Maternal preconceptional nutrition leads to variable fat deposition of adult offspring mice (C57BL/6JBom)

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Abstract

Maternal nutrition during pregnancy or lactation may affect the chance of offspring becoming obese as adults, but little is known about the possible role of maternal nutrition before conception. Here we investigate how variable protein and carbohydrate content of the diet consumed before pregnancy affects fat deposition of offspring mice. Eight week old female mice (C57BL/6JBom) were fed isocaloric low protein (8.4% protein; LP), standard protein (21.5% protein; ST) or high protein (44.2% protein; HP) diets. After eight weeks of feeding, females were mated and fed a standard laboratory chow diet (22.5% protein) throughout periods of mating, gestation, lactation and weaning. Offspring mice were fed the same standard diet until 46 days of age. Then offspring were killed and measures of dissected fat deposits were taken. Fat deposition of the offspring was significantly affected by preconceptional maternal nutrition and the effects differed among sexes. Male offspring deposited most fat when mothers were fed LP diet, whereas female offspring deposited most fat when mothers were fed the ST diet. Our study demonstrates that preconceptional nutrition can have important influence on fat deposition in offspring in mice.

Keywords: protein; maternal diet; fat deposition

Introduction

Early nutritional programming is a concept in which differences in nutritional experience during critical periods of the early life stages program later development of metabolism and different health problems (Lucas, 1998; Patel and Srinivasan, 2002). Evidence of metabolic programming stems from both animal studies and epidemiological studies on humans (Taylor and Poston, 2007; Robinson, 2007). For example, increased preference for high fat foods was developed *in utero* as an effect of protein poor fetal diet in rodents (Ozanne *et al.,* 2004; Bellinger *et al.,* 2004).

Focus has recently been placed on the epigenetic determinants of obesity, especially through the maternal diet during pregnancy and breast feeding (Baker, 2004, Demmelmair and Rosen, 2006). Several studies have shown that early nutrition can cause subsequent health problems in the offspring, and that both pre- and postnatal diet treatments can program later development, metabolism and health. For example, low protein intake of pregnant mothers elevated blood pressure in offspring rats (Langley-Evans *et al.,* 1999), and increased later weight gain in mice offspring due to an elevated intake of high fat foods (Ozanne *et al.,* 2004). In rats (Lucas *et al.,*

1996), it was demonstrated that both pre- and postnatal stages are sensitive periods for nutritional programming, and dietary protein during pregnancy or lactation affected plasma concentrations of cholesterol and triacylglycerols in the offspring.

Although a link between prenatal nutrition and offspring obesity has been established over the past decade, less is known about the consequences of maternal diet and maternal body composition before pregnancy. In rats, blood glucose level of offspring may be affected by maternal body composition before pregnancy and a maternal low protein diet may increase blood glucose of the offspring, when they reach adulthood (Joshi *et al.,* 2003a). Thus, it seems that maternal body composition at the time of conception may affect metabolism and the obesity risks of the offspring. Considering that adult health might be programmed early in life, it is interesting to study the nutritional basis for effects of maternal diet on the offspring. Previous studies have investigated how a variety of nutritional compounds including fish oils (Hartvigsen *et al.,* 2003; Joshi *et al.,* 2003b), vitamins (Javaid *et al.,* 2006; Watson and McDonald, 2007), minerals (Hostetler *et al.,* 2003) and macronutrients (Saldana *et al.,* 2004; Moore and Davies, 2005) may affect offspring phenotype, but most studies have concerned protein composition in the mother's diet during pregnancy or lactation and obesity risk of offspring (Taylor and Poston, 2007; Bellinger *et al.,* 2004; Gorski *et al.,* 2006; Howie *et al.,* 2009).

In this study, we investigate how variation in protein to carbohydrate balance of maternal food before mating affects fat deposition of adult offspring mice.

Materials and methods

Housing and general procedures

Housing and general procedures are described in Mortensen *et al.* (2010).

Experimental diets

Three types of custom made experimental diets and a standard laboratory chow diet (dry, in pellet form) were purchased from Brogaarden /Lynge, Denmark) and manufactured by Altromin GmbH Company (Lage, Germany). The experimental diets were isocaloric and similar in composition, except for a variation in the ratio of protein to carbohydrate (Table 1). Breeding mice/rat diet Altromin 1314 was used as the standard laboratory chow diet. Before the mice were purchased, they were fed with a NIH-31 diet, with 18% protein, 4% fat, 5% fibre and 8% ash content.

