

Exposure of neonatal rats to maternal cafeteria feeding during suckling alters hepatic gene expression and DNA methylation in the insulin signalling pathway

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Abstract Nutrition in early life is a determinant of life-long physiological and metabolic function. Diseases that are associated with ageing may, therefore, have their antecedents in maternal nutrition during pregnancy and lactation. Rat mothers were fed either a standard laboratory chow diet (C) or a cafeteria diet (O) based upon a varied panel of highly palatable human foods, during lactation. Their offspring were then weaned onto chow or cafeteria diet giving four groups of animals (CC, CO, OC, OO $n = 9-10$). Livers were harvested 10 weeks post-weaning for assessment of gene and protein expression, and DNA methylation. Cafeteria feeding post-weaning impaired glucose tolerance and was associated with sex-specific altered mRNA expression of peroxisome proliferator activated receptor gamma and components of the insulin signalling pathway (*Irs2*, *Akt1* and *IrB*). Exposure to the cafeteria diet during the suckling period modified the later response to the dietary challenge. Post-weaning cafeteria feeding only down-regulated *IrB* when associated with cafeteria feeding during suckling (group OO, interaction of diet in weaning and lactation $P = 0.041$). Responses to cafeteria diet during both phases of the experiment varied between males and females. Global DNA methylation was altered in the liver following cafeteria feeding in the post-weaning period, in males but not females. Methylation of the *IrB* promoter was increased in group OC, but not OO

($P = 0.036$). The findings of this study add to a growing evidence base that suggests tissue function across the lifespan a product of cumulative modifications to the epigenome and transcriptome, which may be both tissue and sex-specific.

Keywords Programming · Epigenetics · Insulin signalling · Cafeteria diet

Introduction

The concept of the developmental origins of health and disease founded on the observation that low birthweight and other sequelae of maternal undernutrition are associated with cardiovascular disease and type-2 diabetes, is strongly supported by animal experiments (McMullen and Langley-Evans 2010). Variation in the quality or quantity of nutrient provision during pregnancy has a strong programming effect upon long-term health and well-being in the offspring of rodents, pigs, sheep and non-human primates (McMullen and Langley-Evans 2010).

Much of the work to date that has investigated the impact of maternal nutrition as a programming factor that determines physiological and metabolic function has focused upon maternal undernutrition. Food restriction (Vickers et al. 2003), protein restriction (Langley and Jackson 1994) and iron deficiency (Gambling et al. 2003) in rodent pregnancy all elicit similar phenotypes in the resulting offspring, including cardiovascular dysfunction (Yates et al. 2008; Torrens et al. 2009) impaired glucose homeostasis (Fernandez-Twinn et al. 2005) and renal impairments (Langley-Evans et al. 1999). A smaller body of work has shown that maternal over-feeding, generally with high-fat diets, has similar programming effects in rats

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and mice (Samuelsson et al. 2008; Shankar et al. 2008). Work in our laboratory has demonstrated that feeding a cafeteria diet (a varying menu of highly palatable human foods) can impact upon metabolic function, with changes in glucose homeostasis as determined by intraperitoneal glucose tolerance test (Akyol et al. 2012). This work established that foetal life is not the only period of sensitivity to the maternal diet. Feeding a cafeteria diet during lactation in the rat also altered responses to a glucose load and had powerful effects upon feeding and other behaviours (Wright et al. 2011a, b).

The most favoured explanation for associations between early dietary exposures and later physiological and metabolic function is that early diet impacts upon epigenetic marks and hence gene expression (Szyf 2009; Lillycrop and Burdge 2011). There is good evidence from animal models of undernutrition during foetal life that maternal diet can alter the epigenome, particularly DNA methylation, and this may establish changes in gene expression that permanently modify tissue structure or reset the responses to dietary and age-related challenges that occur later in life (Sinclair et al. 2007; Lillycrop et al. 2007; Bogdarina et al. 2010). Exposure to high-fat diets has been shown to alter DNA methylation and histone marks in rodents, non-human primates and primates, with the brain being particularly sensitive to dietary influences (Seki et al. 2012; Carlin et al. 2013; Langie et al. 2013; Jacobsen et al. 2012). The theory that epigenetic marks are solely vulnerable to nutritional programming in foetal life is now discounted as the epigenome responds to dietary cues at all life stages.

Whilst there is strong evidence of altered DNA methylation patterns across the whole genome in response to variation in maternal nutrition during pregnancy, it is not clear how wide the “window” for epigenetic programming in response to nutrition may be. Certainly, there is some evidence that a degree of plasticity remains into the pubertal growth phase in rats, as epigenetic marks on the peroxisome proliferator activated receptor alpha promoter respond to the folate content of the diet during this period (Burdge et al. 2009). With a long period of sensitivity to diet, it is of interest to determine whether the state of the epigenome following one set of dietary stimuli, for example in foetal life, can influence the response of the epigenome to a stimulus at a later stage.

