ± 35 vs. 371 ± 23, and 844 ± 40 vs. 918 ± 59 nCi/g in NF or OF rats, respectively; $P > 0.05$).

Postnatal overfeeding induces a hyperactivity of the HPA axis during development and exposes adult animals to greater basal and stress-induced glucocorticoid signals throughout their lifespan. In OF compared with NF rats, adrenal weight was not changed at P8 and P14 and was significantly increased at P21, whereas adrenal corticosterone content was not modified at P8 and was enhanced at P14 and P21. Overfeeding did not change circulating ACTH and corticosterone at P8, whereas it induced significant increases at P14 and P21 (Fig. 3). CRH mRNA concentrations in the PVN decreased with age, with significant lower values in OF rats compared with NF rats. OF rat PVN GR mRNA levels were increased at P8 and P14 (Fig. 4).

Adult OF rats presented with a significant adrenal hypertrophy (58.4 ± 3.1 vs. NF 47.9 ± 2.4 mg, $P < 0.05$) and an increased adrenal corticosterone content (56.1 ± 4.0 vs. NF 37.7 ± 5.1 pmol/mg tissue, $P < 0.05$). Urinary free corticosterone concentrations were significantly elevated in adult OF rats at the time of peak (Fig. 5). When exposed to a mild psychological stress adult NF and OF rats showed similar kinetic patterns of corticosterone response (Fig. 5). However, OF rats displayed increased corticosterone secretion during the ascending phase of corticosterone hypersecretion (repeated-measures ANOVA t0–t30: $P < 0.05$; peak value: 1,220 ± 75 vs. NF 900 ± 110 nmol/l, $P < 0.01$). CRH mRNA concentrations in the PVN were not different in adult OF rats compared with NF rats (114.9 ± 2.3 vs. NF 107.4 ± 4.8 nCi/g, $P > 0.05$), whereas PVN GR mRNA levels were decreased (60.0 ± 6.5 vs. NF 87.5 ± 7.9 nCi/g, $P < 0.05$).

Neonatal overfeeding programs AT glucocorticoid sensitivity through an upregulation of local GR and 11β-HSD-1 mRNA levels. Overfeeding induced a significant increase in GR mRNA concentrations in both EAT and MAT of P21 rats. The nutritional manipulation did not

TABLE 2
Circulating metabolic parameters in fed P8, P14, P21, and adult NF ($n = 10$) or OF ($n = 9$) rats killed between 1400 and 1600

	NF	OF
Age P8		
Glucose (mmol/l)	7.6 ± 0.1	7.9 ± 0.2*
Insulin (pmol/l)	110 ± 23	170±28*
Insulin-to-glucose ratio	14.5 ± 3.0	21.4 ± 3.7*
FFAs (g/l)	0.24 ± 0.02	0.23 ± 0.02*
Leptin (ng/ml)	5.95 ± 1.11	13.84 ± 2.19†
Age P14		
Glucose (mmol/l)	7.4 ± 0.1	9.1 ± 0.3†
Insulin (pmol/l)	27 ± 10	118 ± 23‡
Insulin-to-glucose ratio	2.1 ± 0.5	12.7 ± 2.3‡
FFAs (g/l)	0.14 ± 0.01	0.17 ± 0.01*
Leptin (ng/ml)	1.79 ± 0.18	7.25 ± 0.70†
Age P21		
Glucose (mmol/l)	7.7 ± 0.2	8.1 ± 0.1*
Insulin (pmol/l)	75 ± 25	212 ± 37§
Insulin-to-glucose ratio	9.1 ± 2.6	25.7 ± 4.4§
FFAs (g/l)	0.09 ± 0.01	0.14 ± 0.01*
Leptin (ng/ml)	2.43 ± 0.32	7.49 ± 0.95‖
Adults		
Glucose (mmol/l)	6.6 ± 0.2	7.5 ± 0.3‡
Insulin (pmol/l)	90 ± 11	128 ± 13‡
Insulin-to-glucose ratio	13.6 ± 1.5	17.0 ± 1.3*
FFAs (g/l)	0.09 ± 0.01	0.17 ± 0.01†
Total cholesterol (mmol/l)	1.88 ± 0.09	1.92 ± 0.09*
HDL cholesterol (mmol/l)	0.97 ± 0.06	0.90 ± 0.04*
Triglycerides (mmol/l)	1.43 ± 0.18	1.54 ± 0.16*
Leptin (ng/ml)	7.7 ± 1.0	13.6 ± 1.5§

Data are means ± SE. The experiment was repeated once with the same consistent results. *NS; †$P < 0.0001$, ‡$P < 0.05$, §$P < 0.01$, ‖$P < 0.001$ vs. NF rats.

