# Developmental programming of adult adrenal structure and steroidogenesis: effects of fetal glucocorticoid excess and postnatal dietary omega-3 fatty acids

**Brendan J Waddell[1], Maike Bollen[1], Caitlin S Wyrwoll[1], Trevor A Mori[2]** and **Peter J Mark[1]**

[1]School of Anatomy and Human Biology and [2]School of Medicine and Pharmacology, The University of Western Australia, 35 Stirling Highway, Crawley, Perth, Western Australia 6009, Australia

(Correspondence should be addressed to B J Waddell; Email: bwaddell@anhb.uwa.edu.au)

## Abstract

Fetal glucocorticoid excess programs a range of detrimental outcomes in the adult phenotype, at least some of which may be due to altered adult adrenocortical function. In this study, we determined the effects of maternal dexamethasone treatment on offspring adrenal morphology and function, as well as the interactive effects of postnatal dietary omega-3 (n-3) fatty acids. This postnatal dietary intervention has been shown to alleviate many of the programming outcomes in this model, but whether this is via the effects on adrenal function is unknown. Dexamethasone acetate was administered to pregnant rats (0·75 μg/ml drinking water) from day 13 to term. Cross-fostered offspring were raised on either a standard or high-n-3 diet. Adrenal weight (relative to body weight) at 6 months of age was unaffected by prenatal dexamethasone, regardless of postnatal diet, and stereological analysis showed no effect of dexamethasone on the volumes of adrenal components (zona glomerulosa, zona fasciculata/reticularis or adrenal medulla). Expression of key steroidogenic genes (*Cyp11a1* and *Star*) was unaffected by either prenatal dexamethasone or postnatal diet. In contrast, adrenal expression of *Mc2r* mRNA, which encodes the ACTH receptor, was higher in offspring of dexamethasone-treated mothers, an effect partially attenuated by the Hn3 diet. Moreover, stress-induced levels of plasma and urinary corticosterone and urinary aldosterone were elevated in offspring of dexamethasone-treated mothers, indicative of enhanced adrenal responsiveness. In conclusion, this study shows that prenatal exposure to dexamethasone does not increase basal adrenocortical activity but does result in a more stress-responsive adrenal phenotype, possibly via increased *Mc2r* expression.

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## Introduction

A poor fetal environment and the associated restriction of fetal growth have been consistently linked to adverse phenotypic outcomes in adult offspring, with increased risk of elevated blood pressure, insulin resistance and disturbances in adrenal function (for reviews see Gluckman & Hanson (2004) and Seckl & Holmes (2007)). Nutritional perturbations, placental dysfunction and fetal glucocorticoid excess are all recognized as key determinants of a poor fetal environment, and their interactive effects likely program the adult phenotype. The foetus is normally protected from high maternal glucocorticoid levels by the 'placental glucocorticoid barrier', in which the enzyme 11β-hydroxysteroid dehydrogenase type 2 (HSD11B2) metabolizes active glucocorticoids (cortisol or corticosterone) during transplacental passage (Yang 1997, Burton & Waddell 1999, Mark *et al*. 2009, Wyrwoll *et al*. 2009). Accordingly, treatment of pregnant rats with carbenoxolone, an inhibitor of HSD11B2, increases the passage of maternal corticosterone to the foetus and programs hyperglycemia and cardiovascular abnormalities in adult offspring (Lindsay *et al*. 1996). Similarly, treatment of mothers with synthetic glucocorticoids (which bypass the placental glucocorticoid barrier) programs adverse outcomes in adult offspring of several species (Benediktsson *et al*. 1993, Nyirenda *et al*. 1998, Smith & Waddell 2000, Holness & Sugden 2001, Wintour *et al*. 2003, O'Regan *et al*. 2004, 2008, Wyrwoll *et al*. 2006, 2007, 2008, Sloboda *et al*. 2007, de Vries *et al*. 2007). In humans, increased fetal and placental glucocorticoid exposure is thought to occur in a number of clinical settings. These include maternal administration of synthetic glucocorticoids for treatment of threatened preterm delivery, and reduced placental HSD11B2 in pregnancies complicated by intrauterine growth retardation (Kajantie *et al*. 2003) and pre-eclampsia (Causevic & Mohaupt 2007).

