

The effect of gestational undernutrition on maternal weight change and fetal weight in lines of mice selected for different growth characteristics

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Abstract

The present study investigated whether the genetic growth characteristics (fast or slow growing, lean or fat) of a mother influences her ability to partition nutrients to developing offspring. A total of sixty-one pregnant mice of three selected lines were used: fast-growing, relatively fat (FF, *n* 19); fast-growing, relatively lean (FL, *n* 23); and normal growth, relatively lean (NL, *n* 19). On day 1 of pregnancy, mice were given either *ad libitum* access to food (control (C): *n* 32) or pair-fed at 80% of C intake (restricted (R): *n* 29). Feed intake and dam weight were measured daily. The weight of the mouse, organs, mammary tissue and the weight of fetuses and placentas were determined at day 18 of gestation. Overall, R dams gained less than half the weight of C dams during gestation. NL dams gained the most weight, and FF dams gained the least weight ($P < 0.001$). R dams in the fast-growing lines mobilised significantly more body fat during gestation than the NL line ($P < 0.001$) and had a greater reduction in mammary tissue growth. The relative weight of the litter increased in R dams of the FF line but was reduced in both the lean lines. Undernutrition reduced fetal and placental weight, and reduced placental efficiency in all the lines. The reduction was least in the FF line and greatest in the FL line. The data suggest that selection of animals for different growth characteristics alters their response to undernutrition during pregnancy, the relatively fat line was better able to buffer its offspring from the effects of undernutrition than the lean lines, regardless of their underlying rate of growth.

Key words: Gestational undernutrition: Fetal weight: Growth characteristics: Mice

The impact of undernutrition in pregnancy, particularly on the developing fetus, has been the subject of much experimental interest in recent years. In general, nutrient restriction during early development has long-lasting consequences for the offspring including reduced birth weights, alterations in fat metabolism^(1–3), hypothalamic–pituitary–adrenal axis reactivity^(4,5), changes in muscle structure and function^(6,7), reduced reproductive capacity^(8–11) and behavioural effects^(12–16). Some of these effects can be permanent resulting in altered health profiles, for example increased obesity and hypertension in adulthood^(17–20), and can be transgenerational in their effects⁽²¹⁾. These findings have obvious implications for human health and development, but more recently there has also been concern about how this may have an impact on the development, productivity and welfare of animals kept for agriculture.

The data from animal studies suggest that genetic lines or breeds of animals may have differing abilities to cope with periods of undernutrition. For example, Scottish

Blackface and Suffolk ewes have different placental characteristics and better placental efficiency (in terms of weight of fetus supported per g of placenta) is seen in the Scottish Blackface breed⁽²²⁾. The Scottish Blackface breed has been adapted to surviving in a harsh environment and also has been subjected to considerably less selection pressure for rapid lean tissue growth compared to the Suffolk. Preliminary data suggest that the birth weight of prenatally undernourished lambs of different breeds are differentially affected⁽²³⁾, and these lambs show differential behavioural responses after birth⁽²⁴⁾. Elsewhere, a study comparing two lines of ewes selected for management under good and poor nutritional conditions, respectively, demonstrated that ewes selected under conditions of limited nutrition were better able to protect their fetuses from the effect of a period of undernutrition⁽²⁵⁾. These data suggest that there may be considerable genotype differences in the impact of undernutrition in pregnancy on subsequent offspring development. To address this in more detail, in the present study, we

Abbreviations: C, control; FF, fast-growing, relatively fat mice; FL, fast-growing, relatively lean mice; NL, normal growth, relatively lean mice; R, restricted; TRARS, thiobarbituric acid-reactive substances.

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made use of the marked variation in growth available in different long-term selected lines of mice^(26–28) to ask whether the growth characteristics of the mother affected her ability to protect her developing fetuses from the consequences of undernutrition *in utero*. We hypothesised that lines selected for rapid lean tissue growth would prioritise their own body tissues at the expense of their fetuses more than mothers from slower growing lines. As our interest in the present study was primarily to examine the impact of selection for rapid lean tissue growth over normal growth patterns, we used lines of mice of similar leanness, but with different growth potential. Further, in order to separate the effects of leanness *per se* from that of rapid growth, we used a third line that also showed rapid growth but was significantly fatter than the other rapid growth line.

