

# The effects of dietary fatty acid composition in the post-sucking period on metabolic alterations in adulthood: can $\omega$ 3 polyunsaturated fatty acids prevent adverse programming outcomes?

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## Abstract

Early life nutrition is important in the regulation of metabolism in adulthood. We studied the effects of different fatty acid composition diets on adiposity measures, glucose tolerance, and peripheral glucocorticoid (GC) metabolism in overfed neonatal rats. Rat litters were adjusted to a litter size of three (small litters (SLs)) or ten (normal litters (NLs)) on postnatal day 3 to induce overfeeding or normal feeding respectively. After weaning, SL and NL rats were fed a  $\omega$ 6 polyunsaturated fatty acid (PUFA) diet (14% calories as fat, soybean oil) or high-saturated fatty acid (high-fat; 31% calories as fat, lard) diet until postnatal week 16 respectively. SL rats were also divided into the third group fed a  $\omega$ 3 PUFA diet (14% calories as fat, fish oil). A high-fat diet induced earlier and/or more pronounced weight gain, hyperphagia, glucose intolerance, and hyperlipidemia in

SL rats compared with NL rats. In addition, a high-fat diet increased *11 $\beta$ -hsd1* (*Hsd11b1*) mRNA expression and activity in the retroperitoneal adipose tissue of both litter groups compared with standard chow counterparts, whereas high-fat feeding increased hepatic *11 $\beta$ -hsd1* mRNA expression and activity only in SL rats. SL and a high-fat diet exhibited significant interactions in both retroperitoneal adipose tissue and hepatic *11 $\beta$ -HSD1* activity. Dietary  $\omega$ 3 PUFA offered protection against glucose intolerance and elevated GC exposure in the retroperitoneal adipose tissue and liver of SL rats. Taken together, the results suggest that dietary fatty acid composition in the post-sucking period may interact with neonatal feeding and codetermine metabolic alterations in adulthood.

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## Introduction

Obesity, particularly visceral obesity, is related to metabolic diseases such as insulin resistance, hyperlipidemia, hypertension, type 2 diabetes, and nonalcoholic fatty liver disease (Boden 2011). Numerous clinical and experimental evidence indicates that the early nutritional status of an organism may be a programming factor for the development of obesity and metabolic syndrome later in life (Ong & Loos 2006, Symonds *et al.* 2009), particularly malnutrition or overnutrition during the prenatal period. Elevated glucocorticoid (GC) activity has been proposed as a possible mediator of permanent obesity and metabolic syndrome programming in adults (Metges 2009).

The enzyme *11 $\beta$ -hydroxysteroid dehydrogenase type 1* (*11 $\beta$ -HSD1* (*HSD11B1*)) provides sufficient intracellular GC levels by converting inactive GC to active GC (corticosterone or cortisol), amplifying local GC action in the adipose tissue and liver (Odermatt & Nashev 2010). Evidence from animal

models and clinical studies confirmed that overexpression of *11 $\beta$ -HSD1* in the adipose tissue or liver is associated with metabolic disorders (Masuzaki *et al.* 2001, Morton *et al.* 2004b). By contrast, A-ring reductases (*5 $\alpha$ -* and *5 $\beta$ -reductase*) inactivate GCs in the liver (Livingstone *et al.* 2000), and elevated hepatic *5 $\alpha$*  reductase type 1 (*5 $\alpha$ R1*) and *5 $\beta$ R* activity is associated with insulin resistance and obesity (Westerbacka *et al.* 2003, Tomlinson *et al.* 2008). Therefore, the activities of these enzyme systems may codetermine GC availability. When studying postnatal overfeeding induced by small litter (SL) rearing in rats, Boullu-Ciocca *et al.* (2005) found that these rats developed hypothalamic-pituitary-adrenal (HPA) axis hyperactivity and higher *11 $\beta$ -hsd1* mRNA expression in the visceral adipose tissue, accompanied by obesity, insulin resistance, and hyperleptinemia in adulthood. Moreover, we (Hou *et al.* 2011) confirmed that a similar experimental setup induced peripheral tissue-specific alterations in *11 $\beta$ -hsd1* mRNA expression and activity and *5 $\alpha$ R1* and *5 $\beta$ R* mRNA expression at puberty, which could

contribute to elevated tissue-specific GC exposure and aggravate the development of metabolic dysregulation in adults. Importantly, maturation of the rodent neuroendocrine system occurs mainly during the postnatal period, in contrast to primates, in which it occurs during the third trimester (Matthews 2002).

Dietary fatty acids are important regulators of gene expression, acting as intracellular messengers or ligands for transcription factors (Lichtenstein & Schwab 2000, Riccardi *et al.* 2004, Tierney *et al.* 2011) and are implicated in the development of metabolic syndrome. Studies have shown that local GC metabolism is influenced not only by cytokines and hormones but also by different dietary fatty acid approaches, such as composition, dose, and duration of the treatment applied in different studies (Bermudez & Sinclair 2009, Donahue *et al.* 2011). A diet high in saturated fatty acids increases *11 $\beta$ -hsd1* gene expression in the retroperitoneal adipose tissue of rats (Vara *et al.* 2010) and whole-body *11 $\beta$ -HSD1* activity in humans (Stimson *et al.* 2007). By contrast, a safflower oil diet (rich in polyunsaturated fatty acids (PUFAs)) reduces plasma corticosterone and adipose tissue *11 $\beta$ -HSD1* activity (Man *et al.* 2011). Dietary fish oil (rich in  $\omega$ 3 PUFAs) for 6 months after birth prevents hypertension and hyperleptinemia in rats programmed by fetal GC exposure (Wyrwoll *et al.* 2006). Taken together, these observations strongly suggest the existence of an association between local GC metabolism and nutritional status or fatty acid composition.