The present study utilised tissue from the study previously reported by Akyol et al. (2012), as a preliminary investigation to establish the possible mechanistic basis of programming by maternal over-feeding in the lactation period. Specifically, we investigated the hypothesis that the observed metabolic phenotype is linked to cafeteria diet-induced changes in the insulin signalling pathway that are not just responses to current diet. The study also aimed to

establish whether the epigenome is sensitive to maternal over-feeding during lactation or post-weaning.

Materials and methods

Experimental protocol and sample preparation

This paper describes a study which utilised a subset of tissue harvested from rats aged 3 months that had been subject to either maternal cafeteria diet feeding or a standard commercial chow diet during suckling. The full experiment which generated the tissue has been previously published (Akyol et al. 2012). The protocol is described briefly below.

The animal experiments were performed under licence from the Home Office in accordance with the 1986 Animals (Scientific Procedures) Act. Virgin female Wistar rats (aged 3 weeks) were fed standard laboratory chow diet (Harlan UK) from weaning until mating at age 10 weeks. On giving birth, all of the resulting litters were culled to a maximum of 8 pups (4 males and 4 females where possible, randomly selected) and were randomly allocated to be fed either a control chow diet alone ($n = 11$, chow control; C) or a control chow diet alongside an obesogenic random selection of highly energetic and palatable human foods ($n = 12$, cafeteria diet; O), as previously described by Akyol et al. (2009, 2012). All litters were weaned at 3 weeks of age and then half of the offspring from each litter were weaned onto the standard chow diet, and the remaining weanlings were allocated to receive chow and cafeteria diet. There were four groups of animals considered in this study: control in both lactation and post-weaning (CC); control in lactation, cafeteria post-weaning (CO); cafeteria during lactation, control post-weaning (OC) and cafeteria diet in both lactation and post-weaning (OO).

At the age of 13 weeks, all offspring were subject to an intraperitoneal glucose tolerance test, following an 18-h fast, and were then culled (Akyol et al. 2012). Liver was rapidly dissected and snap-frozen in liquid nitrogen before storage at -80°C . RNA was prepared from frozen tissue using Qiagen AllPrep kits according to the manufacturers instructions. DNA was extracted using Qiagen DNeasy kits. Protein extractions were performed by homogenising ground sample with 5 volumes of extraction buffer (150 mM NaCl, 50 mM HEPES, 2.5 mM EDTA, 10 % glycerol, 1 % Triton, 1 mM Na_3VO_4 , 10 mM NaF). After addition of 2 volumes SDS mix (glycerol, 1 M Tris/HCl, 10 % SDS, 0.154 g/ml dithiothreitol 2:1.25:4:1 v:v:v:v), the samples were centrifuged at 13,000 rpm for 10 min at 4°C . Protein content of supernatant was determined using the method of Lowry et al. (1951). Protein, DNA and RNA

were all prepared from the same samples, with only one liver per litter selected for this study.

Quantitative real-time PCR

The transcription of a number of target genes was determined by quantitative real-time PCR. The targets included the insulin receptor (*IrB*) and the glucose transporter *Glut2* (Kahn 1997) as markers of the insulin signalling pathway (insulin receptor substrate 2; *Irs2* and the alpha serine/threonine-protein kinase; *Akt1* had been previously measured in the same tissues, Akyol et al. 2012). The expression of peroxisome proliferator activated receptor alpha (*PPARa*) was determined as this transcription factor regulates hepatic glucose metabolism (Viana Abranches et al. 2011). Peroxisome proliferator activated receptor gamma (*PPARg*) expression was also measured as the effects of *PPARg* on adipocytes increase insulin sensitivity and the production of cytokines that regulate glucose homeostasis (Floyd and Stephens 2012). Expression of acetyl CoA carboxylase 1 (*Acc1*) and carnitine-palmitoyl transferase 1a (*Cpt1a*) was determined as these are downstream targets of *PPARa* (Menendez-Gutierrez et al. 2012).

RNA was reverse transcribed using Moloney murine leukaemia virus (MMLV) reverse transcriptase (Promega, UK). Real-time PCR primers were designed for *PPARa*, *PPARg*, *Glut2*, *IrB*, *ACC1* and *Cpt1a* using Primer Express software (version 1.5; Applied Biosystems) from the RNA sequence and were purchased from Sigma (UK). The primer sequences were as follows: Rat *PPARa* Fwd: CCACGAAGCCTACCTGAAGAAGCT Rvr: ACAAAGGCGGATTGTTGCT; Rat *PPARg* gamma Fwd: GAATACCAAAGTGCGATCAAAGTAGA Rvr: GGCCATGAGGGAGTTTGAA G; Rat *Glut2* Fwd: TCAGCCAGCCTGTGTATGCA Rvr: TCCACAAGCAGCACAGAGACA; Rat *IrB* Fwd: GGAT TATTGTCTCAAAGGGCTGAA Rvr: CGTCATACTCAC TCTGATTGTGCTT; Rat *ACC1* Fwd: CCATTGGTATTG GGGCTTAC Rvr: CCGACCAAGGACTTTGTTG; Rat *Cpt1a* Fwd: CATTACAAGGACATGGGCAAGTT Rvr: T GTCCTCCCTTCTCGGAAGA. Primers were ordered from MWG Biotech, (Germany). Primer sequences for the housekeeping gene (cyclophilin) are published elsewhere (Austin and Langley-Evans 2011). Real-time PCR was performed using a Lightcycler 480 PCR machine (Roche, UK) and SYBR green, as previously described (Swali et al. 2012). Expression of the genes of interest was normalised to the housekeeping gene, expression of which was unaltered in response to the dietary treatments.