Animals, materials and methods

The entire protocol was reviewed and approved by the local experiments and ethics committee (Scottish Agricultural College, Edinburgh, UK), and specific procedures were done under an Animal (Scientific Procedures) Act 1986 Project Licence granted by the Home Office.

Mouse lines

Our initial studies found that some of the inbred mouse lines^(26,28) displaying the required growth characteristics for the study had poor fertility. For example, in one line, only one of the twenty mice seen with a vaginal plug after mating was found to be pregnant at day 18 gestation, and in a second line, eleven of the twenty dams produced less than five pups per litter. Therefore, to improve fertility, hybrid lines were created by mating two or more inbred lines with the required growth characteristics. The lines created were as follows: a fast growth, relatively fat (FF) hybrid line by mating Dummersdorf high and Raleigh high inbred lines (mean adult body weight = 68.4 g); a fast growth, lean line (FL) by mating Roslin high and Edinburgh High inbred lines (mean adult body weight = 43.8 g); and a normal growth, relatively lean line (NL: mean adult body weight = 29.9 g) by reciprocal matings between two high growth, lean lines (Roslin high and Edinburgh high) and two low growth, lean lines (Roslin low and Edinburgh low). For details of the development of the parent lines used to create the hybrid lines used in the present study, see Bünger *et al.*⁽²⁶⁾. The mice used in the present study were F1 crosses of these hybrid lines.

Animals and treatments

A total of seventy-one virgin female mice of three different hybrid lines were used in the study (twenty-four FF, twenty-three FL and twenty-four NL). The mice were housed in a group of six together with a stud male of the

same genotype as themselves. They were checked daily for the presence of vaginal plugs and females with plugs were weighed and placed into individual plastic MB1 cages (45 × 28 × 13 cm; North Kent Plastics Limited, Rochester, UK) with sawdust and paper nesting material. Within the line, the mice were matched for weight (within 10%), and then one of the pair received *ad libitum* access to food (control: C), whereas the other was pair-fed at 80% of *ad libitum* intake (restricted: R). A known weight of food (a standard breeding pelleted diet: Rat and Mouse Number 3: Breeding, Special Diet Services, Witham, Essex, UK supplying 20.2% digestible crude protein and 12.4 MJ/kg DM digestible energy) was offered to the C mice every morning and refusals were weighed the following day. R mice were then offered 80% of the intake of their paired C mouse that day, so the intake of R mice tracked 1 day behind the *ad libitum* intake of C mice. All the mice had *ad libitum* access to clean, fresh drinking-water.

The mouse facility was maintained with controlled lighting (12 h light, 12 h dark) and at a constant temperature of 21°C. All the mice were housed in the same room for the duration of the study.

Measurements

Of the mice, sixty-one were found to be pregnant (nineteen FF: eight C, eleven R; twenty-three FL: twelve C, eleven R; nineteen NL: twelve C, seven R) at day 18 gestation. The mice were weighed daily throughout gestation until day 18 when they were killed by placing into a CO₂ chamber. Once death was confirmed, the gravid uterus was exposed, removed and weighed. The number and location of pups, including mummified or partially reabsorbed fetuses, within each horn, were recorded, and then each pup and its associated placenta were removed. Fetal weight, crown–rump length (CRL), measured with digital callipers, and placental weight were recorded for each pup. Fetal ponderal index (fetal weight/CRL³) and placental efficiency (g of fetus supported/g placenta) were calculated for each pup. The maternal mammary tissue, liver, heart, kidneys, spleen and fat pads (intrascapular and gonadal) were all dissected out and weighed. The ovaries were removed from the uterine horns and the number of ovulations per ovary was determined. Conception rate (number of fetuses/number of ovulations) was calculated for each dam.

The maternal conceptus-free body was weighed and then freeze-dried for the determination of body composition as described previously^(29,30).

