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Programming of Rat Muscle and Fat Metabolism by *in Utero* Overexposure to Glucocorticoids

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In utero **overexposure to glucocorticoids may explain the association between low birth weight and subsequent development of the metabolic syndrome. We previously showed that prenatal dexamethasone (dex) exposure in the rat lowers birth weight and programs adult fasting and postprandial hyperglycemia, associated with increased hepatic gluconeogenesis driven by elevated liver glucocorticoid receptor (GR) expression. This study aimed to determine whether prenatal dex (100 μg/kg per day from embryonic d 15 to embryonic d 21) programs adult GR expression in skeletal muscle and/or adipose tissue and whether this contributes to altered peripheral glucose uptake or metabolism.** ***In utero*** **dex-exposed rats remained lighter until 6 months of age, despite some early catch-up growth. Adults had smaller epididymal fat pads, with a relative increase in muscle size. Although glycogen storage was reduced in quadriceps, 2-deoxyglucose uptake into extensor digitorum longus muscle was increased by 32% ($P < 0.05$), whereas uptake in other muscles and adipose beds was unaffected by prenatal dex. GR mRNA was not different in most muscles but selectively reduced in soleus (by 23%, $P < 0.05$). However, GR mRNA was markedly increased specifically in retroperitoneal fat (by 50%, $P < 0.02$). This was accompanied by a shift from peroxisomal proliferator-activated receptor γ1 to γ2 expression and a reduction in lipoprotein lipase mRNA (by 28%, $P < 0.02$). Adipose leptin, uncoupling protein-3 and resistin mRNAs, muscle GLUT-4, and circulating lipids were not affected by prenatal dex. These data suggest that hyperglycemia in 6-month-old rats exposed to dexamethasone** ***in utero*** **is not due to attenuated peripheral glucose disposal. However, increased GR and attenuated fatty acid uptake specifically in visceral adipose are consistent with insulin resistance in this crucial metabolic depot and could indirectly contribute to increased hepatic glucose output. (*Endocrinology* 144: 999–1007, 2003)**

NUMEROUS EPIDEMIOLOGICAL STUDIES have shown an association between low birth weight and the development in later life of features of the metabolic syndrome and thus a higher risk of coronary heart disease (1). In particular, elevated plasma triglycerides, hyperglycemia, hyperinsulinemia, and an increased incidence of type 2 diabetes have been noted in low-birth-weight adults (2–6). The mechanisms for the putative pathophysiological link between *in utero* growth retardation and adult insulin resistance have not yet been established, but fetal malnutrition (7) and *in utero* overexposure to glucocorticoids (8) have been proposed as possible mediators of permanent programming of cardiovascular and metabolic characteristics.

Glucocorticoids are important in the prenatal development of various organ systems and elevated glucocorticoid levels during pregnancy are associated with *in utero* growth retardation (9, 10). Rats exposed to dexamethasone (dex) during the third week of pregnancy become hypertensive (11, 12), hyperglycemic, glucose intolerant, and hyperinsulinemic in adulthood (13).

Hyperglycemia in these offspring could arise through failure of the inhibition of hepatic glucose output by insulin (14) and/or the attenuation of glucose uptake by the insulin-regulated glucose transporter (GLUT-4) in skeletal muscle and adipose tissue (15). The pattern of glucose intolerance (raised fasting and postprandial glucose levels) (13) suggest that both components may be abnormal. Increased hepatic gluconeogenesis is probably explained by permanent elevation in expression of hepatic phosphoenolpyruvate carboxykinase in dex-treated offspring (13). The effects of prenatal glucocorticoid overexposure on glucose uptake and metabolism in muscle and adipose have not been investigated.

Changes in local glucocorticoid action may mediate the observed metabolic changes in dex-programmed offspring through altered level of glucocorticoid receptor (GR) expression. Widespread permanent GR up-regulation has been reported in the offspring of protein-restricted rat mothers, which also exhibit insulin resistance and hypertension (16), whereas GR is elevated in the liver (13) but down-regulated in the hippocampus of adult dex-treated offspring (12, 17). This tissue-specific pattern probably explains the combination of increased circulating corticosterone levels (because of impaired hippocampal negative feedback to the hypothalamic-pituitary-adrenal axis) and glucocorticoid-dependent increase in phosphoenolpyruvate carboxykinase in the dex model. If GR is also altered in tissues contributing to peripheral glucose uptake, then the consequences may vary according to adipose depot and skeletal muscle fiber type. Type I and II muscle fibers as found predominantly in soleus and extensor digitorum longus (EDL) muscles, respectively (18), differ with respect to absolute levels of GR expression (19). Intraabdominal or visceral fat is particularly sensitive to glucocorticoids because GR is expressed at a higher level in this depot (20) and is less responsive to the antilipolytic

Abbreviations: Ab, Antibody; dex, dexamethasone; dNTP, deoxynucleotide triphosphate; 2-DOG, 2-deoxyglucose; EDL, extensor digitorum longus; GR, glucocorticoid receptor; LDM, low-density microsomal; LPL, lipoprotein lipase; NEFA, nonesterified fatty acid; PPAR, peroxisome proliferator activated receptor; RT, reverse transcription; UCP-3, uncoupling protein 3.

effects of insulin than sc fat (21). Lipid accumulation in visceral fat is specifically associated with other features of the metabolic syndrome in man (22), but the expression of muscle and fat GR in dex-programmed offspring has yet to be reported.

In this study, we aimed to determine whether deranged skeletal muscle and white adipose tissue metabolism contributes to the insulin resistance syndrome induced in adult rats following prenatal overexposure to glucocorticoids and whether this is attributable to dysregulation of GR expression. We administered dex or vehicle to pregnant rats during the third week of gestation and studied the muscle and fat phenotypes of adult offspring.