A Cayman Chemicals DNA Methylation EIA kit was used to estimate crude global DNA methylation in the samples. This assay measures 5-methyl-2'-deoxy cytidine in DNA. Methylation-specific real-time PCR was performed for *PPARa*, *PPARg* and *IrB* using the SABiosciences

EpiTect Methyl II PCR Primer assay, following the manufacturers instructions.

Western blotting

Protein expression was determined using Western blot, as described previously (Ryan et al. 2012). Primary antibodies were *PPARa* (Cayman Chemicals), *PPARg* (Santa Cruz), *Glut2* (Millipore) and insulin receptor beta (cell signalling). Expression was normalised against beta-actin (Sigma) as a housekeeper. For *Glut2*, *IrB*, *PPARa* and *PPARg*, the secondary antibody was anti-rabbit, and for beta-actin, it was anti-mouse (both from Amersham). Signal was quantified using ECL Select (Amersham) developed on Hyperfilm (Amersham).

Statistical analysis

All data are presented as mean \pm SEM. Data were analysed by three-way analysis of variance (SPSS 20) to examine the influence of offspring sex, maternal diet during lactation and offspring diet from weaning until 3 months of age. $P \leq 0.05$ was accepted as statistically significant.

Results

The original experiment which generated the tissues for this study (Akyol et al. 2012) evaluated the impact of cafeteria diet prior to and during pregnancy, in addition to considering the effects of the diet during lactation upon glucose homeostasis in the offspring. As the current study used a subset of groups from the original study, the glucose tolerance data from those groups were reanalysed to focus on the interaction between maternal diet during lactation and post-weaning diet (Fig. 1). After adjustment for sex, there was a significant interaction of the diet in lactation and post-weaning ($P = 0.039$). Compared to group CO, glucose tolerance was significantly impaired by cafeteria feeding in both periods (OO). Glucose tolerance was not significantly different to group CC in any of the groups, although group CO cleared an i.p. glucose load moderately faster.

Our previously published work suggested that exposure to cafeteria diet at key stages of life altered expression of two components of the insulin signalling pathway (*Irs2* and *Akt1*, Akyol et al. 2012). Confining the analysis to just the four groups of rats in this study confirmed the sensitivity of these genes to cafeteria diet as *Irs2* was strongly up-regulated by post-weaning cafeteria diet in females only (2.64-fold, $P < 0.001$, Table 1). *Akt1* expression was up-regulated by both cafeteria diet in lactation and post-weaning, but again

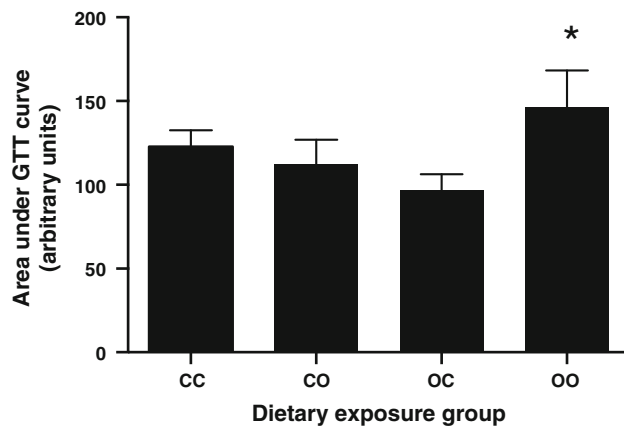


Fig. 1 Area under the curve from glucose tolerance test. Data are shown as mean \pm SEM for 9–10 observations per group. Data are shown for males and females combined (no significant effect of sex) and adjusted for sex. *indicates significantly different to OC ($P < 0.05$). Glucose tolerance area under the curve was influenced by an interaction of lactation and post-weaning diet ($P = 0.039$). Reanalysis of data from Akylou et al. (2012)

Table 1 Impact of cafeteria diet during lactation and post-weaning on mRNA expression

Gene	<i>P</i> effect of sex	Lactation fold change (OC and OO vs. CC and CO)	<i>P</i> effect of cafeteria diet during lactation	Post-weaning fold change (CO and OO vs. CC and OC)	<i>P</i> effect of cafeteria diet post-weaning
<i>Irs2</i>	0.002	−1.29	NS	1.63 [§]	0.043
<i>Akt1</i>	NS	2.45 ^{*e}	0.008	−1.51 ^{*e}	NS
<i>IrB</i>	NS	−1.04 ⁺	NS	−1.12 ⁺	NS
<i>Glut2</i>	NS	1.06	NS	1.07	NS
<i>PPARa</i>	0.019	−1.29	0.038	1.10	NS
<i>Cpt1a</i>	0.026	1.30	0.059	1.06 [§]	NS
<i>Acc1</i>	NS	1.05	NS	1.17	NS
<i>PPARg</i>	0.002	−1.04	NS	1.27 [§]	NS