Data analysis

To analyse the variable total body fat deposits, an analysis of covariance model was used with maternal diet and mouse gender as factors. When testing the mass of total fat deposits, we used mouse wet mass minus the removed fat deposits as the covariate. Throughout all these analyses, litter size was added as a covariate in the initial model and removed again if nonsignificant, which was always the case. If we found significant interactions between the maternal diet factor and gender, we conducted a new test on each of the genders, separately. Homogeneity of variances

Table 1. Composition of three types of experimental diets and one standard laboratory chow diet (g/100 g dry mass). The experimental diets, low protein (LP), standard protein (ST) and high protein (HP) varied in protein and carbohydrate concentrations, where other ingredients were kept similar. Main ingredients: casein, cornstarch, wheat starch, sucrose, sunflower oil, cellulose powder, a mineral mix (K_2CO_3, Coal.Chalk, NaCl, $MgCl_2$, K_2HPO_4, Fe_2O_3, $FeSO_4$, $MnSO_4$, NaF, KI, Na_2SeO_3, Na_2MoO_4, $CoCl_2$, $ZnCO_3$, $CuSO_4$) and a vitamin mixture (C 1000, Altromin).

Diets	LP	ST	HP	STlab
Crude protein %	8.4	21.5	44.2	22.5
Carbohydrates %	60.0	47.2	26.3	50.5
Crude fat %	7.2	7.2	7.1	5.0
Crude fibre %	5.0	5.1	5.4	4.5
Ash %	5.0	5.1	5.1	6.5
Energy (kcal/g)	3.3	3.3	3.5	3.0

was tested using Levene's test (P>0.05). If this assumption was violated, we tried using one of the following transformations log(x), √x, 1/√x, x^2 or $1/x^2$. If variances were still unequal, we tested the two genders individually. Detailed differences of various fat deposits were tested using analysis of variance (ANOVA). Differences between feeding treatments in each variable were analyzed using Student's t-test. All statistics were done using JMP 7 (SAS Institute Inc., Cary, USA).

Results

Females feeding on the different diets did not consume different amount of energy (ANOVA, $F_{2,23}$ = 0.09, P=0.92) or mass (ANOVA, $F_{2,23}$ = 1.36, P=0.28) over the first 3 weeks of the preconceptional period. However, there was a large difference in the amount of consumed carbohydrates (g/day±SE.: LP=1.52±0.05, standard protein (ST)=1.21±0.03, high protein (HP)=0.63±0.03; ANOVA, $F_{2,23}$=120.7, P<0.0001) and protein (g/day±SE: LP=0.21±0.007, ST=0.55±0.01, HP=1.06±0.04; ANOVA, $F_{2,23}$=506.2, P<0.0001).

Although maternal diet did not affect body length (ANOVA, $F_{2,68}$=0.21, P=0.81) or body wet mass (ANOVA, $F_{2,68}$=0.73, P=0.49) of male offspring (Figure 1), we found significant effects on length (ANOVA, $F_{2,51}$=3.50, P=0.04) and body wet mass (ANOVA, $F_{2,51}$=3.87, P=0.03) of female offspring. Females of mothers fed LP diets were significantly shorter and lighter than those whose mothers had been fed ST diet or HP diet (Figure 1a,b).

Fat deposits

The total mass of fat deposits (subcutaneous, genital and kidney) varied positively with the covariate total fat-free mass of the mice (Table 2). We found a significant interaction between gender and maternal diet on total body fat (Table 2), which reflects that female offspring of mothers fed the ST diet had the largest fat deposits while male offspring had the most fat

