Postnatal Programming of Glucocorticoid Metabolism in Rats Modulates High-Fat Diet–Induced Regulation of Visceral Adipose Tissue Glucocorticoid Exposure and Sensitivity and Adiponectin and Proinflammatory Adipokines Gene Expression in Adulthood

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OBJECTIVE—Alterations of the perinatal environment, which lead to increased prevalence of the metabolic syndrome in adulthood, program an upregulation of systemic and/or adipose tissue glucocorticoid metabolism (11β-hydroxysteroid dehydrogenase type 1 [11β-HSD-1]-induced corticosterone reactivation). We hypothesized that postnatal programming could modulate high-fat diet–induced adipose tissue dysregulation in adulthood.

RESEARCH DESIGN AND METHODS—We compared the effects of chronic (since weaning) high- or low-fat diet in postnatally normofed (control) or overfed (programmed) rats.

RESULTS—Postnatal programming accentuated high-fat diet–induced overweight, insulin resistance, glucose intolerance, and decrease in circulating and epididymal adipose tissue adiponectin. Neither manipulation altered liver function. Postnatal programming or high-fat diet increased systemic corticosterone production, which was not further modified when both manipulations were associated. Postnatal programming suppressed high-fat diet–induced decrease in mesenteric adipose tissue (MAT) glucocorticoid sensitivity and triggered high-fat diet–induced increase in MAT glucocorticoid exposure, subsequent to enhanced MAT 11β-HSD-1 gene expression. MAT tumor necrosis factor (TNF)-α, TNF-receptor 1, interleukin (IL)-6, resistin, and plasminogen activator inhibitor-1 mRNAs were not changed by high-fat feeding in control rats and showed a large increase in programmed animals, with this effect further enhanced by high-fat diet for TNF-α and IL-6.

CONCLUSIONS—Our data show for the first time that postnatal manipulation programs high-fat diet–induced upregulation of MAT glucocorticoid exposure, sensitivity, and inflammatory status and therefore reveal the pivotal role of the environment during the perinatal period on the development of diet-induced adipose tissue dysregulation in adulthood. They also urge the need for clinical trials with specific 11β-HSD-1 inhibitors. ***Diabetes*** **57:669–677, 2008**

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Received for publication 14 September 2007 and accepted in revised form 28 November 2007.

Published ahead of print at http://diabetes.diabetesjournals.org on 5 December 2007. DOI: 10.2337/db07-1316.

11β-HSD-1, 11β-hydroxysteroid dehydrogenase type 1; AUC, area under the curve; C/EBPs, CCAAT/enhancer-binding proteins; CHF, control high-fat diet; CLF, control low-fat diet; EAT, epididymal adipose tissue; FFA, free fatty acid; GR, glucocorticoid receptor; IL, interleukin; MAT, mesenteric adipose tissue; PAI, plasminogen activator inhibitor; PHF, programmed high-fat diet; PLF, programmed low-fat diet; PPAR, peroxisome proliferator–activated receptor; TNF, tumor necrosis factor; TNF-R1, TNF receptor 1.

Increase in glucocorticoids secretion and/or action is believed to play a major causative role in obesity and its associated complications. Indeed, glucocorticoids have well-characterized deleterious effects on adipose tissue development and regulation, in particular at a visceral level, and on glucose metabolism and blood pressure (1). Obesity is characterized by reduced cortisol secretion (2), suggesting that increased glucocorticoid exposure or sensitivity in obese patients occurs at a tissular level. Increased concentrations of 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD-1), the enzyme that catalyzes the local reactivation of circulating inert cortisone (11-dehydrocortisosterone in rodents) to cortisol (corticosterone) have been found in adipose tissue of obese patients (3–6) and are closely related to adipose tissue accumulation and features of insulin resistance (5,6). Polymorphic variability at the 11β-HSD-1 locus is associated with greater BMI, waist circumference, waist-to-hip ratio, and insulin resistance in children (7) and with increased waist-to-hip ratio in adults (8). Transgenic mice with adipocyte-targeted 11β-HSD-1 overexpression developed a visceral obesity that was exaggerated by a high-fat diet (9). Mice with disruption of 11β-HSD-1 in all tissues or mice with adipocyte-targeted 11β-HSD-2 (the 11β-HSD subtype that catalyzes the conversion of active to inactive glucocorticoids) overexpression have reduced weight gain on high-fat diet with improved glucose tolerance and insulin sensitivity, as well as decreased expression of proinflammatory cytokines such as resistin and tumor necrosis factor (TNF)-α (10,11). All the observations above strongly suggest that local glucocorticoid metabolism, in particular 11β-HSD-1, is a key determinant of obesity and its metabolic complications. However, the relationship between increased fat intake, which is known in humans to be, at least in part, responsible for the burden of obesity in developed countries (12), and 11β-HSD-1 is not clear. Morton et al. (13) have reported that chronic (18 weeks) high-fat feeding in mice induced downregulation of 11β-HSD-1 in both subcutaneous and visceral adipose tissue that was more pronounced in metabolic disease–prone (C57BL/6J) than in metabolic disease–resistant (A/J) mice. Drake et al. (14) showed that a chronic high-fat diet in Wistar rats induced transient downregulation of adipose 11β-HSD-1 mRNA and activity, which disappeared after 20 weeks. Pharmacological inhibition of 11β-HSD-1 in C56BL/6J mice with high-fat diet–induced obesity im-

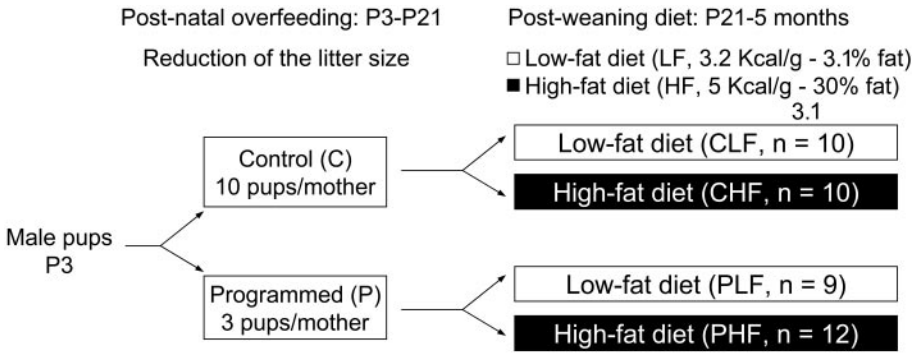

FIG. 1. Experimental protocol.

proved body weight and the biological markers of the metabolic syndrome (15).

