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RESEARCH ARTICLE

Metabolic effects of a high-fat diet post-weaning after low maternal dietary folate during pregnancy and lactation

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Scope: Investigate the influence of low-folate supply during pregnancy and lactation on obesity and markers of the metabolic syndrome in offspring, and how provision of a high-fat diet post weaning may exacerbate the resultant phenotype.

Methods and results: Female C57Bl/6 mice were randomized to low or normal folate diets (0.4 or 2 mg folic acid/kg diet) prior to and during pregnancy and lactation. At 4 wk of age, offspring were randomized to high- or low-fat diets, weighed weekly and food intake assessed at 9 and 18 wk old. Adiposity was measured at 3 and 6 months. Plasma glucose and triacylglycerol (TAG) concentrations were measured at 6 months.

Maternal folate supply did not influence adult offspring body weight or adiposity. High-fat feeding post weaning increased body weight and adiposity at 3 and 6 months ($p > 0.001$). Maternal low folate lowered plasma glucose ($p = 0.010$) but increased plasma TAG ($p = 0.048$). High-fat feeding post weaning increased plasma glucose and TAG ($p = 0.023$, $p = 0.049$ respectively). Offspring from folate-depleted (but not folate-adequate) dams had 30% higher TAG concentration when fed the high-fat diet from weaning ($p = 0.005$ for interaction).

Conclusion: Inadequate maternal folate intake has long-term effects on offspring metabolism, manifested as increased circulating TAG, particularly in offspring with high-fat intake post weaning.

Keywords:
Adiposity / DOHaD / High-fat diet / Maternal folate intake / Metabolic syndrome

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1 Introduction

The developmental origins of health and disease (DOHaD) hypothesis proposes that exposures during early life modulate the risk of developing noncommunicable diseases in adulthood. Indeed, there is substantial evidence for an association between lower birth weight and increased risk of type-2 diabetes, coronary heart disease, and hypertension, which has been attributed to poor nutrition in utero [1]. These observations indicate the potential for a degree of plasticity during development, through which the fetal phenotype may be altered in response to environment cues [2] in ways that may prepare it for the anticipated postnatal environment [3].

The "predictive adaptive response" hypothesis proposes that mismatch between the environment anticipated by the fetus, based on early (in utero) environmental exposures,

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Abbreviations: DOHaD, Developmental origins of health and disease; **MS**, metabolic syndrome; **SI**, small intestine; **TAG**, triacylglycerol

and the environment encountered postnatally may predispose to the early development of disease [4–6]. Therefore the adverse consequences of inadequate nutrient supply during early developmental may be exacerbated by over-nutrition postnatally.

Many studies report detrimental effects of low-folate status before and during pregnancy on offspring health. Effects include increased risk of neural tube defects [7,8], and low birth weight [9,10]—the latter may be a predictor for metabolic disease in later life [11]. Recently, folic acid supplementation (or folic acid supplementation with iron and zinc) during pregnancy was observed to reduce metabolic syndrome (MS) development in children at 6 to 8 years of age in a generally undernourished population [12]. Conversely, in an Indian population, higher maternal erythrocyte folate concentrations during pregnancy predicted higher offspring adiposity and increased insulin resistance at 6 years of age [13]. Furthermore, folate supplementation in pregnant rats modified growth patterns and metabolic response to fasting in adult offspring [14], providing further evidence that maternal folate intake during pregnancy affects metabolic health in offspring. Previously, we observed that mice born to low-folate fed dams tended to be heavier in adulthood compared with controls (McKay et al. unpublished data). Given the global reach of the present obesity epidemic and important etiological contribution made by obesity to the development of insulin resistance, MS [15], and a range of noncommunicable diseases [16], identification of modifiable factors associated with predisposition to obesity is of considerable public health importance. Maternal diet, specifically maternal folate supply, may be particularly important in this respect because of its potential to benefit long-term offspring health through developmental programing.

We hypothesized that low-folate supply during pregnancy may contribute to the development of obesity and MS in offspring, and that these effects would be exacerbated by provision of a high-fat diet post weaning. To test this hypothesis, we measured body mass and adiposity and markers of MS in mice born to mothers given low or adequate folate intakes during pregnancy and lactation and which had been fed high- or low-fat diets post weaning.

2 Materials and methods

2.1 Animal housing, husbandry, and dietary intervention

All animal procedures were approved by the Newcastle University Ethics Review Committee and the UK Home Office (Project license number 60/3979). Animals were housed in the Comparative Biology Centre (Newcastle University) at 20–22°C and with 12-h light and dark cycles. Fresh water was available ad libitum. The study was a 2 × 2 factorial design with 2 levels of maternal folate supply (low and adequate) and 2 levels of dietary fat (high and low) from weaning of the offspring. Female C57BL/6J mice were allocated randomly to a low-folate (0.4 mg folic acid/kg diet) or normal-folate (2 mg folic acid/kg diet) diet (6 g/day; described previously [17]; apart from folic acid content, these two diets were identical), and maintained on this diet for 4 wk prior to mating. Mating trios (two females; one male) were provided with 6 g/day/mouse of females' allocated diet. Upon presence of a vaginal plug, pregnant females were recaged and provided with 10 g/day of allocated diet throughout pregnancy. All dams carried at least one pregnancy to term, but not all litters survived. All failed litters survived less than 24 h. Dams that gave birth to failed litters were allowed a 14-day recovery period before a male mate was reintroduced. No more than three pregnancies/dam were allowed. In the case of successful litters, diet quantity was increased to 20 g/dam/day at 2 k postpartum.

