Developmental programming of adult hyperinsulinemia, increased proinflammatory cytokine production, and altered skeletal muscle expression of SLC2A4 (GLUT4) and uncoupling protein 3

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Abstract

Fetal glucocorticoid excess programs detrimental effects in the adult phenotype including hyperleptinemia and aberrant glycemic control. In this study, we determined the interactive effects of maternal dexamethasone (Dex) treatment and postnatal dietary ω-3 (n-3) fatty acids on adult proinflammatory cytokine production and skeletal muscle expression of genes central to glucose handling and fatty acid metabolism. Dex acetate was administered to pregnant rats (0.75 μg/ml drinking water) from day 13 to term. Offspring of treated and control mothers were cross-fostered to mothers on either a standard (Std) or high n-3 (Hn3) diet, and remained on these diets postweaning. Adult offspring exposed to Dex in utero exhibited fasting hyperinsulinemia when raised on the Std diet but not when raised on the Hn3 diet. Dex also programmed increased plasma tumour necrosis factorα and interleukin 1β (IL-1β), but the increase in IL-1β was also prevented by the Hn3 diet. In skeletal muscle, expression of insulin regulated Slc2a4 (formerly known as GLUT4) was elevated (up to 15-fold) after Dex in utero, and this resulted in elevated intracellular, but not membrane-associated, SLC2A4 protein. Fetal glucocorticoid excess also reduced adult skeletal muscle Ucp3 expression in all offspring, whereas skeletal muscle expression of both Ppard and Pparg1a were increased in females but not males. In conclusion, our data show that fetal glucocorticoid excess programs adult hyperinsulinemia and increased proinflammatory cytokine production. Related changes in the skeletal muscle Slc2a4, Ucp3, and Ppard indicate that fetal glucocorticoid excess disturbs adult glucose/fatty acid transport and metabolism.


Introduction

An increased incidence of hypertension, insulin resistance, obesity, and dyslipidemia, collectively referred to as the metabolic syndrome, has been linked to low birth weight, an indicator of a poor fetal environment (Gluckman & Hanson 2004). This association is thought to reflect the impact of early developmental disturbances that program adverse outcomes in adult life (Gluckman & Hanson 2004). The latter may manifest as a predisposition for disease states, such as that which occurs in rats following fetal glucocorticoid excess (Seckl 2001) or maternal undernutrition (Gluckman & Hanson 2004). Disturbances in glycemic control are a feature of the programmed adult phenotype in several animal models; for example, fetal growth restriction induced by uterine artery ligation in rats produces glucose-intolerant offspring with impaired insulin signaling and altered hepatic gluconeogenesis (Vuguin et al. 2004). Moreover, treatment of pregnant mothers with either dexamethasone (Dex) or carbenoxolone, an inhibitor of 11β-hydroxysteroid dehydrogenase, increases fetal glucocorticoid exposure and leads to offspring hyperglycemia, glucose intolerance, hyperinsulinenia, and insulin resistance (Nyirenda et al. 1998, Cleasby et al. 2003, O’Regan et al. 2004). The similar effects of Dex and carbenoxolone suggest that fetal glucocorticoid exposure per se, rather than indirect effects of glucocorticoids on maternal physiology, is the primary stimulus for these programming outcomes. Nevertheless, the precise mechanisms by which fetal glucocorticoid excess programs aberrant glycemic control in offspring remain obscure.

Proinflammatory cytokines are recognized as key drivers of insulin resistance, particularly that associated with obesity (for review see Shoelson et al. 2006), yet their role in the etiology of programmed disturbances in glucose handling remains unexplored. Alterations in skeletal muscle insulin regulated Slc2a4 expression and/or function may also be central to programmed disturbances in glycemic control, since prenatal ethanol exposure in rats (Chen & Nyomba 2003) and nutrient restriction in sheep (Gardner et al. 2005) both reduce Slc2a4 expression in glucose-intolerant offspring. Maternal calorie restriction has also been shown to compromise insulin-induced translocation of SLC2A4 to the plasma membrane (Thamotharan et al. 2005).