Clinical and experimental evidence show that changes in the environment during the perinatal period can affect regulation of both metabolic and hormonal axes in adulthood, leading to a programming of metabolic and cardiovascular diseases (16). In humans, intrauterine growth retardation is followed by increased prevalence of cardiovascular, metabolic, and neuroendocrine disorders, in particular elevated plasma cortisol concentrations, in later life (17). In subjects born with intrauterine growth retardation, the catch-up growth phenomenon, which favors visceral fat deposition, increases risk for insulin resistance and subsequent diabetes later in life by promoting obesity in adulthood (18). Prenatal dexamethasone exposure resulted in glucose intolerance, hyperinsulinemia, high blood pressure, and exaggerated cortisol response to stress in nonhuman primate offspring (19). We have shown that postnatal overfeeding in rats, obtained by reducing the size of the litter in the immediate postnatal period, a time crucial for endocrine maturation such as late gestation in humans, is followed in adulthood by a moderate overweight status, significant metabolic disturbances comparable with those described in the metabolic syndrome, and upregulation of basal and stress-induced systemic glucocorticoid secretion and of adipose tissue exposure and sensitivity (20). We hypothesized that postnatal programming of glucocorticoid metabolism in rats could modulate high-fat diet–induced adipose tissue dysregulation in adulthood. Therefore, we compared in adult control or postnatally programmed rats fed from weaning either with low- or high-fat diet body composition and systemic metabolic and hormonal indexes. We also studied adipose tissue hormonal and inflammatory parameters.

RESEARCH DESIGN AND METHODS

All experimental procedures were approved by the local animal care and use committee. Wistar rats (Janvier, Le Genest St. Isle, France) were housed under standard conditions of light (12-h light/dark cycle; lights on at 0600 h) and temperature (22–24°C), with free access to tap water and standard pellet diet. Virgin females were time mated. Figure 1 depicts the experimental protocol. At postnatal day 3, male pups from nine litters were mixed and randomly redistributed (10 newborns for normofed rats [controls] or to 3 pups for overfed rats [programmed]) among the 9 mothers. Animals were subsequently left undisturbed, except for weighing and cage cleaning at postnatal days 8 and 14. Rats were weaned at postnatal day 21 and given either low-fat (3.2 kcal/g; lipids 3.1%, protein 16.1%, carbohydrates as corn starch 23.0% and as saccharose 37.0%, vitamines and minerals 5.0%, moisture 15.8%; SAFE, Villemoisson-sur-Orge, France) or high-fat (5 kcal/g, lipids as lard 30.0%, protein 16.1%, carbohydrates as saccharose 37.0%, vitamines and minerals 5.0%, moisture 11.9%; SAFE) diet ad libitum.

Basal and dynamic studies. At the adult age, six randomly selected rats per group were placed in metabolic cages for a 2-week acclimation period; then, urine was collected for corticosterone measurements. All adult rats (aged 5 months) were fasted overnight and injected intraperitoneally with 1.5 mg/g 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. One week later, fed rats were killed by rapid stressless decapitation between 1400 and 1600 h. 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 until at −70°C until assay. Liver and white fat pads were weighed and frozen or paraffin embedded. Tissues were stored at −70°C until further processing.

Assays. Corticosterone was assayed in urine after dichloromethane extraction using a radioimmunoassay, as previously described (20). Plasma glucose and free fatty acids (FFAs) were measured using enzymatic methods (Biomérieux, Marcy l'Etoile, France, and Oxoid, Dardilly, France, respectively). Plasma insulin and adiponectin were assayed using radioimmunoassay (Linco Research, St. Charles, MO).

In situ hybridization and morphological analysis. Sections (12 or 20 μm) were cut in a cryostat microtome at −20°C in liver or in 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 at previously described (21). Glucocorticoid receptor (GR), 11β-HSD-1, TNF-α, TNF–receptor 1 (TNF-R1), interleukin (IL)-6, resistin, adiponectin, PEPCK, plasminogen activator inhibitor (PAI)-1, and peroxisome proliferator–activated receptor (PPAR)γ antisense probes were generated by in vitro transcription in the presence of ^{35}S-uridine triphosphate (Perkin Elmer, Paris, France) from cDNAs inserted into pPCR script and corresponding to bases 1617-2150, 18-271, 24-272, 998-1284, 727-1004, 6-186, 54-355, 174-531, 1346-1788, and 1080-1587 of their respective mRNAs. 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 into nCi/g using the ^{14}C standards. Adipocytes surface was measured on counterstained paraffin adipose tissue sections (six randomly chosen fields per depot per rat).

Statistical analysis. Data are presented as means ± SE. Statistical analysis was performed with the Statview analysis program using two-way ANOVA followed by Fisher's multiple comparison post hoc test. The Kolmogorov-Smirnov test was used to compare adipocyte surfaces. Area under the curve (AUC) for plasma glucose, insulin, and FFAs was calculated using the trapezoidal method. Linear regression analysis was performed to identify correlates of adipose tissue 11β-HSD-1, and covariance analysis (SPSS software; SPSS, Chicago, IL) was performed for variables found to be $P < 0.05$ to determine their independent relationship with adipose tissue 11β-HSD-1.