Data are shown as fold change (−denotes down-regulation). *P* values for lactation and post-weaning effects are univariate analyses. Interaction terms are not shown in this table, see legend Fig. 2, but are indicated by [§] (sex \times post-weaning, $P < 0.05$), ^e (lactation \times post-weaning, $P < 0.05$) and ^{*} (sex \times lactation \times post-weaning, $P < 0.05$)

only in females (interaction sex \times post-weaning diet \times lactation diet $P < 0.05$, Table 1). The effects of cafeteria diet were not observed when the diet was fed in both the lactation and post-weaning periods; hence, expression was increased in groups OC (6.8-fold) and CO (2.9-fold), but not OO. *Irs2* expression was correlated with the area under the glucose tolerance curve ($r = 0.450$, $P = 0.006$). Expression of two other components of the insulin signalling pathway indicated that

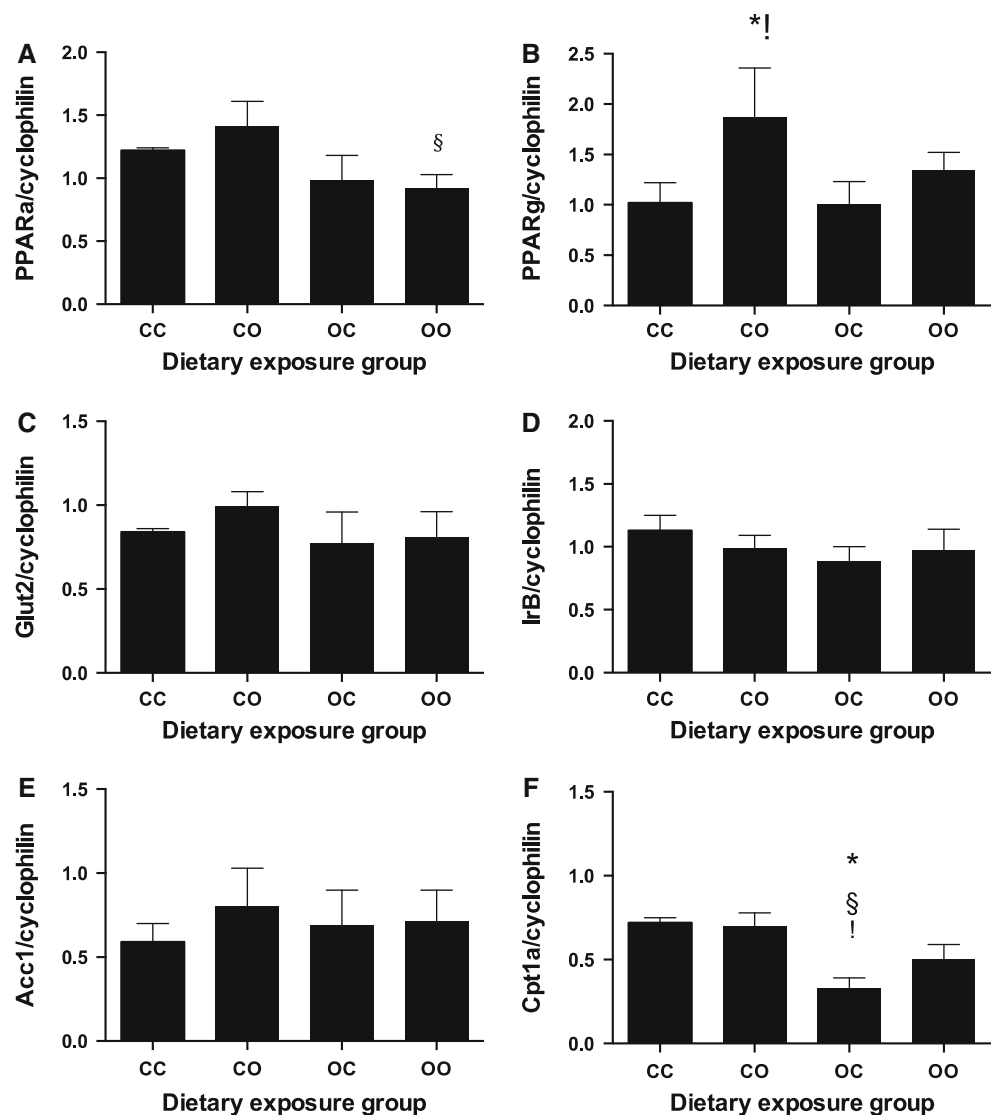
Glut2 expression was not influenced by cafeteria feeding (Figs. 2d, 3d), but *IrB* expression was influenced by sex, lactation diet and post-weaning diet (interaction $P = 0.041$, Table 1; Figs. 2c, 3c). In females, it was only the animals suckled by cafeteria fed mothers that were sensitive to post-weaning cafeteria diet, but these OO females had reduced (20 % lower) expression of *IrB* compared to CC.