Therefore, the present study tested the hypothesis that disturbances in glycemic control in adult offspring after fetal
glucocorticoid excess are accompanied by increased proinflammatory cytokines and changes in the expression of key genes that influence glucose handling. Specifically, we measured fasting blood glucose, insulin, tumour necrosis factor alpha (TNFα), IL-1β, and IL-6 in adult male and female offspring of mothers treated with Dex. The skeletal muscle expression of SLC2A4 mRNA and protein, including its subcellular localization, and peroxisome proliferator-activated receptor (PPAR)-α, PPAR-γ coactivator-1α (PPARGC1A), and uncoupling protein 3 (UCP3) mRNAs, were also determined. These genes were targeted because PPARδ is a key regulator of fuel metabolism (Brunmair et al. 2006), the effects of which involve coactivation by PPAR.GC1A (Finck & Kelly 2006), whereas UCP3 promotes fatty acid oxidation (MacLellan et al. 2005) and glucose uptake (Huppertz et al. 2001), and limits mitochondrial damage via effects on lipid peroxidation (Brand et al. 2002). The interactive effects of a postnatal, high ω-3 fatty acid (high n-3, Hn3) diet were also determined, since our previous studies show that this dietary intervention markedly reduces other adverse phenotypic outcomes programmed by fetal glucocorticoid excess (Wyrwoll et al. 2006, 2007).

Materials and Methods

Animals and diets

Nulliparous albino Wistar rats aged between 8 and 10 weeks were obtained from the Animal Resources Center (Murdoch, Australia) and maintained under controlled lighting and temperature as previously described (Burton & Waddell 1994). Two isocaloric, semi-pure diets were used in this study, each formulated with identical ratios of protein, carbohydrate, fat, and salt, but with markedly different n-3 fatty acid contents as previously described (Wyrwoll et al. 2006). The semi-pure diets were manufactured by Speciality Feeds (Glen Forrest, Australia) and were sterilized by γ-irradiation. Ten days before mating, half the females were placed on one of the two semi-pure diets (standard (Std) or Hn3), whilst the others remained on normal rat chow (Speciality Feeds). All rats consumed acidified water and food ad libitum. All procedures involving animals were approved by the Animal Ethics Committee of The University of Western Australia.

Rats were mated overnight and the day on which spermatozoa were present in a vaginal smear was designated day 1 of pregnancy. Dex acetate (Sigma Chemical Co.) was administered in the drinking water (0-75 μg/ml) from day 13 of pregnancy until birth in half the mothers on normal rat chow. Previous studies show that Dex acetate administered via maternal drinking water results in consistent, dose-dependent reductions in birth weight (Smith & Waddell 2000). In this particular cohort, birth weight was reduced by 24 and 25% in males and females respectively (Wyrwoll et al. 2006). Within 24 h of birth, all pups from control (Con) and Dex-treated mothers were cross-fostered to a mother on either a Std diet or Hn3 diet. Cross-fostering resulted in four treatment groups (Con/Std, Con/Hn3, Dex/Std and Dex/Hn3), and pups remained with their foster mothers until weaning, at which point male and female offspring were caged separately and remained on their allocated diets (Std or Hn3).

Blood and tissue collection

At 6 months of age, animals were fasted overnight, then anesthetized with halothane/nitrous oxide, and a blood sample obtained from the dorsal aorta. Blood was collected using two different methods: into a heparinized tube and centrifuged at 1300 × g for 5 min to obtain plasma; and into an unheparinized tube and incubated at room temperature for 1 h before centrifugation at 1000 × g for 10 min to obtain serum. Blood samples were stored at −20 °C until subsequent analysis. Gastrocnemius muscle samples were collected, snap frozen in liquid nitrogen and stored at −80 °C until analysis.