RESULTS

Postnatal programming accentuates high-fat diet–induced overweight, insulin resistance, and glucose intolerance and does not significantly alter liver function. Table 1 shows the results of body measurements and of basal- and glucose-stimulated circulating metabolic parameters. Compared with control low-fat diet (CLF) rats, control high-fat diet (CHF) and programmed low-fat diet (PLF) rats showed a comparable increase in body weight and EAT and MAT masses, which were further enhanced in programmed high-fat diet (PHF) animals. Compared with control rats, postnatal programming or high-fat feeding alone increased mean adipocyte sur-

TABLE 1
Effect of postnatal overfeeding and high fat diet on body and fat pads weight, adipocyte surface, and circulating metabolic parameters

	CLF	CHF	PLF	PHF
n	10	10	9	12
Body weight (g)	512 ± 7	549 ± 15*	573 ± 15†	610 ± 9§‖
EAT weight (mg/g body wt)	9.6 ± 0.4	12.9 ± 0.7*	11.7 ± 0.5†	14.5 ± 0.6§‖
MAT weight (mg/g body wt)	12.7 ± 0.3	16.3 ± 1.3	16.7 ± 1.0†	21.2 ± 1.3§‖
EAT adipocyte surface (μm^2)	3,028 ± 188	3,821 ± 205*	3,494 ± 185†‡	5,546 ± 363§‖
MAT adipocyte surface (μm^2)	2,189 ± 66	2,840 ± 196*	2,510 ± 185†‡	4,036 ± 274§‖
Fasting circulating parameters				
Plasma glucose (mmol/l)	5.4 ± 0.1	5.0 ± 0.1	5.2 ± 0.2	5.3 ± 0.2
Plasma insulin (pmol/l)	27 ± 3	114 ± 15.*	93 ± 18†	170 ± 21§‖
Insulin-to-glucose ratio	4.9 ± 0.6	22.6 ± 2.9*	18.3 ± 4.1†	31.5 ± 4.0§
Plasma FFAs (g/l)	0.42 ± 0.05	0.73 ± 0.07*	0.75 ± 0.1†	1.14 ± 0.11§‖
Intraperitoneal glucose tolerance test				
AUC plasma glucose ($mmol \cdot l^{-1} \cdot min^{-1}$)	8.0 ± 0.1	9.3 ± 0.3*	9.5 ± 0.1†	10.8 ± 0.2§‖
AUC plasma insulin ($pmol \cdot l^{-1} \cdot min^{-1}$)	69 ± 11	194 ± 17*	133 ± 17†‡	247 ± 14§‖
AUC plasma FFAs ($g \cdot l^{-1} \cdot min^{-1}$)	0.10 ± 0.02	0.21 ± 0.03*	0.22 ± 0.1†	0.30 ± 0.03§‖

Data are means ± SE. Adipocyte surface results are presented as the mean ± SE of averaged values obtained in each rat. Statistical analysis was performed by two-way ANOVA followed by Fisher's post hoc test and by the Kolmogorov Smirnov test for adipocyte surface. *$P < 0.05$, CHF vs. CLF; †$P < 0.05$, PLF vs. CLF; ‡$P < 0.05$, PLF vs. CHF; §$P < 0.05$, PHF vs. PLF; ‖$P < 0.05$ PHF vs. CHF.

face in both EAT and MAT, with this effect significantly more pronounced for high-fat diet (CHF vs. PLF in EAT and MAT, $P < 0.0001$). When both experimental paradigms were associated, adipocyte surface showed a larger increase.

In fasted animals, glycemia was not different between groups, whereas insulinemia, plasma FFAs, and insulin-to-glucose ratio were enhanced in CHF and PLF rats compared with CLF rats and were further increased in PHF rats. Intraperitoneal glucose tolerance test confirmed that high-fat feeding or postnatal programming induced glucose intolerance, insulin resistance, and dyslipidemia. Indeed, the AUC for plasma glucose, insulin, and circulating FFAs increased in CHF and PLF rats compared with that in CLF rats. AUCs for plasma glucose and FFAs were comparable in CHF and PLF animals, whereas the AUC for plasma insulin was greater in CHF compared with that in PLF rats. When programmed rats were fed with a high-fat diet, AUCs for plasma glucose, insulin, and plasma FFAs were further increased.

Postnatal programming had opposite effects on liver 11β-HSD-1 and GR mRNA levels. Compared with CLF rats, PLF animals showed increased GR mRNA levels and decreased 11β-HSD-1 mRNA concentrations. High-fat diet decreased liver 11β-HSD-1 mRNA in controls and programmed rats, whereas liver GR mRNA values were significantly decreased only in programmed animals. PEPCK mRNA concentrations were comparable between all groups. Liver PAI-1 mRNA concentrations were not affected by either postnatal programming or high-fat diet but showed a significant increase when both treatments were associated (Fig. 2).

High-fat diet stimulates glucocorticoid synthesis independently of postnatal programming (Fig. 3). Measurements of urinary corticosterone concentrations throughout 24 h indicated an overall effect of rhythm ($F = 17.46, P < 0.0001$) and treatment ($F = 3.43, P = 0.0184$) but no significant interaction between these two parameters (two-way ANOVA: $F = 0.58, P = 0.8873$). In rats fed with control diet, postnatal programming stimulated urinary corticosterone levels ($P = 0.0284$). In control but not programmed animals, high-fat feeding induced an increase in urinary corticosterone output ($P = 0.0094$ and $P = 0.4924$, respectively). As a consequence, urinary corticosterone values were comparable in control or programmed rats fed with high-fat diet ($P = 0.4037$).

