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Prenatal Programming of Metabolic Syndrome in the Common Marmoset Is Associated With Increased Expression of 11 β -Hydroxysteroid Dehydrogenase Type 1

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OBJECTIVE—Recent studies in humans and animal models of obesity have shown increased adipose tissue activity of 11 β -hydroxysteroid dehydrogenase type 1 (11 β -HSD1), which amplifies local tissue glucocorticoid concentrations. The reasons for this 11 β -HSD1 dysregulation are unknown. Here, we tested whether 11 β -HSD1 expression, like the metabolic syndrome, is “programmed” by prenatal environmental events in a nonhuman primate model, the common marmoset monkey.

RESEARCH DESIGN AND METHODS—We used a “fetal programming” paradigm where brief antenatal exposure to glucocorticoids leads to the metabolic syndrome in the offspring. Pregnant marmosets were given the synthetic glucocorticoid dexamethasone orally for 1 week in either early or late gestation, or they were given vehicle. Tissue 11 β -HSD1 and glucocorticoid receptor mRNA expression were examined in the offspring at 4 and 24 months of age.

RESULTS—Prenatal dexamethasone administration, selectively during late gestation, resulted in early and persistent elevations in 11 β -HSD1 mRNA expression and activity in the liver, pancreas, and subcutaneous—but not visceral—fat. The increase in 11 β -HSD1 occurred before animals developed obesity or overt features of the metabolic syndrome. In contrast to rodents, in utero dexamethasone exposure did not alter glucocorticoid receptor expression in metabolic tissues in marmosets.

CONCLUSIONS—These data suggest that long-term upregulation of 11 β -HSD1 in metabolically active tissues may follow prenatal “stress” hormone exposure and indicates a novel mechanism for fetal origins of adult obesity and the metabolic syndrome. *Diabetes* 58:2873–2879, 2009

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The metabolic syndrome and its component features (central obesity, insulin resistance/type 2 diabetes, hypertension, dyslipidemia) have been causally linked to early life events as marked by low birth weight and other features of an adverse intrauterine environment (1,2). Two major etiological hypotheses of the “developmental origins” effects have been proposed: malnutrition and glucocorticoid overexposure (3,4). These mechanisms of “programming” may be linked because maternal undernutrition increases maternal glucocorticoid concentrations and reduces the placental enzymatic barrier to maternal glucocorticoids in rats, thus increasing fetal glucocorticoid exposure (5). Moreover, maternal glucocorticoid administration reduces food intake in rodents (6).

The processes that link intrauterine insults and later risk of the metabolic syndrome are not yet understood. The metabolic syndrome resembles the rare Cushing’s syndrome of circulating glucocorticoid excess, but in uncomplicated metabolic syndrome, plasma cortisol levels are not raised, spawning the suggestion that increased tissue sensitivity to glucocorticoid action may be important in its pathogenesis (7). In the major metabolic organs, tissue sensitivity and exposure to glucocorticoids are determined by the density of intracellular glucocorticoid receptors and the activity of the microsomal enzyme 11 β -hydroxysteroid dehydrogenase type 1 (11 β -HSD1) which catalyzes the regeneration of active cortisol (corticosterone in rodents) from inert cortisone (11-dehydrocorticosterone) (7). 11 β -HSD1 is highly expressed in liver and adipose tissue, where glucocorticoids reduce insulin sensitivity and action (7).

In obese humans and in monogenic obesity in rodents, there is a selective increase in 11 β -HSD1 mRNA and activity in adipose tissues (8–10). Increased 11 β -HSD1 in liver is found in other causes of metabolic syndrome, such as myotonic dystrophy (11). Transgenic overexpression of 11 β -HSD1 selectively in adipose tissue in mice recapitulates all the major features of metabolic syndrome without changes in circulating steroid levels (12), whereas overexpression of 11 β -HSD1 in liver alone produces an attenuated syndrome with insulin resistance, dyslipidemia, and hypertension, but not hyperglycemia or obesity (13). Conversely, 11 β -HSD1 knockout mice are insulin sensitized and resist metabolic syndrome with dietary obesity (14).

However, genetic variation in the *HSD1B1* gene does not associate with obesity (15), suggesting the cause of increased 11 β -HSD1 in adipose tissue in obesity is envi-

TABLE 1
Maternal and offspring characteristics

	Maternal age (years)	Gestational period (days)	Litter size	Male-to-female ratio*	Male birth weight (g)
Control	5.0 \pm 0.3	144.4 \pm 0.7	2.9 \pm 0.2	1.6	32.0 \pm 0.8
Early-Dex	4.4 \pm 0.5	144.7 \pm 1.0	2.8 \pm 0.2	1.8	30.4 \pm 0.7
Late-Dex	4.0 \pm 0.5	145.7 \pm 0.8	2.8 \pm 0.3	2.1	32.0 \pm 1.1

*Data is for litters with at least one male offspring; pure female litters were not considered. Early-Dex, early-gestation dexamethasone; Late-Dex, late-gestation dexamethasone.

ronmentally determined. Although many factors may up-regulate 11 β -HSD1 in the short term, attempts to chronically induce 11 β -HSD1 in adipose tissue by nongenetic approaches have been unsuccessful. In particular, high-fat diets in rodents downregulate 11 β -HSD1 in adipose tissue (16), although this does not appear to occur in humans (17), underlining the importance of relevant models of the human situation.