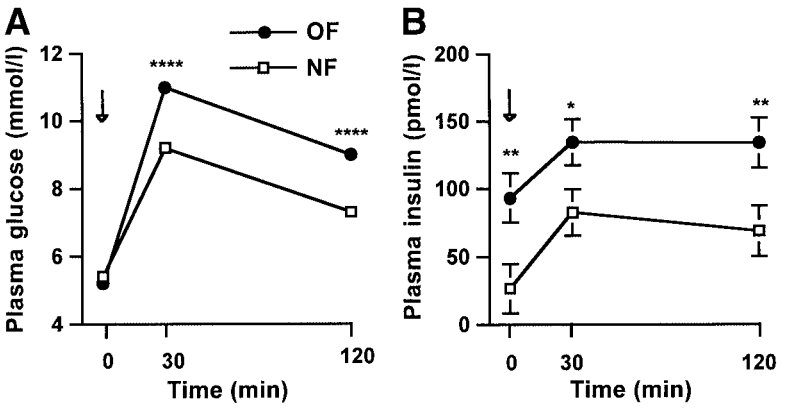

FIG. 2. Changes in plasma glucose and insulin levels (means ± SE) after intraperitoneal glucose loading in adult NF (n = 10) or OF (n = 9) rats. The arrow indicates the time of glucose injection. *P < 0.05, **P < 0.01, ****P < 0.0001 vs. NF animals.

affect 11β-HSD-1 mRNA concentrations in EAT and MAT (Fig. 6).

GR mRNA concentrations were significantly enhanced in MAT compared with EAT in both NF or OF adult animals. Compared with NF rats, OF animals had higher GR mRNA concentrations in EAT and MAT. In NF rats, 11β-HSD-1 mRNA levels were lower in MAT compared with EAT. OF animals showed an opposite pattern of 11β-HSD-1 mRNA distribution with higher concentrations in MAT than in EAT (Fig. 6). Changes in 11β-HSD-1 mRNA expression in AT were mainly located in the stromal portion of the tissue (not shown).

DISCUSSION

Our experiments demonstrate for the first time that postnatal overfeeding associates an acceleration of the maturation of the HPA axis and, in adulthood, a permanent upregulation of the HPA axis and an increased AT glucocorticoid sensitivity. We show that overfeeding accelerates the dynamic changes that characterize the development of the HPA axis in rats, that is, the progressive decrease in PVN CRH mRNA expression and increase in circulating ACTH and corticosterone. This phenomenon was accompanied by an upregulation of AT GR mRNA. In adulthood, neonatally overfed rats presented with moderate increases in basal and stress-induced corticosterone secretion and striking changes in visceral AT glucocorticoid metabolism, that is, enhanced GR and 11β-HSD-1 mRNA levels. The above-mentioned alterations in the endocrine status of OF rats were accompanied by a moderate overweight condition and significant metabolic disturbances comparable to those described in the metabolic syndrome. Our findings confirm and substantially extend previously published data that show that corticosterone secretion in response to a neurogenic stress was elevated in perinatally overfed rats (18) and that maturation of the adrenocortical rhythm was delayed in underfed animals (19). The model of postnatal overfeeding differs from previously published postnatal manipulations in rats (11–14) in that *1*) it uses a mild exclusively environmental stimulus and does not involve any pharmacological treatment, *2*) the changes of corticosterone status found in adults parallel those of developing animals, *3*) it associates systemic and AT changes in glucocorticoid metabolism, and *4*) it induces the metabolic syndrome in adulthood. As a consequence, the experimental paradigm of neonatal overfeeding in rats is an interesting model that could shed light on some of the pathophysiological mechanisms underlying obesity and its associated complications in humans. It is known that obese patients have mild increased basal and stress-induced glucocorticoid synthesis (2,20) and enhanced GR mRNA and 11β-HSD-1 mRNA and activity in stromal and adipocyte compartments of AT (20–24), which correlate with several features of the metabolic syndrome such as central adiposity, fasting glucose, insulin, insulin resistance (25), and AT expression of plasminogen activator inhibitor type 1 mRNA (26).