High-fat diet–induced stimulation of MAT 11β-HSD-1 gene expression is triggered by postnatal programming (Fig. 4). In EAT, GR mRNA values were increased in programmed rats compared with those in control rats, whereas high-fat diet did not change GR expression. In MAT, GR mRNA values were increased in programmed rats compared with those in control rats; high-fat diet significantly decreased GR mRNA concentrations in control rats, whereas this effect was lost in programmed animals. Compared with those in control rats fed low-fat diet, postnatal programming or high-fat diet did not affect EAT 11β-HSD-1 mRNA levels. In MAT, high-fat diet did not change 11β-HSD-1 mRNA values in control rats; postnatal programming enhanced 11β-HSD-1 mRNA expression and demonstrated a significant interaction with high-fat diet (two-way ANOVA effect of programming × diet: $F = 7.97$, $P = 0.0072$) to further stimulate 11β-HSD-1 mRNA concentrations.

Postnatal programming regulates adipose tissue PPARγ gene expression in an opposite manner in EAT and MAT. PPARγ mRNA levels decreased in EAT and increased in MAT of postnatally programmed animals (4.4 ± 0.7 and 5.2 ± 0.6 nCi/g, respectively; $P = 0.0017$ and $P = 0.0308$ vs. EAT or MAT of control rats, respectively). High-fat feeding did not change PPARγ expression in control (6.9 ± 0.9 and 2.5 ± 0.3 nCi/g in EAT or MAT, respectively; $P > 0.05$ vs. low-fat diet) or in postnatally programmed animals (5.0 ± 0.5 and 6.3 ± 0.6 nCi/g in EAT or MAT, respectively; $P > 0.05$ vs. low-fat diet).

Postnatal programming and high-fat diet associate to decrease circulating adiponectin. Compared with those in control animals, circulating adiponectin concentrations were decreased by postnatal programming or high-fat diet. Two-way ANOVA analysis revealed that there was a significant interaction ($F = 5.30$, $P = 0.0262$) between postnatal programming and high-fat diet in decreasing plasma adiponectin. In EAT, but not MAT, high-fat feeding or postnatal programming decreased adiponectin mRNA levels, with this effect more marked in PHF animals (Fig. 5).

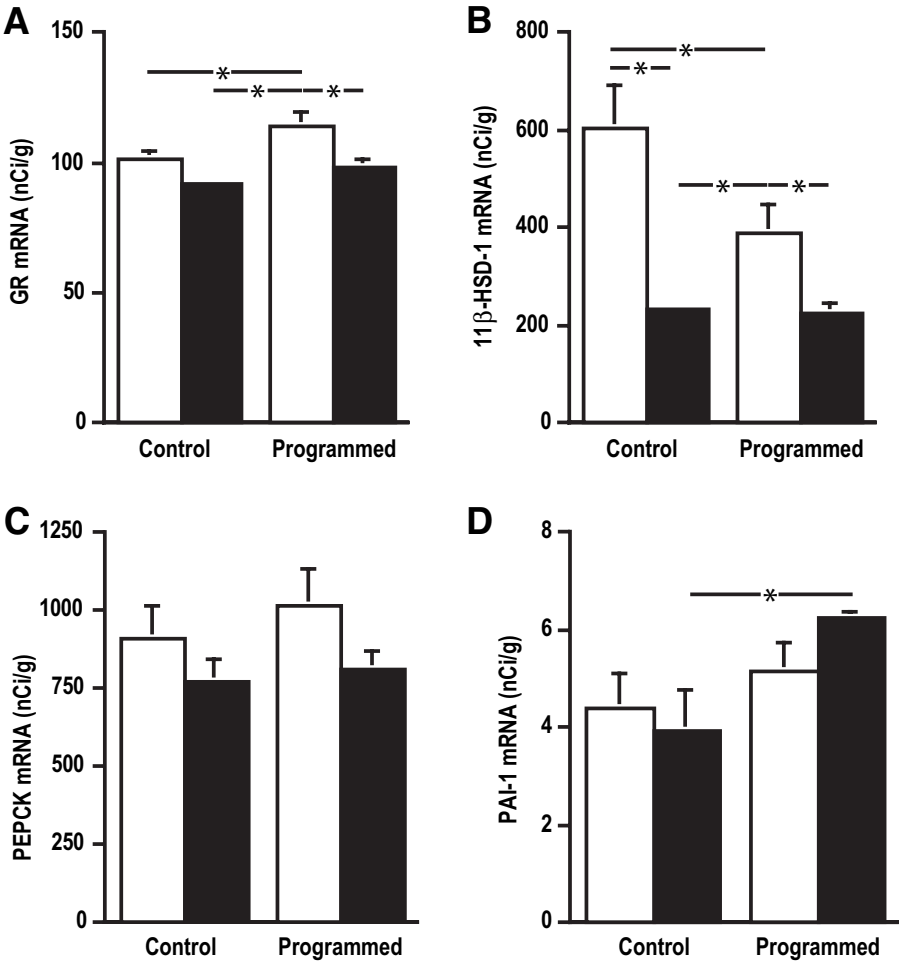

FIG. 2. Semiquantitative analysis of liver GR (*A*), 11β-HSD-1 (*B*), PEPCK (*C*), and PAI-1 (*D*) mRNA in control or programmed rats fed low-fat (□) or high-fat (■) diet (n = 10, 10, 9, and 12, respectively). Data are means ± SE. Statistical analysis was performed by two-way ANOVA followed by Fisher's post hoc test. *$P < 0.05$.