In this study, we investigated the effects of three diets with different fatty acid compositions on adiposity measures, glucose tolerance, and peripheral GC metabolism in neonatal rats induced to overfeed by rearing in SLs.

## Materials and Methods

### Animals

All the studies were approved by the University Committee on Use and Care of Animals and overseen by the Unit for Laboratory Animal Medicine at Nanjing Medical University (ID: 2008031801). Sprague Dawley rats (Nanjing, Jiangsu, China) were maintained under controlled light (0600–1800 h) and temperature ( $22 \pm 2$  °C) conditions with free access to tap water.

### Experimental design

The experimental setup was similar to Boullu-Ciocca's study (Boullu-Ciocca *et al.* 2008). Briefly, female rats were time-mated, and at postnatal day 3, male pups were redistributed to litter sizes of three (SLs) or ten (normal litters (NLs)) to induce early postnatal overfeeding or normal feeding respectively (Velkoska *et al.* 2008, Rodrigues *et al.* 2009). After weaning (postnatal day 21), the rats from the NLs were fed a soybean oil diet (dietary fat was soybean oil rich in  $\omega$ 6 PUFAs; NL group) or a diet high in saturated fats

(NL-HF group), and the rats from the SLs were fed a soybean oil diet (SL group), a diet high in saturated fat (SL-HF group), or a fish oil-rich diet (dietary fat was fish oil rich in  $\omega$ 3 PUFAs; SL-FO group) until postnatal week 16 (W16). The diets (Slac, Shanghai, China) and their nutrient composition are shown in Table 1 (Note: the soybean oil diet was often referred to as 'standard chow' in previous studies (Reeves *et al.* 1993, Brighenti *et al.* 2011)). All animals were housed three per cage postweaning and fed *ad libitum*. Body weight, body length, tail length, and food intake were monitored throughout life. The animals were killed at W3, W6, and W16 between 0830 and 1000 h after fasting overnight.

### Serum and tissue collection

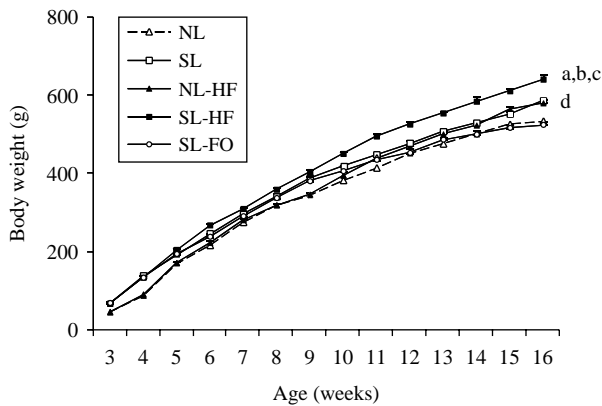
Rats were anesthetized with chloral hydrate (300 mg/kg body weight, i.p.) at 0830 h after overnight fasting (12 h) and blood samples were obtained from the right ventricle. The blood was centrifuged (2000 g, 4 °C, 15 min) and the separated serum stored at  $-70$  °C for subsequent determination of biochemical parameters, serum insulin, corticosterone, and leptin. Kidney, liver, and epididymal and retroperitoneal fat pads were dissected immediately. The kidney and fat pads were weighed, and all tissues were snap-frozen in liquid nitrogen and kept at  $-80$  °C until gene expression and activity analysis.

### Biochemical analysis

Total triglycerides (TGs) and total cholesterol (TC) in the serum were measured using an Olympus AU400 analyzer with enzymatic reagents (Olympus America, New York, NY, USA). Serum corticosterone (Cayman, Ann Arbor, MI, USA) and leptin (Millipore, Billerica, MA, USA) were measured by ELISAs. The limit of sensitivity was 40 pg/ml for corticosterone and 0.04 ng/ml for leptin, and the intra-assay coefficients of variation for corticosterone and leptin were 7–9 and 2–3% respectively.

**Table 1** Purified diet formula and composition (weight (%))

	Soybean oil diet (%)	Fish oil diet (%)	High-fat diet (%)
Casein	18.9	18.9	18.9
L-Cystine	0.3	0.3	0.3
Corn starch	48.3	48.3	39.3
Maltodextrin	3.3	3.3	3.3
Sucrose	13.0	13.0	13.0
Cellulose	4.7	4.7	4.7
Mineral mix	4.3	4.3	4.3
Vitamin mix	1.1	1.1	1.1
Soybean oil	6.0	0.0	6.0
Fish oil	0.0	6.0	0.0
Lard	0.0	0.0	9.0
Total	100.0	100.0	100.0
Energy (kcal/100 g)	392.6	392.6	438.2



**Figure 1** Effect of different diets on body weight from W3 to W16. Data are mean  $\pm$  s.e.m.,  $n=6-9$  in each group. <sup>a</sup> $P<0.05$  vs NL at every time point, <sup>b</sup> $P<0.05$  vs SL from W6 to W16, <sup>c</sup> $P<0.05$  vs NL-HF at every time point, and <sup>d</sup> $P<0.05$  for SL vs NL at every time point. The body weight of NL-HF rats were higher than NL rats at W15 and W16; there was no difference between SL-FO rats' body weight and NL rats from W12 to W16.