Materials and Methods

Maintenance and prenatal treatment of animals

Nulliparous 200- to 250-g female Wistar rats were maintained under controlled lighting (lights on 0700–1900 h) and temperature (22 C), with *ad libitum* access to food and water. They were time mated on d 0 of pregnancy and littered on d 22. Pregnant females were injected sc with 100 μg/kg dex in 0.9% saline containing 4% ethanol (dex mothers) or vehicle (saline mothers) each morning on d 15–21 of pregnancy inclusive. Pups were weighed after birth and litters culled to eight, retaining males in preference. After weaning at 3 wk, two male offspring per litter were selected randomly and caged as sibling pairs. All animal procedures were carried out under the terms of the UK Animals (Scientific Procedures) Act 1986.

Plasma measurements

Measurement of the plasma concentration of various metabolites and hormones was carried out at 6–7 months of age, either in trunk blood collected at time of death during *ad libitum* feeding (leptin) or in tail-tip samples obtained after overnight food deprivation, either at 0900 h or 30 min after rats were given 2 g/kg glucose in water by gavage. Plasma leptin concentration was determined by ELISA (Crystal Chem Inc., Chicago, IL). Triglycerides, total cholesterol, high-density lipoprotein cholesterol and nonesterified fatty acids (NEFA) were determined using kits (Wako Pure Chemical Industries Ltd., Osaka, Japan) or Roche Diagnostics Ltd., Lewes, UK). Plasma [glucose] was determined using the hexokinase method (Sigma, Poole, UK).

Uptake of radiolabeled 2-deoxyglucose (2-DOG) into tissues

Seven- to 8-month-old rats (n = 9/group) were deprived of food overnight, weighed, and anesthetized with halothane in a nitrous oxide/oxygen mixture between 0800 h and 1130 h. Polythene cannulae were inserted into the right femoral artery and vein under local anesthetic. Lightly restrained rats were allowed to recover from anesthesia for a minimum of 2 h. Animals prepared in this way exhibit minimal physiological signs of stress (23).

Glucose uptake into muscles and fat depots was measured using the method of Sokoloff *et al.* (24), adapted as described previously (23, 25). The measurement was initiated with a 30-sec iv injection of tracer (200 μCi/kg 2-deoxy-D-[2,6-^{3}H]glucose, 43 Ci/mmol; Amersham Pharmacia Biotech, Buckinghamshire, UK) with 0.5 g/kg glucose in 0.9% saline (1.5 ml total). Fourteen timed arterial blood samples were collected over 45 min. Rats were then killed and tissues dissected and weighed within 10–15 min.

Tracer concentration and total glucose were determined as described (25). Weighed portions of muscle and fat from the left hindlimb were digested at 50 C overnight in 1 ml Soluene-350 (Packard Bioscience, Groningen, The Netherlands) and ^{3}H content measured by scintillation analysis. Tissue-specific deoxyglucose uptake was calculated, using an adapted operational equation derived from the method of Sokoloff *et al.* (24) but with the incorporation of the lumped constants of Ferre *et al.* (26) and the whole-body kinetic parameters of Jenkins *et al.* (27).

Assay of muscle glycogen

Portions of quadriceps muscle removed from *ad libitum*-fed 6-month-old rats were powdered under liquid nitrogen and homogenized in 30% KOH. Glycogen was quantified, as described (28, 29).

Isolation of RNA

RNA was isolated from tissues removed from *ad libitum*-fed 6-month-old rats using TriZol (Invitrogen, Life Technologies, Inc., Paisley, UK). The integrity of total RNA was assessed using ethidium bromide after agarose gel electrophoresis.

DNA templates

The pGEM-T Easy vector containing a 186-bp length of rat GR exon two, derived by 5′-rapid amplification of cDNA ends PCR from rat thymus RNA (30) (courtesy Dr. Karen Chapman) was linearized with NCo-I, the complementary antisense RNA strand (383 bp) was generated using SP6 RNA polymerase. The p-TRI-B-actin-125-rat plasmid containing a rat β-actin DNA insert (Ambion, Inc., Austin, TX) was linearized using BamH-I and transcribed using SP6 polymerase. Because of a base mismatch, ribonuclease digestion under these conditions results in the generation of a 108-bp hybrid from a 218-bp probe, rather than the 125-bp indicated in the company literature.

cDNA templates were prepared by RT-PCR for uncoupling protein 3 (UCP-3) (236bp) from total muscle RNA; and leptin (511 bp), resistin (360 bp), lipoprotein lipase (LPL) (361 bp), and peroxisome proliferator activated receptor γ (PPARγ) (347 bp) using total RNA extracted from adipose tissue. Oligonucleotide primers were designed to target rat mRNA sequences of interest of between 200 and 500 bp and purchased from TAGN (Newcastle-Upon-Tyne, UK). These were based on published sequences (BLAST, NCBI, NLM, MD) (31). For PPARγ, primers were designed such that two protected bands of differing size would be generated from a single riboprobe on a Rnase protection gel, corresponding to the PPARγ2 isoform and total PPARγ mRNA. UCP-3: TTGGCCTCTACGACTCTG and GACACCTTTCCCTGAACC. LPL: ACTGCCACTTCAACCACAG and CCCAATACTTCGACCAGG. PPARγ: AGATTTGAAAGAAGCTGTGAACC and TGTGAACGGGATGTCGTCTTCATAG. Leptin: CCAAAACCCTCATCAAGACC- and GTCCAACTGTTGAAGAATGTCCC. Resistin: TGTGCCCTGCTGCTGAGCTCTC and GCTAGTGACGGTTGTGCCTTC.