An important question that arises from our observations is what is the primary defect responsible for the overfeeding-induced hormonal and metabolic disturbances. Our finding showing that hypothalamic CRH mRNA levels were altered in OF P8 rats, at a time at which neither body weight nor metabolic parameters were affected, strongly suggests that changes in the central components of the HPA axis represent the primary target of the nutritional manipulation, even though plasma ACTH and corticosterone levels were not changed. Indeed, we have previously reported that the functional maturation of the peripheral components of the HPA axis in the developing rat is scheduled by CRH (27). We found that overfeeding was accompanied by a decrease in hypothalamic CRH mRNA levels and an increase in GR mRNA levels, suggesting that the negative glucocorticoid feedback appears earlier in OF rats. Several mechanisms may be involved for the accelerated maturation of the central components of the HPA axis. It has been suggested that shortage of nutrients is directly responsible for the postnatal decreased adrenal cortex activity associated with intrauterine growth retardation or prematurity with fetal growth inhibition in humans (28). It is conceivable that an excess of nutrients could have per se a stimulatory effect on the maturation of the HPA axis. Alternatively, the increased circulating leptin levels found in overfed developing rats, which are presumably related to the increased caloric intake (29) and/or to the enhanced adipose mass, could play a role. Indeed, it has been demonstrated that chronic leptin injection in developing rats accelerates the maturation of the glucocorticoid feedback (30). The increased basal and stress-stimulated corticosterone secretion in adult OF rats may be, at least in part, related to the diminished hypothalamic GR expression leading to a decreased sensitivity to the negative glucocorticoid feedback. The increase in circulating FFAs could represent an additional mechanism responsible for the hyperactivity of the HPA axis of OF

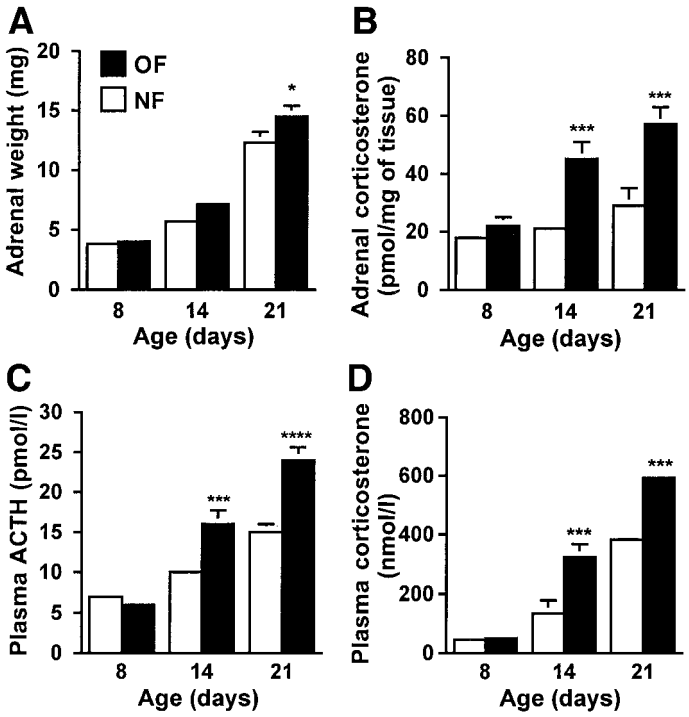

FIG. 3. Evolution of adrenal weight (*A*), adrenal corticosterone content (*B*), circulating ACTH (*C*), and corticosterone (*D*) concentrations during development in NF (n = 10) or OF (n = 9) rats. Values are means ± SE *P < 0.05, ***P < 0.001 vs. NF animals.