Of the 145 offspring that survived until weaning (mean 22 days postpartum), 105 mice were recaged and allocated randomly to a low- or high-fat diet (Supporting Information Table 1; manufactured by Harlan Laboratories, product codes TD.09506 and TD.09507 respectively). Because of the different fat contents, these diets were not isocaloric, that is, the high-fat diet was more energy dense than the low-fat diet. The divergent folate intake applied during pregnancy and lactation was not continued in the postweaning dietary regime. The remaining 40 siblings were killed at weaning. Fresh water and allocated diet were available ad libitum. Mice were weighed weekly. Food intake was measured at 9 and 18 wk of age by weighing food hoppers before and after a 7-day interval without refilling. Food intake per mouse was calculated as the mean of the total amount of food consumed divided by the number of animals per cage. We aimed to select at random nine females and nine males from each of the four dietary regimes for MRI scanning but only eight females were available for the low-folate, low-fat group. A total of 71 mice were selected for measurement of adiposity by MRI scanning at 13 and at 26 wk of age. Offspring were killed at 28 wk of age for tissue analysis.

2.2 MRI scanning for adiposity assessment

Whole body ^{1}H MRS spectra and whole body MRI were performed as described by So et al. [18], to measure adiposity at 3 and 6 months of age. Anesthesia was induced and maintained by gaseous isoflurane (1–2% in oxygen), with heart rate monitoring throughout the scanning procedure. MR images were obtained on a horizontal bore Varian Inova 7T system (Varian Inc., CA, USA) and 72 mm diameter quadrature volume coil (Rapid GmbH, Wuerzburg, Germany). A standard spin-echo MRI sequence was employed with repetition time 2200 ms, echo time 20 ms, matrix size 256 × 256, field of view 48 × 48 mm, 1 average and 48 contiguous slices of 2-mm thickness. Mice were returned to cages after the procedure for recovery from the anesthetic. Total percentage adiposity was calculated as described previously [19].

2.3 Sample collection

Dams were anesthetized using gaseous isoflurane (mean 22 days postpartum), blood removed by cardiac puncture and animals killed by cervical dislocation. Blood was allowed to clot, centrifuged for 10 min, 10 000 × *g*, at 4°C, and serum collected and stored at −80°.

At 28 wk of age, offspring were anesthetized using gaseous isoflurane, blood removed by cardiac puncture, and animals killed by cervical dislocation. Blood was collected in 1.3 mL pediatric blood glucose tubes (NaF/EDTA) (Greiner bio-one, 459085) and centrifuged at 300 × *g* for 15 min at 4°C to separate plasma. Plasma and cell fractions were stored separately at −80°C. The liver, cecum, right kidney, left kidney, spleen, and heart were removed and weighed. The small intestine (SI) and colon lengths were measured. The SI and colon were opened longitudinally and, after removing and discarding contents and washing with PBS, were weighed empty. All tissues were snap frozen in liquid nitrogen and stored at −80°C.

2.4 Dam serum folate concentrations

Frozen serum samples were sent on dry ice to BEVITAL (Laboratoriebygget, Bergen, Norway) for analysis for serum folate concentrations using a microbiological assay.

2.5 Plasma metabolites measurement

Plasma glucose and triacylglycerol (TAG) were measured with commercial kits (Randox Laboratories Ltd., Crumlin, Northern Ireland, UK, GL2623, TR210) using an automated spectrophotometric method on a Cobas Mira clinical analyzer (Roche Diagnostics, Welwyn Garden City, UK). All samples were analyzed in parallel with the respective quality controls provided by the supplier on the same day and in the same assay.

2.6 Image analysis

A total of 139 MRI scans were taken on 70 mice, that is, two scans per mouse (one mouse was removed from the study before second scan due to severe weight loss resulting from tooth curling). Of the remaining 69 scan pairs, MRI scan quality was insufficient for at least one image from six pairs, which were removed from analysis. MRI scans were obtained for 63 mice at both 3 and 6 months, generating 126 MRI scans, which were analyzed using the fat analysis tool software (courtesy of Dr. Pawel Tokarczuk, Experimental MRI Centre, University of Cardiff) to calculate fat volume (mL). All scans were analyzed independently by two researchers who developed, validated, and adhered to a set of Guidelines (Xie L. et al. Unpublished). The Guidelines aimed to be maximally inclusive of areas of fat but minimize inclusion of breathing movements captured on the images. Fat mass (g) was calculated by multiplying fat volume by fat density [20]. Lean mass was calculated by subtracting the fat mass from total body weight. Data generated by the independent researchers were found to be highly correlated, with no statistical difference between the two total means for fat mass (researcher 1; mean = 15.179 g fat (SEM = 0.422) researcher 2; mean = 15.170 g fat (SEM = 0.422) $p = 0.9880$. A mean of the independent measurements obtained by the two researchers was used for analysis.

2.7 Statistical analysis

All statistical analyses were carried out using SPSS statistics Version 19. All data distributions were examined by the Kolmogorov–Smirnov test and were normally distributed. Pearson Chi-square test was used to assess the total number of pregnancies and incidences of reproductive failure (i.e. either miscarriage or postpartum litter death) in relation to maternal folate supply. Analysis of variance was used to examine the effect of maternal diet on litter size, maternal serum folate concentrations, and body weight of pups at weaning. A separate analysis of variance was used to examine the effects of sex, maternal diet, and postweaning diet of the adult offspring, and the interaction between these fixed factors, on body and organ weights and dimensions, food and energy intake, body composition measured by MRI and plasma metabolites. Linear regression analysis was used to investigate relationships between food intake and body weight and composition and between plasma glucose and TAG concentrations. $p < 0.05$ was considered statistically significant.