#### Intraperitoneal glucose tolerance test

The intraperitoneal glucose tolerance test (IPGTT) was performed as described previously (Chen *et al.* 2008). Briefly, at W6 and W16, rats were fasted overnight. A blood sample was then taken from a tail vein and the rats were injected i.p. with 2.0 g D-glucose (50% stock solution in saline)/kg body weight. Blood samples were drawn at 30-, 60-, and 120-min intervals after the glucose injection, and glucose levels were measured by a glucose meter (Accu-Chek; Roche).

#### Quantitative real-time PCR

Total RNA was isolated from the adipose tissue and liver using TRIzol (Invitrogen) according to the manufacturer's instructions and quantified spectrophotometrically by the OD260. The integrity of total RNA was assessed using agarose gel electrophoresis, and cDNA was synthesized using M-MLV reverse transcriptase (Promega) with 1.0  $\mu$ g of the RNA sample as recommended by the manufacturer. PCR

amplification using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) primers with a subset of the cDNA samples confirmed successful reverse transcription. Real-time PCR was performed using the SYBR GREEN ABI Prism 7500 sequence detector with the following program: 50 °C for 2 min, 95 °C for 10 min, 40 cycles of 95 °C for 15 s, and 60 °C for 1 min. The mRNA levels were normalized to GAPDH. Data were analyzed using the  $2^{-\Delta\Delta ct}$  method (Pfaffl 2001). Primer sequences were as follows: 11 $\beta$ -HSD1, forward 5'-GAA GAA GCA TGG AGG TCA AC-3', reverse 5'-GCA ATC AGA GGT TGG GTC AT-3'; 5 $\alpha$ R1, forward 5'-CTG TTT CCT GAC AGG CTT TGC-3', reverse 5'-GCC TCC CCT GGG TAT CTT GT-3'; 5 $\beta$ R, forward 5'-GCC TTT AAG CCT GGA GAG GAA-3', reverse 5'-ACG TGG CAC ACA GAT TTG ATT-3'; GAPDH, forward 5'-CAA GTT CAA CGG CAC AGT CAA-3', reverse 5'-TGG TGA AGA CGC CAG TAG ACT C-3'.

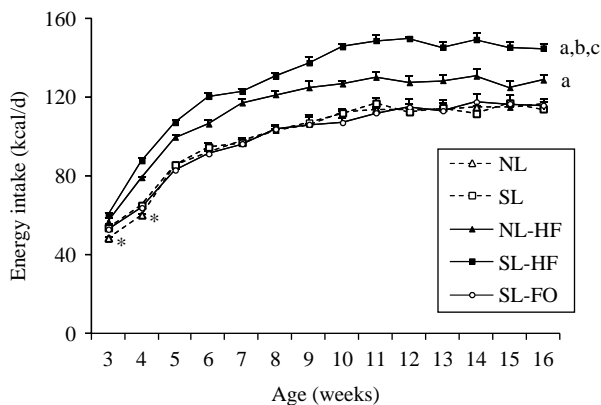
#### 11 $\beta$ -HSD activity assay

To estimate the 11 $\beta$ -HSD1 protein levels, 11 $\beta$ -HSD1 activity was measured in adipose and hepatic tissues in the direction of the dehydrogenase reaction, as it is more stable *in vitro* than in the reductase direction (Lakshmi & Monder 1988). 11 $\beta$ -HSD1 enzyme activity was measured as described previously (Buren *et al.* 2007, Hou *et al.* 2011). Briefly, tissue was homogenized in homogenization buffer (10% glycerol, 300 mM NaCl, 1 mM EDTA, and 50 mM Tris, pH 7.7) containing 1 mM dithiothreitol and then centrifuged at 4 °C. The protein concentration was determined using a Pierce BCA protein assay kit with BSA as the standard (Thermo Fisher Scientific, Rockford, IL, USA). Each sample was analyzed in duplicate using an internal control. Samples of adipose tissue (0.5 mg protein/ml) or liver (10  $\mu$ g protein/ml) homogenate were incubated with 2 mM NADP and 100 nM 1,2,6,7-[<sup>3</sup>H]<sub>4</sub>-corticosterone (Amersham). After incubation in a shaking water bath at 37 °C for 1 or 2 h (liver and adipose tissues respectively), the reaction was interrupted and the steroids extracted with ethyl acetate and

**Table 2** Adiposity measurements at W3, W6, and W16 (g)

	NL	SL	NL-HF	SL-HF	SL-FO
W3 <sup>a</sup>					
RAT weight	0.13 $\pm$ 0.01	0.23 $\pm$ 0.01*	–	–	–
EAT weight	0.14 $\pm$ 0.02	0.20 $\pm$ 0.02*	–	–	–
W6					
RAT weight	1.55 $\pm$ 0.22	1.98 $\pm$ 0.44*	1.57 $\pm$ 0.11 <sup>†</sup>	2.26 $\pm$ 0.14 <sup>*,†,‡</sup>	1.90 $\pm$ 0.87 <sup>*,‡</sup>
EAT weight	1.55 $\pm$ 0.13	1.80 $\pm$ 0.21*	1.54 $\pm$ 0.10 <sup>*,†</sup>	1.81 $\pm$ 0.06 <sup>*,‡</sup>	1.77 $\pm$ 0.23 <sup>*,‡</sup>
W16					
RAT weight	6.2 $\pm$ 0.6	8.9 $\pm$ 0.9*	9.9 $\pm$ 0.9*	11.4 $\pm$ 1.4 <sup>*,†,‡</sup>	7.4 $\pm$ 1.3 <sup>†,‡</sup>
EAT weight	6.0 $\pm$ 0.3	8.1 $\pm$ 1.1*	9.2 $\pm$ 0.8*	10.9 $\pm$ 1.1 <sup>*,†,‡</sup>	6.1 $\pm$ 1.3 <sup>†,‡</sup>