Statistics

The effects on maternal measurements were determined using the restricted maximum likelihood procedure⁽³¹⁾ in GenStat 11.1, fitting litter size and the interaction between

hybrid line and treatment. Because of the significant differences in body weight between hybrid lines, maternal organ weights were expressed as a proportion of body weight. A repeated measures restricted maximum likelihood procedure was used for maternal weight change and food intake. For pup measures, a similar model was used as for the dam measures but dam identity was also fitted as a random variable in the model.

Results

Litter size and pregnancy rates

Overall, there was no significant effect of treatment on pregnancy rate (proportion of mice seen with a vaginal plug that were pregnant at day 18 gestation: 88.9% C and 82.9% R). However, the pregnancy rate for FL dams was 100% compared with 79.2% in the FF and NL groups (Fisher's exact probability, $P=0.05$). There was a significant interaction between hybrid line and treatment on pregnancy rate in the two affected lines: four of the five non-pregnant dams were C in the FF group, whereas five of the five non-pregnant dams were R in the NL group (Fisher's exact probability, $P=0.05$).

Litter sizes of viable pups ranged from seven to twenty-eight pups. There was a significant effect of hybrid line on litter size (Table 1; $P<0.001$) but no effects of treatment or interactions between hybrid line and treatment. The effect of hybrid line on litter size appeared to be due to both an increased ovulation rate in the heavier dam lines (mean number of ovulations: FF = 24.61, FL = 21.23, NL = 14.01, $SED = 0.93$, $Wald = 136.41$, $df = 2$, $P<0.001$) and an increased conception rate in the FF dams (mean conception rate: FF = 0.91, FL = 0.81, NL = 0.87, $SED = 0.03$, $Wald = 9.90$, $df = 2$, $P=0.007$). There were no significant differences between treatment groups in ovulation or conception rates.

Partially reabsorbed or mummified fetuses were found in all the groups except the R dams of the NL line. Significantly more mummified fetuses were found in the FF (63%) and FL (65%) litters compared with NL litters (26%: Fisher's exact $P=0.05$ and 0.015 , respectively). In both the FL and NL lines, there tended to be more mummified fetuses in the C group compared with the R group (FL = 10/12 *v.* 5/11 pregnancies, Fisher's exact $P=0.09$; NL = 5/12 *v.* 0/7 pregnancies, Fisher's exact $P=0.10$), but this relationship was not seen in the FF line (5/8 *v.* 7/11 pregnancies, $P=0.66$).

Maternal weight change and food intake at day 18 of gestation

There were significant effects of litter size, hybrid line and treatment on proportional maternal weight change through gestation (Fig. 1), but no significant interactions between hybrid line and treatment. Overall, NL mice gained a

Table 1. Effects of hybrid line and treatment on litter size, maternal body weight, the net change in body weight over 18 d of pregnancy and body composition at day 18 of gestation (Mean values and standard deviations)

Variable	FF						FL						NL						Effect*
	C			R			C			R			C			R			
	Mean	SD		Mean	SD		Mean	SD		Mean	SD		Mean	SD		Mean	SD		
Litter size	23.25	3.99		21.45	2.77		17.92	1.78		16.36	3.59		11.42	2.61		13.00	1.41		Line: $Wald = 124.2$, $P<0.001$
Conceptus-free body wt (g)	67.24	7.68		47.59	7.78		43.84	5.15		37.16	2.66		29.99	2.76		30.78	2.45		Interaction: $Wald = 15.61$, $P<0.001$ †
Maternal change in body wt (g)	13.85	4.71		5.29	3.75		3.00	3.59		4.45	2.63		0.55	2.02		1.25	2.20		Interaction: $Wald = 23.15$, $P<0.001$
Fat (g)	7.77	1.72		3.17	2.70		4.02	0.94		1.59	0.83		1.71	0.79		2.33	1.63		Interaction: $Wald = 10.80$, $P=0.005$
Fat-free mass (g)	56.10	4.28		43.50	6.41		40.70	4.93		36.64	2.44		29.17	2.74		29.36	2.16		Interaction: $Wald = 14.28$, $P<0.001$
Fat%	12.43	1.93		7.01	4.34		9.41	2.31		4.37	1.93		6.12	2.57		7.44	4.77		Interaction: $Wald = 6.23$, $P=0.044$

FF, fast-growing, relatively fat mice; FL, fast-growing, relatively lean mice; NL, normal growth, relatively lean mice; C, control; R, restricted.