Here, we have explored the early life antecedents of metabolic syndrome and especially 11 β -HSD1 expression in metabolic tissues in a nonhuman primate model (the common marmoset monkey) of fetal programming.

RESEARCH DESIGN AND METHODS

Experiments were conducted in accordance with the European Communities Council directive of 24 November 1986 (86/EEC) and were approved by the Lower Saxony Federal State Office for Consumer Protection and Food Safety, Germany. Adult common marmoset monkeys (*Callithrix jacchus*) were obtained from the breeding colony of the German Primate Center in Göttingen, Germany. Breeding and experimental animals were housed in pairs in wire mesh cages (76 \times 50 \times 125 cm) under controlled and standardized conditions (lights on 7:00 A.M.; lights off 7:00 P.M.; temperature 26 \pm 1.5°C; relative humidity 60–80%; air exchange six to eight times per hour). Animals were fed a pelleted marmoset diet (Spezialdiäten; ssniff, Soest, Germany; www.ssniff.de) ad libitum. This diet (Mar V3843; ssniff) contained, per kilogram of dry matter, 26% crude protein, 7% fat, 2.5% crude fiber, 43.8% starch and saccharides, 1% calcium, 0.7% inorganic phosphate, 3,000 IU vitamin D3, and 15.6 megajoules (MJ) digestible energy. In addition, in the morning each animal was offered 30 g of a mash that contained 21% crude protein, 14% fat, 10% crude fiber, 41% starch and saccharides, 0.95% calcium, and 0.67% organic phosphate in the dry matter and 5,500 IU vitamin D3 and 17.9 MJ digestible energy per kilogram. In the afternoon, each animal received 30 g of clean cut fruits or vegetables. Water was always available.

Time mating and dexamethasone administration. Plasma progesterone concentration was regularly measured in the breeding females for ovarian cycle determination and to detect pregnancy. During the adaptation period, animals were given intramuscular injections of a prostaglandin F2 α analog (Estrumate, 2.5 μ g cloprostenol per animal; Essex-Tierarznei, Munich, Germany) on day 14 of the luteal phase to prevent pregnancy. After adaptation, plasma progesterone concentration was measured in samples taken every 1–2 days between day 7 and day 13 of the follicular phase, and ovulation was assumed to have taken place if plasma progesterone level increased from <10 to >15 ng/ml. The day of ovulation was considered as day 0, and pregnancy was confirmed by ultrasound examination after 4 and 8 weeks.

Although marmoset monkeys have a fully functional HPA (hypothalamic-pituitary-adrenal) axis, they exhibit relatively high physiological cortisol concentrations (18). Therefore, to determine a suitable administration dose of dexamethasone, we undertook a pilot study in which pregnant marmosets were administered a range of doses of dexamethasone, between 0.05 and 10 mg/kg per day orally. Dexamethasone tablets (0.5, 1.5, or 4 mg; Jenapharm, Jena, Germany) were dissolved in 0.4 ml of tap water and mixed with 1.6 ml NutriCal (Albrecht, Aulendorf, Germany). The mixture was taken voluntarily by all animals. We found 1 or 5 mg/kg dexamethasone per day effectively suppressed maternal endogenous cortisol production without adverse effects, whereas 10 mg/kg induced abortion and caused symptoms of glucocorticoid deficiency after cessation of dexamethasone administration. We used 5 mg/kg dexamethasone per day in all subsequent experiments. This dose is significantly higher than those shown to produce programming effects in rodents (19) or vervet monkeys (20), and it reflects the relative resistance of the marmoset to glucocorticoid action (18). Pregnant marmoset monkeys ($n = 10$ per group) were given dexamethasone or vehicle either early in pregnancy or

during late gestation. In marmosets, the average expected full-term gestation is 145 days. With early administration (early dexamethasone), dexamethasone was given daily for 7 days during late first trimester (gestation day 42–48) to target the maturational stage of neurogenesis in this primate (21), and therefore a putative sensitive period for inducing acute central effects with long-term consequences. For late administration (late dexamethasone), dexamethasone was administered daily for 7 days in the late second trimester (gestation day 90–96) to mimic the therapeutic use of glucocorticoids in humans to promote fetal maturity in mothers at risk for preterm delivery, and therefore of particular translational relevance.

At birth, the neonates were sexed and weighed. To ensure that the breeding pair could bring up the young, one infant was removed in cases of triplet litters. No surplus baby food was given to the newborn monkeys. The offspring were kept with their parents until weaning at 6 months of age. One male offspring from each litter was recruited into the study groups.