3 Results

3.1 Effects of maternal dietary folate supply on reproductive performance, reproductive success, and litter size

Figure 1 summarizes overall reproductive performance of females for both dietary groups. There was no significant difference between groups for total number of pregnancies/dam (Pearson Chi-square p value = 0.435) and the mean number of days spent on maternal diet for dams with successful pregnancies did not differ between low and normal folate groups ($p = 0.415$). However, females fed the low-folate diet were more likely to experience later reproductive failure, that is, either miscarriage or postpartum litter death, compared with females fed the folate-adequate diet (Pearson Chi-square $p = 0.006$). Low maternal folate intake had no significant effect on litter size (Table 1, $p = 0.742$) but resulted in a 6% reduction in offspring body weight at weaning (Table 1, $p = 0.011$).

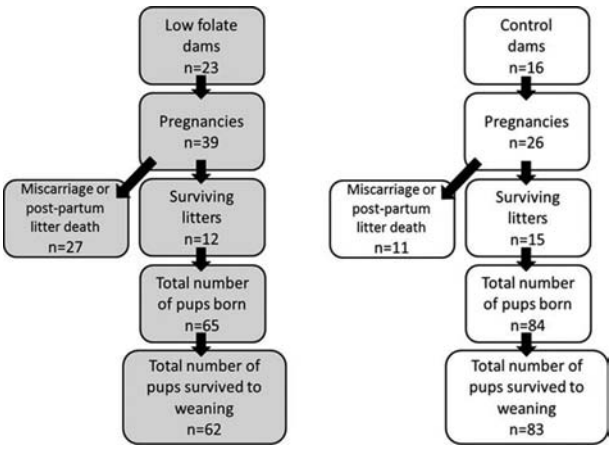

Figure 1. Summary of the reproductive performance of female mice fed low- or normal-folate diets for 4 wk before, and throughout, pregnancy and lactation.

3.2 Effects of dietary folate on maternal serum folate concentrations

Dams fed a low-folate diet had significantly reduced serum folate concentrations (by 70%) compared with mice fed the folate-adequate diet (Table 1, $p < 0.001$).

3.3 Effect of sex, maternal folate supply, and fat content of diet fed from weaning on growth and organ dimensions in adult offspring

There were no significant differences in body weight of offspring on day of randomization to the postweaning diets for those mice derived from low vs. normal folate dams or for those mice randomized to low- or high-fat diets post-weaning ($p = 0.828$ and $p = 0.542$ respectively, Fig. 2).

At 13 and 26 wk of age (time points at which adiposity was assessed by MRI scanning), offspring body weight was unaffected by maternal folate supply ($p = 0.800$ and $p = 0.420$ respectively, Fig. 2). However, as expected, high-fat feeding postweaning resulted in significantly heavier mice at 13 and 26 wk of age ($p < 0.001$ at each time-point, Fig. 2). At baseline, 13 and 26 wk of age, male mice were significantly heavier than females ($p = 0.001$ for each time-point, data not shown).

At death (age 28 wk), male mice were significantly heavier, and had significantly heavier organs (with the exception of the spleen) compared with females (Table 2). Males also had significantly longer SI and colons than females (Table 2). Low maternal folate supply did not influence body weight at death or organ dimensions in adult offspring (Table 2). As expected, high-fat feeding post weaning increased body weight significantly at death in adult offspring compared with mice fed the low-fat diet ($p < 0.001$). Furthermore, weight and length of the SI were significantly increased in high-fat fed mice, as were the colon and right kidney (Table 2). No significant interactions were found between sex, maternal folate supply, or postweaning dietary fat content (data not shown).

3.4 Effect of maternal folate supply, fat content of diet fed from weaning, and sex on food and energy intake in offspring

Food intake was significantly greater in male mice at 9 and 18 wk of age compared with female mice (Fig. 3A), and corresponded with significantly increased energy intake in males at both time points (49.4 and 50.6 kJ/day respectively) compared with females (42.3 and 45.6 kJ/day respectively, $p = 0.003$ for both tests, Fig. 3B). Folate content of maternal diets had no effect on offspring food or energy intake at 9 or 18 wk (data not shown, $p > 0.05$). Despite eating significantly less food (g/day) (Fig. 3C), mice fed the high-fat diet had a higher energy intakes compared with low-fat fed mice at 9 and 18 wk (significant ($p = 0.039$) at 18 wk, Fig. 3D). In addition, there was a significant interaction between postweaning diet and sex at 9 and 18 wk for food and energy intakes. At 9 and 18 wk of age, female mice appeared to be insensitive to the energy density of the diet and ate similar quantities of food, whether offered the low- or high-fat diet (Table 3). In contrast, male mice ate more food when offered the low-fat diet compared with the high-fat diet (27 and 22% greater at 9 and 18 wk respectively, Table 3). As a consequence, female energy intakes were considerably greater on the high-fat diet compared with the low-fat diet whereas male energy intakes were similar on both diets (Table 3).

Table 1. Effect of maternal folate supply on maternal serum folate concentration, litter size, and body weight of pups at weaning

	Low folate	Normal folate	*p*
Dams			
N	12	15	
Mean (SE) maternal serum folate (nmol/L)	23.1 (9.55)	77.7 (8.18)	<0.001
Mean (SE) litter size at birth (pups/dam)	5.4 (0.41)	5.6 (0.37)	0.742
Offspring			
N	62	79*	
Mean (SE) body weight of pups at weaning (g)	7.3 (0.15)	7.8 (0.13)	0.011

*Although 83 pups survived to weaning, body weight data were missing for four pups.