* $df = 2$ throughout.

† Line x treatment interaction throughout.

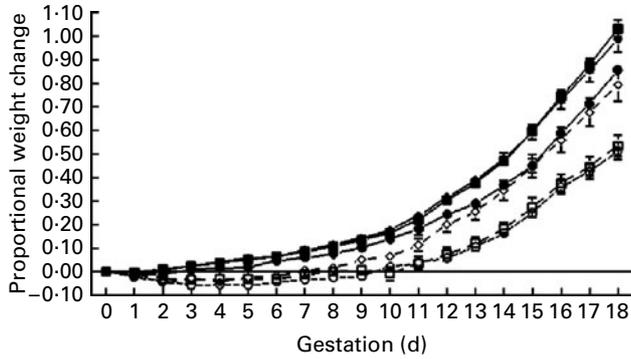


Fig. 1. Effect of hybrid line and nutritional treatment on mean maternal proportional weight gain (with standard errors) throughout gestation (relative to their starting weight) for FF (○, ●), FL (□, ■) and NL dams (◇, ◆) allowed either *ad libitum* feed intake (C –○, ■, ◆) or pair-fed 80% of *ad libitum* intake (R –○, □, ◇). C, Control; R, restricted; FF, fast-growing, relatively fat mice; FL, fast-growing, relatively lean mice; NL, normal growth, relatively lean mice.

significantly greater proportion of their starting weight during pregnancy than either of the fast growth lines (FF = 0.154, FL = 0.180, NL = 0.249, SED = 0.036, Wald = 24.39, df = 2, $P < 0.001$), which was apparently due to the greater weight gain in the NL-R dams compared with R dams of other lines, and the lower relative weight gain of FF dams (Fig. 1). Despite receiving 80% of the food of C animals, R dams gained less than half as much weight in gestation, proportional to their starting weight, as C dams (C = 0.274, R = 0.115, SED = 0.022, Wald = 54.35, df = 1, $P < 0.001$).

Food intake was scaled for body weight to compensate for the differences in maternal weight, and only the effects of litter size and hybrid line were investigated as R dams were fed a fixed amount depending on the intake of their matched C dam. Maternal food intake increased significantly through gestation (Fig. 2; $P < 0.001$). Both litter size ($P < 0.001$) and hybrid line had a significant effect on maternal intake/g of body weight. NL dams ate more food than the faster growing lines (food intake (g/g body

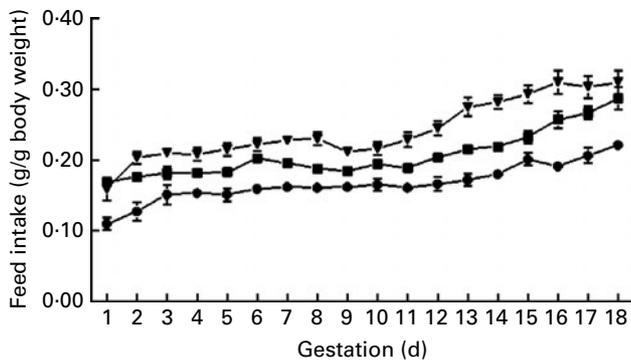


Fig. 2. Effect of hybrid line on mean maternal feed intake (with standard errors) throughout gestation, scaled for differences in maternal starting weight. ●, FF-C; ■, FL-C; ▼, NL-C. C, Control; R, restricted; FF, fast-growing, relatively fat mice; FL, fast-growing, relatively lean mice; NL, normal growth, relatively lean mice.

weight): FF = 0.155, FL = 0.177, NL = 0.213, SED = 0.011, Wald = 46.89, df = 2, $P < 0.001$).