High-fat diet accentuates postnatal programming–induced inflammation in MAT. Compared with that in control rats fed low-fat diet, expression of TNF-α, TNF-R1, resistin, and PAI-1 genes in EAT was unaffected by either postnatal programming or high-fat diet (two-way ANOVA, effect of programming and effect of diet: $F = 1.17$, $P = 0.28$; $F = 0.16$, $P = 0.69$; $F = 0.83$, $P = 0.37$; $F = 2.51$, $P = 0.12$; $F = 0.33$, $P = 0.86$; $F = 1.71$, $P = 0.20$; $F = 2.48$, $P = 0.12$; and $F = 0.04$, $P = 0.85$ for TNFα, TNF-R1, resistin, or PAI-1 mRNA, respectively), whereas IL6 mRNA values were not changed by high-fat diet (two-way ANOVA, effect of diet: $F = 1.11$, $P = 0.30$) and increased by postnatal programming (2.21 ± 0.35 vs. 1.06 ± 0.15 nCi/g in PLF or CLF, respectively [$P = 0.0098$]; 1.77 ± 0.25 vs. 0.98 ± 0.28 nCi/g in PHF or CHF, respectively [$P = 0.036$]). In MAT of control rats, postnatal programming, but not high-fat diet, stimulated TNF-α, TNF-R1, IL-6, Res, and PAI-1 mRNAs. When programmed rats were given high-fat diet, TNF-α and IL-6 mRNAs were further enhanced (Fig. 6).

Correlates of MAT 11β-HSD-1. Simple linear regression analysis showed that MAT 11β-HSD-1 correlated with MAT TNF-α, IL-6, PPARγ, TNF-R1, GR, and resistin mRNAs but not with 24-h urinary corticosterone. Analysis of covariance indicated that the postnatal manipulation accounted for all the interactions between GR, 11β-HSD-1, and adipokine expression found in programmed rats. When considering the effect of high-fat diet, we found that the interactions between 11β-HSD-1 and the proinflammatory cytokines were independent from diet. In addition, we demonstrated that part of the effects of GR on 11β-HSD-1 expression was dependent on high-fat diet (Table 2).

DISCUSSION

Our data demonstrate that postnatal programming accentuates adipose tissue deposition and metabolic disturbances induced by chronic high-fat feeding. Because we previously demonstrated that postnatal programming induced at adulthood moderates increase in basal corticosterone secretion and a striking stimulation of glucocorticoid signaling in MAT (20), we first focused on these two parameters. We found that high-fat feeding increased corticosterone production, as demonstrated by the increased urinary corticosterone concentrations. Such stimulation of adrenal glucocorticoid secretion has already been described both in laboratory animals and in humans (22,23). However, high-fat feeding–induced stimulation of systemic glucocorticoid levels was comparable in control and programmed animals, suggesting that the potentiation of adipose tissue deposition and metabolic alterations induced by high-fat diet in programmed rats was not subsequent to the increase in circulating glucocorticoids. Indeed, it has been hypothesized that local glucocorticoid metabolism in adipose tissue, rather than systemic glucocorticoid status, plays a pivotal role in obesity and its associated complications. We found that chronic high-fat diet did not affect 11β-HSD-1 in EAT or MAT of control rats, consistent with the report of Drake et al. (14), but that

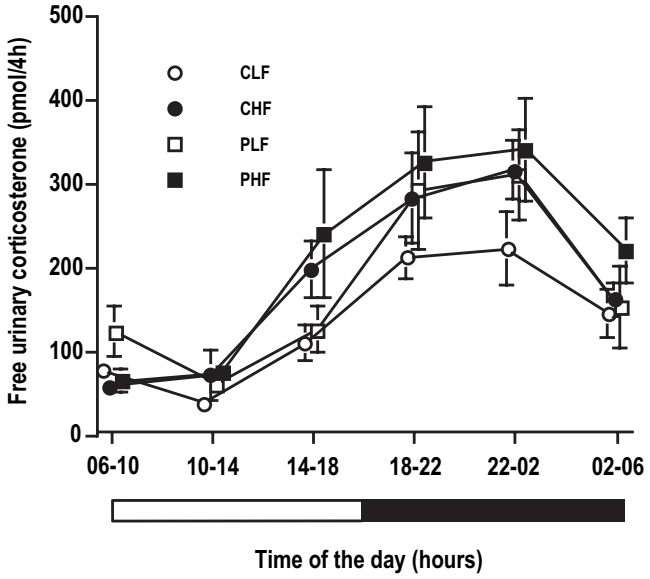

FIG. 3. Nycthemeral free urinary corticosterone concentrations in control and programmed rats fed low- or high-fat diet. Data are means ± SE (n = 6 per group). Two-way ANOVA analysis indicated an overall effect of time (F = 17.46, P < 0.0001) and treatment (F = 3.43, P = 0.0184) but no significant interaction between these two parameters (F = 0.58, P = 0.8873). In rats fed with low-fat diet, postnatal programming stimulated urinary corticosterone levels (P = 0.0284). In control but not programmed animals, high-fat feeding induced an increase in urinary corticosterone output (P = 0.0094 and P = 0.4924, respectively). As a consequence, urinary corticosterone values were comparable in control and programmed rats fed with high-fat diet (P = 0.4037).

postnatal programming triggered MAT 11β-HSD-1 overexpression in response to high-fat diet and reversed high-fat diet–induced decrease in MAT GR mRNA expression. It has been suggested that interindividual variation in the ability of humans to regulate adipose 11β-HSD-1 in response to a diet high in saturated fat (Western diet) may play a role in determining their overall susceptibility to insulin resistance and type 2 diabetes (10). Our data show for the first time that an environmental manipulation such as postnatal overfeeding in rats programs the ability of high-fat diet to upregulate MAT glucocorticoid exposure and sensitivity and, therefore, reveal the pivotal role of the environment during the perinatal period on the subsequent development of diet-induced (Western diet in particular) adipose tissue dysregulation in adulthood. Interestingly, clinical studies in obese patients demonstrated concomitant increases in 11β-HSD-1 and GR expression in MAT (6,24,25), possibly responsible for the enhanced glucocorticoid-induced insulin resistance described in patients with central obesity (26).