Two to 5 μg micrograms RNA in 60 μl ultrapure water were denatured at 65 C 10 min and reverse transcription (RT) undertaken using mixes containing 4 μl first-strand buffer, 1 μl 10 mM mixed deoxynucleotide triphosphates (dNTPs), 2 μl 0.1 M dithiothreitol, 1 μl random priming hexamers, and 2 μl SuperScript II enzyme (Invitrogen, Paisley, UK). Multiples of 10 μl of RT mix were added to 10 μl denatured RNA and incubated at 42 C for 90 min. In subsequent PCR, multiples of 2 μl cDNA, 5 μl 10× PCR buffer, 0.5 μl 10 mM mixed dNTPs, 2 μl each of 10 pg/ml forward and reverse primers, 0.5 μl *Taq* DNA polymerase, and 38 μl ultrapure dH_2O were mixed. Tubes were subjected to 37 cycles of PCR amplification, consisting of 1 min at each of 95 C, the specific annealing temperature for the primers used (53–58 C), and 72 C, followed by a final 10 min at 72 C. Negative RT reactions were regularly performed alongside to reveal genomic contamination. Purified products were ligated into pGEM-T Easy vector and cloned in JM109 *Escherichia coli.* Minipreps of positive colonies, prepared using the Wizard Plus system (Promega Corp., Southampton, UK) were used to verify correct length and orientation of inserts by restriction digestion and agarose gel electrophoresis *vs.* DNA ladders, or chain termination sequencing using [α-^{33}P] dNTPs (55.5 TBq/mmol, Amersham Pharmacia Biotech).

Ribonuclease protection assays

Riboprobes and markers were synthesized using [α-^{32}P]-GTP (111 TBq/mmol, Amersham Pharmacia Biotech) (30), with cold GTP added according to the optimal specific activity for each probe. RNase protection assays were carried out using Hybspeed RPA kits (Ambion, Inc.) and a mixture of RNases A (0.5 U/μl) and T_1 (8 Kunitz units/μl). The amounts of total RNA (5–50 μg) and probe were optimized in preliminary experiments. Protected RNA hybrids (lengths: actin 108 bp, GR 186

bp, UCP-3 163 bp, leptin 394 bp, LPL 315 bp, total PPARγ 239 bp, PPARγ2 171 bp, and resistin 262 bp) were separated on a 4% polyacrylamide gel containing 7 M urea. Results were quantified using a phosphor imager and Aida 2.0 auto image data analyzer software (Raytest Scientific Ltd., Sheffield, UK). Comparison of the relative abundance of test mRNA between treatment groups was made after normalization to actin.

Western blotting

Protein expression of GR and GLUT-4 was determined semiquantitatively in whole lysate and low-density microsomal (LDM) fractions of quadriceps muscle, respectively.

Tissue preparation

For GR, 1–200 mg of each muscle was minced and then autohomogenized in ice-cold 1.05 ml low salt-molybdate buffer (32), containing 20 mM HEPES, 10 mM KCl, 20 mM sodium molybdate, 1 mM EDTA, 1 mM EGTA, 0.1 mM sodium orthovanadate (pH 7.9), 0.2% Nonidet P-40, and 10% glycerol to which 2 μg/ml each of pepstatin A, leupeptin, aprotinin, soybean trypsin inhibitor, and antipain, and 400 μM phenylmethylsulfonyl fluoride (all from Sigma) had been added immediately before use. Homogenates were centrifuged and the supernatants frozen at −20 C overnight.

For GLUT-4 quantitation, LDM fractions were prepared as described (33, 34). Homogenates were centrifuged at 11,400 × *g* for 10 min and the supernatants recentrifuged at 356,000 g for 60 min. The resulting pellets were resuspended in 100–250 μl ice-cold buffer C (20 mM Tris, 255 mM sucrose, 1 mM EDTA, pH 7.4). Protein content was measured by the Bradford method.

Comparison with GR ligand binding

Quantitation of GR protein was improved by standardizing between blots using a bulk preparation of liver cytosol of dex-binding capacity as described (35). The dissociation constant and B_{max} for the preparation were calculated from Scatchard analysis of the data, using the Radlig package (Biosoft, Cambridge, UK). Scatchard analysis of the standard liver cytosol preparation yielded a dissociation constant of 3.5×10^{-9} M and B_{max} (maximal binding capacity) of 6.3×10^{-9} M.

SDS-PAGE and Western blotting

Homogenates were diluted 1:1 in 2× Laemmli buffer (4% sodium dodecyl sulfate, 20% glycerol, 2 mM dithiothreitol, 125 mM Tris, 10% β-mercaptoethanol, pH 6.8, with bromophenol blue) and denatured for 4 min at 95 C. For GR, 60 μg muscle protein samples were electrophoresed alongside lanes containing 4, 14, 26, and 40 μg liver cytosol protein of known binding characteristics. LDM preparations for GLUT-4 (25 μg protein per sample) were electrophoresed alongside quadruplicate lanes containing a bulk preparation of standard quadriceps LDM to control for intergel variation in band intensity. Samples were electrophoresed in triplicate in running buffer (25 mM Tris, 200 mM glycine, and 3.5 mM sodium dodecyl sulfate) at 40 mA. The resolving gel was then presoaked in transfer buffer (25 mM Tris, 200 mM glycine, and 20% methanol) along with enhanced chemiluminescence blotting membrane. Proteins were transferred to the membrane by electroblotting in cold transfer buffer at 250 mA for 2.5–3 h.

Antibody application and visualization

Membranes were agitated overnight at 4 C in 2.5× Tris-buffered saline containing 5% milk powder and 0.1% Tween 20. Dilutions of primary antibody (Ab) GR M-20 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) or RaIRGT anti-GLUT-4 Ab (Biogenesis Ltd., Poole, Dorset, UK) were applied for 2 h, followed by three washes. Antirabbit secondary antibody (Amersham Pharmacia Biotech) was applied for 1 h and washed GR blots were exposed to 1:500 dilutions of anti-GR Ab, followed by 1:5000 dilutions of 2° Ab, yielding single bands at a molecular weight of approximately 95,000. For GLUT-4, antibodies were applied at dilutions of 1:750 and 1:7500, respectively, producing a main band equivalent to a molecular weight of 43,000. Enhanced chemiluminescence (Amersham Pharmacia Biotech) was used to visualize bands.

Quantitation

Film images (MCID-M4 image analysis version 3.0 revised 1.5, Imaging Research, Inc., St. Catharine's, Canada) were captured and band intensity analyzed using Aida. Plots of microgram protein loaded *vs.* GR band intensity for the liver cytosol were used to determine the amount of liver cytosol protein that would yield the GR band intensity in each muscle lane, and thus B_{max} for GR in each quadriceps. Mean band intensity between groups was used to compare GLUT-4 expression.