RAT, retroperitoneal adipose tissue; EAT, epididymal adipose tissue. Data are expressed as mean  $\pm$  s.e.m.,  $n=6-9$  in each group. \* $P<0.05$  vs NL at the same time point, <sup>†</sup> $P<0.05$  vs SL at the same time point, and <sup>‡</sup> $P<0.05$  vs NL-HF at the same time point. <sup>a</sup>Measured before randomization to feeding groups.



**Figure 2** Effect of different diets on energy intake from W3 to W16. Data are mean  $\pm$  S.E.M.,  $n=6-9$  in each group. <sup>a</sup> $P<0.05$  vs NL at every time point, <sup>b</sup> $P<0.05$  vs SL at every time point, and <sup>c</sup> $P<0.05$  vs NL-HF at every time point. \* $P<0.05$  vs SL and SL-FO at the corresponding time point.

dried, dissolved in ethanol, separated by thin-layer chromatography (TLC; mobile phase chloroform:ethanol (92:8)), and exposed to a phosphorimager tritium screen (GE Healthcare Europe, Freiburg, Germany). The TLC plates were then scanned and quantified using a Typhoon scanner (GE Healthcare Europe).  $11\beta$ -HSD1 activity was expressed as the percentage conversion of corticosterone into 11-dehydrocorticosterone.

#### Statistical analysis

Results are expressed as mean  $\pm$  S.E.M. Significant differences between SLs and NLs of rats at W3 were analyzed by unpaired *t*-tests. Significant differences between groups of rats at W6 and W16 were analyzed by two-way ANOVA followed by a *post hoc* Bonferroni test. Body weight, food energy intake, and serum glucose during IPGTT were analyzed using two-way ANOVA with repeated measures, followed by a *post hoc* Bonferroni test.  $P<0.05$  was considered significant.

## Results

#### Body weight gain, energy intake, and adipose tissue weight

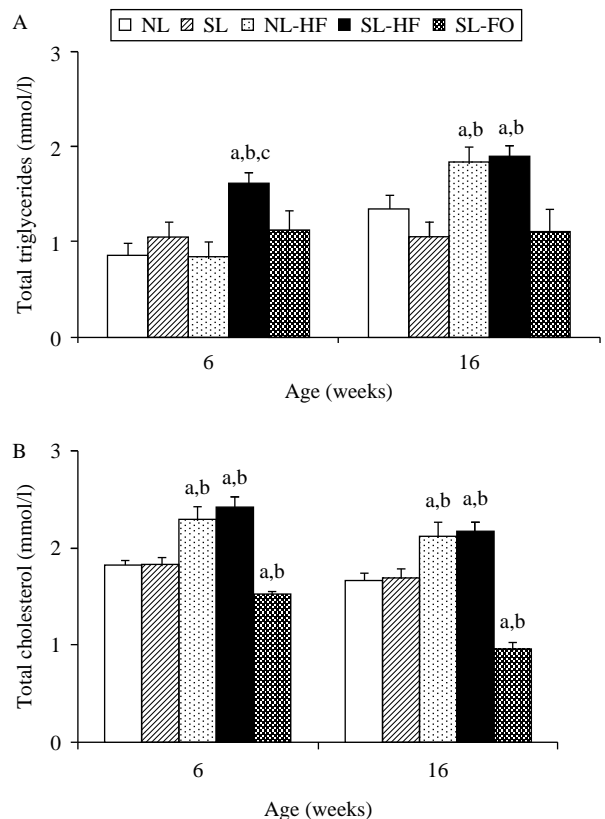
SL rearing induced a significant increase in weight gain compared with NLs by postnatal W2 ( $41.3 \pm 2.3$  vs  $27.8 \pm 2.3$  g,  $P<0.01$ ). The difference in body (Fig. 1) and fat pad (retroperitoneal and epididymal) weight persisted until W16 (Table 2). After 3 weeks on a high-fat diet, body weight and retroperitoneal fat pad weight of SL-HF rats were higher than that of SL rats, and these effects persisted until W16. SLs showed significant interactions with high-fat diet with regard to body weight ( $F=5.033$ ,  $P<0.05$ ). By contrast, the weight gain of SL-FO rats, as well as their fat pad weight, was less than that of SL rats, and from W12 to W16, the SL-FO rats did not differ significantly from NL rats. NL-HF rats had greater body

weight and fat pad weight than NL rats at W15 and W16, but they weighed less than SL-HF rats during the experimental period. No differences were found in body and tail length or kidney weight among the groups (data not shown).