Despite clear evidence for glucocorticoid programming of the adult phenotype, the underlying mechanisms remain poorly understood. Human studies linking low birth weight to an adverse phenotype in adult offspring have suggested


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that adrenal hyperactivity may contribute to several features of the programmed cardiometabolic phenotype (i.e. hypertension, insulin resistance, hyperleptinemia, etc.; Phillips 2007). Indeed, several animal models suggest that fetal glucocorticoid excess programs offspring adrenal hyperactivity (for reviews see O'Regan *et al.* (2001) and Kapoor *et al.* (2008*b*)), but whether this reflects increased basal adrenocortical activity and/or an enhanced adrenal responsiveness to stress is uncertain. This distinction is important but can be difficult to assess because effectively all blood sampling interventions induce some degree of stress.

In the present study, we used unbiased stereolological analysis to quantify adrenal morphology in adult offspring of mothers treated with dexamethasone during pregnancy. Because quantitative adrenal morphology reflects long-term, functional activity, this approach provides an integrated picture of basal adrenocortical function over time, with increased volume reflective of enhanced adrenocortical activity. In addition, we measured urinary levels of corticosterone and aldosterone and adrenal expression of key genes that promote adrenal steroidogenesis. The interactive effects of a postnatal, high-omega-3 fatty acid diet were also assessed because our previous studies show that this dietary intervention prevents several of the adverse phenotypic outcomes of maternal dexamethasone treatment (Wyrwoll *et al.* 2006, 2007). It is not known, however, whether this beneficial effect of postnatal omega-3 fatty acid supplementation involves altered adrenal function.

## Materials and Methods

### *Animals and diets*

Nulliparous albino Wistar rats aged between 8 and 10 weeks were obtained from the Animal Resources Centre (Murdoch, Australia) and maintained under controlled lighting and temperature as previously described (Burton & Waddell 1994). Two isocaloric, semi-pure diets were 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 Specialty Feeds (Glen Forrest, Australia) and were sterilized by γ-irradiation. Ten days before mating, half of the females were placed on a semi-pure diet containing either standard (Std) or high-omega-3 fatty acid (Hn3) levels, while the others remained on normal rat chow (Specialty 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 as day 1 of pregnancy. Dexamethasone acetate (Sigma Chemical Co.) was administered in the drinking water (0·75 μg/ml) from day 13 of pregnancy until birth in half of the mothers on normal rat chow. This route of dexamethasone administration results in consistent, dose-dependent reductions in birth weight (Smith & Waddell 2000) and avoids possible stress-related changes induced by sham injections in control mothers. In this particular cohort, dexamethasone treatment reduced birth weight by 24 and 25% in males and females respectively, but there was no change in sex ratio or postnatal survival (Wyrwoll *et al.* 2006). Within 24 h of birth, pups from control (Con) and dexamethasone-treated (Dex) mothers were cross-fostered to a mother on either a Std or Hn3 diet, with litter size standardized to 10. 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 given their allocated diets (Std or Hn3).

### *Blood and tissue collection*

At 6 months of age, one male and one female were randomly chosen from each litter ($n=6$–8 per group) and placed in a metabolic cage for a 24-h urine collection. Animals were then replaced in their home cage for several days before a blood sample (from the dorsal aorta), and both adrenals were collected (under halothane/nitrous oxide anesthesia). After weighing, one adrenal was snap frozen in liquid nitrogen and stored at −80 °C, while the other was immerse fixed in Histochoice tissue fixative (Amresco, Solon, OH, USA) and processed for routine paraffin histology.

### *Stereological analysis*

Unbiased stereological analyses were used to estimate the volumes of the adrenocortical zones and the adrenal medulla according to the Cavalieri principle (Howard & Reed 1998). The zona glomerulosa and adrenal medulla were readily identified morphologically, and the area between these regions was considered as a single zone (zona fasciculata–reticularis) because the adult rat does not possess a conventional zona reticularis expressing *Cyp17a1* (Pelletier *et al.* 2001, Pignatelli *et al.* 2006). Rather, the reticularis cells are thought to contribute to corticosterone synthesis (Bell *et al.* 1979). One adrenal from each animal was processed for routine paraffin histology, and 7-μm serial sections obtained for the whole adrenal. The initial section was selected randomly from among the first 20; then, this and every subsequent 20th section throughout the entire adrenal were analyzed on an Olympus BX50 microscope adapted with an automated stage and using the Stereo Investigator software (MBF Bioscience, Williston, VT, USA). For each section, the number of points overlying the respective adrenal zonal components was counted, and the resultant volumes were adjusted for shrinkage by the measurement of erythrocyte diameter.