Statistics

Data are mean ± SEM. For comparison between dex and saline-treated offspring, *t* tests, or Mann-Whitney rank sum tests were used, as appropriate. Two-way ANOVA was used to analyze data in which a second variable was involved, with *post hoc* analysis by *t* test or Tukey's test. Significance was set at P of 0.05 or less.

Results

Birth phenotype

Birth weight was reduced in rats given prenatal dex (saline 6.96 ± 0.07 g, dex 5.21 ± 0.05 g, n = 60 and 54, $P < 0.001$), whereas the number of pups per litter was unaltered (saline 12.0 ± 2.2, dex 10.8 ± 1.6, n = 5 per group, $P = 0.67$), consistent with previous reports (12, 13).

Body and tissue weight

Dex-treated offspring remained significantly lighter than controls from birth until 160 d of age (Fig. 1). Whereas dex offspring were on average 25% lighter at birth, they were only 14–16% lighter from 2 months of age onward, *i.e.* incomplete catch-up growth had occurred.

Table 1 shows the weight of three muscles and the epididymal fat pad at 7–8 months of age. All were lighter in dex-treated offspring, consistent with lower overall body weight. However, after correction for body weight, the epididymal fat pad in dex rats was still 17% lighter than con-

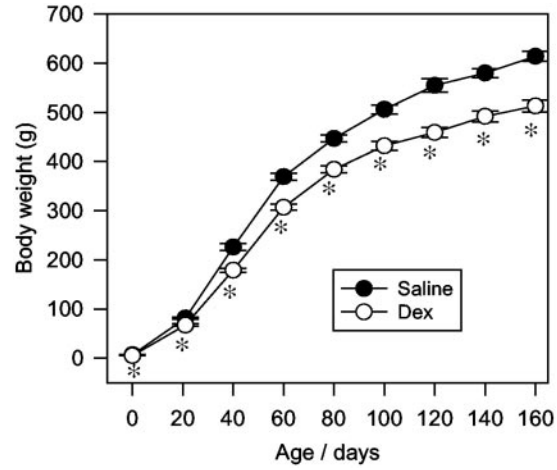

FIG. 1. Weight gain of dex and saline-treated offspring. Mean body weight between birth and 160 d of age (grams) (n = 9 and 13, respectively). By two-way ANOVA, $P < 0.001$ for effect of age and prenatal treatment and interactions between the two. *Post hoc:* *, $P < 0.05$ dex weight *vs.* control at each age. Evidence of partial catch-up growth is apparent in dex-treated animals.

TABLE 1. Adult weights of the epididymal fat depot and muscles of varying fiber type composition

Tissue	Weight of tissue (g)		Percentage of body mass	
	Saline	Dex	Saline	Dex
EDL muscle	0.24 ± 0.00	0.23 ± 0.01[b]	0.037 ± 0.001	0.041 ± 0.002[a]
Soleus muscle	0.28 ± 0.01	0.26 ± 0.01[a]	0.043 ± 0.001	0.046 ± 0.002
Quadriceps muscle	3.82 ± 0.08	3.48 ± 0.09[a]	0.58 ± 0.02	0.64 ± 0.02[a]
Epididymal fat	8.79 ± 0.50	6.00 ± 0.40[b]	1.32 ± 0.07	1.09 ± 0.05[a]

Measurements expressed in absolute units and as a percentage of body weight at death. By Student's *t* test; [a] $P < 0.05$; [b] $P < 0.001$ *vs.* control.

TABLE 2. Adult plasma leptin and lipids

Plasma parameter	Saline-treated	Dex-treated
Leptin (ng/ml)	5.5 ± 0.8	6.5 ± 0.6
Triglycerides (mM)	1.6 ± 0.1	1.5 ± 0.2
NEFA (mM)	1.0 ± 0.1	0.9 ± 0.1
Total cholesterol (mM)	2.2 ± 0.1	2.6 ± 0.3
HDL-cholesterol (mM)	1.6 ± 0.1	1.8 ± 0.1

Measurements were made in starved (all except leptin) or *ad libitum*-fed (leptin only) 6-month-old rat offspring. HDL, High density lipoprotein. n = 5–12 per group, all $P > 0.05$ by Student's *t* test.

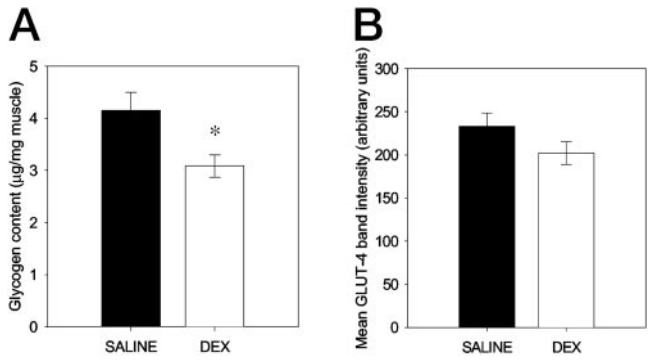

FIG. 2. Glycogen storage and GLUT-4 content of quadriceps muscle of 6-month-old dex- and saline-treated rats. A, Glycogen content (microgram per milligram muscle) (n = 20 and 18, respectively). *, $P = 0.01$ *vs.* control. B, GLUT-4 protein in LDM fraction of muscle, estimated using Western blotting; n = 10 per group.

trols. A similar trend was seen in other adipose depots. In contrast, EDL and quadriceps were, respectively, 10% and 7% heavier in rats exposed to prenatal dex, but soleus muscle weights were not significantly different.

Plasma parameters

Fasting plasma glucose was not significantly different between groups (saline 5.42 ± 0.11 mM, dex 5.63 ± 0.25 mM; n = 9 per group) but was higher in dex offspring 30 min after an oral glucose load (saline 9.18 ± 0.18 mM, dex 10.62 ± 0.52 mM; $P = 0.02$), as previously recorded (13). Plasma concentrations of leptin (nonfasting) and lipid parameters (fasting) did not differ between dex- and saline-treated offspring (Table 2).