The mRNA expression of *PPARa* differed markedly between male and female animals (lower in females) but was not significantly influenced by diet post-weaning. Exposure to cafeteria diet in lactation reduced *PPARa* expression ($P = 0.038$), as shown by the significant effect in males of group OO (Table 1; Figs. 2a, 3a). Similarly, *PPARg* was expressed at a lower level in females than males (25 % lower, Table 1; Figs. 2b, 3b), and specifically, in males of group CO, expression was increased (1.37-fold) by feeding the cafeteria diet post-weaning (sex \times post-weaning diet interaction $P = 0.05$). Given the significant effect of the lactation diet upon *PPARa* expression, the mRNA expression of the downstream targets *Acc1* and *Cpt1a* was evaluated. Whilst *Acc1* expression was unaffected by sex or diet at either stage of life (Table 1; Figs. 2e, 3e), *Cpt1a* expression (Table 1; Figs. 2f, 3f) was influenced by the interaction of sex and the post-weaning diet. In males of group OC, expression was significantly lower (55 %) than in all other groups, whilst in females of the same group, expression tended to be higher (significant compared to OO). Surprisingly, there was no correlation between expression of *PPARa* and either of the downstream targets, which may reflect the differential effects of the dietary exposures upon protein expression.

As shown in Table 2, global DNA methylation was significantly influenced by the post-weaning diet. In males but not females (interaction of sex \times post-weaning diet $P = 0.018$), post-weaning cafeteria diet significantly reduced global methylation. Methylation-specific PCR found no significant effects of cafeteria diet upon *PPARa*, where little methylation was detected in any of the groups (data not shown). There was some evidence that exposure to the cafeteria diet during lactation, followed by weaning onto chow, increased methylation of *PPARg* (group OC), but this was not statistically significant ($P = 0.069$, Table 2). In contrast, there was a clear sensitivity of the *IrB* promoter to effects of diet upon methylation. As with *PPARg*, the OC group exhibited greater methylation than in all other groups (interaction of lactation and post-weaning diet $P = 0.036$).

Figure 4 shows the expression of *PPARa*, *PPARg*, *Glut2* and *IrB* at the protein level. There were no significant effects of sex, maternal diet during lactation or offspring diet from weaning upon these proteins.

Fig. 2 mRNA expression of insulin signalling pathway-related genes in male rat liver at 3 months of age. Data are shown as mean \pm SEM for 4–5 observations per group. *indicates significantly different to CC ($P < 0.05$), §significantly different to CO ($P < 0.05$), †significantly different to OC ($P < 0.05$). **a** *PPARa* expression was different in males and females ($P = 0.019$) and was influenced by exposure to cafeteria diet during lactation ($P = 0.038$). **b** *PPARg* expression was different in males and females ($P = 0.002$) and was influenced by the interaction of sex and cafeteria diet post-weaning ($P = 0.050$). **c** *Glut2* expression was not influenced by sex or dietary exposures. **d** *IrB* expression was influenced by the interaction of sex, diet in lactation and diet post-weaning ($P = 0.041$). **e** *Acc1* expression was not influenced by sex or dietary exposures. **f** *Cpt1a* expression was influenced by sex ($P = 0.026$) and the interaction of sex and post-weaning diet ($P = 0.023$)



Discussion

Cafeteria feeding (Rothwell and Stock 1979) promotes obesity by overcoming the ability of rodents to regulate energy intake, as it provides a diet with high palatability and novelty. Our study replicated the original protocol and cafeteria feeding involved the addition of a constantly varied panel of high-sugar/high-fat foods to standard laboratory chow diet. This protocol was adopted as the literature (Buettner et al. 2007) demonstrates consistent evidence that a metabolic phenotype similar to that observed in the human can be induced. The combination of high fat with high sodium, sugars, saturated fat and cholesterol and low in fibre and micronutrients makes the protocol more closely aligned with dietary patterns observed in the humans than conventional purified high-fat diets used in rodent studies (Crozier et al. 2006; Kant 2004). It is considered the most effective tool for modelling

the effects of “non-prudent” dietary patterns and diet-induced obesity in humans (Sampey et al. 2011).

The study examined two key hypotheses, and the data generated support the assertion that maternal cafeteria feeding during lactation programmes metabolic function through long-term alteration of expression of the insulin signalling and other metabolic pathways. We have also confirmed that the epigenome may be sensitive to this form of dietary challenge during the suckling phase in rodents. This corresponds with work demonstrating that high-fat diets can impact upon histone acetylation and DNA methylation in rodents and macaques (Aagaard-Tillery et al. 2008, Vucetic et al. 2010, 2011).