**Table 1** PCR primers, $MgCl_2$ concentrations, annealing temperatures and PCR product sizes for each gene analyzed by routine quantitative RT-PCR

| Gene | Primer sequence[a] | $MgCl_2$ (mM) | Primer (μM) | AT (°C) | Product size (bp) |
|---|---|---|---|---|---|
| *Mc2r* | F: 5′-ATCTGCAGTTTGGCCATTTC-3′<br>R: 5′-GCAATCACAGACAGGCTGAA-3′ | 2 | 0·5 | 59 | 187 |
| *Hsd11b2* | F: 5′-GATGTTCCCCTCGCCTGAA-3′<br>R: 5′-ATGAGCAGTGCAATAGCTGCCTTG-3′ | 3 | 0·25 | 59 | 349 |
| *Cyp11a1* | F: 5′-GCTGGAAGGTGTAGCTCAGG-3′<br>R: 5′-CGCTCCCCAAATACAACACT-3′ | 4 | 0·25 | 60 | 143 |
| *Star* | F: 5′-GCAGCAGGCAACCTGGTG-3′<br>R: 5′-GGCTGCCAAAGACCATCA-3′ | 3 | 0·2 | 60 | 246 |
| *Rpl19* | F: 5′-CTGAAGGTCAAAGGGAATGTG-3′<br>R: 5′-CCTCCTTGCACAGAGTCTTGA -3′ | 3 | 0·125 | 52 | 194 |

[a]Forward primer sequence is indicated by F, and reverse primer sequence is indicated by R.

### *Measurement of urinary and plasma steroids*

Total urinary corticosterone and aldosterone were measured by enzyme immunoassays (EIAs) validated for direct measurements in diluted urine (Cayman Chemical Co., Ann Arbor, MI, USA). The intra-assay coefficient of variation for the corticosterone EIA was 6% and for the aldosterone EIA was 4%. Urinary steroid levels were expressed relative to those of creatinine, which were determined on a Technicon Axon Analyzer using Technicon reagents and methodology (Bayer Diagnostics). Plasma levels of total corticosterone were measured by a radioimmunoassay kit (MP Biomedicals, Orangeburg, NY, USA) developed specifically for use with rat plasma. The intra-assay coefficient of variation for this assay was 4%.

### *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 cleanup kit (MoBio Laboratories Inc., Solana Beach, CA, USA). Gene-specific primers for rat *Cyp11a1*, *Mc2r* and *Hsd11b2* were designed using Primer3 software (http://frodo.wi.mit.edu/primer3; Rozen & Skaletsky 2000) and were positioned to span introns when present. *Star* primers were derived from those previously reported (Ronen-Fuhrmann *et al.* 1998), and ribosomal *Rpl19* was used as an internal control (Orly *et al.* 1994). For each gene, the PCR primer sequences are shown in Table 1 with $MgCl_2$, primer concentrations, annealing temperatures and PCR product sizes. External standards were generated from regular PCR products and tenfold serial dilutions of the PCR product made in RNase-free water ($1–10^7$-fold dilutions). Quantitative PCR was performed in 10-μl reaction volumes using the Rotorgene 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,

**Table 2** Body weight, combined adrenal weight (absolute (mg) and expressed relative to body weight (mg/kg BW)) and plasma corticosterone levels in 6-month-old offspring of control and dexamethasone-treated mothers. Offspring were raised from birth on semi-pure diets with either standard (Std) or high-omega-3 fatty acids (Hn3). Values are the mean±S.E.M. ($n=8$ per group, except Male Con/Std, $n=7$)