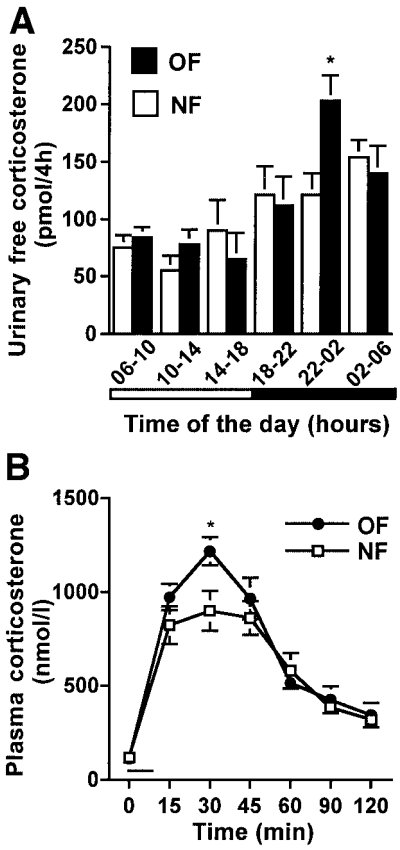

FIG. 5. *A*: Nycthemeral free urinary corticosterone concentrations in NF or OF (n = 6/group) adult rats. Values are means ± SE. *P < 0.05 vs. NF animals. *B*: Plasma corticosterone levels during exposure to stress in NF or OF adult rats (n = 7/group). Values are means ± SE. The solid black line represents the period of exposure to stress. *P < 0.05 vs. NF animals.

rats because it is known that FFAs stimulate basal and stress-induced ACTH and corticosterone secretion (31). Alternatively, changes in hypothalamic vasopressin activity could participate in the increased corticosterone secretion of OF rats.

Our findings demonstrate that, in addition to the systemic hyperactivity of the HPA axis, OF rats presented with alterations of AT glucocorticoid metabolism, particularly in the visceral AT. We found that, in OF rats compared with controls, 11β-HSD-1 mRNA levels were upregulated in MAT and downregulated in EAT (which is nonvisceral AT), suggesting the existence of an amplification of intra-adipose glucocorticoid levels in visceral AT because there is a parallelism between 11β-HSD-1 activity and mRNA levels (23). Consistent with the demonstration that glucocorticoids are potent secretagogues of leptin from AT (32), we found that plasma leptin levels in OF rats were disproportionately elevated. Interestingly, although rats were consuming a standard diet, the above-mentioned changes were accompanied by glucose intolerance and lipid disturbances, without evidence of hepatic dysfunction, and a marker of AT insulin resistance, that is, increased circulating levels of FFAs, both under a basal state and after intraperitoneal glucose loading. Taken together with the findings that adult OF rats were moderately overweight, the above-mentioned observation further stresses the pivotal role of glucocorticoid metabolism in fat as a source of metabolic abnormalities. Transgenic mice with adipocyte-targeted 11β-HSD-1 overexpression developed a visceral obesity that was exaggerated by a high-fat diet and exhibited high blood pressure, insulin-resistant diabetes, hyperlipidemia, and, in particular, increased levels of circulating FFAs (33,34). Mice knockout for 11β-HSD-1 presented with adipocyte insulin sensitization, reduced high-fat feeding–induced body weight gain, visceral fat enlargement, and lipid alterations (35) and showed attenuated starvation-induced activation of hepatic glucose-6-phosphatase and PEPCK and stress-induced hyperglycemia (36). Selective inhibition of 11β-HSD-1 decreased blood glucose concentrations in spontaneously hyperglycemic KKA^{Y}, *ob/ob*, and *db/db* mice and circulating FFAs in *ob/ob* mice (37,38).

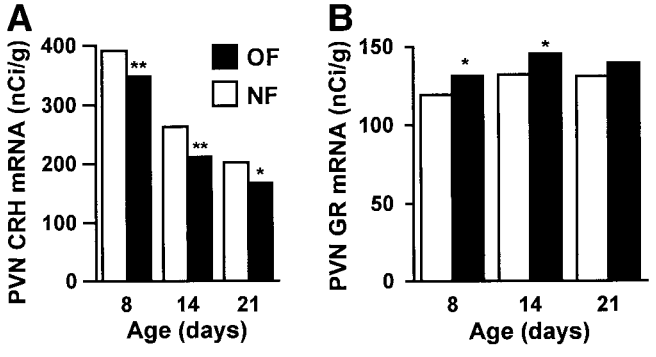

FIG. 4. Semiquantitative analysis of CRH (*A*) and GR (*B*) mRNA expression in the hypothalamic PVN during development in NF (n = 10) or OF (n = 9) rats. Values are means ± SE. *P < 0.05, **P < 0.01 vs. NF animals.