Measurement of blood glucose, insulin, TNFα, IL-1β, and IL-6

Serum glucose was measured with an automated Technicon Axon Analyzer (Bayer Diagnostics) using a hexokinase method. The between-batch precision was 3-1 and 2-4% at a glucose level of 4-9 and 16-8 mmol/l respectively. Plasma insulin levels were determined by RIA (Linco Research, St Charles, MO, USA) according to the manufacturer’s instructions. All samples were analyzed in a single assay, for which the intra-assay coefficient of variation was 3-4%. Plasma levels of TNFα, IL-1β, and IL-6 were measured by a rat cytokine LINCOp lex kit (Linco Research). All samples were analyzed in a single assay, and the intra-assay coefficients of variation were 4-8, 3-6, and 4-2%.

Measurement of mRNA expression by quantitative RT-PCR analysis

Total RNA was extracted from tissue samples using TRI Reagent (Molecular Research Center, Cincinnati, OH, USA) and extracted RNA was treated using the Ambion DNA-free kit (Austin, TX, USA) to remove contaminating genomic DNA. RNA (1 μg) was then reverse transcribed at 55 °C for 50 min using MMLV RT (Promega) according to the manufacturer’s instructions. The resultant cDNA was purified using the UltraClean PCR Clean-up Kit (MoBio Laboratories Inc., Solana Beach, CA, USA). The cDNA primers for rat SLC2A4, Ucp3, Ppard, and Pparγ1α were designed using Primer3 software (http://frodo.wi.mit.edu/cgi-bin/primer-er3/ primer3-www.cgi), and were positioned to span introns; ribosomal Sd1g11 was used as an internal control (Orly et al. 1994). For each gene, the PCR primer sequences are shown in Table 1 along with MgCl2 and primer concentrations, annealing temperatures, and PCR product sizes. External standards were generated from regular PCR products and


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Table 1. PCR primers, MgCl₂ concentrations, annealing temperatures, and PCR product sizes for each gene analyzed by routine quantitative RT-PCR.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer sequencea</th>
<th>MgCl₂ (mM)</th>
<th>Primer (µM)</th>
<th>AT (°C)</th>
<th>Product size (bp)</th>
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<tr>
<td>Slec2A4</td>
<td>F: 5'-GCT GTC GCT GCC CTT CTC TC-3'</td>
<td>2</td>
<td>0-25</td>
<td>59</td>
<td>166</td>
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<tr>
<td></td>
<td>R: 5'-TGG ACG CTC TCT TCC CAA CT-3'</td>
<td></td>
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<tr>
<td>Ppargc1a</td>
<td>F: TCT GGA ACT GCA GGC CTA ACT C</td>
<td>4</td>
<td>0-25</td>
<td>60</td>
<td>96</td>
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<tr>
<td></td>
<td>R: GCA AGA GGG CTT CAG CTT TG</td>
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<tr>
<td>Ppard</td>
<td>F: 5'-GAG GGG TGG TGA AAG GGC TTC TT-3'</td>
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<td>0-5</td>
<td>60</td>
<td>101</td>
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<tr>
<td></td>
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<tr>
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<td>F: 5'-CTG TGG ATG CCT ACA GAA GCA CCA TC-3'</td>
<td>2-5</td>
<td>0-5</td>
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<td>116</td>
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<tr>
<td></td>
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<tr>
<td>Sdtg11</td>
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<tr>
<td></td>
<td>R: 5'-GGA CAG AGT CTT GAT CTC-3'</td>
<td></td>
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</table>

*aForward primer sequence indicated by F, reverse primer sequence indicated by R.