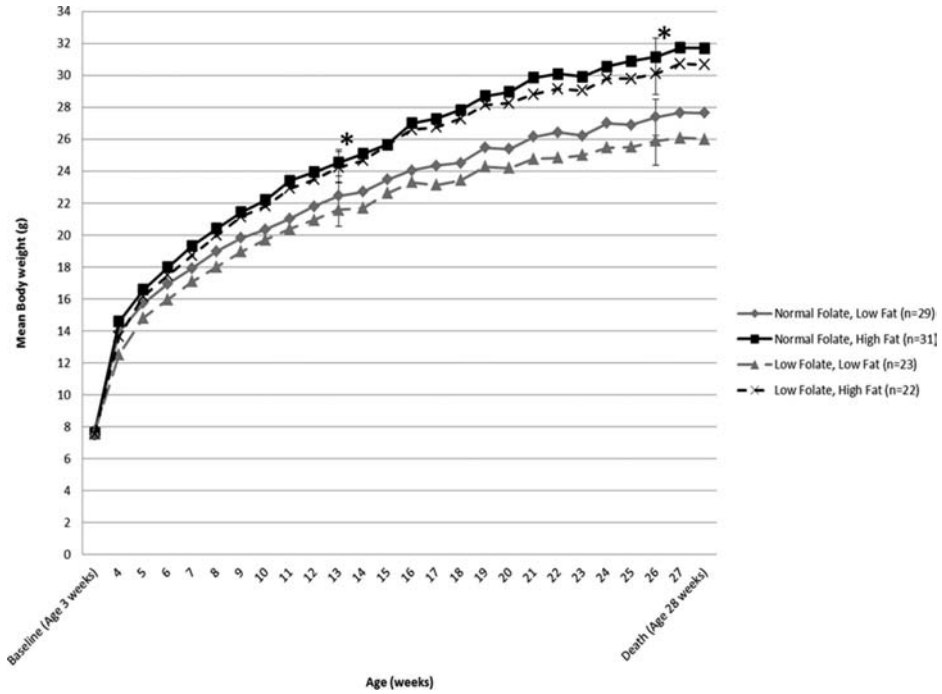

Figure 2. Growth curves from weaning of offspring grouped by dietary regime.

3.5 Effect of sex, maternal folate supply, and fat content of diet fed post weaning on body composition of offspring at 3 and 6 months

Female mice had significantly lower lean and fat masses at 3 and 6 months of age compared with males (Fig. 4, $p < 0.001$). Folate content of the maternal diet during pregnancy and lactation had no effect on lean or fat mass in adult offspring at 3 or 6 months of age (Fig. 4). However, high-fat feeding from weaning caused increased fat, but not lean, mass in adult offspring at 3 and 6 months (Fig. 4, $p < 0.001$ at both time points for fat mass).

3.6 Effect of sex, maternal folate supply, and fat content of diet fed post weaning on plasma glucose and TAG concentrations in adult offspring

Male mice had significantly higher plasma glucose concentrations compared with females (Fig. 5A, $p = 0.034$). Adult offspring of low folate fed dams had significantly lower plasma glucose concentrations compared with those born to dams fed the folate-adequate diet (Fig. 5A, $p = 0.010$) but had higher plasma TAG concentrations (Fig. 5B, $p = 0.048$).

Feeding a high-fat diet from weaning increased plasma glucose and TAG concentrations compared with feeding the low-fat diet (Fig. 5A and B, $p = 0.023$ and $p = 0.049$ respectively). Importantly, there was a highly significant ($p = 0.005$) interaction between maternal folate supply and postweaning dietary fat intake on plasma TAG concentrations (Fig. 5C), which was not evident for plasma glucose concentration. For offspring of normal folate fed dams, there was no effect of postweaning dietary fat content on plasma TAG concentration. However, offspring from folate-depleted dams had 30% higher TAG concentration when high-fat post weaning than for those fed the low-fat diet (Fig. 5C).

4 Discussion

Evidence suggests that inadequate folate intake during pregnancy produces adverse effects on offspring in rodents [21, 22], and that inadequate maternal folate before and during pregnancy increases the risk of neural tube defects in humans (reviewed in [23]). Depending on the degree of undernutrition, reducing folate supply during pregnancy can cause spontaneous abortion, teratogenic effects in offspring, and reduced litter number in rodents [21, 24]. Here, we fed dams mildly folate-depleted diets (0.4 mg folic acid/kg diet), which we have reported produces viable litters [17, 25] without evidence of teratogenic effects but may reduce litter size compared with a control (2 mg folic acid/kg diet) diet [25]. In this respect, our observations may be relevant to human pregnancies/lactations in which the mothers have low, but not deficient, folate status. In the present study, we did not detect reduced litter size, but the low-folate diet was detrimental to reproductive success. Compared with control pregnancies, there was a 64% excess of miscarriage or postpartum litter death for low folate fed dams, suggesting that folate supply decreased capacity to carry pregnancy to term or rear pups effectively. Previous studies have reported lower