Both SL-HF and NL-HF rats exhibited higher energy intake compared with their counterparts from W3 to W16, and SL-HF rats had a higher energy intake than NL-HF rats throughout the study period. SLs and a high-fat diet showed a significant interaction for general energy intake ( $F=84.11$ ,  $P<0.01$ ). Although SL and SL-FO rats exhibited a higher energy intake than NL rats at W3 and W4, no difference was found in food energy intake among NL, SL, and SL-FO rats from W5 to W16 (Fig. 2).

#### Serum lipids

No difference was observed in serum TGs (Fig. 3A) and TC (Fig. 3B) between NL and SL rats from W6 to W16. Serum TG levels were increased in SL-HF rats compared with SL rats from W6, and a high-fat diet and SLs showed a significant interaction with regard to TGs ( $F=4.899$ ,  $P<0.05$ ). TG levels in NL-HF rats were increased at W16, whereas TG levels in SL-FO rats were unchanged compared with



**Figure 3** Effect of different diets on serum levels of total triglycerides (A) and total cholesterol (B) at W6 and W16. Data are mean  $\pm$  S.E.M.,  $n=6-9$  in each group. <sup>a</sup> $P<0.05$  vs NL, <sup>b</sup> $P<0.05$  vs SL, and <sup>c</sup> $P<0.05$  vs NL-HF.

NL or SL during the experimental period (Fig. 3A). The TC levels of NL-HF and SL-HF rats were higher than the levels in their counterparts at W6 and W16 respectively, whereas fish oil decreased TC levels compared with NL and SL rats during the experimental period (Fig. 3B).

#### Glucose homeostasis

IPGTT confirmed that SLs and the high-fat diet induced glucose intolerance with a pronounced interaction at W6 ( $F=5.365$ ,  $P<0.05$ ), and this effect persisted to W16 ( $F=4.457$ ,  $P<0.05$ ). The area under the curve (AUC) for plasma glucose increased in SL-HF rats already at W6, which indicated higher insulin resistance early in development (Fig. 4C). At W16, the AUC was increased in SL, SL-HF, and NL-HF rats compared with NL rats and was highest in the SL-HF rats (Fig. 4D). By contrast, the AUC in SL-FO rats was decreased compared with SL rats and did not differ from that of NL rats.

#### Serum leptin and corticosterone

As shown in Table 3, serum leptin mirrored adiposity measures very well. Both SL and the high-fat diet resulted in elevated serum leptin levels. By contrast, the fish oil diet decreased serum leptin compared with SL and NL-HF rats, but serum leptin was elevated compared with NL rats at W16.

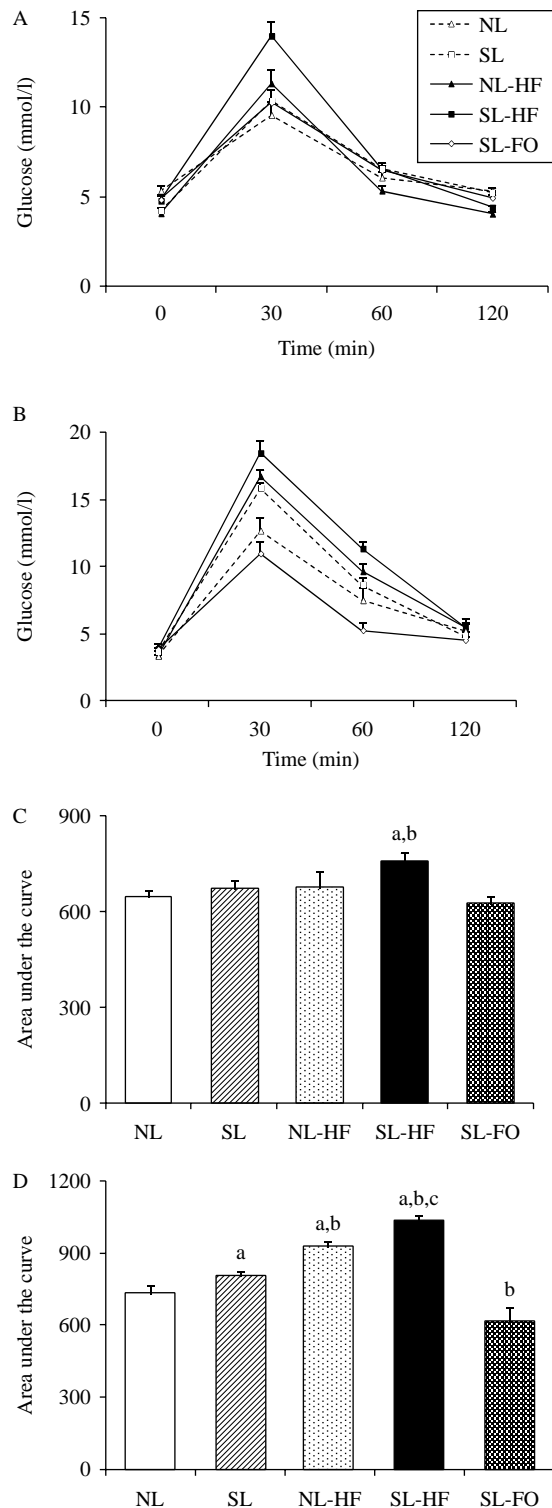
Serum corticosterone levels were elevated in SL rats compared with NL rats at W16. A high-fat diet had no effect on serum corticosterone levels in normal or SL rats. Moreover, we found a nonsignificant tendency for decreased serum corticosterone levels in SL-FO rats compared with SL rats (Table 3).