tenfold serial dilutions of the PCR product were made in R.Nase-free water (1- to 10⁷-fold dilutions). Quantitative PCR was performed in 10 µl reaction volumes using the Rotor-Gene 3000 system (Corbett Research, Sydney, Australia) with primer concentrations as specified in Table 1. Immolase enzyme (0-5 U; Bioline, Alexandria, Australia), and 1/40 000 dilution of stock SYBR Green (Molecular Probes, Eugene, OR, USA) per reaction. The PCR cycling conditions included an initial denaturation at 94 °C for 7 min followed by up to 50 cycles at 94 °C for 1 s; an annealing temperature (specified in Table 1) for 15 s; and 72 °C for 5 s. In each case, melt-curve analysis from 70 to 99 °C showed a single PCR product that was confirmed to be the correct size and sequence by gel electrophoresis and sequence analysis respectively (data not shown). Fluorescence values were analyzed, standard curves constructed using the RotorGene software (Corbett Research, Sydney, Australia), and all samples standardized against the internal control (Sdtg11).

Western blot analysis

Procedures from Takeuchi et al. (1998) and Huisamen et al. (2001) were used to obtain protein from both the intracellular compartment and the membrane fraction of gastrocnemius muscle samples. Homogenates (2 vol ice-cold 20 mM Tris-HCl (pH 7-5), 330 mM sucrose, 2 mM EDTA, 0.5 mM EGTA, 1 mM phenylmethylsulphonyl fluoride (PMSF), 1/100 (v/v) Protease cocktail) were centrifuged at 1000 g for 10 min at 4 °C to remove particulate matter. The supernatant was centrifuged at 20 000 g for 2 h at 4 °C and the resultant supernatant contained the intracellular compartment. The remaining pellet was then suspended in an equal volume of 20 mM Tris-HCl (pH 7-5), 1% (v/v) Triton X-100, 2 mM EDTA, 0.5 mM EGTA, 1 mM PMSF, 1/100 (v/v) Protease cocktail, and centrifuged at 20 000 g for 2 h at 4 °C. The resultant supernatant contained the membranous fraction of the sample. Protein fractions were resolved by SDS-PAGE using the Bio-Rad Mini-PROTEAN 3 System. SeeBlue prestained molecular weight standards were included on each gel to determine protein band molecular weight. Proteins were transferred to Hybond C+ Super membrane (Amersham) by electroblotting using a trans-blot system (Bio-Rad Laboratories). Non-specific binding was blocked by incubation with Tris-buffered saline with Tween-20 (TBS–TWEEN; 0-1 M Tris, 0-15 M NaCl, 0-1% Tween-20, (pH 7-5)) containing 5% non-fat milk powder for 1 h at room temperature, and the membranes were exposed overnight at 4 °C to the primary antibody diluted with TBS–TWEEN (1:250 for Slec2A4; 1:5000 for β-actin). The SLC2A4 antibody was purchased from Biogenesis Poole (Dorset, UK) and the β-actin antibody from Sigma. Membranes were then washed in three changes of TBS–TWEEN and incubated at room temperature for 1 h with a horseradish peroxidase-conjugated secondary antibody (1:10 000 goat anti-mouse for β-actin; 1:10 000 goat anti-rabbit for SLC2A4; Santa Cruz Biotechnology, Santa Cruz, CA, USA). Immunoreactive bands were visualized using a chemiluminescence detection kit (SuperSignal Substrate; Pierce Chemical, Rockford, IL, USA) and resultant images were quantified using a Kodak Image Station and Kodak Molecular Imaging Software v.4.0.4 (Eastman Kodak Company). Values for SLC2A4 in the intracellular compartment are expressed relative to β-actin, and those for the membrane fraction are expressed relative to those of the paired intracellular compartments.