Plasma glucose and triglycerides. At 6, 12, 18, and 24 months of age, oral glucose tolerance tests were carried out after an overnight (15 h) fast. Glucose was given in a solution of 500 g/l glucose and 300 g/l gum Arabic (to increase viscosity and prevent spillage). All animals took the glucose solution voluntarily. Plasma glucose concentrations from fasting and samples taken 20 min after the oral glucose load (2 g/kg) were measured with the Ectachem DT60 system (Ortho-Clinical Diagnostics, Johnson & Johnson, Rochester, NY), and A1C was determined using a Bio-Rad Micromat II (Bio-Rad, Perth, U.K.). Triglycerides were measured enzymatically with Infinity triglycerides liquid stable reagent (ThermoTrace; Alpha Laboratories, Hampshire, U.K.). We were unable to measure plasma insulin concentrations because of the lack of a reliable assay for marmoset insulin. Urine samples were collected in the morning by placing animals in collecting cages, and cortisol and creatinine concentrations were determined by liquid chromatography–tandem mass spectrometry as previously described (22).

Tissue processing. At 4 months of the age, animals had liver biopsies under general anesthesia with ultrasound guidance, and samples were stored at –80°C for subsequent analysis. The offspring were killed at 24 months of age. Animals were anesthetized by intramuscular injections of alphaxalone (6.75 mg/kg) plus alphadolone (Saffan 2.25 mg/kg; Schering-Plough Animal Health, Welwyn Garden City, U.K.), glycopyrroniumbromide (Robinul 0.02 mg/kg; Riemser, Greifswald, Germany), and diazepam (0.25 mg/kg; Ratiopharm, Ulm, Germany) and killed by an intracardiac overdose of ketamine. Tissues were quickly collected, snap-frozen in liquid nitrogen, and stored at –80°C for subsequent analyses.

Real-time PCR. RNA was extracted from biopsy or necropsy samples using TRIzol reagent (Life Technologies, Invitrogen, Paisley, U.K.). cDNA was created, and real-time PCR was performed as previously described (20) to quantify phosphoenolpyruvate carboxykinase (PCK1), 11 β -HSD1, and glucocorticoid receptor- α mRNA expression. The following primers and probes were used in PCR: PEPCK 5'-GCTGGATGTCAGAGGAGGATTTTGTAG-3' (forward), 5'-CCATGCTGAATGGGATGACGTACATG-3' (reverse), and 5'-6-FAM-CAATGCCAGGTTCCCGGGGTG-TAMRA-3' (probe); 11 β -HSD1 5'-CCTCAGCGCACTACATTGTGGC-3' (forward), 5'-GAGCATGTCTAGTC-TCCCATGAGC-3' (reverse), and 5'-6-FAM-GGAAGACATGGCCTTCGCAGAGC-TAMRA-3' (probe); glucocorticoid receptor- α 5'-CATTGTCAAGGGGAAGG-AACTC-3' (forward), 5'-GATTTTCAACCCTTCATGCATAGAA-3' (reverse), and 5'-6-FAM-TTTGTTCAGTTGATAAAACCGCTGCCAGTTCT-TAMRA-3' (probe). 18S rRNA primers and probes (Applied Biosystems, Cheshire, U.K.) were used to normalize the transcript levels. A standard curve for each primer-probe set was generated in triplicate by serial dilution of cDNA that was pooled from several subjects. Each sample was run in duplicate, and the mean values of the duplicates were used to calculate the transcript level from the standard curve. The results are expressed as arbitrary units.

11 β -HSD1 activity. Under in vitro conditions, 11 β -HSD1 is bidirectional, and the more stable dehydrogenase reaction was measured as previously described (23). In brief, samples were homogenized in Krebs buffer at pH 7.4, and then 600 μ g/ml protein was incubated at 37°C with 2 mmol/l NADP and

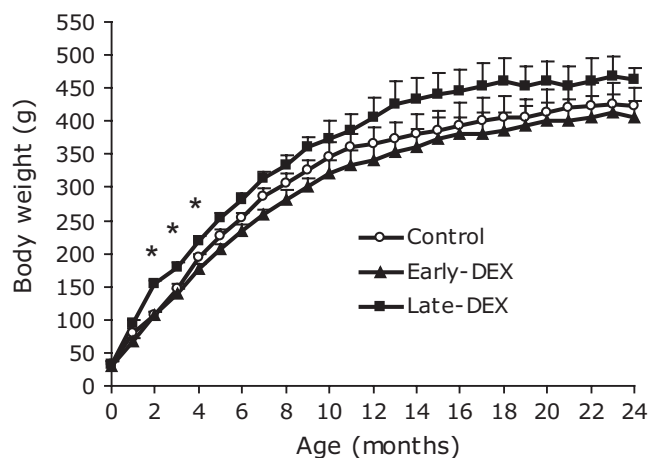


FIG. 1. Effect of prenatal dexamethasone on offspring body weight. Graph shows body weight at different ages in offspring of mothers that received vehicle (Control), or dexamethasone during early (Early-DEX) or late (Late-DEX) pregnancy. * $P < 0.05$ compared with control animals.

1,2,6,7- $^3\text{H}_4$ cortisol (100 nm) for 24 h. The conversion of $^3\text{H}_4$ cortisol to $^3\text{H}_4$ cortisone was quantified in the samples after separation by high-performance liquid chromatography with online liquid scintillation detection.