#### Hepatic $11\beta$ -hsd1 mRNA expression and enzyme activity

Hepatic  $11\beta$ -hsd1 mRNA expression in rats in SLs was higher than the expression in rats from NLs at W3 ( $15.5 \pm 1.8$  vs  $8.5 \pm 1.8$ ,  $P<0.05$ ) and a similar difference persisted until W16 (Fig. 5A). Compared with SL rats,  $11\beta$ -hsd1 mRNA expression was increased in SL-HF rats but decreased in SL-FO rats at W16. No difference in  $11\beta$ -hsd1 mRNA expression was found between NL-HF rats and NL rats (Fig. 5A). Moreover, the pattern of hepatic  $11\beta$ -HSD1 enzyme activity paralleled  $11\beta$ -hsd1 gene expression in all groups, and SL and the high-fat diet exhibited a significant interaction with regard to hepatic  $11\beta$ -HSD1 enzyme activity ( $F=22.49$ ,  $P<0.01$ ; Fig. 5B).

#### $11\beta$ -Hsd1 mRNA expression and enzyme activity in adipose tissue

As shown in Fig. 6A,  $11\beta$ -hsd1 mRNA expression was increased in SL rats compared with NL rats. Feeding rats a high-fat diet induced a pronounced increase in  $11\beta$ -hsd1 mRNA expression in the retroperitoneal adipose tissue of



**Figure 4** Effect of different diets on intraperitoneal glucose tolerance test (A and B) and areas under the curve (C and D) at W6 (A and C) and W16 (B and D). Data are mean  $\pm$  s.e.m.,  $n=6-9$  in each group. <sup>a</sup> $P<0.05$  vs NL, <sup>b</sup> $P<0.05$  vs SL, and <sup>c</sup> $P<0.05$  vs NL-HF.

**Table 3** Serum leptin and corticosterone levels at W16

	NL	SL	NL-HF	SL-HF	SL-FO
Leptin (ng/ml)	13.0±1.0	18.0±0.2*	18.8±1.8*	21.9±1.1* <sup>a,†,‡</sup>	15.1±1.5* <sup>a,†,‡</sup>
Corticosterone (ng/ml)	10.2±0.8	14.0±0.7*	10.2±1.0 <sup>†</sup>	15.5±0.6* <sup>‡</sup>	12.6±0.8*

Data are expressed as mean±S.E.M.,  $n=6-9$  in each group. \* $P<0.05$  vs NL, <sup>†</sup> $P<0.05$  vs SL, and <sup>‡</sup> $P<0.05$  vs NL-HF.

both SL-HF and NL-HF rats compared with their counterparts fed standard chow.  $11\beta$ -HSD1 activity was similar to gene expression data, and SL and the high-fat diet showed a significant interaction with regard to adipose tissue  $11\beta$ -HSD1 activity ( $F=18.45$ ,  $P<0.01$ ). Similar to the liver,  $11\beta$ -*hsd1* mRNA expression and enzyme activity in adipose tissue was decreased in SL-FO rats compared with SL rats (Fig. 6B). In epididymal adipose tissue,  $11\beta$ -*hsd1* mRNA expression and enzyme activity did not differ significantly between different diet groups (data not shown).

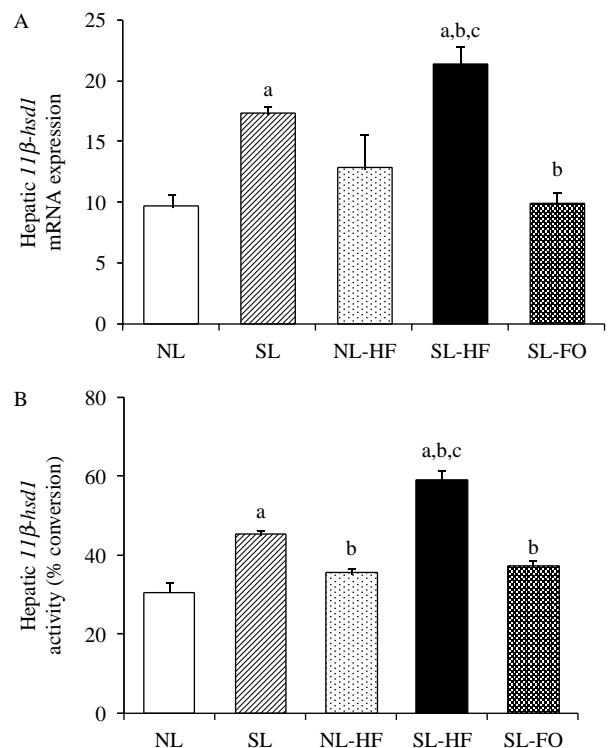
#### Hepatic A-ring reductase mRNA expression

No group difference was found in hepatic  $5\alpha$ R1 mRNA expression (data not shown). Hepatic  $5\beta$ R mRNA expression was 27% lower in SL rats than in NL rats ( $P<0.05$ ) but increased in SL-HF (50%,  $P<0.05$ ) and NL-HF rats (62%,  $P<0.05$ ) compared with SL and NL respectively. For SL-FO rats, hepatic  $5\beta$ R mRNA expression was not significantly altered compared with NL or SL rats at W16 (data not shown).