| | | | | | *P* values | |
|---|---|---|---|---|---|---|
| | Con/Std | Dex/Std | Con/Hn3 | Dex/Hn3 | Treatment | Diet |
| Male offspring | | | | | | |
| Body weight (g) | 543±13 | 461±16 | 583±25 | 484±24 | <0·001 | NS |
| Adrenal weight (mg) | 55±4 | 53±5 | 75±7 | 55±3 | 0·020 | 0·033 |
| Adrenal weight (mg/kg BW) | 103±8 | 116±11 | 128±8 | 115±9 | NS | NS |
| Corticosterone (ng/ml) | 255±200 | 1006±394 | 268±131 | 689±120 | <0·001 | NS |
| Female offspring | | | | | | |
| Body weight (g) | 310±12 | 265±14 | 329±9 | 291±9 | 0·001 | NS |
| Adrenal weight (mg) | 78±5 | 58±11 | 88±4 | 74±4 | <0·001 | 0·007 |
| Adrenal weight (mg/kg BW) | 252±11 | 225±17 | 274±20 | 259±2 | NS | NS |
| Corticosterone (ng/ml) | 81±32 | 467±161 | 37±9 | 615±307 | 0·006 | NS |

Eugene, OR, USA) per reaction. The PCR cycling conditions included an initial denaturation at 94 °C for 10 min followed by up to 45 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 of the correct size and sequenced by gel electrophoresis and sequence analysis (data not shown). Fluorescence values were analyzed, standard curves constructed using the Rotorgene software, and all samples standardized against the internal control (*Rpl19*).

### *Statistical analysis*

All data are expressed as mean±S.E.M., with each litter representing an *n* of one. All variables were analyzed by ANOVAs (initially three-way to account for variation due to sex, prenatal treatment and postnatal diet) followed by *post hoc* LSD tests where appropriate (Snedecor & Cochran 1989). When the interaction between sources of variation was statistically significant ($P<0{\cdot}05$), analyses of subsets of data were made by further ANOVAs as appropriate.

## Results

### *Adrenal weight and morphology*

Offspring total body and adrenal weights each varied with sex, prenatal treatment and diet (all $P<0{\cdot}001$). Most notably, both were lower in offspring of dexamethasone-treated mothers, and so, after adjustment for body weight, all the treatment and diet effects on adrenal weight were lost (see Table 2). Relative adrenal weight was approximately twofold greater in females than in males irrespective of treatment or diet (overall sex effect, $P<0{\cdot}001$). Similarly, quantitative analysis of adrenal zone volumes by stereology showed highly significant sex effects (females>males) for total volume and all component volumes (each $P<0{\cdot}001$), with the exception of absolute volume of zona glomerulosa. Therefore, separate analyses were conducted for each sex, and in both cases, these showed that after adjustment for body weight, prenatal dexamethasone did not affect the volume of either zona fasciculata–reticularis or zona glomerulosa (see Fig. 1 for male data; data for females not shown). Interestingly, the relative volume of the zona glomerulosa was slightly greater ($P=0{\cdot}014$) in male offspring raised on the high-n-3 diet regardless of prenatal treatment.

### *Adrenal expression of* Mc2r, Hsd11b2, Star *and* Cyp11a1

Adrenal *Mc2r* mRNA expression was higher in females than in males ($P<0{\cdot}001$), and there were significant overall sex–treatment ($P=0{\cdot}02$) and treatment–diet ($P<0{\cdot}001$) interactions. Therefore, separate analyses were conducted for male and female offspring, and these revealed a highly significant stimulatory effect of prenatal dexamethasone on adrenal *Mc2r* expression in both male ($P=0{\cdot}007$) and female ($P<0{\cdot}001$) offspring (Fig. 2a). Moreover, there was a significant treatment–diet interaction ($P=0{\cdot}001$) in females, such that *Mc2r* expression was increased by prenatal dexamethasone only in those females raised on the Std diet (Fig. 2a). Adrenal expression of *Hsd11b2* mRNA also varied significantly with sex (females>males; $P=0{\cdot}01$), prenatal treatment (Dex>Con; $P<0{\cdot}001$) and postnatal diet (Hn3>Std; $P<0{\cdot}001$; Fig. 2b). Adrenal expression of two key genes involved in steroidogenesis (*Star* and *Cyp11a1*) was not affected by either prenatal treatment or postnatal diet (data not shown).