Hepatic PEPCK activity. PEPCK activity was measured in liver cytosolic fraction homogenates from 24-month-old marmosets, as described previously (19). In short, the cytosolic fraction (1 mg) was assayed in 1 ml buffer containing 50 mmol/l HEPES (pH 6.5), 50 mmol/l sodium bicarbonate, 1 mmol/l MgCl_2 , 0.25 mmol/l NADH, 1.0 mmol/l phosphoenolpyruvate, and 1.5 units malate dehydrogenase. The absorbance of NADH was determined at 340 nm at 30°C. After a stable signal was obtained, the reaction was initiated by addition of 0.15 mmol/l 2'-deoxyguanosine 5'-diphosphate. After 3 min of

incubation, a decrease in absorbance was measured. A reaction mixture without bicarbonate was used as a negative control.

Area under the glucose versus time curve. The area under the curve glucose versus time was the product of mean glucose concentration during the oral glucose tolerance test (mmol/l) and the exact time interval (min) between application of the glucose solution and blood sampling thereafter.

Statistics. All data are the means \pm SEM. Data were compared using one- or two-way ANOVA followed by Newman-Keuls or Bonferroni post hoc multiple comparisons test, where appropriate. Values were considered significantly different at $P < 0.05$.

RESULTS

Litter size and body weight. There were no significant differences in maternal age. It was not possible to accurately quantify maternal food intake under the social housing conditions used, but dexamethasone did not significantly affect maternal weight gain during pregnancy. Similarly, there were no differences in gestational period or litter size among the dexamethasone administration groups (Table 1). Dexamethasone, given in either early or late gestation, had no significant effect on offspring birth weight. The offspring of mothers that received dexamethasone late in pregnancy showed higher rates of weight gain postnatally compared with controls, and, overall, late-administration dexamethasone had a significant effect on offspring body weight across the 24 months ($P = 0.04$), although by 24 months of age, there were no statistically significant differences in body weight by dexamethasone administration group (Fig. 1).

Plasma glucose and triglycerides. The offspring in the three groups had similar plasma fasting and reactive glucose concentrations at 6, 12, or 18 months of age (Table 2).

TABLE 2
Offspring plasma glucose, A1C, and triglycerides

	Control	Early-DEX	Late-DEX
6 months			
Fasting glucose (mmol/l)	6.5 \pm 0.3	7.3 \pm 0.6	6.2 \pm 0.7
OGTT 20-min glucose (mmol/l)	11.7 \pm 1.2	12.4 \pm 0.9	11.8 \pm 0.9
AUC _{0-20min} glucose	181.8 \pm 11.6	190.1 \pm 13.1	180.7 \pm 14.5
A1C (%)	4.4 \pm 0.3	4.7 \pm 0.7	4.6 \pm 0.2
Fasting triglycerides (mmol/l)	0.68 \pm 0.05	0.71 \pm 0.10	0.78 \pm 0.06
Urine cortisol ($\mu\text{g}/\text{mg}$ creatinine)	11.7 \pm 2.1	11.1 \pm 1.0	13.4 \pm 6.0
12 months			
Fasting glucose (mmol/l)	7.3 \pm 0.5	7.4 \pm 1.0	7.9 \pm 0.7
20-min glucose (mmol/l)	11.4 \pm 1.0	12.1 \pm 1.4	13.3 \pm 1.3
AUC _{0-20min} glucose	187.8 \pm 10.2	177.3 \pm 16.7	214.7 \pm 18.5
A1C (%)	4.6 \pm 0.1	4.6 \pm 0.2	4.9 \pm 0.5
Fasting triglycerides (mmol/l)	0.81 \pm 0.10	0.67 \pm 0.04	0.90 \pm 0.14
Urine cortisol ($\mu\text{g}/\text{mg}$ creatinine)	7.3 \pm 1.4	6.7 \pm 0.8	7.0 \pm 1.2
18 months			
Fasting glucose (mmol/l)	7.5 \pm 0.9	7.9 \pm 0.8	8.9 \pm 1.2
20-min glucose (mmol/l)	11.0 \pm 1.1	12.1 \pm 1.7	12.4 \pm 1.6
AUC _{0-20min} glucose	186.4 \pm 10.0	184.7 \pm 15.5	218.2 \pm 26.9
A1C (%)	4.6 \pm 0.2	4.8 \pm 0.4	4.8 \pm 0.5
Fasting triglycerides (mmol/l)	0.81 \pm 0.12	0.98 \pm 0.21	2.12 \pm 0.67
Urine cortisol ($\mu\text{g}/\text{mg}$ creatinine)	7.4 \pm 1.4	4.1 \pm 0.5	5.6 \pm 1.0
24 months			
Fasting glucose (mmol/l)	7.5 \pm 0.7	7.6 \pm 0.6	9.4 \pm 0.7*
20-min glucose (mmol/l)	11.9 \pm 1.0	11.8 \pm 0.6	13.3 \pm 1.1
AUC _{0-20min} glucose	194.2 \pm 8.9	200.0 \pm 13.5	237.9 \pm 12.6
A1C (%)	4.5 \pm 0.2	4.7 \pm 0.3	5.2 \pm 0.5
Fasting triglycerides (mmol/l)	1.39 \pm 0.56	1.36 \pm 0.13	2.18 \pm 0.28
Urine cortisol ($\mu\text{g}/\text{mg}$ creatinine)	9.6 \pm 2.1	6.8 \pm 1.4	9.2 \pm 1.5

* $P < 0.05$ is relative to control or early-gestation dexamethasone. AUC, area under the curve in oral glucose tolerance test; Early-Dex, early-gestation dexamethasone; Late-Dex, late-gestation dexamethasone; OGTT, oral glucose tolerance test.