From a metabolic perspective, the animals most affected by the cafeteria diet were in the OO group. The slower clearance of a glucose load in this group is likely to be explained by their lower *IrB* expression. Clearly, changes in expression of the insulin signalling pathway (*IrB*, *Irs2*

Fig. 3 mRNA expression of insulin signalling pathway-related genes in female rat liver at 3 months of age. Data are shown as mean \pm SEM for 4–5 observations per group. *indicates significantly different to CC ($P < 0.05$), §significantly different to CO ($P < 0.05$), †significantly different to OC ($P < 0.05$). For full legend see Fig. 2

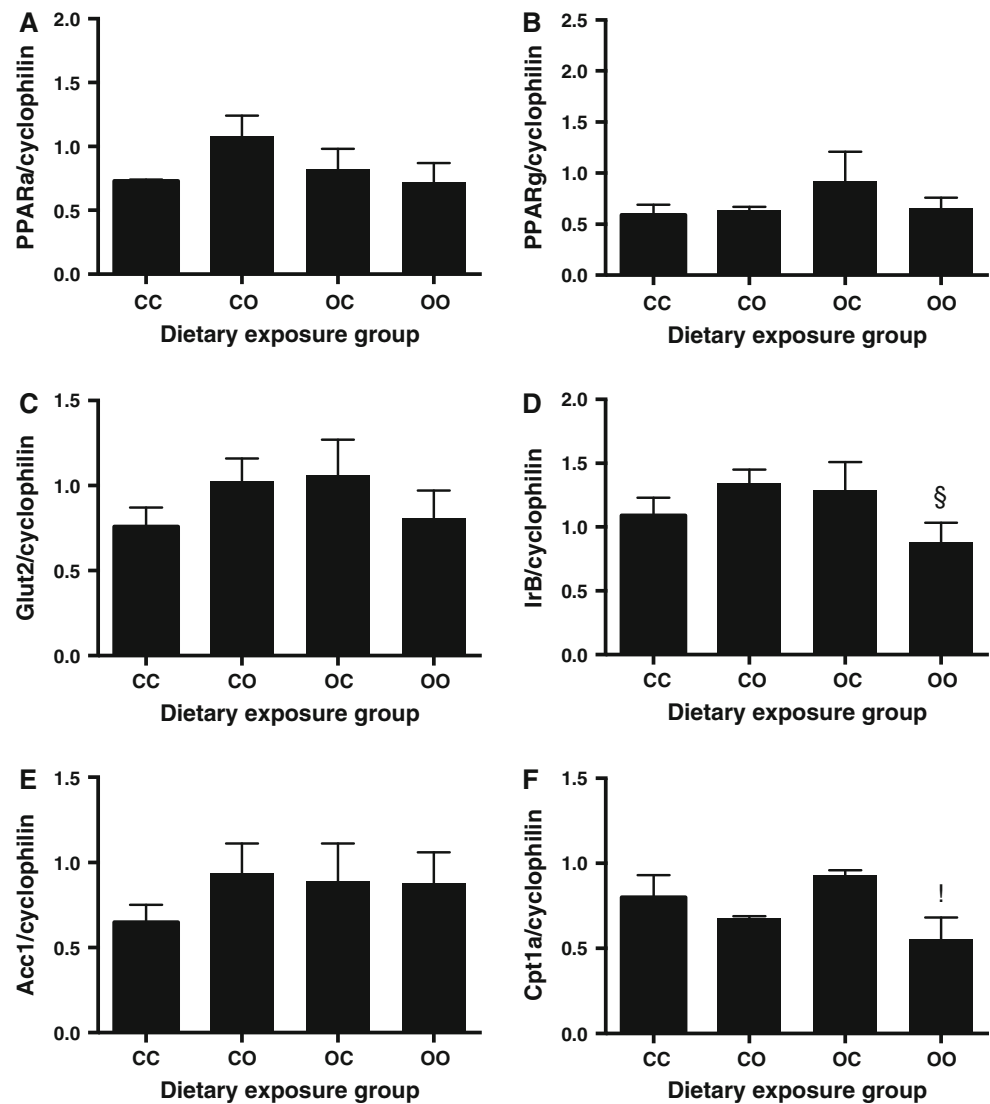


Table 2 DNA Methylation in liver of 3-month-old rats

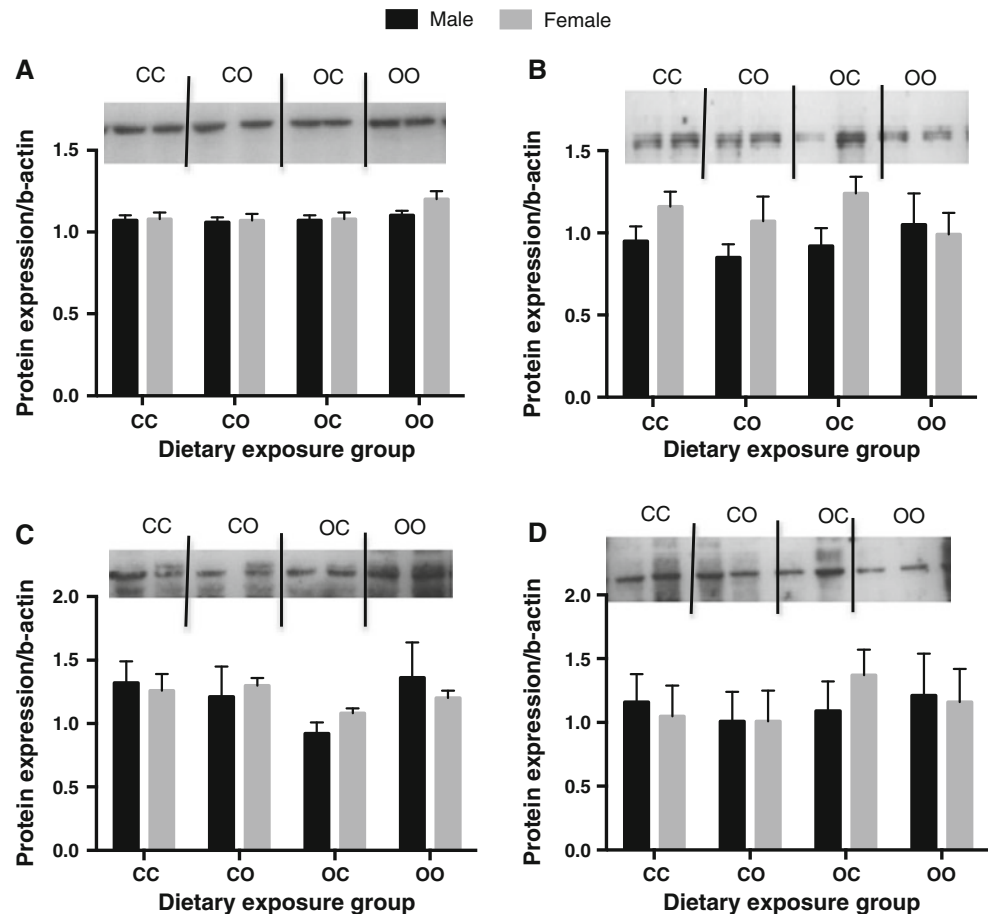
	Dietary exposure groups							
	CC		CO		OC		OO	
	Male	Female	Male	Female	Male	Female	Male	Female
Global methylation (pg MDC/ μ g DNA)	190 \pm 11	174 \pm 12	154 \pm 4	176 \pm 4	166 \pm 12	160 \pm 6	120 \pm 11	212 \pm 5
Methylation PPAR γ (%)	0.47 \pm 0.17	0.42 \pm 0.20	0.61 \pm 0.38	0.48 \pm 0.23	9.24 \pm 6.63	9.19 \pm 6.62	0.27 \pm 0.04	0.21 \pm 0.04
Methylation IrB (%)	0.60 \pm 0.14	0.54 \pm 0.12	1.43 \pm 0.68	0.91 \pm 0.68	13.1 \pm 7.72	13.1 \pm 7.68	0.46 \pm 0.05	0.49 \pm 0.02

Data are shown as mean \pm SEM for 5–6 observations per group. Global methylation was influenced by the interaction of sex and post-weaning diet ($P = 0.018$). Methylation of PPAR γ was not influenced by sex or diet at any stage. IrB methylation was significantly influenced by the interaction of diet in lactation and weaning ($P = 0.036$), with univariate influences of lactation ($P = 0.058$) and post-weaning ($P = 0.054$) diets approaching significance. MDC-5-methyl-2'-deoxy cytidine

and *Akt1*) are contributing to the metabolic response to the diet, and a role for *Glut2* has been excluded. As previously reported (Akyol et al. 2012), the differences in metabolic response to glucose are more likely to be due to this