The pivotal role of the low-grade inflammatory status in obesity on vascular and metabolic dysregulations and adipose tissue development (27–29) prompted us to investigate the expression of the mRNAs encoding factors involved in distinct functions (insulin resistance: TNF-α, TNF-R1, resistin, and IL-6 [30]; adipose tissue insulin sensitization and differentiation: adiponectin and PPARγ [30]; and atherosclerosis: PAI-1 [31]). It is surprising to note that, under our experimental conditions, high-fat feeding alone, although responsible for a marked insulin resistance state and for decreased circulating adiponectin and EAT adiponectin gene expression, did not affect the inflammatory parameters measured in adipose tissue. However, it should be stressed that our high-fat diet contained 30% lipids, a value relatively low compared with those (45–60%) used in studies designed to investigate the effects of Western-type diet on inflammatory parameters (27). Our data provide the first evidence that postnatal programming increased the expression of MAT TNF-α, TNF-R1, IL-6, and resistin mRNAs and decreased EAT and circulating adiponectin concentrations, further emphasizing the deleterious consequences of postnatal programming. In addition, high-fat diet was able to potentiate the programming-induced increases in MAT TNF-α and IL-6 mRNAs and interacted with postnatal programming to further decrease plasma adiponectin levels. Such changes could explain, at least in part, the associated increased glucose intolerance and insulin-resistance state. It has recently been demonstrated that mice with reduced TNF-α bioavailability (induced by partial inactivation of TNF-α–converting enzyme) have reduced high-fat diet–induced glucose intolerance and insulin resistance (32) and that adipose tissue macrophages recruited during diet-induced obesity overexpress IL-6 (33). Overexpression of adiponectin in *ob/ob* mice normalized insulin sensitivity and glucose tolerance and reduced systemic and adipose tissue inflammation (34). Our data also show that postnatal programming and high-fat diet stimulated PAI-1 gene expression in liver and MAT. Whereas the stimulation of PAI-1 gene expression in liver and adipose tissue could be subsequent to the increase in circulating insulin concentrations (35), changes in adipose tissue PAI-1 mRNA levels could be directly controlled by enhanced 11β-HSD-1 mRNA expression. We have previously shown that 11β-HSD-1–driven cortisone reactivation regulates PAI-1 in adipose tissue of obese women (36).

The factors responsible for the postnatal programming–and/or high-fat diet–induced increase of MAT glucocorticoid metabolism are not clear. It is established that glucocorticoids stimulate 11β-HSD-1 gene expression (37). We previously demonstrated that postnatal overfeeding induced MAT GR mRNA upregulation, which was detectable as soon as day 21 of life and preceded that of 11β-HSD-1, suggesting that a diet-induced programming of GR gene expression, possibly of epigenetic origin (38), was the primary phenomenon (20). Such a phenomenon may account for the reversal of the decrease in MAT GR mRNA expression found in programmed rats fed high-fat diet. Regarding the effects of high-fat diet, we found that the interactions between 11β-HSD-1 and the various adipokines measured were independent of diet, suggesting either the existence of common regulatory mechanisms or regulatory interactions (39,40). However, it is not clear whether changes in adipose 11β-HSD-1 expression in response to high-fat diet are a consequence or a cause of the local microinflammatory syndrome. Indeed, glucocorticoids are widely used as anti-inflammatory agents. It has been demonstrated that dexamethasone treatment decreases basal and lipopolysaccharide-induced mRNA expression and secretion of IL-1β, TNF-α, and monocyte chemoattractant protein-1 in J774.1 macrophages (41). As a consequence, it could be suggested that the accentuation of 11β-HSD-1 overexpression found in MAT of PHF animals is a counterregulatory phenomenon subsequent to the local microinflammatory syndrome induced by high-fat diet (42). It is established that in human adipose tissue, TNF-α and FFAs, which are natural ligands of PPARγ, stimulate 11β-HSD-1 activity through a modulation of 11β-HSD-1 gene transcription (43,44). Because, in addition to MAT GR mRNA, MAT TNF-α mRNA and circulating