The maternal utilisation of food to support increased growth was calculated as the ratio of weight gained per g of food eaten. Maternal utilisation of food for growth increased significantly over pregnancy (Fig. 3; $P < 0.001$) and was significantly affected by litter size ($P < 0.001$), line (FF = 0.016, FL = 0.021, NL = 0.038, SED = 0.0052, Wald = 30.43, df = 2, $P < 0.001$) and treatment (C = 0.032, R = 0.018, SED = 0.0032, Wald = 19.24, df = 1, $P < 0.001$). There was also a significant time × hybrid line × treatment interaction (Fig. 3; $P < 0.001$) as restricted NL dams utilised feed for growth at a faster rate, and restricted FL at a slower rate, over the last half of pregnancy.

Maternal weight and body composition at day 18 of gestation

The maternal conceptus-free body weight at day 18 of gestation was significantly affected by both hybrid line (FL > FF > NL, $P < 0.001$) and treatment (C > R, $P < 0.001$). However, there was a significant interaction between line and treatment ($P < 0.001$, Table 1) with a greater difference in maternal weight between C and R dams in the FF hybrid line (19.14 g or 29.2% of C maternal body weight) compared with 7.45 g (15.2%) in FL and a slight increase of 0.7 g (–2.6%) in NL. The control FF dams had a net weight gain over the course of their pregnancy (Table 1), whereas there was little net weight gain in the lean hybrid lines. The R dams of both the fast-growing hybrid lines had a net weight loss but the same effect was not seen in the NL line. The weight loss in R dams was due to a reduction in both the amount of fat and the fat-free mass of the maternal body (Table 1). There were, however, significant line × treatment interactions for all body composition measures (Table 1) with R dams of the fast-growing lines having a 50% reduction in body fat, in comparison to a slight increase in the R dams of the normal growth line.

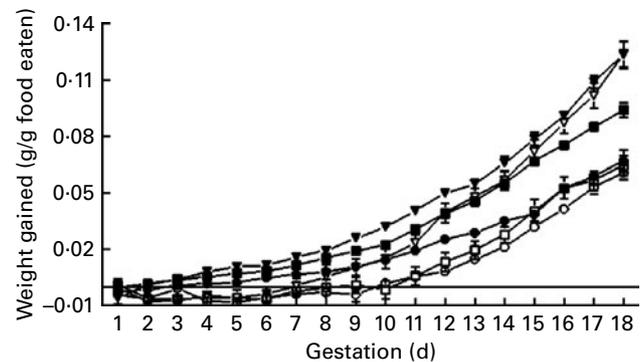


Fig. 3. Effect of hybrid line and nutritional treatment on mean maternal feed utilisation (g weight gained/g food eaten; with standard errors) to support growth throughout gestation. ●, ○, FF; ■, □, FL; ▼, ▽ NL. FF, fast-growing, relatively fat mice; FL, fast-growing, relatively lean mice; NL, normal growth, relatively lean mice.

Maternal organ weights

The effect of nutritional restriction and hybrid line on the proportional weight of the maternal organs is shown in Table 2. Overall, heart, kidney and liver were a greater proportion of maternal weight in the two lean lines of mice than those in the FF hybrid line. Nutritional restriction in gestation increased the proportional weight of these organs in the fast-growing lines but had only a small effect on the NL hybrid line. Spleen weights as a proportion of maternal weight were equivalent in all lines and reduced by gestational nutritional restriction in all the lines.

The gonadal fat pad, but not the intrascapular pad, tended to be proportionally heavier in the FF hybrid line dams compared with the leaner lines (Wald = 5.75, $df = 2$, $P = 0.056$), and both fat pads tended to be reduced in weight by maternal undernutrition in gestation, although this effect was small in the NL line (Table 2). Mammary tissue as a proportion of maternal weight was greatly affected by the nutritional restriction in all the hybrid lines with reductions as a proportion of maternal body weight of 46, 54 and 37% for the FF, FL and NL lines, respectively (Table 2; Wald = 61.96, $df = 1$, $P < 0.001$). The total weight of the litter supported as a percentage of maternal body weight was significantly influenced by both line and treatment (Table 2; $P < 0.001$). However, there was a significant interaction between line and treatment with undernutrition reducing the weight of the litter as a proportion of maternal weight in the lean lines (FL and NL) and increasing it in the FF line (Table 2; Wald = 21.95, $df = 2$, $P < 0.001$).