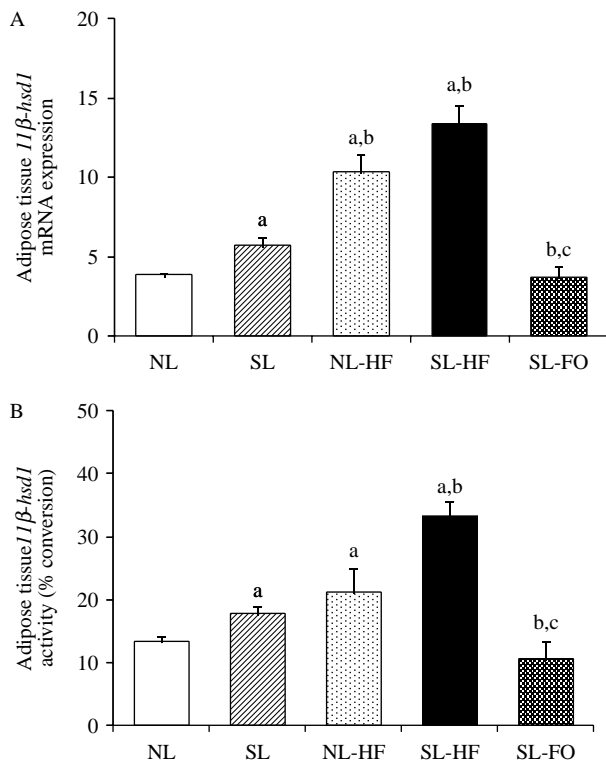
## Discussion

Previous studies have shown that, in rats, neonatal over-feeding induced by SL rearing results in adulthood onset of obesity, insulin resistance, hyperlipidemia, hyperleptinemia, and GC metabolism abnormalities (Boullu-Ciocca *et al.* 2005, Plagemann *et al.* 2009, Prior & Armitage 2009, Hou *et al.* 2011). Our study aimed to investigate whether postweaning diets with different fatty acid compositions could aggravate or ameliorate peripheral GC metabolism abnormalities and systemic metabolic disturbances in neonatally overfed rats. We found that a high-fat diet induced early-onset hyperphagia, obesity, insulin resistance, hyperlipidemia, and high circulating leptin. The effect of the high-fat diet in SL rats was earlier and more pronounced compared with NL rats. In SL-FO rats, the  $\omega$ 3 PUFA-rich diet prevented the increase in body weight and adipose tissue weight, lowered cholesterol, and improved glucose metabolism without reducing energy intake. The novel finding in this study was that  $11\beta$ -*hsd1* mRNA expression and enzyme activity were increased in both the hepatic and the visceral adipose tissue of SL-HF rats but decreased in SL-FO rats compared with SL rats. These data suggest that postweaning dietary fatty acid composition may potentially interact with metabolic and hormonal axes maturation and formation and codetermine the metabolic outcome, partly by regulating local GC action.

A high-fat diet may induce obesity in mice (Kozawa *et al.* 2011), rats (Mullen *et al.* 2009), and humans (Boden 2011). Consistent with previous reports, the high-fat diet induced visceral adipose gain and obesity in both NL-HF and SL-HF rats. However, SL-HF rats exhibited a greater rate of fat gain as well as hyperlipidemia and insulin resistance after 3 weeks on the diet, and the body weight and visceral adipose tissue weight measured for SL-HF rats were much higher than in other groups due to a significant interaction between SLs and the high-fat diet in the upregulation of serum TGs, body weight, and adipose tissue weight gain. These data suggest that rats that are overfed during lactation are more susceptible to high-fat stimulation after weaning, and this may lead to abnormal metabolic dysregulation compared with normally fed rats. Such divergent nutritional exposure during early development can reprogram the response to a particular nutritional environment, as reported previously in rat



**Figure 5** Effect of different diets on  $11\beta$ -*hsd1* mRNA expression (A) and enzyme activity (B) in the liver at W16. Data are mean±S.E.M.,  $n=6-9$  in each group. <sup>a</sup> $P<0.05$  vs NL, <sup>b</sup> $P<0.05$  vs SL, and <sup>c</sup> $P<0.05$  vs NL-HF.



**Figure 6** Effect of different diets on *11β-hsd1* mRNA expression (A) and enzyme activity (B) in retroperitoneal adipose tissue at W16. Data are mean  $\pm$  S.E.M.,  $n=6-9$  in each group. <sup>a</sup> $P < 0.05$  vs NL, <sup>b</sup> $P < 0.05$  vs SL and <sup>c</sup> $P < 0.05$  vs NL-HF.

offspring exposed to gestation stress (Tamashiro & Moran 2010) and maternal malnutrition (Minana-Solis & Escobar 2007). By contrast, perinatal mice fed a normal diet are resistant to high-fat diet-induced hyperphagia, obesity, and insulin resistance (Gallou-Kabani *et al.* 2007). These observations indicate that the perinatal period represents a critical time frame during which metabolic regulatory set points may be modified and that persistent overnutrition (e.g. high-fat diet) may further increase the incidence of obesity in rats exposed to overfeeding during lactation.