Statistical analysis

All data are expressed as mean± s.e.m., with each litter representing an n of one. In all cases, each experimental group had an n of 6–8. All variables were analyzed by ANOVAs (one-, two- or three-way as appropriate to account for variation due to sex, maternal treatment, and postnatal diet) followed by post hoc least significant difference (LSD) tests (Snedecor & Cochran 1989). When the interaction between the sources of variation was statistically significant, further analyses of subsets of data were made by ANOVA or unpaired t-tests. The relationship between skeletal muscle Ppard and UCP3 expression was assessed by analysis of covariance (Snedecor & Cochran 1989).
Results

Blood insulin and glucose concentrations

Fasting plasma insulin was increased twofold in both male and female offspring exposed to Dexamethasone in utero and consuming a Std diet postnatally (P<0.01; Fig. 1a). In addition, there was a significant interaction between Dex exposure in utero and postnatal diet (P<0.01) such that the elevation in plasma insulin was not observed in Dex-exposed offspring consuming a postnatal Hn3 diet. Offspring serum glucose did not vary due to either maternal treatment or postnatal diet (Fig. 1b).

Plasma levels of TNFα, IL-1β, and IL-6

Plasma TNFα varied significantly with maternal treatment (P<0.001; Fig. 2a), with male and female offspring of Dex-treated mothers showing elevated plasma TNFα (up to 82% higher) irrespective of postnatal diet. Plasma IL-1β also varied with diet (P<0.001) and prenatal treatment (P<0.001), and there was significant interaction between these factors (P<0.01). Because of this significant interaction term, Dex effects were assessed separately for each sex and diet by unpaired t-tests. These pairwise comparisons showed that maternal Dex treatment programmed elevated plasma IL-1β in male (threefold increase, Fig. 2b) and female (2.8-fold increase) offspring raised on a Std diet, but not in those offspring raised on the Hn3 diet. Moreover, control male (but not female) offspring consuming the Hn3 diet had lower (P<0.05) plasma IL-1β than control animals on a Std diet. For plasma IL-6, there were smaller overall diet (P<0.001) and maternal Dex treatment (P<0.05) effects, but no significant interaction between these factors.

Skeletal muscle Slc2a4 expression and subcellular localization

Skeletal muscle SLC2A4 mRNA expression was substantially elevated (up to 15-fold; P<0.01; Fig. 3a) in all offspring exposed to Dex in utero, and this effect was greater in females than males (P<0.01). Western blot analysis revealed that skeletal muscle SLC2A4 protein was also increased in male and female offspring of Dex-treated mothers, but only in the intracellular compartment (P<0.01; Fig. 3b). SLC2A4 protein localized to the plasma membrane fraction was not affected by either maternal Dex treatment or postnatal diet.

Skeletal muscle Ucp3, Ppard, and Ppargc1a mRNA expression

Skeletal muscle UCP3 mRNA expression was markedly reduced (by as much as 75%) in both male and female offspring of Dex-treated mothers (P<0.01; Fig. 4), but was...
unaffected by the postnatal Hn3 diet. By contrast, skeletal muscle expression of both *Ppard* and *Ppargc1a* were increased in female (but not male) offspring of Dex-treated mothers (*P*! 0.01 and *P*! 0.05; Fig. 4). The *Ucp3* expression was positively related to *Ppard* expression in all offspring of control and Dex-treated mothers (*P*! 0.01 in each case), but the slope of this association was more than tenfold lower in offspring of Dex-treated mothers (F value for regression coefficients Z 85 and 34 for males and females respectively; both *P*! 0.001; see Fig. 5).

**Discussion**

The key findings of this study were that Dex exposure in utero programmed hyperinsulinemia and markedly increased plasma levels of proinflammatory cytokines in adult offspring. These effects occurred without increased adiposity and were accompanied by a striking increase in skeletal muscle *Slc2a4* mRNA expression and accumulation of *SLC2A4* protein in the intracellular compartment. Prenatal Dex also dramatically reduced skeletal muscle *Ucp3* expression and altered the relationship between *Ppard* and *Ucp3* expression. Raising animals from birth on a diet enriched with n-3 fatty acids prevented programmed increases in plasma insulin and IL-1β, but did not correct disturbances in skeletal muscle *Slc2a4*, *Ppard*, and *Ucp3* expression.