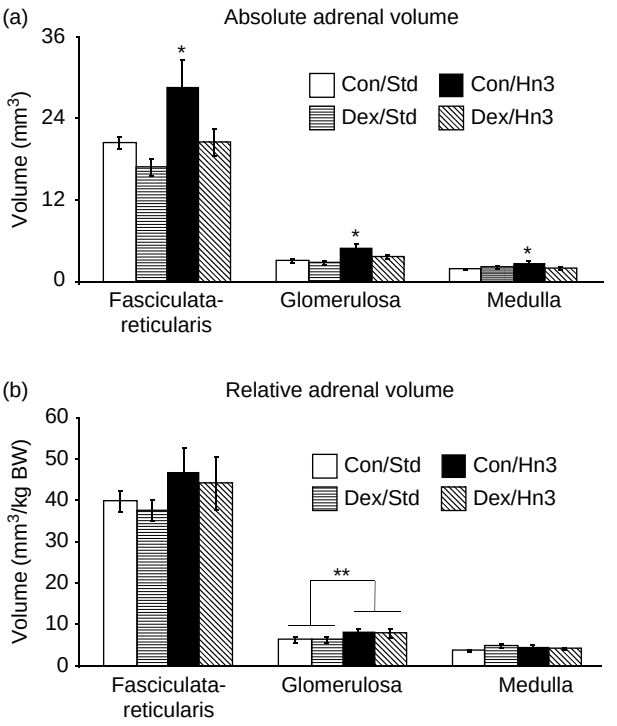


**Figure 1** Volumes of adrenal gland components (zona glomerulosa, zona fasciculata–reticularis and adrenal medulla) measured by unbiased stereology in male offspring of control (Con) and dexamethasone-treated (Dex) mothers. Offspring were raised on either a standard (Std) or a high-omega-3 fatty acid (Hn3) diet from birth. Values are the mean±S.E.M. ($n=6$ per group except for Con/Std, $n=5$) of (a) absolute volume ($mm^3$) and (b) relative volume ($mm^3$ per kg body weight). Each data set were analyzed by three-way ANOVA (with sex, prenatal treatment and postnatal diet as sources of variation). With the exception of absolute medulla volume, there was a highly significant sex effect ($P<0{\cdot}001$, females>males) for each adrenal component (both absolute and relative), but the treatment effects were similar between the sexes, and so only data from males are shown. *$P<0{\cdot}05$ compared with corresponding value in all other groups; **Significant diet effect for zona glomerulosa volume ($P=0{\cdot}014$, two-way ANOVA) was observed.