Fetal and placental weight

The effects of hybrid line and nutritional treatment on fetal and placental weights are shown in Table 3. Maternal undernutrition significantly reduced the weight and length of the fetus, the weight of the placenta and placental efficiency (fetal:placental weight ratio). Ponderal indices were increased with undernutrition, as fetuses were relatively longer and thinner than their *ad libitum*-fed counterparts (Table 3, Wald = 9.06, $df = 1$, $P = 0.003$). There were also significant effects of hybrid line on all traits except ponderal index. In general, FF dams gave birth to heavier and longer fetuses attached to more efficient placentae than other lines, and FL line had the heaviest and least efficient placentae (Table 3).

Discussion

Overall, our data suggested that there were significant hybrid line effects in the maternal response to pregnancy and that undernutrition had different effects within line in terms of maternal maintenance of pregnancy, maternal weight gain and relative changes in organ weights.

In general, in proportion to their starting body weight, the normal growth line dams ate more food, gained more weight and utilised more feed for growth than the fast-growing lines. However, the control FF dams, despite their lower feed intake and utilisation, had a net weight gain during pregnancy that was not seen in the other lines. The R dams gained much less weight than the C dams and converted less feed into growth. This effect was greatest in the FF line but was much less marked in the NL dams. There were significant effects of line and treatment on the growth of reproductive tissues (mammary, fetus and placenta), but no significant interactions between line and treatment. Undernutrition decreased fetal and placental weights and placental efficiency in all the lines and increased ponderal index. The greatest effects of undernutrition on mammary weight, fetal weight, length, ponderal index and placental efficiency were seen in the FL line and the least effect on fetal dimensions, and placental efficiency in the FF animals, NL lines were intermediate. Placenta and mammary weight were reduced least by undernutrition in the NL line compared with the other lines. Thus, although the mothers of different growth lines differed in their ability to spare their own tissues from the effects of undernutrition, they were not able to buffer their fetuses from a 20 to 30% reduction in weight by day 18 of gestation. However, there was variation between lines in the severity of effect of undernutrition, with the offspring of the FF dams being least, and those of the FL dams most, affected. In general, the data support our hypothesis that the offspring of fast-growing, lean lines of animals will be disproportionately affected by gestational undernutrition in comparison to more slow-growing or fatter lines. The data suggest that it is the dual combination of fast growth and leanness that is important as the fast-growth, fat line was less affected by undernutrition.

There were no treatment effects on the maintenance of pregnancy, litter size, embryo or fetal survival, although there were significant genotype effects. In previous studies of farm animals, both high^(32,33) and low^(34,35) nutritional planes after conception have been associated with reduced embryo survival. However, in sheep, this appears to be related to maternal maturity as effects of high nutrition reduced conception in adolescent but not mature ewes⁽³⁶⁾ and is also influenced by the timing of nutritional manipulations in pigs⁽³⁷⁾. Our data suggest that, in the mouse, a relatively mild nutritional stressor, imposed immediately after conception in virgin mice, has no impact on dam fertility. However, larger litter sizes were associated with heavier maternal body weight, as has been previously demonstrated in sheep⁽³⁸⁾. In addition, larger litter sizes are also associated with an increase in leanness in sheep and mice^(39–41), although in the present study the largest litter sizes were in the line with greatest fatness. Fetal mortality was higher in the fast-growing lines (FF and FL). However, as these lines also had the largest litters, this may have been primarily due to uterine

Table 2. Effect of gestational nutrition on the proportional weight of maternal organs and tissues and total litter weight, scaled for hybrid line differences in maternal weight, at day 18 of gestation (Mean values and standard deviations)