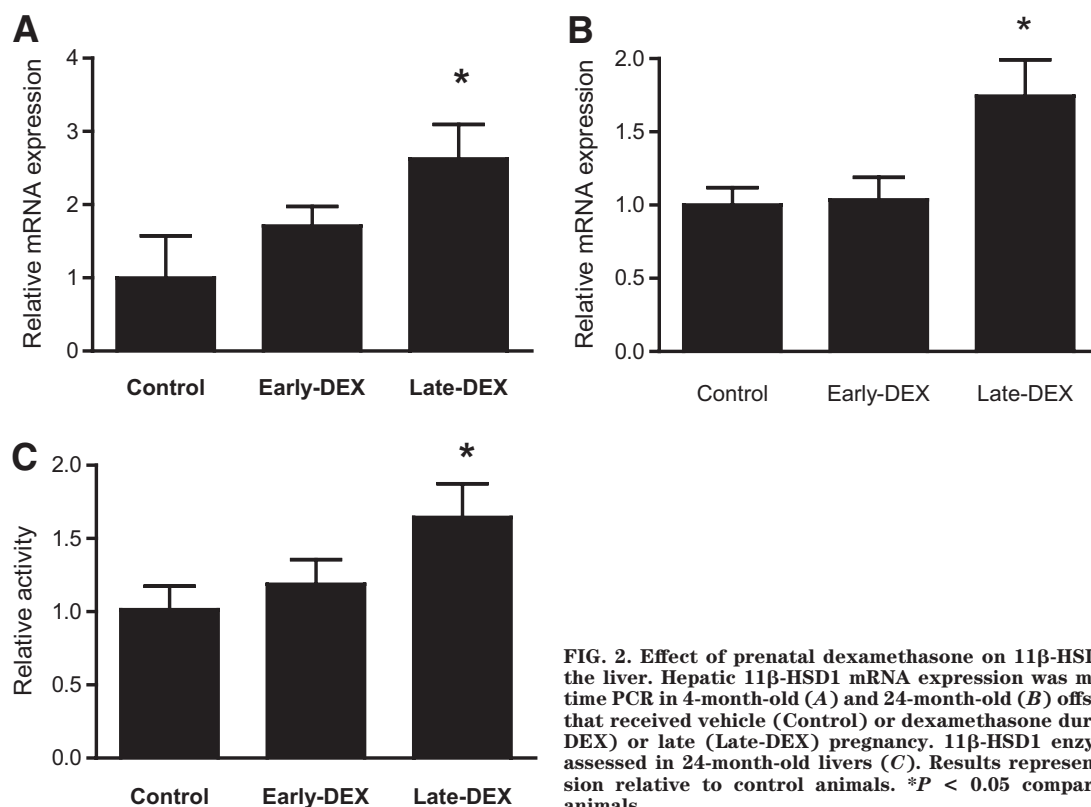


FIG. 2. Effect of prenatal dexamethasone on 11 β -HSD1 expression in the liver. Hepatic 11 β -HSD1 mRNA expression was measured by real-time PCR in 4-month-old (A) and 24-month-old (B) offspring of mothers that received vehicle (Control) or dexamethasone during early (Early-DEX) or late (Late-DEX) pregnancy. 11 β -HSD1 enzyme activity was assessed in 24-month-old livers (C). Results represent mRNA expression relative to control animals. * $P < 0.05$ compared with control animals.

However, by 24 months, marmosets that were exposed to dexamethasone during late pregnancy had higher fasting plasma glucose concentration than controls (Table 2). Although 20-min (postload) plasma glucose concentrations did not significantly differ, there was a strong trend for increased area under the curve for glucose in Late-dexamethasone animals compared with controls ($P = 0.056$). Fasting triglycerides also tended to be higher in late-dexamethasone than in control offspring at 24 months ($P = 0.06$). Early administration of dexamethasone did not alter plasma glucose (fasting or reactive) or triglyceride concentrations in the offspring (Table 2).

The effect of prenatal dexamethasone on 11 β -HSD1 expression in key metabolic tissues. Early dexamethasone administration did not affect expression of 11 β -HSD1 mRNA in the liver, pancreas, or adipose tissue of the offspring. In contrast, offspring of mothers that received late administration of dexamethasone had significantly higher 11 β -HSD1 mRNA levels in the liver at 4 months of age, and this persisted into adulthood (Fig. 2). Similarly, these animals showed increased 11 β -HSD1 mRNA expression in the pancreas and subcutaneous, but not peritoneal, adipose tissue (Fig. 3). In control 2-year-old marmosets, 11 β -HSD1 mRNA expression was nearly 2.5-fold higher in subcutaneous than peritoneal adipose tissue. This difference rose to fivefold in animals that had received dexamethasone in late gestation.