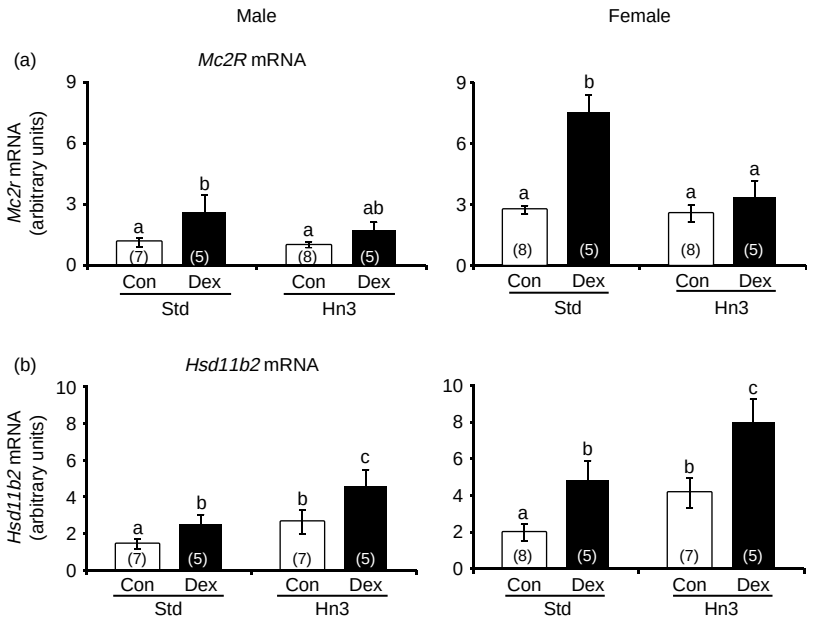


**Figure 2** Adrenal expression of (a) *Mc2r* and (b) *Hsd11b2* mRNAs in 6-month-old offspring of untreated (Con; open bars) and dexamethasone-treated (Dex; filled bars) mothers. Offspring were raised on either a standard (Std) or a high-omega-3 fatty acid (Hn3) diet from birth. Values are the mean ± S.E.M.; the *n* value for each group is shown in parentheses. Data were analyzed by three-way ANOVA (sex, treatment and diet), and overall, there were significant sex (females > males) and treatment (Dex > Con) effects for both genes. For adrenal *Mc2r* expression, there was a significant treatment–diet interaction ($P<0{\cdot}001$) in female offspring. Adrenal *Hsd11b2* mRNA expression also varied with postnatal diet ($P<0{\cdot}001$). Within each sex, values without common notations differ significantly ($P<0{\cdot}05$, LSD test).

### *Urinary and plasma steroid levels*

Despite similar steroidogenic enzyme expression levels and adrenal volume, offspring of dexamethasone-treated mothers had higher urinary corticosterone levels compared with controls (overall treatment effect $P<0{\cdot}01$; see Fig. 3), but there was no effect of postnatal diet. As expected, urinary corticosterone production was substantially higher (4·8-fold overall) in females than in males ($P<0{\cdot}001$). Urinary aldosterone production was also elevated after prenatal dexamethasone ($P=0{\cdot}01$ for ANOVA on all male and female data) and was markedly higher in females (sevenfold, $P<0{\cdot}001$). Plasma corticosterone levels were also higher in anesthetized offspring of dexamethasone-treated mothers regardless of postnatal diet (males: $P<0{\cdot}001$; females: $P=0{\cdot}006$; see Table 2).

## Discussion

This study demonstrates that prenatal dexamethasone exposure does not program increased adrenocortical volume, either total or zone-specific, or elevated adrenal expression of key genes involved in steroidogenesis in adult offspring. Importantly, however, prenatal dexamethasone did program increases in stimulated urinary corticosterone and aldosterone (after overnight isolation) and plasma corticosterone levels (under anesthesia), suggestive of heightened adrenal responsiveness to stress. Consistent with these observations, adrenal expression of *Mc2r* mRNA, which encodes the ACTH receptor, was also elevated in offspring of dexamethasone-treated mothers. These effects of prenatal dexamethasone were generally similar in offspring raised on a Hn3 diet, suggesting that the prevention of various programmed outcomes by this dietary intervention is not mediated by changes in adrenal function.

Chronic adrenal hyperactivity is classically associated with increases in both adrenal volume (Ulrich-Lai *et al.* 2006, Raone *et al.* 2007) and expression of key genes involved in steroidogenesis, most notably *Star* and *Cyp11a1* (Lehoux *et al.* 1998). Exposure to dexamethasone prenatally had no effect on either total adrenal volume or that of the zona fasciculata–reticularis in adult offspring. The fasciculata and reticularis regions were considered as a single zone, because the adult rat does not possess a conventional zona reticularis expressing *Cyp17a1* (Pelletier *et al.* 2001, Pignatelli *et al.* 2006) and the reticularis cells contribute to corticosterone synthesis (Bell *et al.* 1979). The absence of a programmed increase in adrenal volume or expression of steroidogenic genes indicates that basal adrenal steroidogenesis was not chronically up-regulated