The mechanisms responsible for the regulation of 11β-HSD-1 in AT are not completely understood. It is interest-

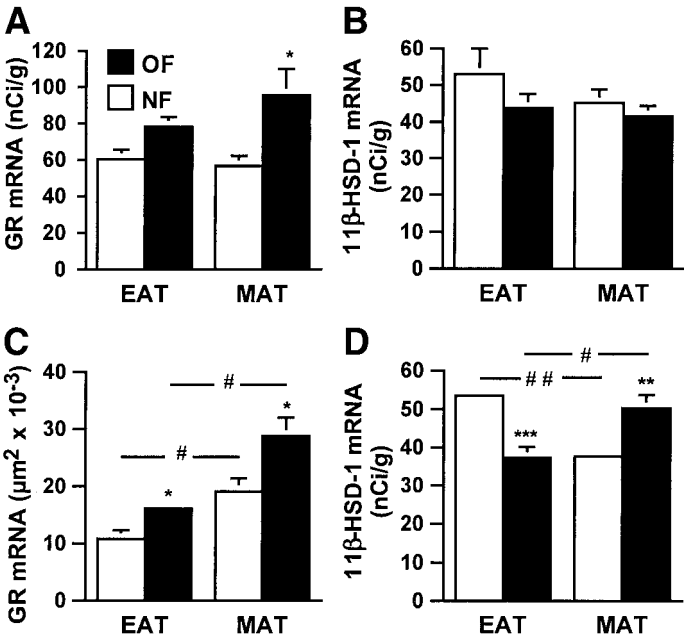

FIG. 6. Semiquantitative analysis of GR (*A* and *C*) and 11β-HSD-1 (*B* and *D*) mRNA expression in EAT and MAT of NF ($n = 10$) or OF ($n = 9$) P21 (*A* and *B*) or adult (*C* and *D*) rats. Values are means ± SE. *$P < 0.05$, **$P < 0.01$, ***$P < 0.005$ vs. NF animals. #$P < 0.05$, ##$P < 0.01$ between fat pads. Note that mRNA levels in *A*, *B*, and *D* were measured on film autoradiograms and are expressed as nanocuries per gram, whereas mRNA levels in *C* were measured on emulsion-dipped tissue sections and are expressed as the hybridized surface.

ing to notice that alterations in AT GR mRNA were present as soon as P21 in visceral AT of OF rats, whereas the dysregulation of AT 11β-HSD-1 appeared later, suggesting that changes in 11β-HSD-1 expression were subsequent to the increased circulating corticosterone concentrations and the enhanced AT GR, because it is established that glucocorticoids stimulate 11β-HSD-1 expression (4). The increase in AT GR mRNA in face of an upregulated HPA axis is a surprising finding because it is admitted that circulating glucocorticoids downregulate GR mRNA (39), consistent with the decreased PVN GR mRNA levels found in OF rats. Such an upregulation of GR mRNA expression has been described in the liver (8) and in the retroperitoneal AT (40) of adult rats exposed to dexamethasone during late pregnancy. It is known that rat GR mRNA shows high variability at the 5′ end, reflecting tissue-specific differences in promoter activity, and that first exon usage in the hippocampus is altered by perinatal environmental manipulations (41). Therefore, increased expression of AT GR mRNA in OF rats may reflect induction of an AT-specific promoter.

Adult OF rats presented with a significant overweight condition associated with hyperphagia and increased circulating leptin. Plagemann et al. (42,43) reported that postnatal overfeeding induces alterations of central nervous system neuropeptides involved in food intake (galanin) and satiety (cholecystokinin). Our findings that developing and adult OF rats had increased HPA axis activity could, at least in part, explain the concomitant alterations in food intake, because it is known that glucocorticoids have a stimulatory effect on the biosynthesis and the metabolic action of neuropeptide Y (44) and on the expression of the mRNAs coding for the neuropeptide Y receptors type 1 and 5 in the ventromedial hypothalamus (45). Indeed, glucocorticoids have been proposed to represent a counterregulatory hormone of leptin action and may participate in the state of leptin resistance found in obesity (46).

In conclusion, our findings demonstrate a potent and long-term effect of neonatal overfeeding that can program major changes in the development of both endocrine and metabolic regulatory mechanisms. This observation emphasizes the pivotal role of the glucocorticoid environment during the perinatal period on the subsequent development of metabolic alterations in adulthood. Thus, the experimental paradigm of postnatal overfeeding in rats is a powerful animal model to study, in infants, the pathophysiology of the glucocorticoid-induced programming of metabolic axes and, in adulthood, the endocrine and metabolic effects of manipulations, which are known to worsen (high-fat feeding) or to improve (11β-HSD-1 inhibitors or peroxisome proliferator–activated receptor-γ agonist treatment) the metabolic syndrome.

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