Our observation of adult hyperinsulinemia programmed by fetal glucocorticoid excess is consistent with previous reports (Lindsay et al. 1996, Nyirenda et al. 1998), but unlike these we did not observe fasting hyperglycemia, suggestive of a less severe phenotype in our model. On the other hand, we observed marked increases in plasma levels of TNFα and IL-1β in offspring of Dex-treated mothers, raising the possibility that programmed hyperinsulinemia occurs within an increased inflammatory state. A causal link between obesity-related inflammation and insulin resistance is well recognized (for review see ref Shoelson et al. 2006), but intriguingly our programmed phenotype exhibits normal adiposity (Wyrwoll et al. 2006). This suggests that fetal glucocorticoid excess may program adipocyte gene expression without effects on adiposity per se, at least not when animals are raised on a low-fat (5%) diet. Indeed, we have identified increases in adipose expression of leptin (Wyrwoll et al. 2006), angiotensin-converting enzyme, and renin (Wyrwoll et al. 2007) in adult offspring of Dex-treated mothers. Such changes may reflect, in part, a programmed increase in exposure of adipose tissue to glucocorticoids, since several reports show that fetal glucocorticoid excess programs hyperactivity of the

![Figure 3](https://example.com/image1.png)  **Figure 3** Skeletal muscle (a) *SLC2A4* mRNA expression and (b) protein abundance in plasma membrane and intracellular fractions in female rat offspring. See legend of Fig. 1 for abbreviations. Values are the mean ± S.E.M. (*n*= 6–8 per group). Data were analyzed by three-way ANOVA followed by post hoc LSD tests. There was a significant prenatal treatment effect for both mRNA (*P*< 0.01) and intracellular protein abundance (*P*< 0.05). Within each panel, values without a common notation (a or b) differ significantly (*P*< 0.05, LSD test).

![Figure 4](https://example.com/image2.png)  **Figure 4** Skeletal muscle (a) *Ucp3*, (b) *Ppard*, and (c) *Ppargc1a* mRNA expression in male and female offspring. See legend of Fig. 1 for abbreviations. Values are the mean ± S.E.M. (*n*= 6–8 per group). Data were analyzed by three-way ANOVA followed by post hoc LSD tests. There was a significant prenatal treatment effect for *Ucp3* (*P*< 0.01), and significant interaction (*P*< 0.05) between sex and prenatal treatment for both *Ppard* and *Ppargc1a*. Within each panel, values without a common notation (a or b) differ significantly (*P*< 0.05, LSD test).
hypothalamic–pituitary–adrenal (HPA) axis in adult offspring (Kapoor et al. 2006). Indeed, we have preliminary data showing an apparent heightened corticosterone response to stress in offspring of Dex-treated mothers in this cohort (Mark et al. 2007). Increased circulating proinflammatory cytokine levels are also likely to reflect production by non-adipocyte sources, including macrophages within adipose tissue (Trayhurn & Wood 2004). Importantly, elevated proinflammatory cytokine levels can also disturb lipid homeostasis and cardiovascular function, and so regardless of their precise origin we propose that the programmed increases in circulating TNFα and IL-1β may contribute to several aspects of the programmed cardiometabolic phenotype.