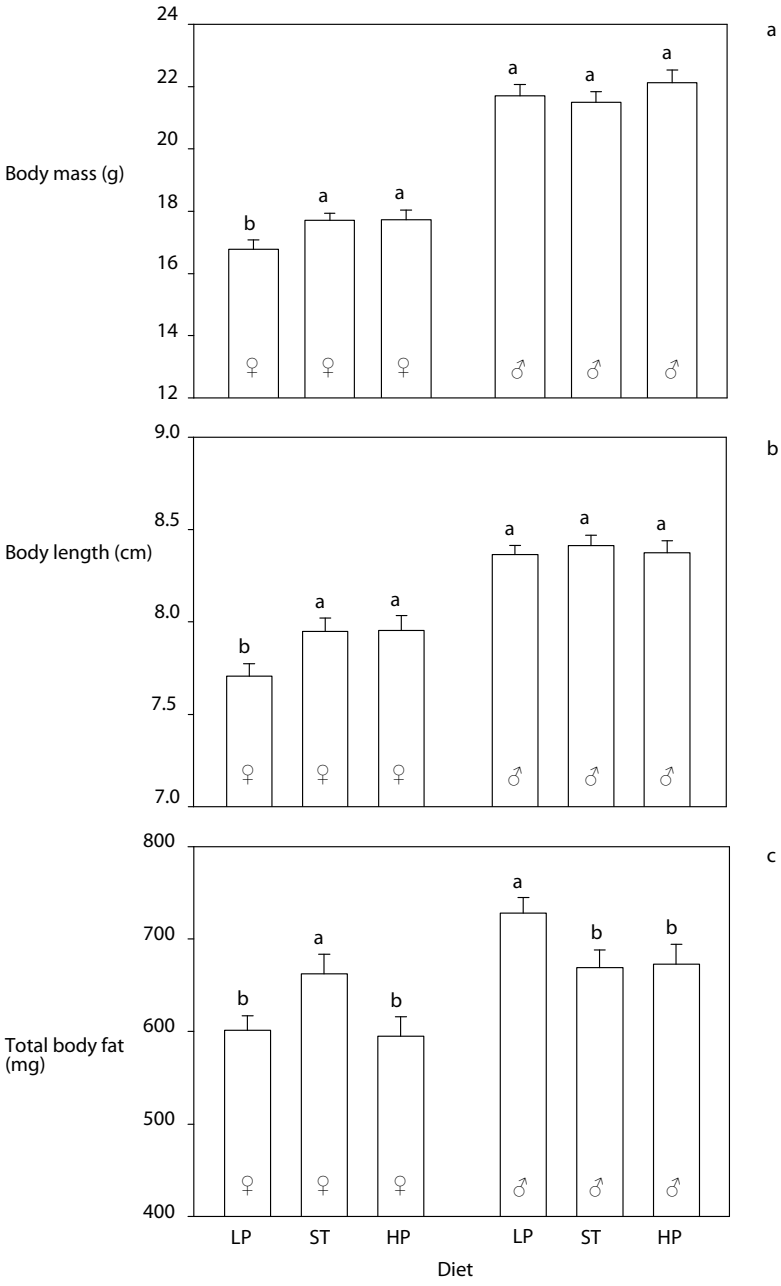

Figure 1. Effects of maternal preconceptional diet on (a) body mass, (b) total body fat and (c) body length of offspring at 46 days of age (mean±SE). Maternal experimental diets were given eight weeks before mating and a standard laboratory diet were fed to females and offspring during mating, lactation, weaning and to offspring during their entire lives. Letters indicate differences across maternal feeding treatments within each gender using Student's t*-test.*

Table 2. ANCOVA on total body fat, in offspring with maternal diet and mouse gender as factors. Covariates were included to adjust variables for total size or mass of the mice. In all analysis, litter size was used as a covariate in the initial model but it was never significant and was therefore removed in the final model.

Source of variation	DF	F	*P*-value
Total fat analysis			
Gender	1	0.32	0.57
Maternal diet	2	2.90	0.06
Gender × maternal diet	2	3.75	0.03
Wet mass minus total fat	1	15.45	<0.0001
Total fat – males			
Maternal diet	2	5.14	0.008
Wet mass minus total fat	1	29.96	<0.0001
Total fat – females			
Maternal diet	2	3.87	0.03
Wet mass minus total fat	1	0.62	0.43

when maternal diet was LP diet (Figure 1c). The separate analysis on each gender confirmed a significant effect of maternal diet on both males and females (Table 2). As this model adjusted for total fat-free mass, the result indicates that it is the relative amount of fat and not total body mass of the mice that was affected by maternal nutrition. This effect is also illustrated by the fact that the groups having significantly highest fat deposits (Figure 1c) did not have a significantly higher body mass (Figure 1a).

Focusing on the separate sections of fat deposits, females had more subcutaneous fat and kidney fat when mothers were fed the ST diet compared to LP and HP diets. No significant difference in genital fat was found (Table 3). The largest effect of maternal nutrition in males was found in

Table 3. Effects of maternal nutrition on fat deposits (mg). Maternal diets were low protein (LP), standard protein (ST) and high protein (HP) and they were fed to females for eight weeks until conception.

Gender	Female			Male		
Maternal diet	**LP**	**ST**	**HP**	**LP**	**ST**	**HP**
Subcutaneous fat mass	319.6±8.6^{a}	360.3±15.7^{b}	317.9±11.5^{a}	329.5±7.9^{a}	296.7±7.4^{b}	296.2±7.7^{b}
Genital fat mass	174.3±5.6	187.5±6.2	175.9±8.2	275.1±6.8	254.1±9.7	257.6±10.5
Kidney fat mass	107.4±3.2^{ab}	114.4±2.7^{a}	100.8±4.0^{b}	123.2±3.2	118.2±3.8	119.1±4.5

^{a,b} Letters indicate differences across maternal feeding treatments within each gender using Student's *t*-test. The test was only made if the initial ANOVA test found an overall significant effect of maternal nutrition.

subcutaneous fat mass, while differences in genital and kidney fat deposits were non-significant (Table 3).