The changes in 11 β -HSD1 mRNA expression were functionally significant because 11 β -HSD1 activity assays showed late-dexamethasone animals had parallel increases in 11 β -HSD1 activity in their livers (Fig. 2). Moreover, this was associated with significantly increased mRNA expression and activity of PEPCK1 (Fig. 4), the rate-limiting enzyme of gluconeogenesis and an important target for glucocorticoids and for glucocorticoid programming in rodents (19). In contrast to prenatal dexametha-

sone programming in rats (19), neither early nor late administration of dexamethasone had any effect on glucocorticoid receptor mRNA levels in any of the tissues examined (Fig. 5). Prenatal dexamethasone administration did not significantly affect urinary cortisol concentrations, suggesting that the induction of 11 β -HSD1 in metabolic tissues is not attributable to elevated circulating glucocorticoids per se (Table 2).

DISCUSSION

Here, we demonstrate, in a nonhuman primate model, that prenatal dexamethasone administration causes permanent tissue-specific changes in expression of 11 β -HSD1, a gene that has emerged as an important player in the pathogenesis of obesity and the metabolic syndrome. Thus, prenatal dexamethasone exposure was associated with elevated 11 β -HSD1 expression in key metabolic organs, such as liver, pancreas, and subcutaneous fat, but not visceral adipose tissue. The changes in 11 β -HSD1 expression were seen when dexamethasone was administered selectively in late gestation, congruent with the critical window of sensitivity for programming key target organs involved in glucose metabolism in various other animal models (3,19,20). As in the African vervet monkey (20), but unlike rodent programming models (19), prenatal dexamethasone exposure was not associated with a significant reduction in marmoset birth weight. The reasons for this discrepancy are unclear, but perhaps reflect the fact that birth weight is a relatively crude measure of organ growth, and antenatal insults may alter offspring physiology without necessarily having a significant effect on birth weight. In most rodent models, in which antenatal glucocorticoids reliably reduce birth weight, glucocorticoid exposure typically extends over a relatively larger fraction of the fetal period (i.e., 5–6 days of the 21–22 day rat gestation) than