Table 2. Effects of sex, maternal folate, and postweaning fat diets on body weights and organ dimensions at 28 wk of age in adult offspring

n	Sex			Maternal diet			Postweaning diet		
	Male mean (SE) 55	Female mean (SE) 50	*p* Value	Low folate mean (SE) 45	Normal folate mean (SE) 60	*p* Value	High-fat mean (SE) 53	Low-fat mean (SE) 52	*p* Value
Body weight (g)	33.8 (0.65)	24.3 (0.67)	**<0.001**	28.4 (0.70)	29.3 (0.61)	0.387	31.0 (0.66)	26.7 (0.66)	**<0.001**
Liver weight (g)	1.69 (0.064)	1.095 (0.066)	**<0.001**	1.33 (0.070)	1.456 (0.061)	0.178	1.476 (0.065)	1.312 (0.065)	0.078
Small intestine weight (g)	1.13 (0.038)	0.96 (0.039)	**0.003**	1.07 (0.041)	1.01 (0.036)	0.310	1.11 (0.039)	0.96 (0.039)	**0.011**
Small intestine length (cm)	30.2 (0.24)	28.1 (0.24)	**<0.001**	29.1 (0.26)	29.3 (0.22)	0.573	29.5 (0.24)	28.8 (0.24)	**0.037**
Cecum weight (g)	0.41 (0.012)	0.28 (0.013)	**<0.001**	0.36 (0.014)	0.33 (0.012)	0.110	0.34 (0.013)	0.36 (0.013)	0.736
Colon weight (g)	0.17 (0.005)	0.15 (0.005)	**0.001**	0.15 (0.006)	0.17 (0.005)	0.097	0.17 (0.005)	0.15 (0.005)	**0.003**
Colon length (cm)	6.2 (0.09)	5.5 (0.09)	**<0.001**	5.7 (0.10)	5.9 (0.09)	0.126	5.8 (0.09)	5.9 (0.09)	0.794
Right kidney weight (g)	0.195 (0.003)	0.148 (0.003)	**<0.001**	0.176 (0.003)	0.168 (0.003)	0.061	0.176 (0.003)	0.167 (0.003)	**0.039**
Left kidney weight (g)	0.183 (0.003)	0.143 (0.003)	**<0.001**	0.165 (0.003)	0.162 (0.003)	0.563	0.167 (0.003)	0.159 (0.003)	0.096
Spleen weight (g)	0.11 (0.004)	0.10 (0.005)	0.194	0.11 (0.005)	0.10 (0.004)	0.090	0.11 (0.005)	0.10 (0.005)	0.846
Heart weight (g)	0.22 (0.006)	0.18 (0.007)	**<0.001**	0.20 (0.007)	0.20 (0.006)	0.545	0.20 (0.007)	0.20 (0.007)	0.796

p values highlighted in bold represent statistically significant ($p < 0.05$) differences between dietary groups.

Table 3. Interaction between sex and post weaning dietary fat content on intakes of food (g/day) and of energy (kJ/day)

	n	9 Weeks						18 Weeks					
		Food intake (g/day)	SE	P for interaction	Energy intake (kJ/day)	SE	P for interaction	Food intake (g/day)	SE	P for interaction	Energy intake (kJ/day)	SE	P for inter-action
High fat													
Male	28	2.629	0.11	**0.002**	47.3	2.26	**0.019**	2.675	0.088	**0.009**	50.2	1.55	**0.026**
Female	25	2.531	0.115		45.6	2.34		2.608	0.092		49.0	1.63	
Low fat													
Male	27	3.333	0.108		51.5	2.26		3.269	0.09		50.6	1.59	
Female	25	2.52	0.111		38.9	2.34		2.722	0.092		42.3	1.63	

p values highlighted in bold represent statistically significant ($p < 0.05$) differences between dietary groups.