Muscle glycogen and GLUT-4

As shown in Fig. 2, glycogen storage in quadriceps of 6-month-old adults was reduced by approximately 26% in the prenatal dex group. There was no significant effect of prenatal dex on GLUT-4 protein content of quadriceps muscle.

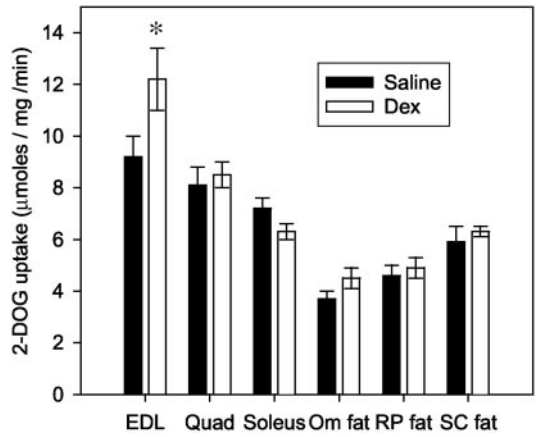

FIG. 3. 2-DOG uptake by muscles and fat depots in 7- to 8-month-old dex- and saline-treated rats (micromoles per milligram tissue per minute) over the 45 min following bolus administration of glucose and tracer (n = 9 per group). By two-way ANOVA, there were significant effects of tissue ($P < 0.001$) and prenatal treatment ($P < 0.05$) on peripheral 2-DOG uptake, with interactions between the two variables ($P < 0.05$). *Post hoc:* 2-DOG uptake differed between EDL and quadriceps or soleus muscle ($P < 0.05$) and sc fat and retroperitoneal (RP) or omental (Om) fat ($P < 0.05$). Between treatment groups, *, $P < 0.05$ *vs.* control tissue.

Uptake of radiolabeled 2-DOG into muscle and fat

Uptake of 2-DOG tracer by three different muscles and three fat depots was measured in the presence of a glucose load. As shown in Fig. 3, 2-DOG uptake per milligram tissue in muscle was generally greater than in adipose tissue, uptake by EDL muscle was greater than quadriceps or soleus and sc fat imported more 2-DOG than retroperitoneal or omental fat. Prenatal dex administration resulted in a 32% increase in uptake of 2-DOG by EDL muscle but had no effect in other muscles or fat depots.

GR expression in skeletal muscle and adipose tissue

In muscle, as shown in Fig. 4, GR mRNA expression was unaffected by prenatal dex in EDL but down-regulated by 23% in soleus muscle. There was no significant difference in GR mRNA in quadriceps muscle, but the trend in mRNA was intermediate between that of EDL and soleus. GR protein, as estimated by Western blot, could be examined only in the larger quadriceps muscles but showed similar trends as mRNA.

GR mRNA was up-regulated by 50% in retroperitoneal fat, but there was no effect of prenatal dex on GR mRNA in sc fat (Fig. 4).