Dietary fatty acids are important regulators of gene expression, acting as intracellular messengers or ligands for transcription factors (Lichtenstein & Schwab 2000, Riccardi *et al.* 2004, Tierney *et al.* 2011) and cause a pronounced tissue-specific regulation of *11β-HSD1* in rodents (Morton *et al.* 2004a, Drake *et al.* 2005, Vara *et al.* 2010). In agreement with previous findings (Shin *et al.* 2011), *11β-hsd1* mRNA expression and enzyme activity was increased in retroperitoneal adipose tissue from rats fed a high-fat diet, regardless of litter size. Notably, hepatic *11β-HSD1* activity was persistently higher in SL rats compared with NL rats, and SL and the high-fat diet exhibited a significant interaction with regard to *11β-HSD1* activity, whereas no changes in hepatic *11β-HSD1* expression and enzyme activity was seen in NL-HF rats, which are similar to the majority of animal

models of obesity (Morton *et al.* 2004b, Drake *et al.* 2005). This inconsistency may be explained by different time frames of exposure to overnutrition (Chen *et al.* 2009) and implies that the key set point of *11β-HSD1* in the liver is the perinatal period. In addition, nutritional experience in early development may determine the gene response to circumstances later in life (Bouchard 2008). The overexpression of *11β-HSD1* in the liver of transgenic mice results in insulin resistance, dyslipidemia, hypertension, and fatty liver. Therefore, the persistent increase in hepatic *11β-HSD1* activity in SL and SL-HF rats may induce early-onset and more severe obesity, as well as other features of metabolic dysregulation.

Although both  $\omega 3$  PUFAs and  $\omega 6$  PUFAs exhibit antiobesity effects, products derived from  $\omega 6$  PUFAs are more potent mediators of inflammation than similar products derived from  $\omega 3$  PUFAs (Kang 2003) and a higher dietary  $\omega 6:\omega 3$  PUFA ratio increases the risk of obesity (Ailhaud *et al.* 2008). Thus, we selected a fish oil diet rich in  $\omega 3$  PUFAs to test the possibility of preventing the programming outcome in overfed neonatal rats. We found that  $\omega 3$  PUFAs in the diet downregulated *11β-HSD1* expression and enzyme activity in the adipose tissue and liver, which is in line with ameliorated glucose tolerance and the prevention of excess adipose tissue accumulation. This finding is consistent with the well-documented effect of  $\omega 3$  PUFA-rich diets against body fat gain in rats (Belzung *et al.* 1993) and mice (Ruzickova *et al.* 2004). We found that the fish oil diet prevented the development of metabolic disorders without reducing energy intake and body growth and development. Thus,  $\omega 3$  PUFAs have an effect on tissue function that is not dependant on calorie restriction. Reduced local GC activation by  $\omega 3$  PUFAs may play a key role in reducing adverse programming outcomes later in life, which might be a useful strategy for improving early programming effects following GC excess.

It is worth noting that we did not include a group of rats from NLs fed a fish oil diet in this study. Interestingly, there is at least one study reporting that a fish oil diet may reduce adipose weight gain in normal fed rats (Wyrwoll *et al.* 2006), and it is plausible that *11β-HSD1* enzyme activity levels are altered in those rats as well. However, the effect of  $\omega 3$  PUFAs on GC metabolism in normal size litter fed rats still needs to be studied.

The regulation of *11β-HSD1* by dietary fatty acids may involve peroxisome proliferator-activated receptor- $\gamma$  (PPAR $\gamma$ ) and PPAR $\alpha$  and CCAAT enhancer binding protein  $\alpha$  (C/EBP $\alpha$ ) (Vara *et al.* 2010, Vagnerova *et al.* 2011). PPARs are known to downregulate the expression of *11β-HSD1* (Berger *et al.* 2001, Srivastava 2009) and have a high affinity for PUFAs compared with saturated fatty acids, whereas a high-fat diet may increase the expression of C/EBP $\alpha$ , a key transcription factor for *11β-HSD1* (Kim & Park 2008). However, the details of dietary fatty acid regulation of *11β-HSD1* need to be further studied *in vitro*.

The A-ring reductases are the main inactivating enzymes for GC in the liver (Livingstone *et al.* 2000). Studies of obese

or insulin-resistant animals (Livingstone *et al.* 2000) and humans (Tsilchorozidou *et al.* 2003, Baudrand *et al.* 2011) have revealed higher expression of hepatic 5 $\alpha$ -reductase. In our study, 5 $\beta$ R mRNA expression was increased following a high-fat diet, independent of litter size. This alteration might be due to compensatory activation following a high-fat diet, independent of nutritional status, similar to Drake's report (Drake *et al.* 2005). The compensation may prevent circulating GC levels from increasing with a high-fat diet. Notably, 5 $\beta$ R mRNA expression seemed to be maintained in SL-FO rats compared with NL rats, with decreased circulating GC levels, though this was not significant. These data suggest a recovery of the GC conversion enzyme in SL-FO rats, which could be one of the mechanisms by which  $\omega$ 3 PUFAs prevent metabolic disease after postnatal overfeeding. However, little is known about the regulation of A-ring reductases by  $\omega$ 3 PUFAs until now, and the specific mechanisms involved in this process require additional study.

In conclusion, overfed neonatal rats had increased sensitivity to a high-fat diet from post-suckling to adulthood and presented early-onset hyperphagia, obesity, insulin resistance, and hyperlipidemia. By contrast, a  $\omega$ 3 PUFA-rich diet prevented programmed excess adipose accumulation and insulin resistance in early overfed rats. Tissue-specific regulation of GC-converting enzymes may underlie this process. This finding raises the possibility that dietary supplementation with  $\omega$ 3 PUFAs may provide a viable therapeutic option in humans for preventing and/or reducing adverse programming outcomes later in life.

### 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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