Variable (%)	FF				FL				NL				SED	Effects
	C		R		C		R		C		R			
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD		
Kidney	1.02	0.20	1.28	0.10	1.35	0.24	1.64	0.10	1.58	0.08	1.56	0.12	0.11	Line: $P < 0.001$ Treatment: $P = 0.004$ Interaction: $P = 0.034^*$
Liver	6.08	1.25	6.59	0.45	8.70	0.54	7.51	0.42	8.45	0.45	8.06	0.70	0.46	Line: $P < 0.001$ Treatment: $P = 0.024$ Interaction: $P = 0.006^*$
Heart	0.32	0.12	0.52	0.07	0.45	0.07	0.68	0.12	0.68	0.07	0.62	0.06	0.05	Line: $P = 0.015$ Treatment: $P < 0.001$ Interaction: $P < 0.001^*$
Spleen	0.33	0.08	0.32	0.04	0.38	0.03	0.32	0.06	0.33	0.04	0.25	0.06	0.04	Treatment: $P < 0.001$
Gonadal fat	1.56	0.94	0.69	0.88	0.60	0.24	0.27	0.17	0.38	0.21	0.41	0.18	0.36	Line: $P = 0.056$ Treatment: $P = 0.12$ Interaction: $P = 0.13^*$
Intra-scapular fat	0.31	0.11	0.22	0.07	0.33	0.09	0.24	0.11	0.27	0.11	0.25	0.08	0.07	Treatment: $P = 0.069$
Mammary	9.24	2.09	5.00	1.91	8.81	1.10	4.05	1.13	8.29	1.59	5.20	1.85	1.02	Line: $P < 0.001$ Treatment: $P < 0.001$
Litter	47.12	11.9	52.77	4.98	61.04	7.96	48.81	11.08	68.28	8.76	54.57	6.67	3.76	Line: $P < 0.001$ Treatment: $P < 0.001$ Interaction: $P < 0.001^*$

FF, fast-growing, relatively fat mice; FL, fast-growing, relatively lean mice; NL, normal growth, relatively lean mice; C, control; R, restricted.
*Line × treatment interaction.

Table 3. Effect of hybrid line and maternal gestational undernutrition on fetal and placental weights at day 18 of gestation (Mean values and standard deviations)

Variable	FF				FL				NL				SED	Effects
	C		R		C		R		C		R			
	Mean	SD												
Fetal wt (g)	1.67	0.162	1.33	0.189	1.40	0.155	0.95	0.148	1.25	0.179	0.93	0.189	0.072	Line: $P < 0.001$ Treatment: $P < 0.001$
% Change Placental wt (g)	0.133	0.024	0.117	0.027	0.138	0.023	0.123	0.024	0.110	0.029	0.107	0.021	0.008	Line: $P = 0.009$ Treatment: $P = 0.008$
% Change Fetal : placental wt ratio	13.38	1.614	12.01	1.662	10.64	1.671	8.08	1.406	11.15	3.141	8.93	2.751	0.858	Line: $P < 0.001$ Treatment: $P < 0.001$
% Change Fetal length (mm)	23.97	1.584	21.99	2.007	22.72	1.261	19.17	1.315	20.92	1.536	18.40	1.741	0.681	Line: $P = 0.004$ Treatment: $P < 0.001$ Interaction: $P = 0.12^*$
% Change Ponderal Index	12.44	2.109	12.85	2.711	12.23	1.769	13.81	2.157	13.54	2.172	15.02	2.740	0.880	Treatment: $P = 0.003$
% Change			3.3				12.9				10.9			

FF, fast-growing, relatively fat mice; FL, fast-growing, relatively lean mice; NL, normal growth, relatively lean mice; C, control; R, restricted.
*Line × treatment interaction.

Effect of gestational undernutrition

crowding rather than a direct effect of the maternal line growth characteristics.