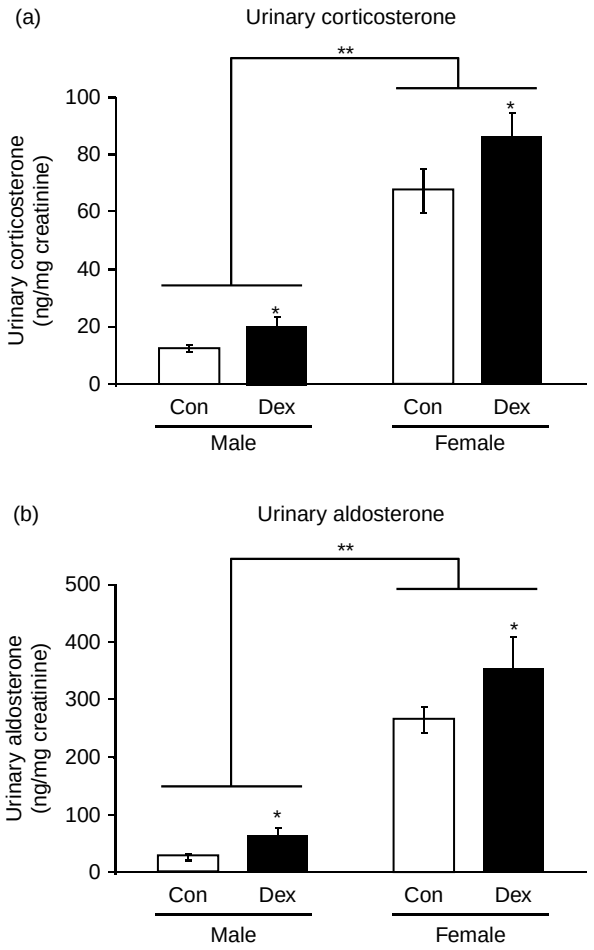


**Figure 3** Urinary production of corticosterone (ng/mg creatinine) in 6-month-old male and female offspring of untreated (Con; open bars) and dexamethasone-treated (Dex; filled bars) mothers. Offspring were raised on either a standard (Std) or a high-omega-3 fatty acid (Hn3) diet from birth. Values are the mean ± S.E.M. Data were analyzed by three-way ANOVA (sex, treatment and diet), and for both steroids, significant variation was attributable to sex and prenatal treatment but not postnatal diet, and there were no significant interactions among these factors. Therefore, pooled data from animals on the different diets are presented ($n=14$–15 per group). *$P<0{\cdot}01$, overall effect of prenatal dexamethasone treatment; **$P<0{\cdot}001$, overall sex effect.

in offspring of mothers treated with dexamethasone. Interestingly, however, plasma corticosterone in anesthetized rats and corticosterone and aldosterone levels in urine obtained from rats housed individually in a metabolic cage overnight were all higher in offspring of dexamethasone-treated mothers. These effects of maternal dexamethasone treatment on offspring steroid production are likely to reflect enhanced adrenal responses to stressful stimuli, since blood collection from the dorsal aorta of anesthetized rats is clearly a major stressor, and previous studies indicate that isolation of rodents in a metabolic cage stimulates adrenal steroid synthesis (Armando *et al.* 2007, Al-Dujaili *et al.* 2009). A programmed increase in responsiveness of the rat adrenal to stress is in line with previous reports showing enhanced activity of the adult HPA axis at both the hypothalamic and pituitary levels after fetal glucocorticoid excess. Thus, Shoener *et al.* (2006) reported elevated expression of hypothalamic CRH in adult offspring of dexamethasone-treated mothers, and perinatal dexamethasone treatment was shown to increase pituitary corticotroph number in female offspring (John *et al.* 2006). Our observation that adrenal *Mc2r* expression is elevated in offspring of dexamethasone-treated mothers suggests that an enhanced HPA reactivity also involves greater responsiveness at the level of the adrenal (i.e. to ACTH). This is consistent with a recent report in the guinea pig showing that prenatal stress resulted in increased adrenal expression of *Mc2r* mRNA in the offspring (Kapoor *et al.* 2008*a*). Interestingly, the high-n-3 diet appeared to negate this increased adrenal expression of *Mc2r*, at least in female offspring, consistent with a previous human study showing that a dietary fish oil supplementation limits the adrenal responsiveness to a mental stress (Delarue *et al.* 2003). Adrenal *Mc2r* expression was also around twofold higher in female offspring compared with male offspring, in line with the female adrenal being generally more responsive to ACTH (Atkinson & Waddell 1997).

The apparent programmed increase in adrenal responsiveness is also consistent with recent observations of heightened blood pressure responses to handling following dexamethasone exposure *in utero*. Thus, O'Regan *et al.* (2008) demonstrated that maternal dexamethasone treatment programmed offspring hypertension when measured by the tail-cuff plethysmography, but not when measured by a non-invasive, telemetry approach. The authors concluded that programmed hypertension represents a greater response to the measurement procedure than a chronic elevation in blood pressure. Indeed, when measured by telemetry, offspring of dexamethasone-treated mothers were slightly hypotensive (O'Regan *et al.* 2008). While this elevated cardiovascular response to stress was linked to increased sympathetic drive, our data suggest that a more responsive adrenal may also contribute to this phenotype.