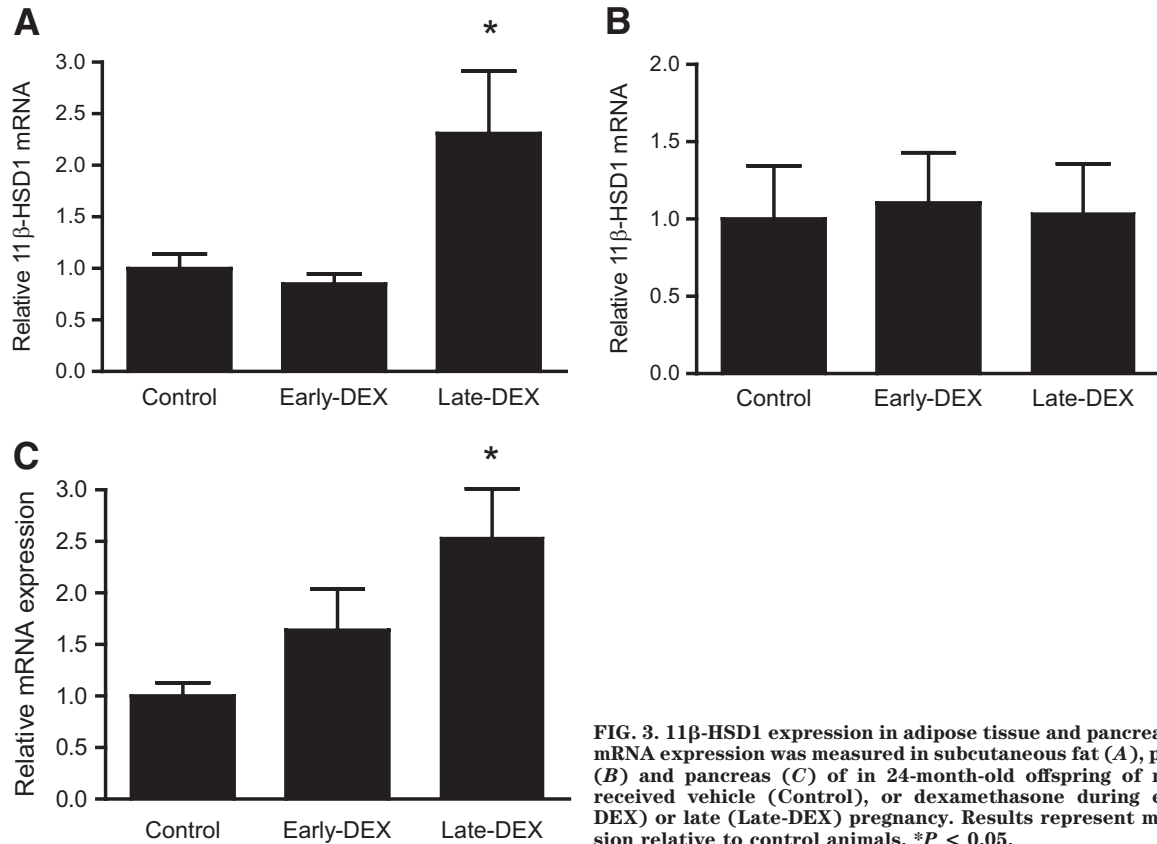


FIG. 3. 11 β -HSD1 expression in adipose tissue and pancreas. 11 β -HSD1 mRNA expression was measured in subcutaneous fat (A), peritoneal fat (B) and pancreas (C) of in 24-month-old offspring of mothers that received vehicle (Control), or dexamethasone during early (Early-DEX) or late (Late-DEX) pregnancy. Results represent mRNA expression relative to control animals. * $P < 0.05$.

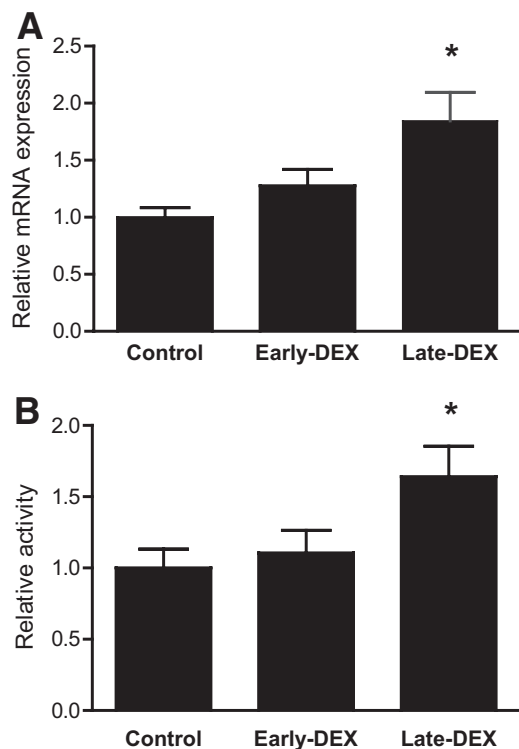


FIG. 4. Effect of prenatal dexamethasone on PEPCK expression in the liver. Hepatic PEPCK mRNA (A) and activity (B) were measured in 24-month-old offspring of mothers that received vehicle (Control) or dexamethasone during early (Early-DEX) or late (Late-DEX) pregnancy. Results represent mRNA expression or enzyme activity relative to control animals. * $P < 0.05$ compared with control animals.

used here (3,19). Interestingly, even long-term antenatal dexamethasone does not inevitably reduce birth weight in humans (24).

The increase in 11 β -HSD1 mRNA expression in animals exposed to dexamethasone in late gestation was observed, at least in the liver, at an early age (4 months) and persisted into adulthood. Marmosets are normally lean, and the animals in our study were killed at a relatively young age. Although these animals did not have a postnatal challenge (such as high-fat feeding) and were not overtly obese, late-gestation dexamethasone administration was associated with mild hyperglycemia and borderline hypertriglyceridemia. Elevated tissue levels of 11 β -HSD1 preceded these metabolic changes, suggesting that these are primary. In support of this notion, a recent study showed that rats born to diabetic mothers, which also develop obesity and insulin resistance, have similar early increases in 11 β -HSD1 mRNA and activity in the liver and adipose tissue (25). Taken together, these data suggest that 11 β -HSD1 is influenced by diverse intrauterine environmental insults, and they may provide a common mechanism in programming the metabolic syndrome. Postnatal overfeeding (by reducing litter size in the immediate neonatal period) in rats has also been shown to result in obesity associated with increased adipose tissue glucocorticoid receptor and 11 β -HSD1 mRNA expression. Interestingly, whereas the change in glucocorticoid receptor was evident as early as postnatal day 21, increased 11 β -HSD1 expression was only seen in adult animals (26).

In marmosets, subcutaneous fat had more 11 β -HSD1 mRNA expression than peritoneal adipose tissue, and late-gestation dexamethasone increased 11 β -HSD1 mRNA expression selectively in the subcutaneous fat depot. Interestingly, a recent human study showed that increased