Most studies of maternal undernutrition have concentrated on fetal outcomes and much less on the impact on the maternal body of experiencing a period of undernutrition during pregnancy. However, recent developments in the control of metabolism and reproduction, e.g. roles played by leptin and ghrelin⁽⁴²⁾, have focused attention on the response of the pregnant female to nutritional signals. Our data suggest that dams of lines that were of normal growth, but genetically lean, were less affected by undernutrition, in terms of maternal weight gain and mobilisation of body reserves, than the other lines, and the FF line was the most affected. It is likely that the different lines have altered neuroendocrine responses to nutritional signals^(30,43) to support their divergent growth patterns. For example, mice divergently selected for body fatness have extreme differences in circulating leptin concentrations when both fed and fasted⁽³⁰⁾. Thus, although these have not been investigated in these hybrid lines, it is likely that leptin concentrations were higher in the FF hybrid line than the FL or NL line. In sheep, the pattern of leptin secretions with altered nutrition is influenced by the degree of fatness of ewes at the start of the nutritional manipulations⁽⁴⁴⁾. Thus, the variation in fatness in our mouse lines at the start of the study may have affected their physiological responses to undernutrition with consequences for their change in weight and efficiency of food utilisation. In addition, recent studies in mice with a targeted knockout for galanin-like peptide, implicated in the neuroendocrine control of metabolism and reproduction, suggest that galanin-like peptide plays a role in the changes in weight gain and adjustments in energy balance with underfeeding⁽⁴⁵⁾. Thus the differential responses of the mice lines to undernutrition and the degree of mobilisation of fat tissue seen in the present study may be indicative of differences in underlying metabolic control.

Leptin has also been shown to affect maternal investment in offspring compared with self-maintenance in Siberian hamsters⁽⁴⁶⁾. In the present study, the NL line showed the least maternal response to undernutrition, as R dams were largely able to maintain their own body tissues (Table 1). These mice also had the lowest relative proportion of body fat. In contrast, the FF line, with the greatest proportion of body fat, showed the greatest mobilisation of body reserves and the most weight loss. The FF line also appeared to invest more heavily in their future offspring when resources were limited than the lean lines as litter weight as a proportion of maternal weight increased with undernutrition and pup weight reduction with undernutrition was least in this group. The data, therefore, suggest that the FF line had a greater ability to alter maternal investment in her developing offspring than the other lines, perhaps due to her greater ability to mobilise fat stores.

Although FF dams appeared able to protect their developing fetuses to some extent from the effects of

undernutrition, this did not extend to the placenta, which showed the greatest proportional reduction in weight in the FF group compared with the other groups. The reduction in placental efficiency with undernutrition, as has been seen previously^(25,47), was least in the FF group, however, and may be related to placental remodelling to improve blood flow and exchange surface area as an adaptation to undernutrition^(47–49). Previous studies in sheep adapted to low nutrition have shown that they are able to maintain placental efficiency with undernutrition in comparison to ewes adapted to good nutrition⁽²⁵⁾, and this was achieved via an alteration in placental structure to facilitate nutrient exchange. Thus, although not investigated in the present study, it seems possible that the placentas of restricted-fed FF dams may have shown greater adaptations to promote transfer of nutrients to the developing fetuses than the placentas of the other lines. In addition, the placentas of the FL line were the heaviest but the least efficient and the most affected by undernutrition, and thus the weight of the fetuses in this line were also the most affected by a restricted diet.

In conclusion, the present study has shown that different lines of mice selected for differing growth characteristics have altered responses to the effects of undernutrition in pregnancy and that this has consequences for the growth of their fetuses. The fast-growing, relatively fat line showed the greatest maternal response to undernutrition, and the slower-growing lean line the least, in terms of the impact on maternal weight gain and fat mobilisation. However, the offspring of the FF line were least affected in terms of a reduction in weight and length, and those of the FL line showed the greatest weight loss. One limitation of the present study is the lack of a normal growth, fat line which could allow complete separation of the impacts of growth and fatness on the response to undernutrition. In particular, additional studies with these lines might help to explain the apparent ability of the normal growth, lean line to withstand reductions in maternal body weight when confronted with undernutrition. Further, whether the differential effect of undernutrition has consequences for the behavioural and physiological development of the pups of different lines, and for their longer-term health, remains to be seen, and the mechanisms by which the different lines show altered responses to limited nutrition are unknown. These data also have consequences for farm animals where there has been intensive selection for animals that show rapid lean tissue growth. It seems likely that these animals will be more affected, in terms of fetal development, if nutrition is limited, than animals that are fatter or are selected for slower growth.

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