Skeletal muscle Slc2a4 mRNA expression and SLC2A4 protein abundance in the intracellular compartment were both markedly increased in programmed offspring. This suggests that these animals require a greater pool of cytoplasmic SLC2A4 for sufficient recruitment to the plasma membrane in response to insulin, and/or that not all of the SLC2A4 cytoplasmic pool can undergo translocation. Indeed, SLC2A4 has been shown to accumulate in dense membrane compartments of muscle in insulin-resistant humans, and does not appear to translocate in response to insulin (Garvey et al. 1998). Similar impairment in SLC2A4 translocation occurs in adipocytes isolated from the s.c. fat of diabetic patients (Maianu et al. 2001), and it has been suggested that impaired ATP synthesis in skeletal muscle of rats following intrauterine growth retardation may compromise energy-dependent SLC2A4 recruitment to the cell surface (Selak et al. 2003). Thamotharan et al. (2005) demonstrated that maternal caloric restriction programmed aberrant SLC2A4 cellular distribution, but in this case skeletal muscle SLC2A4 was concentrated in the plasma membrane rather than the cytoplasm, and further translocation was not induced by insulin. These authors suggested that this aberration reflects heightened basal insulin sensitivity in the face of caloric restriction that leads to subsequent glucose intolerance when postnatal nutrition is adequate (Thamotharan et al. 2005). Regardless of the precise explanation, there appears to be a fundamental difference in the programming effects of in utero caloric restriction and increased glucocorticoid exposure on Slc2a4 expression, yet both lead to glucose intolerance.

Maternal Dex treatment also programmed a striking, tenfold reduction in expression of the mitochondrial protein UCP3 in skeletal muscle of adult offspring. UCP3 is best known for its roles in promoting fatty acid oxidation (MacLellan et al. 2005) and limiting mitochondrial damage by suppression of lipid peroxidation and the production of reactive oxygen species (Vidal-Puig et al. 2000, Brand et al. 2002). But, UCP3 can also stimulate glucose uptake and recruitment of SLC2A4 to the cell surface (Huppertz et al. 2001) and so its decline in programmed offspring may contribute to the observed accumulation of cytoplasmic SLC2A4. Interestingly, reduced skeletal muscle Ucp3 expression has also been observed in diabetic human subjects (Krook et al. 1998, Schrauwen et al. 2001), and a recent report indicates that this is already the case in prediabetic subjects with impaired glucose tolerance (Schrauwen et al. 2006). Such a prediabetic state appears to characterize our programmed phenotype, since offspring of Dex-treated mothers were hyperinsulinemic, yet maintained euglycemia in the fasted state. Interestingly, we observed no effect of either prenatal treatment or postnatal diet on the phosphorylation status of Akt in skeletal muscle, a crucial component of the insulin-signaling pathway (Watson & Pessin 2006), in this study (data not shown). Although this might be interpreted as evidence for normal insulin signaling, importantly, skeletal muscle was obtained from fasted animals and as such would not have been exposed to an insulin challenge. Indeed, the similarity of Akt phosphorylation status is consistent with the euglycemia observed in all groups. Further studies are required to establish whether these animals are indeed insulin resistant, and whether SLC2A4 translocation in response to a glucose load is impaired. At the very least, programmed hyperinsulinemia and the aberrant skeletal muscle phenotype suggest that these offspring are predisposed to adverse outcomes arising from postnatal metabolic challenges such as a high-energy diet. Indeed, previous research clearly shows that hypercaloric diets exacerbate adverse developmental programming effects (Vickers et al. 2000, 2003), including glucose intolerance (Chen & Nyomba 2003).

Female but not male offspring of Dex-treated mothers had increased skeletal muscle expression of both Ppard and Ppargc1a. This gender-specific programming effect is likely due to the interactive effects of estrogen, which has recently been shown to potently stimulate expression of Ppard and its downstream targets in muscle (D’Eon et al. 2005). It is well established that activation of Ppard enhances insulin sensitivity, an effect mediated partly by stimulation of fatty acid oxidation and associated changes in lipid profile (Wang...
Programming of proinflammatory cytokines