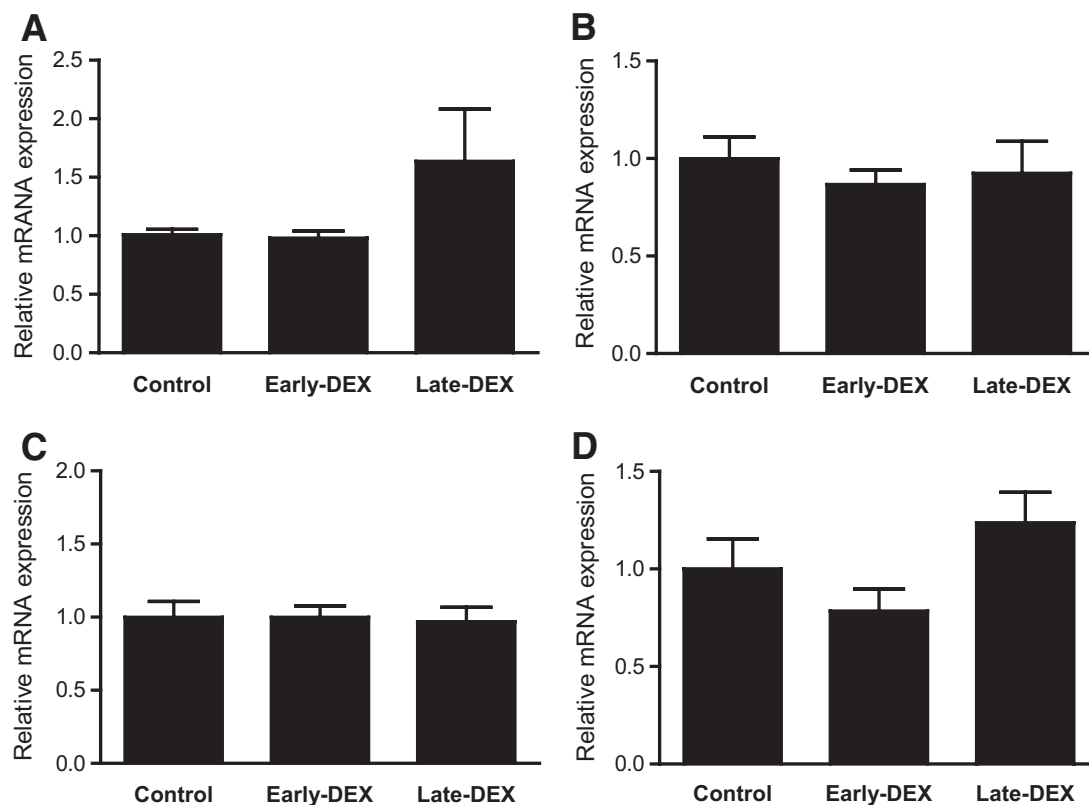


FIG. 5. Effect of dexamethasone on glucocorticoid receptor expression. Tissue glucocorticoid receptor mRNA was measured in liver (A), subcutaneous fat (B), peritoneal fat (C), and pancreas (D) by real-time PCR in 24-month-old offspring of mothers that received vehicle (Control) or dexamethasone during early (Early-DEX) or late (Late-DEX) pregnancy. Results are expressed as mRNA expression relative to control animals.

11 β -HSD1 expression in subcutaneous, but not in visceral, adipose tissue was associated with a worsening of the metabolic syndrome (27). The reasons for the depot-specific effects of late-gestation dexamethasone are unclear. Transcriptional regulation of the *HSD11B1* gene is complex and highly tissue specific, but the mechanisms that underlie this tissue-specific regulation or its dysregulation in obesity are unknown. A number of factors, including the C/EBP family of transcription factors, insulin, proinflammatory mediators, as well as glucocorticoids themselves have been shown to influence 11 β -HSD1 mRNA expression and/or activity (28).

Overactivity of 11 β -HSD1 in the liver might also contribute to the metabolic syndrome because glucocorticoids regulate a number of pathways that control hepatic glucose and lipid metabolism (29). In support of this, the offspring of mothers that were administered dexamethasone during late gestation showed enhanced hepatic activity of PEPCK1, the rate-limiting enzyme of gluconeogenesis, suggestive of increased hepatic glucose output. Similarly, glucocorticoids are known to inhibit insulin secretion (30), and increased activity of 11 β -HSD1 in the pancreas in rodents associates with β -cell dysfunction and may contribute to the pathogenesis of hyperglycemia (31).

In rodent models, in utero exposure to dexamethasone has also been associated with permanent tissue-specific changes in glucocorticoid receptor mRNA expression, downregulation in the hippocampus, and increased expression in adipose tissue and liver (1). However, in the current study, we observed no significant differences in tissue glucocorticoid receptor expression between marmosets that received dexamethasone in utero and con-

trols. The reasons for this discrepancy are unclear, but they are unlikely to be due the fact that New World monkey species have altered glucocorticoid receptor signaling and regulation (18) because a similar observation was made in an Old World species (the vervet monkey) (20). This underlines a possible programming difference in metabolic organs between rodents and primates, with prenatal glucocorticoid exposure targeting primarily glucocorticoid receptors in the former (19,32) but primarily 11 β -HSD1 in the latter.

In summary, antenatal glucocorticoid excess in marmosets causes persistent increases in 11 β -HSD1 expression in adipose tissue and other metabolic organs, suggesting that long-term upregulation of 11 β -HSD1 in adipose tissues may follow prenatal "stress." Because increased adipose or hepatic 11 β -HSD1 has been implicated in the pathogenesis of obesity and the metabolic syndrome, these data indicate, first, a novel mechanism for fetal origins of adult obesity and the metabolic syndrome and, second, a potential mechanism to explain the upregulation of 11 β -HSD1 seen in humans with metabolic syndrome/obesity.

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