It has been proposed that sustained adrenal hyperactivity may drive several features of the programmed cardiometabolic phenotype (Phillips 2007). The present study, however, suggests that in this programming model, adrenal hyperactivity is more likely an intermittent outcome, occurring only at times of stress. Another important consideration in this context is that glucocorticoid actions in target tissues may still be enhanced without adrenal hyperactivity *per se*, since glucocorticoid sensitivity appears to be elevated in various target tissues of programmed offspring. For example, expression of both hepatic (Nyirenda *et al.* 1998) and renal (Wyrwoll *et al.* 2007) glucocorticoid receptors is elevated in adult offspring of dexamethasone-treated mothers, each with apparent downstream effects on glucocorticoid-sensitive genes (i.e. PEPCK in the liver (Nyirenda *et al.* 1998) and RAS genes in the kidney (Wyrwoll *et al.* 2007)).

Changes in adrenal function of offspring following maternal glucocorticoid treatment have also been observed in other species, but there is considerable variation in the onset and nature of these responses. For example, maternal betamethasone treatment in sheep increases offspring pituitary and adrenal responsiveness to CRH/AVP at 1 year of age, yet by 2 and 3 years this effect is lost (Sloboda *et al*. 2007). Similarly, adult offspring of guinea pig mothers treated with β-methasone mostly exhibit adrenal hypoactivity, although interestingly females display adrenal hyperactivity during the oestrous phase of the cycle (Liu *et al*. 2001). While the prevalence of adrenal hypoactivity in these sheep and guinea pig models is at odds with our present observation of increased adrenal responsiveness, there are important differences relating to duration of prenatal glucocorticoid exposure. Specifically, the sheep and guinea pig models employed intermittent glucocorticoid doses at different stages of pregnancy, whereas in our model sustained exposure to dexamethasone occurred over the final third of pregnancy.

Offspring of dexamethasone-treated mothers showed a marked upregulation in the adrenal expression of *Hsd11b2*, and this was further enhanced by the high-n-3 diet. While adrenal *Hsd11b2* expression has been noted previously in the rat (Smith *et al*. 1997) and other species (Yang & Matthews 1995, Ross *et al*. 2000), its physiological significance remains uncertain. The HSD11B2 enzyme catalyzes the conversion of corticosterone to its biologically inert, 11-keto derivative 11-dehydrocorticosterone (11-DHC), and Yang & Matthews (1995) suggested that adrenal HSD11B2 may serve to limit exposure of adrenocortical cells to very high levels of corticosterone and/or provide a source of 11-DHC for reactivation within target tissues by HSD11B1. Therefore, the higher expression of *Hsd11b2* after prenatal dexamethasone may limit any adverse local effects of heightened adrenal responsiveness. The high-n-3 diet also stimulated adrenal *Hsd11b2* expression regardless of prenatal treatment, similar to the effects of this diet on renal *Hsd11b2* expression (Wyrwoll *et al*. 2007). This effect of the Hn3 diet on adrenal and renal *Hsd11b2* may be mediated via suppression of the proinflammatory cytokines (Wyrwoll *et al*. 2008), which are known to inhibit *Hsd11b2* expression in several cell types including kidney epithelial cells (Heiniger *et al*. 2001) and placenta (Chisaka *et al*. 2005). In contrast, the stimulatory effect of prenatal dexamethasone on *Hsd11b2* expression in the adrenal is opposite to its suppressive effect in the kidney (Wyrwoll *et al*. 2007). Further studies are required to establish the mechanisms driving this tissue-specific regulation of *Hsd11b2* by prenatal dexamethasone.

In conclusion, this study shows that maternal dexamethasone treatment does not program increased adrenocortical volume or expression of key genes involved in steroidogenesis in adult offspring, indicative of normal basal adrenocortical activity. By contrast, both stress-induced adrenal steroid production and adrenal expression of *Mc2r* were higher in offspring of dexamethasone-treated mothers, indicative of a more stress-responsive adrenal phenotype.

## Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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