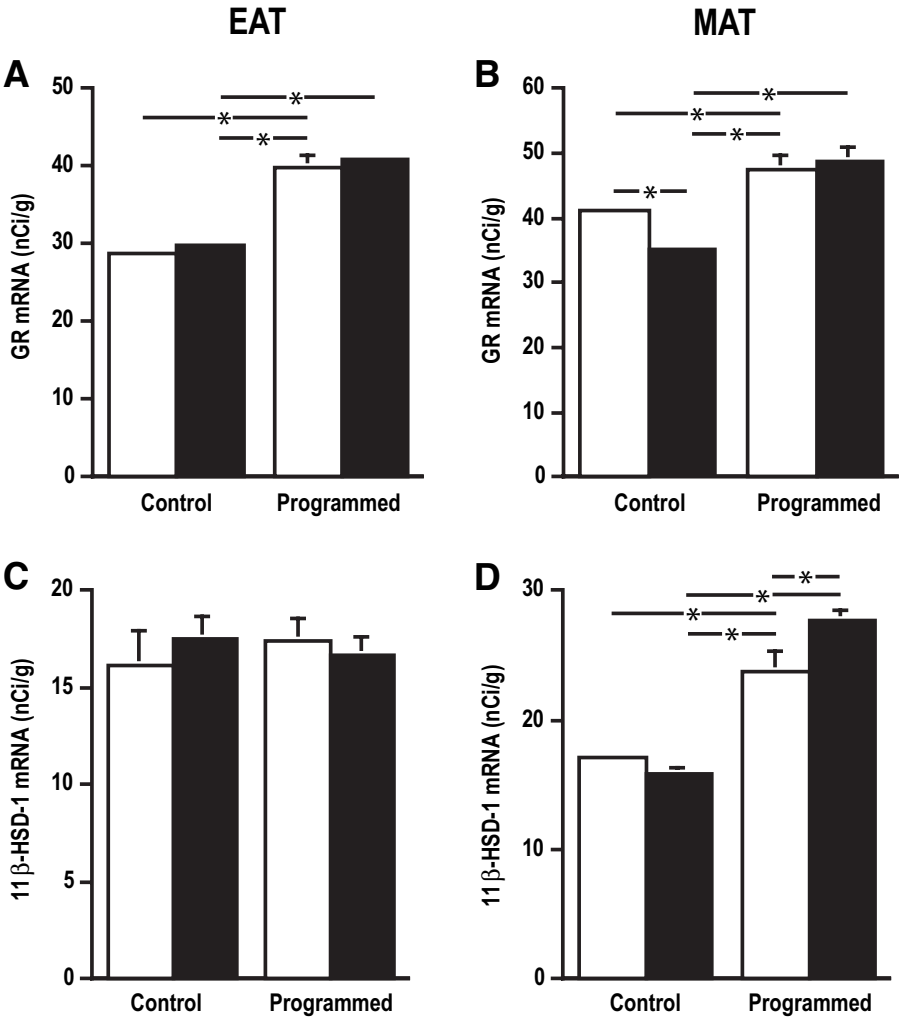

FIG. 4. Semiquantitative analysis of EAT GR (*A*) and 11β-HSD-1 (*C*) mRNA (*left panel*) and MAT GR (*B*) and 11β-HSD-1 (*D*) (*right panel*) mRNA in control or programmed rats fed low-fat (□) or high-fat (■) diet (n = 10, 10, 9, and 12, respectively). Data are means ± SE. Statistical analysis was performed by two-way ANOVA followed by Fisher's post hoc test. *P < 0.05.

FFA concentrations were increased in programmed rats fed with high-fat diet, the hypothesis above could explain, at least in part, the concomitant increase in MAT 11β-HSD-1 mRNA levels. Conversely, it could be suggested that the high-fat diet–induced increase in 11β-HSD-1 expression found in MAT of PHF rats is involved, at least in part, in generating MAT inflammation. Adipose tissue–targeted overexpression of 11β-HSD-1 resulted in decreased adipose tissue adiponectin and increased circulating TNF-α. 11β-HSD-1$^{-/-}$ mice or mice with adipose overexpression of 11β-HSD-2 expressed lower resistin and TNF-α and higher adiponectin mRNAs in adipose tissue (10,11). Reduction of adipose glucocorticoid receptor expression with antisense oligonucleotides in *ob/ob* mice resulted in a decrease in plasma resistin and TNF-α (45). Pharmacological blockade of 11β-HSD-1 in J774.1 macrophages decreased LPS-induced mRNA expression and secretion of IL-1β, TNF-α, and monocyte chemoattractant protein-1 (41). Further experiments investigating, with our experimental model of postnatal overfeeding, the effect of specific inhibitors of 11β-HSD-1, when available, will be useful in understanding these phenomena.

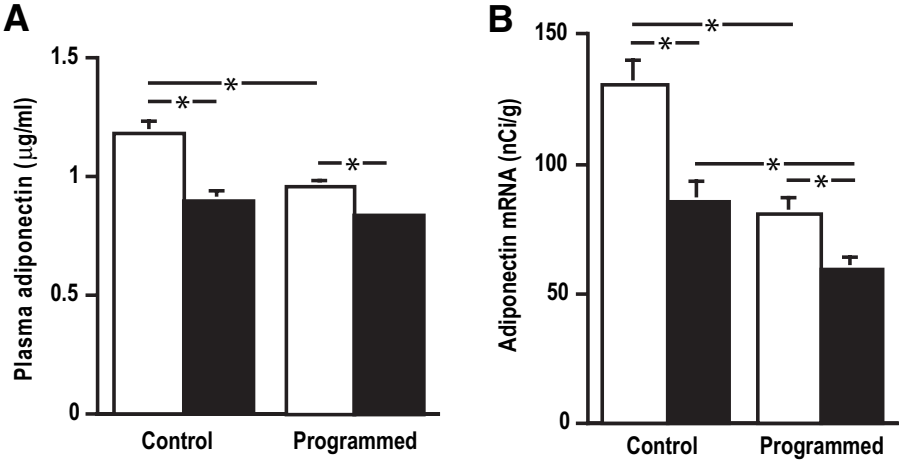

FIG. 5. Circulating adiponectin (*A*) and semiquantitative analysis of EAT adiponectin mRNA in control or programmed rats fed low-fat (□) or high-fat (■) diet (n = 10, 10, 9, and 12, respectively). Data are means ± SE. Statistical analysis was performed by two-way ANOVA followed by Fisher's post hoc test. *P < 0.05.