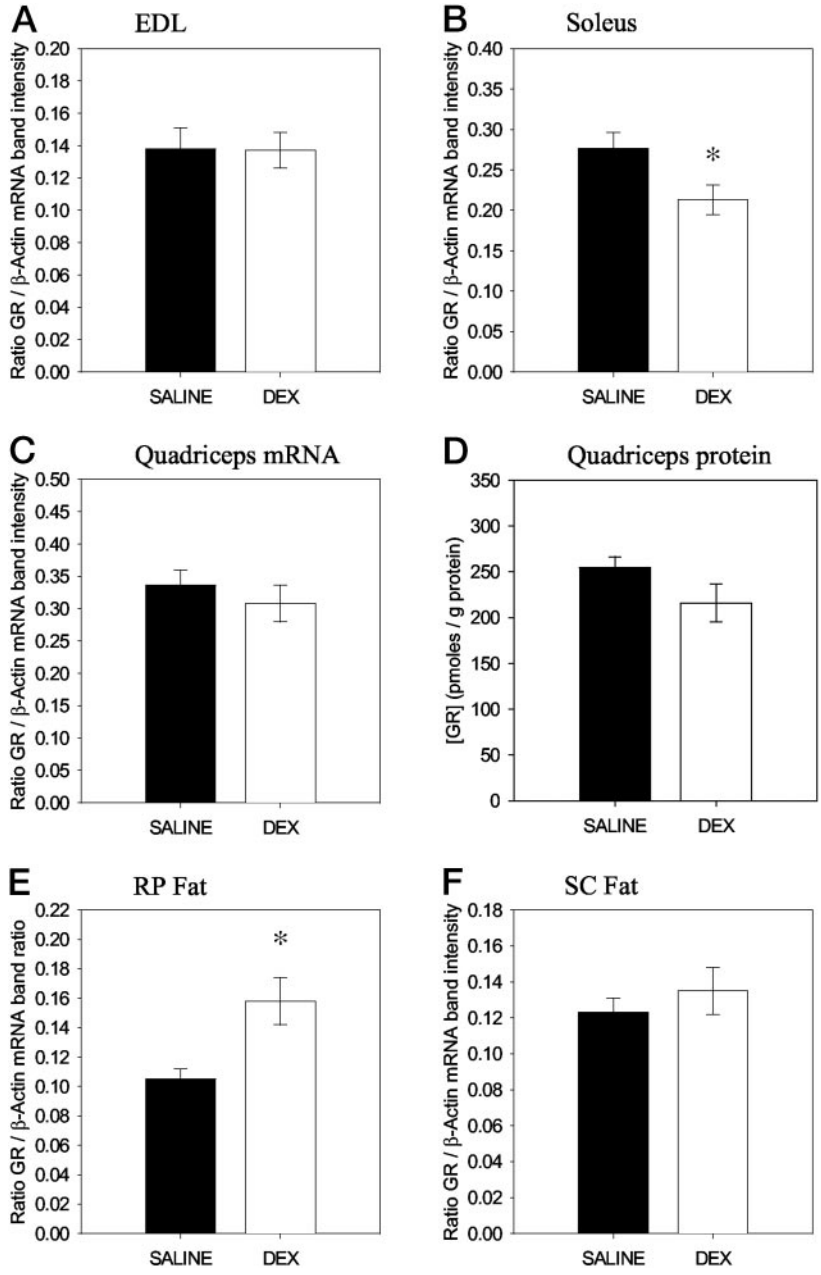

FIG. 4. GR expression in skeletal muscles and white adipose tissue depots of 6-month-old dex- and saline-treated offspring (n = 9–10 per group). A, EDL muscle. B, Soleus. C, Quadriceps. E, Retroperitoneal fat. E, Subcutaneous fat GR mRNA quantified using Rnase protection assays, normalized to β-actin. D, Quadriceps GR protein, measured by Western blotting, quantified by comparison with a standard preparation of liver cytosol of known binding capacity. *, $P < 0.05$ *vs.* control.

Expression of metabolic genes in skeletal muscle and adipose tissue

No effect of prenatal treatment was seen on UCP-3 mRNA in quadriceps muscle or on either leptin or resistin mRNA in retroperitoneal fat (Fig. 5). However, there was a 28% reduction in LPL mRNA in retroperitoneal fat of dex offspring. Although there was no significant change in expression of PPARγ1, PPARγ2, or total PPARγ mRNA in retroperitoneal fat (Fig. 6), there was a trend toward increased PPARγ2 ($P = 0.09$) and a significant shift from PPARγ1 to PPARγ2 expression in this tissue.

Discussion

These investigations revealed effects of prenatal dex on both adult skeletal muscle and white adipose tissue. Adult dex-treated offspring were lighter and leaner than controls, reflecting incomplete catch-up growth, smaller fat pads, and relatively preserved muscle mass. Despite reduced glycogen storage in the quadriceps, we did not find compelling evidence that programed hyperglycemia results from attenuated glucose uptake by these tissues. Indeed, in EDL muscle at least, glucose uptake was increased. However, lipoprotein lipase expression was attenuated in intraabdominal fat, consistent with attenuated insulin-stimulated disposal of lipids into visceral adipose. These changes may be mediated, at least in part, by down-regulation of GR in type I fibers in skeletal muscle and up-regulation of GR and PPARγ 2 in intraabdominal adipose tissue.

Dex-treated rats remained lighter than saline-treated controls until 5–6 months, although partial catch-up growth