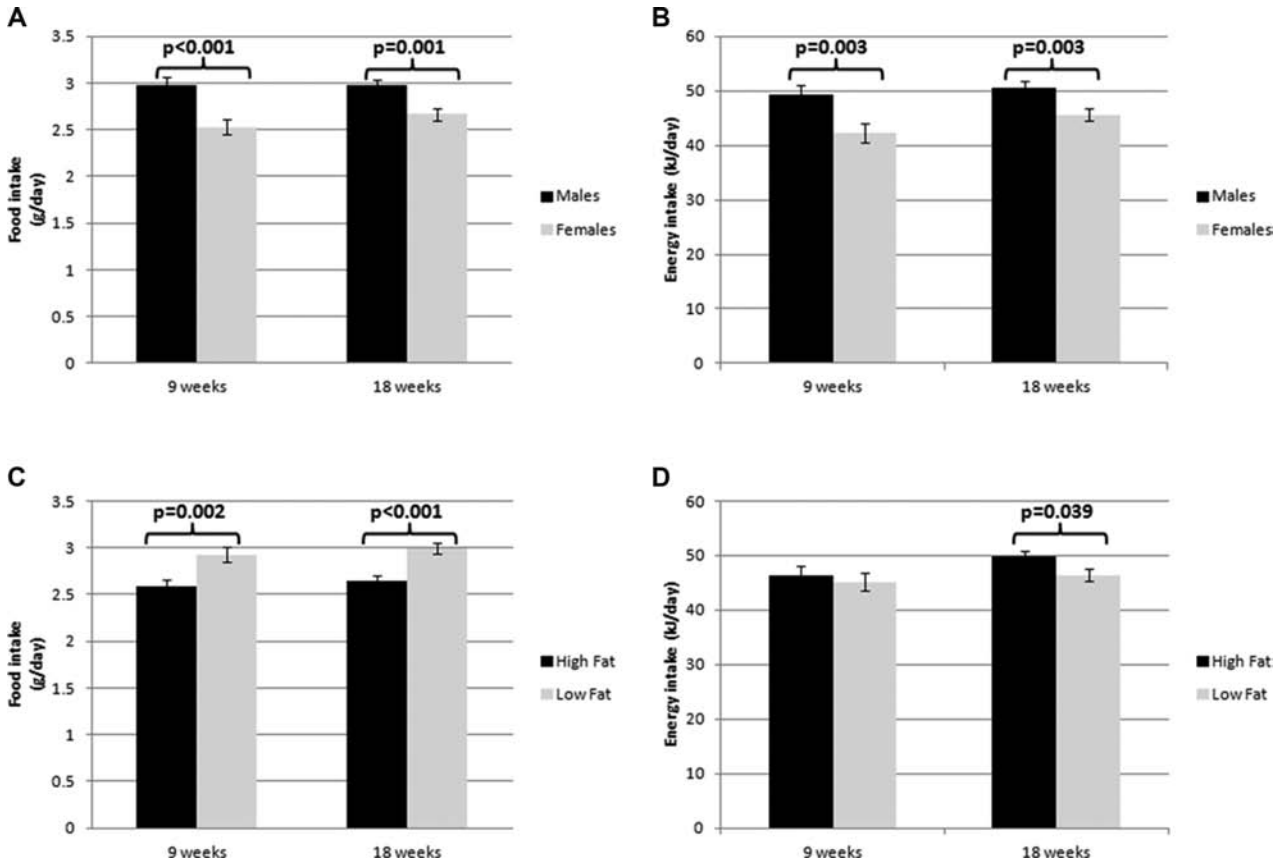

Figure 3. Effect of sex and postweaning dietary fat content on food and energy intake of offspring (bars represent SE). (A) Daily food intake in males ($n = 55$) and females ($n = 50$) at 9 and 18 wk of age. (B) Daily energy intake in males ($n = 55$) and females ($n = 50$) at 9 and 18 wk of age. (C) Effect of postweaning dietary fat content on food intake at 9 and 18 wk of age. (D) Effect of postweaning dietary fat content on energy intake at 9 and 18 wk of age (high fat group $n = 52$, low-fat group $n = 53$).

reproductive success, measured as reduced litter size or increased number of fetal reabsorptions, in animals fed low-folate diets [21, 24, 26, 27] but, to our knowledge, no previous study investigating the influence of maternal folic acid intake has commented on the capacity to carry pregnancies to term or on rearing of pups to weaning. Furthermore, pups born to folate-depleted mothers were 6% lighter at weaning ($p = 0.011$) compared with those born to controls (confirming our previous observation [25]), which may reflect a lower birth weight and/or indicate reduced postpartum growth. Both rodent and human studies have reported that low maternal folate status during pregnancy leads to lower birth weight [24, 26–31]. Given the considerable evidence that low birth weight is associated with increased risk of developing of noncommunicable diseases in adulthood including cardiovascular disease, diabetes, and MS [1], we hypothesize that inadequate maternal folate intake may contribute to the development of such diseases via its influence on birth weight.

Maternal diet during development may affect profoundly the metabolic programing of offspring, resulting in perturbations in metabolic markers, which may be indicative of the development of MS in later life (reviewed in [32]). Here, reduced maternal folate intake during pregnancy and lactation produced divergent effects on MS markers, without any effect on obesity. Adult offspring from folate-depleted dams had lower plasma glucose but increased plasma TAG concentrations (Fig. 5). Folic acid supplementation during pregnancy in rats resulted in increased plasma glucose and TAG concentrations in offspring [14]. In contrast, Chmurzynska et al. reported decreased circulating glucose concentration in 10-wk-old, but not 16-wk-old, rat offspring and lower TAG in 16-wk-old offspring born to folic acid-supplemented dams [33]. When maternal folic acid supplementation was coupled with protein restriction, plasma glucose and TAG were reduced in adult rat offspring [14]. Several factors, including offspring age, dietary differences between studies, and species differences, may underlie the discordance in effects of maternal folate supply on markers of MS in rodents. To resolve these differences, it will be important to explore dose-responses to altered maternal folate supply, as well as reproductive stage, and duration of nutrient manipulation, to understand the potential effects of folate nutrition during early development on the offspring's metabolic health.