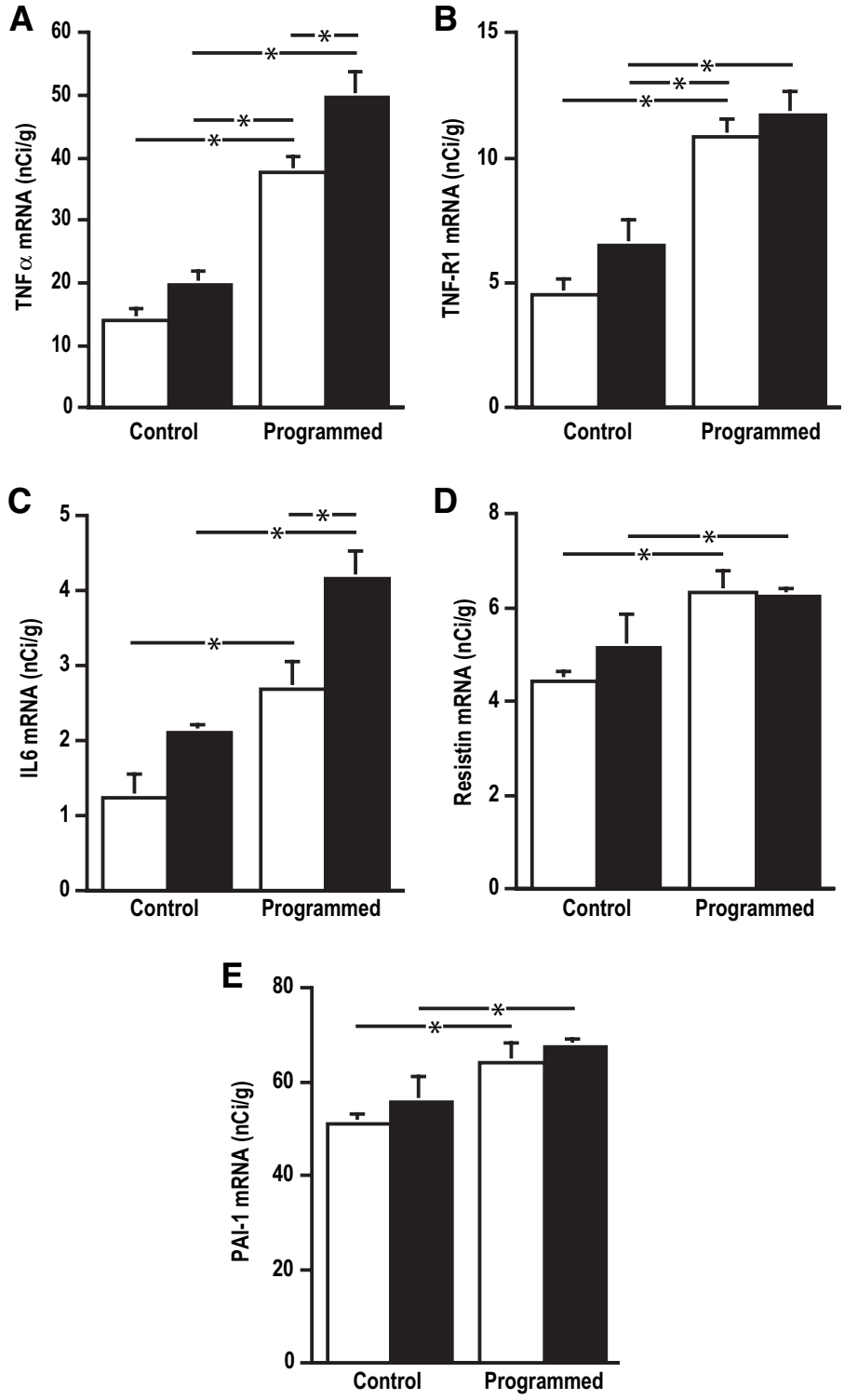

FIG. 6. Semiquantitative analysis of MAT TNF-α (*A*), TNF-R1 (*B*), IL-6 (*C*), resistin (*D*), and PAI-1 (*E*) mRNA in control or programmed rats fed low-fat (□) or high-fat (■) diet (n = 10, 10, 9, and 12, respectively). Data are means ± SE. Statistical analysis was performed by two-way ANOVA followed by Fisher's post hoc test. *$P < 0.05$.

Finally, our data demonstrate that the environmental and/or nutritional regulations of local glucocorticoid metabolism are tissue specific. We found that high-fat feeding downregulated liver GR and 11β-HSD-1 expression, as previously described (14), independently of the postnatal programming. Such changes could represent an adaptive mechanism counteracting metabolic disease, as illustrated by the lack of changes in liver PEPCK mRNA levels in the face of increased postnatal programming– or high-fat diet–induced corticosterone production. Differences in the activity of CCAAT/enhancer-binding proteins (C/EBPs), which are required for adipocytes differentiation and maturation, between liver and adipose tissue could be involved. It has been shown that in liver, C/EBPα is a potent activator of the 11β-HSD-1 gene, whereas C/EBPβ acts as a dominant repressor of C/EBPα-stimulated 11β-HSD-1

TABLE 2
Correlates of MAT 11β-HSD-1

	Simple regression analysis	
	r	*P*
MAT TNFα mRNA	0.698	<0.0001
MAT IL6 mRNA	0.617	<0.0001
MAT PPARγ mRNA	0.595	<0.0001
MAT TNF-R1 mRNA	0.565	<0.0001
MAT GR mRNA	0.590	<0.0001
MAT resistin mRNA	0.509	0.0047
24h urinary corticosterone	0.177	0.31

	Analysis of covariance			
	Covariate: postnatal programming		Covariate: high-fat diet	
	F	*P*	*F*	*P*
TNF-α	1.806	0.186	33.046	<0.001
Covariate	29.366	<0.001	0.103	0.749
IL-6	2.809	0.101	20.319	<0.001
Covariate	50.603	<0.001	0.0	0.997
PPARγ	1.382	0.246	18.382	<0.001
Covariate	50.732	<0.001	1.261	0.268
TNF-R1	0.025	0.876	17.094	<0.001
Covariate	47.153	<0.001	1.203	0.279
GR	1.716	0.197	27.033	<0.001
Covariate	51.158	<0.001	6.047	0.018
Resistin	0.014	0.906	7.814	0.008
Covariate	71.984	<0.001	2.215	0.144

promoter activity (46). Conversely, although both C/EBPα and -β are required for the basal transcriptional activity of 11β-HSD-1 in 3T3-L1, a preadipocyte cell line, C/EBPβ is strongly involved in forskolin-induced stimulation of 11β-HSD-1 gene transcription (47). Interestingly, it has recently been demonstrated that mice with a deletion in the gene for C/EBPβ are protected against diet-induced obesity (48).

In conclusion, our data show for the first time that an environmental manipulation, such as postnatal overfeeding, programs high-fat diet-induced upregulation of MAT glucocorticoid exposure, sensitivity, and inflammatory status and, therefore, reveal the pivotal role of the environment during the perinatal period on the development of diet-induced adipose tissue dysregulation in adulthood. The data also urge the need for clinical trials with specific 11β-HSD-1 inhibitors.

ACKNOWLEDGMENTS

We thank Prof. A. Auquier and Dr. A. Loundou (Unit of Clinical Research, AP-HM, Marseille, France) for their help in statistical analysis.

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