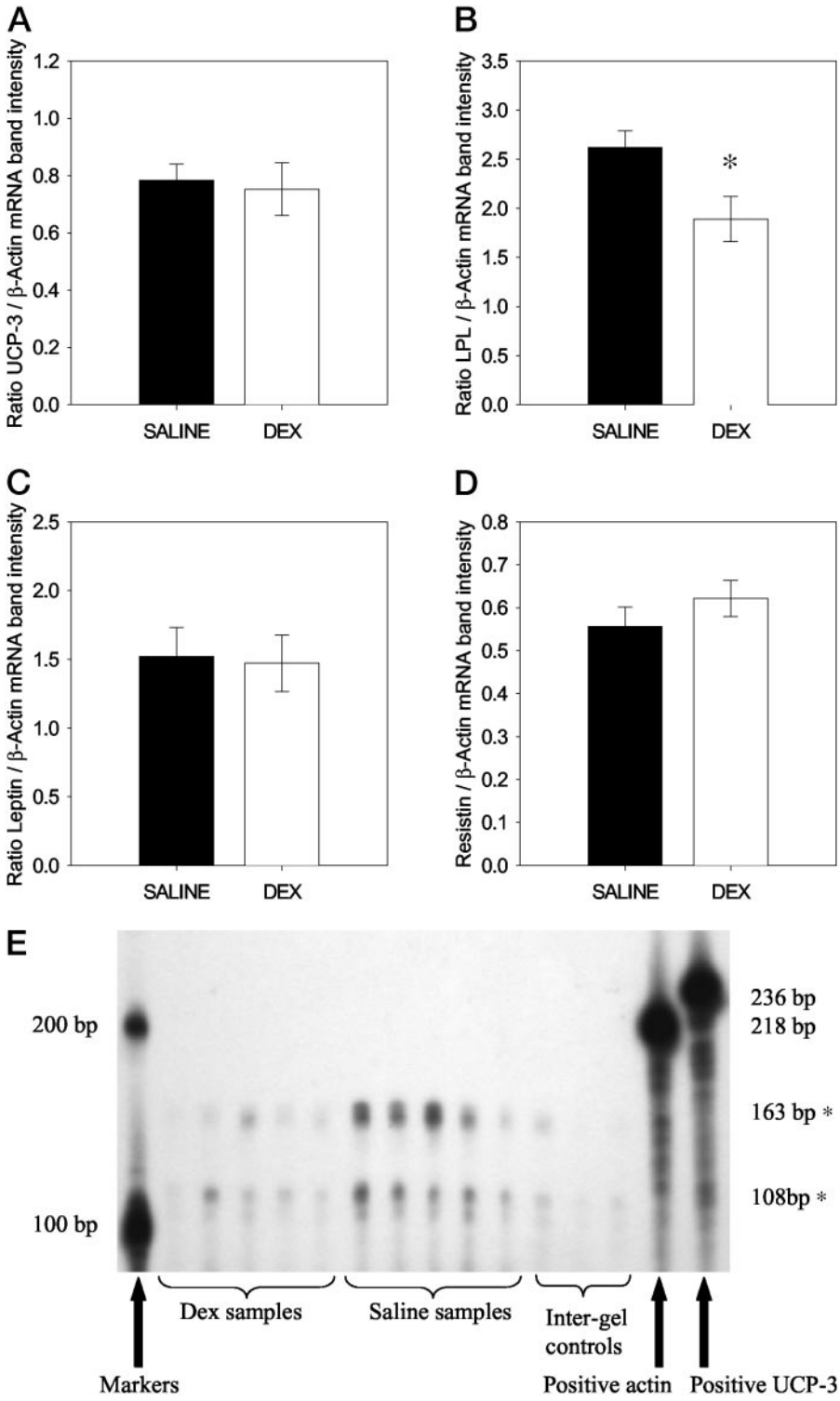

FIG. 5. Expression of metabolic gene mRNA in quadriceps and retroperitoneal adipose tissue of adult dex- and saline-treated offspring, quantified using Rnase protection *vs.* β-actin. A, UCP-3 in quadriceps. B, LPL. C, Leptin. D, Resistin in retroperitoneal fat (n = 9–10 per group). *, $P < 0.02$ *vs.* control. E, Example RNase protection gel for UCP-3 in quadriceps. Positive control (UCP-3 and β-actin full-length probes), RNA marker, intergel control, and sample lanes (dex- and saline-treated offspring) displayed, with lengths of RNA species. Protected bands marked with an *asterisk*.

occurred during the first 2 months of life. Although prior single measurements of body weight in adulthood have not shown this maintained difference (13), this trend is more typical of recent unpublished longitudinal studies in our laboratory in which body weight has been followed up in rat cohorts. Increased muscle mass relative to body weight in dex-treated offspring could imply muscular hypertrophy; however, the marked reduction in the size of epididymal fat pads, and probably other fat depots, suggests that dex-programmed rats are leaner than controls, at least on a standard chow diet.

GR expression was programmed in skeletal muscle by prenatal dex in a fiber type-specific manner. Whereas in EDL muscle, comprising predominantly type II fibers, there was

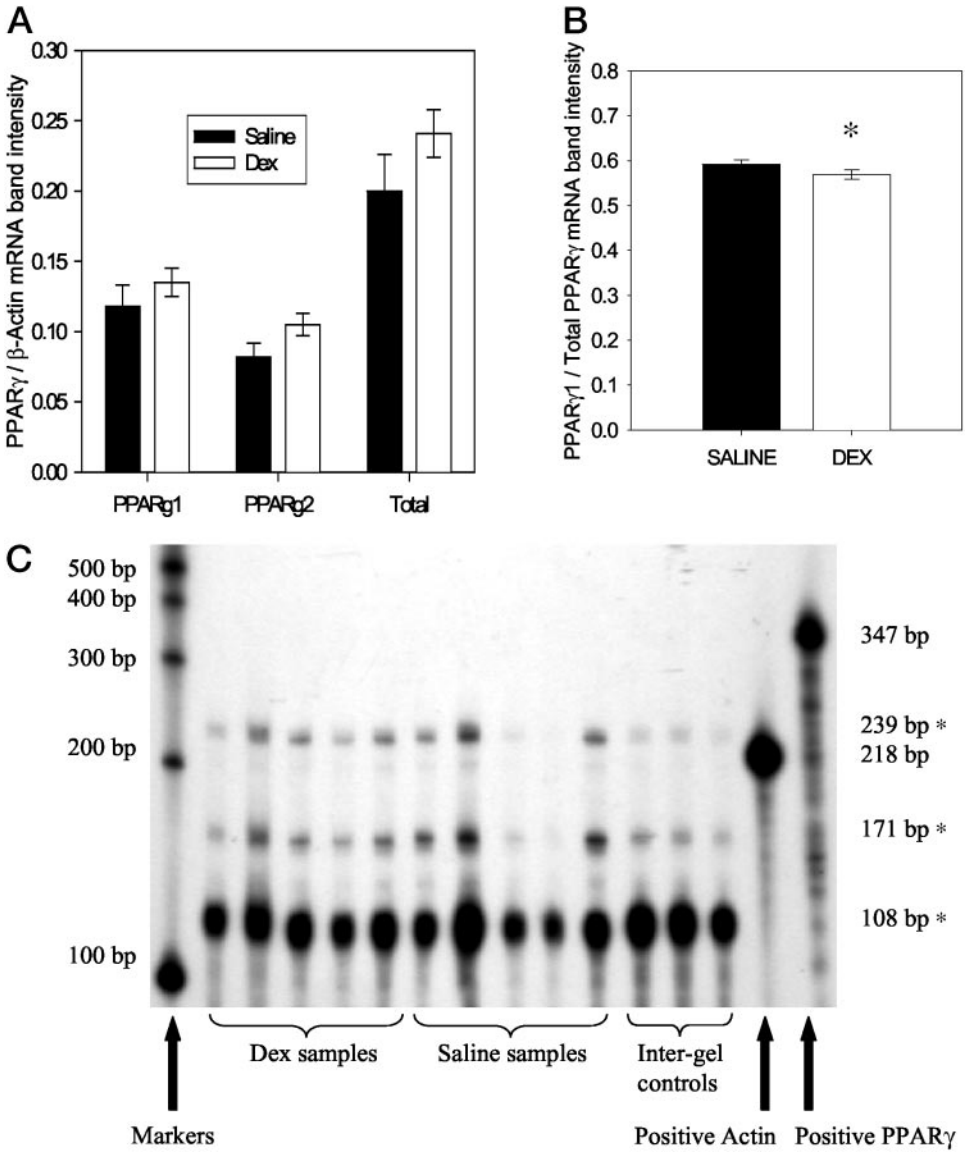

FIG. 6. Level of expression of mRNA of PPARγ isoforms in retroperitoneal fat of dex- and saline-treated offspring, measured by Rnase protection. A, PPARγ1 (PPARg1), PPARγ2 (PPARg2), and total PPARγ mRNA, normalized to β-actin. B, Ratio of PPARγ1 to total PPARγ mRNA expression (n = 10 per group). *, $P < 0.05$ *vs.* control. C, Example RNase protection gel for PPARγ isoforms 1 and 2 in retroperitoneal fat. Positive control (PPARγ and β-actin full-length probes), RNA marker, inter-gel control, and sample lanes (dex- and saline-treated offspring) displayed, with lengths of RNA species. Protected bands marked with an *asterisk:* total PPARγ, 239 bp; PPARγ2, 171 bp; β-actin, 108 bp.