programming of the insulin signalling pathway or glucose metabolism as there were no gross effects of the cafeteria diet upon other aspects of metabolic function. Whilst the post-weaning cafeteria diet promoted fat deposition, there

Fig. 4 Protein expression of insulin signalling pathway-related genes in rat liver at 3 months of age. Data are shown as mean \pm SEM for 4–5 observations per group. All protein expression was normalised to expression of beta-actin. Expression of all proteins, including the housekeeper beta-actin was not influenced by sex or dietary exposures. Blot examples shown are representative of each group. **a** Glut 2, **b** IRB, **c** PPAR α , **d** PPAR γ



was no effect of the diet in lactation on body fatness. Abdominal fat deposition in OC rats was no different to CC, and in CO, there was no different to OO (Akyol et al. 2012). The data critically demonstrate that the transcriptional and metabolic effects seen in OO animals are not just a response to the diet at the time of measurement, as the nature of the response also depended upon the dietary exposure during lactation.

Using a similar protocol to the present study, Bayol et al. (2010) found sex-specific effects of cafeteria diet upon insulin signalling and glucose metabolism in rats. However, in the Bayol study, insulin receptor expression was *increased* by cafeteria feeding at any stage of life in males and *Irs2* was down-regulated by cafeteria diet in females. Effects of the diet on expression of PPAR γ and glucose transporters in adipose tissue were also reported (Bayol et al. 2008). The differences between the current study and the work by Bayol and colleagues are most likely to be explained by variation in the nature of the cafeteria feeding regimens and different timing of exposure (control over litter size and variety of cafeteria foods offered).