et al. 2003, Barish et al. 2006). Similarly, PPARGC1A contributes to insulin-sensitizing effects of PPARD and PPARG activators via its role as a transcriptional coactivator (Finck & Kelly 2006). Therefore, the upregulation of these genes in female offspring of Dex-treated mothers may be a homeostatic response to disturbances in insulin signaling. Moreover, the upregulation of Ppargc1a may be a downstream effect of increased PPARD, since activation of the latter induces Ppargc1a both in vitro and in vivo (Hondares et al. 2007). Activation of Pparg has also been shown to stimulate Ucp3 expression in muscle (Luquet et al. 2003, Wang et al. 2003, Villarroya et al. 2007), and accordingly we observed a highly-significant, positive correlation between skeletal muscle Pparg and Ucp3 mRNA in all offspring regardless of prenatal treatment. Importantly, however, prenatal Dex markedly changed the nature of this relationship, with the slope of the association being around tenfold lower in offspring of Dex-treated mothers. This disturbance in the Pparg–Ucp3 relationship suggests that Dex exposure in early life programs a skeletal muscle phenotype in which the capacity of PPARD to stimulate Ucp3 expression is severely compromised, but how this occurs awaits further study. Moreover, further studies on the developmental emergence of the components of the skeletal muscle phenotype induced by fetal glucocorticoid excess should clarify which aspects are programmed and which reflect homeostatic responses to disturbances in insulin signaling and/or lipid metabolism. In this context, it is noteworthy that all aspects of the programmed skeletal muscle phenotype remained evident in offspring raised on a Hn3 diet, despite the absence of hyperinsulinemia.

The mechanisms by which a Hn3 diet prevents the appearance of glucocorticoid programmed hyperinsulinemia are unclear, but their well-documented, anti-inflammatory effects (Endres et al. 1989, Rallidis et al. 2003, Mishra et al. 2004, Mori & Beilin 2004, Calder 2006) are very likely involved. As discussed above, proinflammatory cytokines are a key driver of insulin resistance (Shoelson et al. 2006), and the programmed increase in IL-1β was prevented by the Hn3 diet. Although programming- and diet-induced changes in IL-6 generally paralleled those for IL-1β, their magnitude was so small that their physiological importance is probably low. Interestingly, animals raised on the Hn3 diet still exhibited the programmed rise in plasma TNFα, possibly due to a mix of the stimulatory and inhibitory effects of n-3 fatty acids on TNFα production by different cell types (Skuladottir et al. 2007). Thus, the differential programming and diet effects on the three cytokines examined likely reflect different cellular origins of each (e.g., adipocytes versus macrophages).

While the present study clearly demonstrates profound effects of maternal Dex treatment on the phenotype of adult offspring, its relevance to developmental programming in humans is uncertain. Importantly, the Dex treatment regimen used in our model covers almost the entire fetal period, considerably longer than the exposure that normally occurs with the clinical use of synthetic glucocorticoids in cases of threatened premature delivery. On the other hand, high levels of maternal stress and the associated hyperactivity of the maternal HPA axis could potentially increase fetal glucocorticoid exposure over an extended period of gestation (Hobel et al. 2008). Indeed, maternal psychosocial stress was recently linked to subsequent disturbances in offspring insulin sensitivity (Entringer et al. 2008). Therefore, further studies are required to determine the extent to which maternal stress and/or disturbances in the placental glucocorticoid barrier (Burton & Waddell 1999) can lead to increased fetal glucocorticoid exposure in humans.

In conclusion, this study shows for the first time that adult hyperinsulinemia programmed by fetal glucocorticoid excess is associated with increased production of the proinflammatory cytokines TNFα, IL-1β, and IL-6. Moreover, maternal Dex treatment also programmed major disturbances in adult skeletal muscle expression of genes that regulate glucose uptake and fuel metabolism, possibly indicative of a prediabetic state. While a postnatal Hn3 diet prevented adverse outcomes with respect to plasma insulin and IL-1β, programmed changes in plasma TNFα and skeletal muscle expression of Slc2a4, Pparg, Ppargc1a, and Ucp3 all remained in place.

Declaration of Interest

The authors have no conflict of interest that would prejudice the impartiality of the study.

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