no difference in GR between groups, GR mRNA was decreased by prenatal dex in soleus muscle, which contains mainly type I fibers. An intermediate trend was apparent in quadriceps, consistent with its mixture of type I and II muscle fibers (18). GR mRNA and protein expression showed identical trends between treatment groups in quadriceps, consistent with predominant transcriptional regulation of the GR gene. The reduced glucocorticoid sensitivity in soleus muscle, and therefore presumably in type I fibers, is of uncertain significance. Replacement of type I with type II fibers may be responsible for the reduction in GR here, as in other insulin resistant states (36) because type I fibers express more GR than type II fibers (19). Importantly, our data showed that type II fibers in EDL and quadriceps muscle do not show the up-regulation of GR expression evident in liver and adipose tissue.

Reduced glycogen storage in quadriceps could imply either attenuated glucose uptake or increased muscular activity in dex-programmed rats. Behavioral testing did not suggest that dex offspring were more active (17). Glucose transport is thought to be the rate-limiting step in glycogen accumulation (37) and is predominantly carried out in muscle through GLUT-4. Transport activity using GLUT-4 is controlled primarily through intracellular trafficking of glucose transporters (38), so it is perhaps not surprising that no difference in total GLUT-4 protein was detected in dex offspring. Indeed, prenatal malnutrition of rats results in altered subcellular distribution of GLUT-4 (39), which would not have been detected by the methods employed here. More persuasively, measurement of 2-DOG uptake into quadriceps and soleus muscle was unaffected by prenatal dex treatment, implying no attenuation of glucose uptake. Indeed, 2-DOG uptake into EDL muscle was slightly increased. Thus, these data do not support a contribution of attenuated glucose uptake into muscle to dex-programmed hyperglycemia.

In retroperitoneal adipose tissue, the combination of increased GR and plasma corticosterone (12) in a glucocorticoid-sensitive adipose depot (20) would be expected to

greatly amplify glucocorticoid-mediated effects. This does not, however, appear to impact on glucose uptake. Uptake of 2-DOG by adipose depots was quantitatively less than into muscle and greatest in the sc rather than intraabdominal depot, consistent with previous reports of relatively higher insulin sensitivity and glucose uptake in sc fat (40). Moreover, no differences in adipose glucose uptake as a result of prenatal treatment was identified. Thus, like muscle, attenuated glucose uptake into fat does not appear to contribute to dex-programmed hyperglycemia. Interestingly, a similar observation has been made in 3-month-old prenatally protein-restricted rats, which show improved glucose uptake into muscle and also adipocytes (39) yet later become profoundly insulin resistant. The link between up-regulation of adipose GR and insulin resistance may lie, however, in the fact that intraabdominal fat depots are important in determining hepatic insulin sensitivity (41).

In the absence of altered glucose uptake in adipose tissue, the significance of up-regulation of GR expression, and mechanisms for reduced fat mass, were further explored by examining several glucocorticoid-regulated genes, which are differentially regulated in insulin resistance. In retroperitoneal fat, leptin mRNA and plasma leptin were unaltered by prenatal dex, despite the reduced epididymal fat pad size and leanness of the animals. This may reflect an altered set point of leptin secretion relative to fat mass promoting satiety. This is supported by the observation that plasma leptin was increased in 1-yr-old rats given dex continuously during their third week of gestation (42), suggesting that this phenotype could deteriorate with age. In addition, LPL mRNA was decreased in adipose in dex-programmed animals, which could contribute to the reduction in size of fat depots through reduced uptake of fatty acids from plasma. Reduced LPL activity has also been recorded in adipocytes from insulin resistant patients (43). No parallel alterations in plasma triglycerides, NEFA, or lipoprotein profile were identified; however, these measurements reflect whole-body lipid turnover, rather than specific output from visceral fat. Because delivery of NEFAs from visceral adipose tissue to the liver is associated with increased hepatic glucose output (44), portal sampling of lipid parameters in dex rats could yield a different pattern. The mechanism of alteration in LPL mRNA is unclear; LPL expression is up-regulated in visceral adipocytes by glucocorticoids (45), suggesting that this is not a direct effect of elevated plasma corticosterone or GR.

An additional consequence of increased GR mRNA in adipose tissue of dex-programmed rats may lie in its effects on adipose differentiation. In this, it may interact with PPARγ (46). Altered expression of PPARγ isoforms, alongside changes in GR, could be involved in mediating expression of metabolic genes in fat. Glucocorticoids predominantly increase the γ1 isoform; thus, the shift in favor of the γ2 isoform is not likely to be a result of hypercorticosteronemia or elevated GR (47). However, PPARγ2 has recently been identified as the isoform most involved in adipocyte differentiation (48), and its expression was increased in fat from obese insulin resistant patients (47).

In summary, fat and muscle metabolism is programmed by prenatal glucocorticoid overexposure. GR expression is programmed in a tissue-specific fashion. It is down-regulated in soleus muscle but unchanged in two other muscles. However, elevated GR expression in visceral adipose with concomitant hypercorticosteronemia suggests markedly increased glucocorticoid action in this depot and consequent local (adipose and hepatic) insulin resistance. Additional changes in adipose, including altered expression of PPARγ isoforms and reduced LPL expression, are unlikely to be mediated directly by increased glucocorticoid action and may either be independently programmed or be indirect consequences of insulin resistance. The absence of measurable attenuation of glucose uptake into fat and muscle suggests that increased hepatic glucose output is most likely to be responsible for the programmed hyperglycemia. Reduced fat deposition is probably explained in part by increased leptin secretion and in part by down-regulation of LPL expression, consistent with a programmed attenuation of fatty acid uptake by visceral fat. Consequent alteration in the supply of NEFAs to the liver could be responsible for increased hepatic glucose output in adult dex-programmed rats.

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