The reanalysis of mRNA expression data in this study showed that *Irs2* was up-regulated in response to post-weaning cafeteria feeding, and we would suggest that this

is likely to be a compensatory response to maintain glucose homeostasis. *Akt1* expression also responded to cafeteria feeding post-weaning, again an adaptation that would be expected to maintain homeostasis in the face of increasing adiposity and dietary challenge. There was clear variation in response dependent upon the lactation exposure. Differential responses to the post-weaning dietary challenge between males and females were noted with respect to the nature of adaptive responses to over-feeding. Males up-regulated PPAR γ rather than the *Irs2/Akt* pathway. This would provide a means of alternative disposal in glucose adipose tissue rather than liver. Sex differences in phenotypic outcomes of *Irs2* (Masaki et al. 2004) and PPAR γ knockout (Duan et al. 2010; Kadowaki et al. 2007) and *Akt1* activation (Foryst-Ludwig et al. 2011; Chen et al. 2013) have been reported previously.

The assessment of global DNA methylation did not note any effects of cafeteria diet during lactation. This may be unremarkable as simple assays of 5-methyl-2'-deoxy cytidine are considerably less sensitive than sequencing based methods that were beyond the scope of this investigation. However, post-weaning diet did influence global methylation, consistent with reports of Vucetic et al. (2010). It is of interest that this effect was seen only in males. Sex

differences in methylation are unexplained, but our observations are consistent with Sinclair et al. (2007) who similarly reported sensitivity of DNA methylation to maternal diet in males, but not females. As crude global methylation changes would be insufficient evidence that the diet impacted upon the genome, it is important to note that *Irf1* was the a specific gene target for which an effect of the diet upon DNA methylation was noted. However, whilst methylation was increased, there was no associated decrease in mRNA expression. In fact, quite the opposite was apparent. Discordance of DNA methylation and mRNA expression has been widely reported (Carone et al. 2010; Zhou et al. 2011). A recent, robust analysis of DNA methylation across the whole genome and genome-wide gene expression changes in response to maternal under-nutrition found that discordance of methylation and gene expression was the norm (Altobelli et al. 2013). The discordance suggests that the interplay between DNA methylation and gene expression is complex. Differences in methylation may only, for example, bring about expression changes during a metabolic challenge or in response to a specific stimulus. Furthermore, the effect of methylation on expression of a specific gene will also depend upon the histone marks associated with that promoter. Liver is not a homogenous tissue and contains many distinct cell types. It is important to note that methylation and expression may differ between different cell types, and this may contribute to the discordance in the data.

A general lack of major effects of the diet during lactation upon methylation of DNA might also result from the window of epigenetic sensitivity being much wider than originally suspected. If the diet fed during weaning can also change epigenetic marks, and possibly in a different manner to earlier phases, then effects of diet in lactation could be masked or erased. Investigation of this would require a different experimental design with earlier time-points to consider the epigenome during periods of transition from one diet to another. The study of Jacobsen et al. (2012) showed that when healthy young men were fed a short-term high-fat diet, there were changes in genome-wide methylation in skeletal muscle, which reversed very slowly and were still present 8 weeks after the withdrawal of the diet. This is consistent with the view that dietary effects on the epigenome at one point in life can determine the response to nutritional cues and the impact of the diet upon the genome at another. With ageing, the nature of the epigenome will reflect an accumulation of methylation patterns that are generated by dietary and environmental exposures across the life course.

It is clear from the data presented in this paper that there was marked discordance between the mRNA and protein measurements, with none of the gene expression changes verified at the protein level. This is not entirely unsurprising

as a number of studies have shown that at most only 40 % of the protein levels in mammalian cells are related to mRNA expression even in homogenous tissues and cultures. (Vogel et al. 2010). Transcription factors, such as PPAR α and PPAR γ , are among a large group of proteins where both mRNA and protein have high rates of turnover (Schwanhäusser et al. 2011). This applies also to cyclins which are apparent targets for nutritional programming in rats (Swali et al. 2011). The discordance of mRNA and protein data in this study could limit the capacity of the study to establish the underlying basis of physiological and metabolic phenotype, but does not lessen the significance of the observed effect of a highly variable, human-type, cafeteria diet upon transcription and the epigenome.

Feeding a cafeteria diet based upon highly palatable human foods to rats during lactation modulates the response of their offspring to the same dietary pattern post-weaning. Programmed changes in hepatic gene expression underpin these differential responses and may be, in part, driven by differences in DNA methylation. The study adds to a growing evidence base that suggests tissue function across the lifespan is determined by cumulative modifications to the epigenome and hence transcriptome, which may be both tissue and sex-specific.

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Conflict of interest None.

Ethical standard All institutional and national guidelines for the care and use of laboratory animals were followed.

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