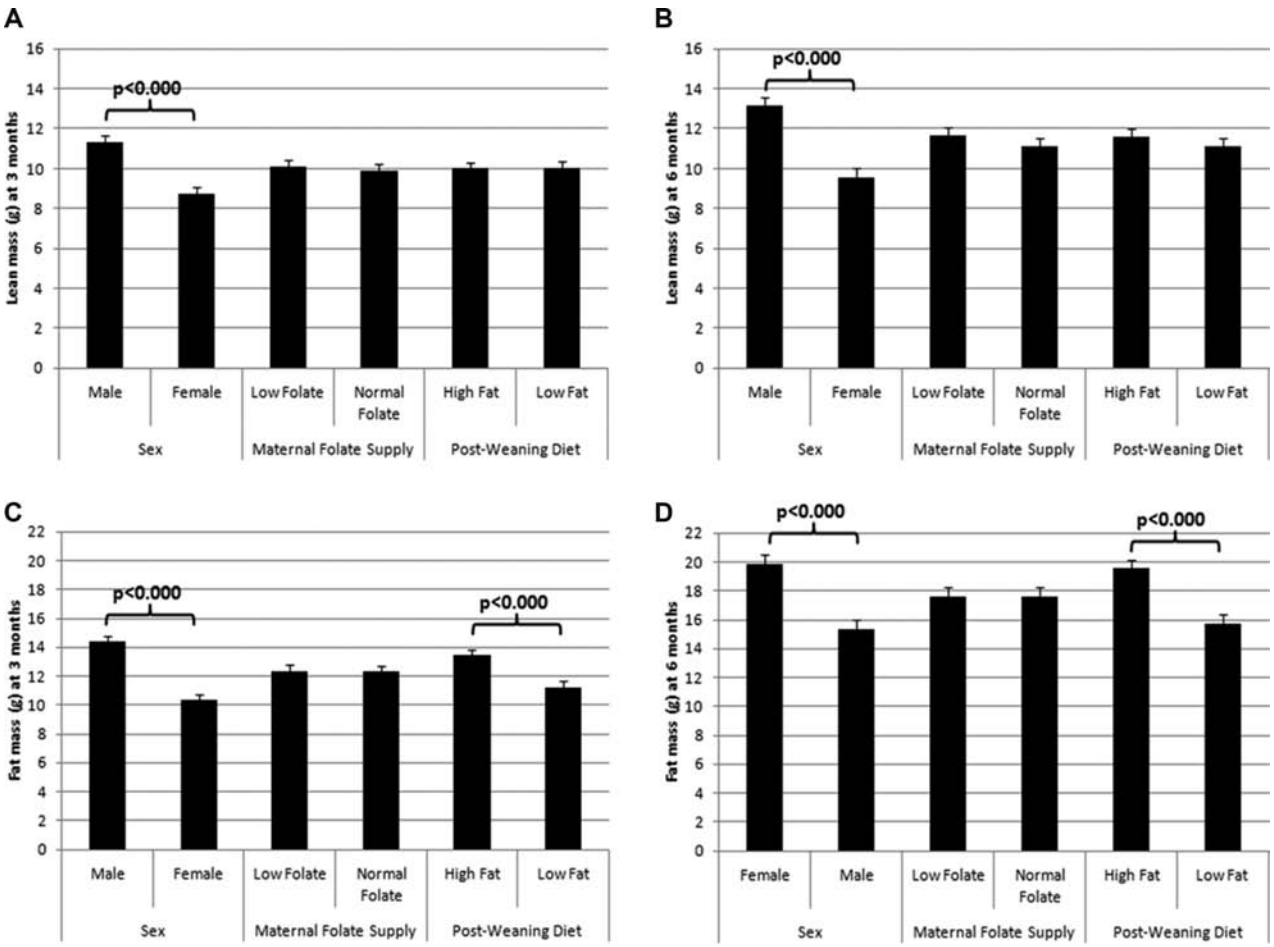

Figure 4. Effects of sex, maternal, and postweaning diets on lean and fat mass in adult offspring. (A) Lean mass at 3 months. (B) Lean mass at 6 months. (C) Fat mass at 3 months. (D) Fat mass at 6 months. (Bars represent SE) (males $n = 32$, females $n = 31$, maternal low folate $n = 29$, maternal normal folate $n = 34$, postweaning high fat $n = 35$, postweaning low fat $n = 28$).

In this study, we made the novel observation that inadequate maternal folate supply resulted in offspring that were more susceptible to the adverse metabolic effects of high-fat feeding. As shown in Fig. 5, feeding a high-fat diet post weaning had no effect on plasma TAG in offspring whose mothers were well nourished in respect of folate. However, offspring whose mothers were folate depleted had increased plasma TAG if high-fat fed post-weaning. To our knowledge there are no comparable published data. However, it is of interest that maternal folate supplementation during pregnancy in rats fed low-protein diets, reduced plasma TAG concentration in offspring fed a high-fat diet post weaning [14, 33].

Maternal folic acid supplementation (5 mg folic acid/kg diet compared with 1 and 2 mg/kg diet respectively) has been reported to decrease body weight in offspring [14, 33], and we previously observed increased offspring body weight at 14 wk of age in response to low maternal folate intake during pregnancy in a pilot study (0.4 mg folic acid/kg diet compared with 2 mg folic acid/kg diet) (McKay et al., unpublished data). However, in the present study we observed no significant ($p > 0.05$) effects of maternal folate depletion on body weight or adiposity in the adult offspring at 3 or 6 months of age. The relatively mild level of folate restriction applied in the current study (used to mimic levels of folate nutrition likely during human pregnancy) may account for these discordant observations.

In this study, there were notable sex differences in ingestive behavior responses to high-fat feeding. Male mice appeared to compensate for the higher energy density of the high-fat diet by reducing the quantity of food consumed while females ate similar weights of both low- and high-fat diets resulting in greater energy intakes when offered the high-fat diet. These findings are in line with the inter-sex differences in response to a high-fat diet reported previously for C57BL/6N mice by Medrikova et al. [34]. The mechanisms responsible for this sex-specific effect on energy intake in response to high-fat feeding are unknown but may be due in part to sex hormones, which appear to play a role in altered appetite regulation between the sexes [35, 36].