Discussion

Diet composition during pregnancy or lactation is known to alter the risk of obesity in adulthood (Howie *et al.,* 2009). However, this study is, to our knowledge, the first experimental demonstration that diet composition supplied to mice before conception will lead to variable fat deposition in adult offspring.

To test for differences in palatability between the diets, we compared food intakes over the first 3 weeks of the experiment. Our results showed that there were no differences in food or energy intake over this period but large differences in protein and carbohydrate intake. This does not, however, rule out the possibility that there were differences in energy intake over the remaining 5 weeks of the experimental period. In an experiment using the same experimental feed but different strain (NMRI), sex and age group of mice, it was found that both energy consumption and final body composition differed (Sørensen *et al.,* 2008). Thus, it is possible that the females in this study had different energy consumption and/or different body composition when conception occurred and such variation might have contributed to the effect on the offspring. An important role of dietary protein density in the diets of mice (Sørensen *et al.,* 2008), as well as humans (Simpson *et al.,* 2003) and non-human primates (Felton *et al.,* 2009), is that it can influence health through its 'leverage' on calorie intake (Simpson and Raubenheimer, 2005). Although further work is needed to disentangle the effects of dietary protein content on energy intake *per se* vs. the balance of ingested macronutrients, our experiments have shown that the balance of macronutrients in foods of preconceptional females has effects on the developmental trajectories of their offspring.

The period of maximum susceptibility for imprinting of future metabolic physiology of offspring is controversial, but there is good evidence of an effect of maternal nutrition during the suckling period. For instance, rat offspring of lean prenatal dams became obese and insulin resistant when raised postnatally by obese dams (Gorski *et al.,* 2006). Other studies have found that vital effects of protein imbalance occur during different periods of pregnancy in animals (Howie *et al.,* 2009), including humans (Villar *et al.,* 1992; Mardonese-Santander *et al.,* 1998). Our study on mice indicates that protein composition in the maternal diet before conception may also be important in determining fat deposition of the offspring. In rats, variable preconceptional diet affected sizes of heart, brain and kidneys in the offspring, but not offspring body mass (Joshi *et al.,* 2003a). We similarly did not find differences in overall body mass, but fat deposition was significantly affected. Hence, it is possible that the rats also had variable fat deposition in response to preconceptional maternal diets (Joshi *et al.,* 2003a), although this was not reported.

In a previous study, in which young mice of an outbred strain were fed the same diet, males on the LP diet had relatively more body fat after 4.5 weeks of feeding than males on other diets (Sørensen *et al.,* 2008). We fed the dams for 8 weeks on the different diets, so body composition of the females by the time of conception may have been different, and we cannot, therefore, discern direct short-term nutritional effects from longer-term nutritional effects that are mediated by

the body composition of the mother. There is some evidence that body composition in itself may mediate effects on offspring. In sheep, for example, offspring from mothers with low body condition score showed mild glucose intolerance compared with offspring from mothers with a normal body condition score (Cripps *et al.*. 2008). This indicates that the variable body fat of the female sheep, results in different glucose intolerance of offspring.

Fat deposition occurs when caloric intake exceeds expenditure. It is, therefore, an interesting question why male mice in this experiment deposited more fat when dams were fed LP diet. Although we did not measure food consumption in the offspring, other studies indicate that variable fat deposition may be due to differences in feeding behaviour (Sørensen *et al.,* 2008). The hypothalamic pathways responsible for appetite regulation develop already *in utero* (Bouret, 2009, Grove *et al.,* 2003, 2005) and continue to develop during lactation in rats (Grove *et al.,* 2003; Rinaman, 2003). An alternative explanation for the susceptibility of male offspring to maternal LP diet may be that energy expenditure is reduced compared to females, either by reduced basal metabolic rate or lower activity levels (Vickers *et al.,* 2003). Although we have no immediate explanation for the gender differences in our study, such interactions between offspring gender and maternal diets are not uncommon and, given the marked biological differences male and female mammals, not unexpected. For example, pregnant rats exposed to a maternal LP diet throughout gestation had female offspring with higher tendency to become obese than controls, but no effect was observed in male offspring (Bellinger *et al.,* 2004).

We only tested one strain of mice, but there may well be variation in how different strains respond to preconceptional maternal nutrition. Pre-pregnant nutrition may be a factor that can induce different susceptibility to overweight and obesity in adulthood. This is important from a management perspective, because unlike genetic effects diet can readily be altered.

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