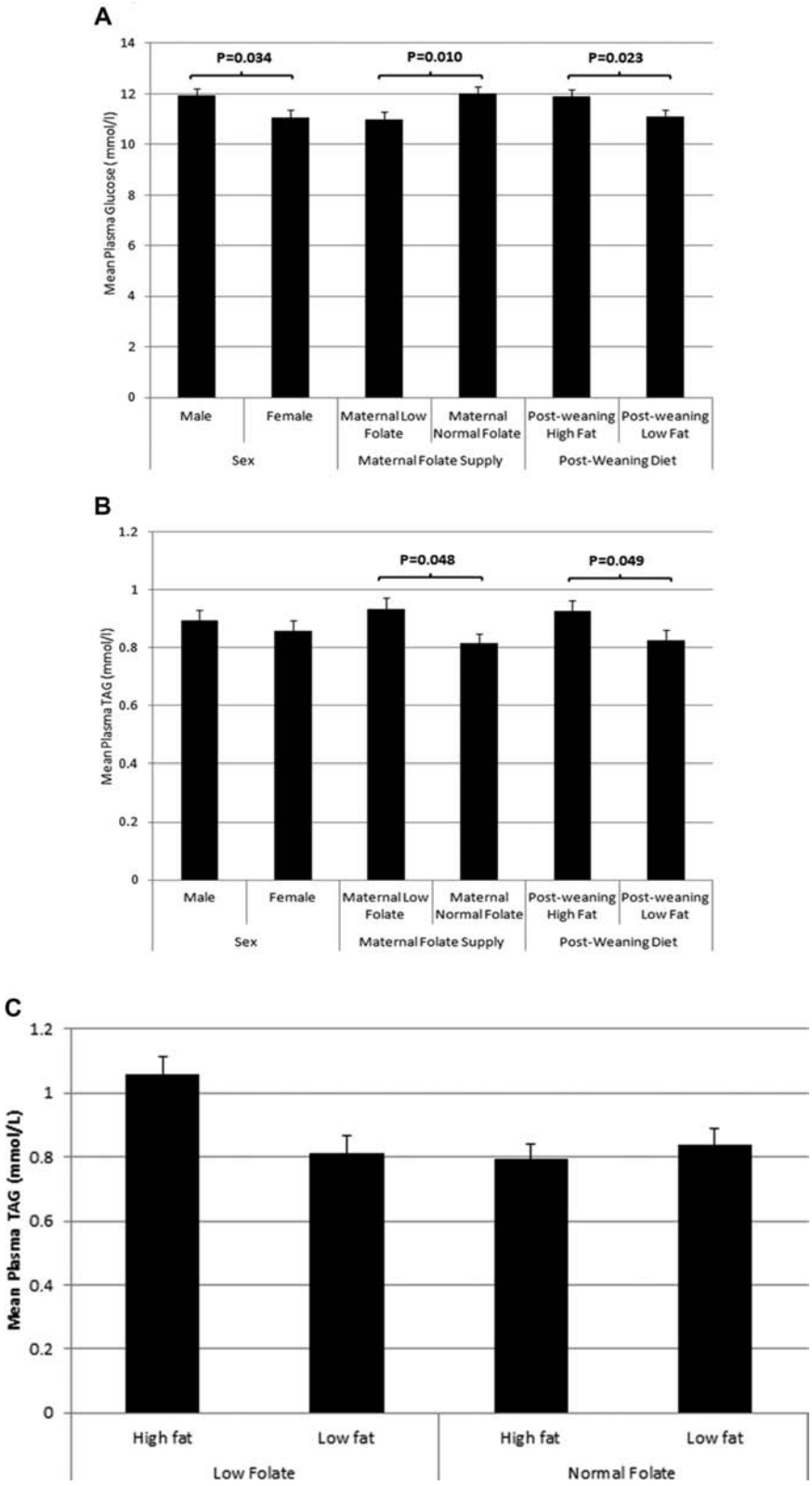

Figure 5. Effects of sex, maternal folate supply, and postweaning dietary fat content on (A) plasma glucose and (B) TAG in adult offspring. (bars represent SE; males $n = 55$, females $n = 50$, maternal low folate $n = 45$, maternal normal folate $n = 60$, postweaning high fat $n = 52$, postweaning low fat $n = 53$). (C) Interaction between maternal folate supply and high-fat feeding from weaning on plasma TAG concentration in adult offspring. ($p = 0.005$ for interaction; normal folate, low fat $n = 29$, normal folate, high fat $n = 31$, low folate, low fat $n = 23$, low folate, high fat $n = 22$).

In conclusion, this study reveals that inadequate intake of folate during pregnancy and lactation has long-term effects on metabolism of the offspring, and these effects are modulated by fat intake post weaning. Importantly, we show that low maternal folate supply results in offspring that are more susceptible to detrimental metabolic effects of a high-fat diet, manifested as increased circulating TAG concentration. Interestingly, this metabolic phenotype in adult offspring occurred without any detectable change in adiposity, suggesting a different etiological origin from the more commonly reported association between maternal undernutrition leading to increased offspring adiposity and to symptoms of MS (reviewed [37]). Several potential mechanisms may be responsible for the long-term metabolic effects on adult offspring of low maternal folate intake during pregnancy and lactation. Our observations suggest that low maternal folate supply altered offspring programing of key pathways regulating metabolism (exemplified by changes in serum glucose and TAG concentrations) and, importantly, influenced how the offspring from low-folate dams responded to a high-fat diet post weaning. Given the important role of folate in one carbon metabolism, and in particular for the conversion of *S*-adenosylhomocysteine to the universal methyl donor *S*-adenosylmethionine, it is possible that such programing effects may occur via epigenetic mechanisms. There is accumulating evidence that folate intake influences epigenetic mechanisms including DNA and histone methylation (reviewed in [38]), which have key roles in regulating gene expression. Since epigenetic patterns are established in utero it is plausible that altered folate nutrition during critical windows of development may impact on epigenetic programing, and thus influence offspring long-term health as observed here. We are investigating this hypothesis in on-going studies. In humans, the adverse effects of poor maternal folate supply on offspring long-term metabolic health may be of particular importance in countries that are undergoing a nutritional transition, where maternal micronutrient (including folate) status is low and where children are exposed increasingly to higher fat "Western-style" diets. Further longer term studies of the benefits of improved maternal folate nutrition on offspring health are warranted.

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