

Energy metabolism of young rats after early postnatal overnutrition

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Early postnatal overnutrition (PNO) induced by restricting litter size in rats leads to increased body-weight (BW) and body-fat gain in later life. PNO rats are used as an animal model of moderate obesity and early hyperinsulinism. We investigated whether the increased adiposity could be due to a decreased energy expenditure. Male newborn Wistar rats were raised in litters of either two (SL) or twelve pups (NL), weaned at 4 weeks of age and subsequently fed *ad libitum*. BW was recorded continuously until 12 weeks of age. Daily energy intake, total daily energy expenditure (EE, measured by indirect calorimetry) and body composition were measured in weaned pups at 5, 8 and 12 weeks of age. SL rats displayed increased BW compared with NL rats from week 2 to 5 and again from week 10 to 12. Lean body mass, body fat and protein content and total EE were increased in SL rats at week 5. The same linear correlation described the relationship between BW and total EE in NL and SL rats. At week 8 to 12 no differences in energy metabolism could be found, but the total fat content was increased in SL rats at week 12. Energy balance, i.e. assimilated energy minus EE, was no different between SL and NL at any time that it was measured. We conclude that although PNO rats display increased adiposity in early life, there seem to be no long-lasting effects on energy metabolism in later life, even if a tendency to increased adiposity can still be detected.

Obesity: Energy expenditure: Small litters: Body composition

Overweight in early life induced by early postnatal overnutrition (PNO) is a risk factor for development of obesity and associated cardiovascular diseases in later life (Martorell *et al.* 2001). Reduction of pre-weaning litter size in rats and mice leads to increased fat deposition of pups during the sucking period and can also result in increased adult body mass and fat content (Knittle & Hirsch, 1968; Faust *et al.* 1980). It has also been shown that rats from small litters (SL) can develop persistent hyperinsulinaemia, increased systolic blood pressure, and hyperleptinaemia (Cryer & Jones, 1980; You *et al.* 1990; Plagemann *et al.* 1992, 1999). Although it is quite clear that rats reared in SL show increased fat deposition at the end of the sucking period, there are controversial reports in the literature as to whether this has a long-lasting effect, and whether SL rats display hyper-, normo- or even hypophagia after weaning (Miller & Personage, 1972; Wurtman & Miller, 1976; Faust *et al.* 1980; Hausberger & Volz, 1984; Aust *et al.* 1985; Lambert & Koeslag, 1992; Plagemann *et al.* 1992). One reason for this could be that the number of pups

considered as 'SL' differed from two to six. On the other hand, many studies compared SL with large litters (sixteen to twenty-four pups) in which pups were undernourished (Knittle & Hirsch, 1968; Oscai & McGarr, 1978; Faust *et al.* 1980). Furthermore, there seems to be a sex effect, with males being more responsive to early PNO than females (Cryer & Jones, 1980; Bassett & Craig, 1988; Lambert & Koeslag, 1992) and strain differences (Hausberger & Volz, 1984). The importance of the genetic background has been emphasised by a recent study, which demonstrated that heterozygous *+/fa* pups raised in small litters showed a greater increase in body fat than wildtype *+/+* pups, although they do not show an overt phenotype under normal feeding conditions (Schmidt *et al.* 2000). Moreover, detailed studies examining the energy metabolism of PNO rats after weaning and in later life are still lacking. It is not known whether permanent changes in energy expenditure (EE) could contribute to development and persistence of obesity in this model. Our objective was therefore to re-evaluate the model of early PNO, especially with regard to energy metabolism.

Abbreviations: BW, body weight; EE, energy expenditure; LBM, lean body mass; NL, normal litter; PNO, postnatal overnutrition; SL, small litter.

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Material and methods

Experimental design

All animal experiments were performed in accordance with the guidelines of the ethics committee of the Ministry of Agriculture, Nutrition and Forestry (State Brandenburg, Germany). Shoe–Wistar rats (12-week-old, Tierzucht Schoenwalde, Schoenwalde, Germany) were housed in a temperature-controlled room (22°C) with a 12 h light–dark cycle and with food and water *ad libitum*. Throughout the experiment, all animals received commercial rat chow (maintenance diet no 1326; Altromin GmbH, Lage, Germany). Male and female rats were housed together for 5 d to mate. During the gestation and lactation periods dams were housed individually. One day after birth, male pups were distributed randomly and assigned to either SL (two pups per litter) or normal litters (NL twelve pups per litter). Pups were weaned at 4 weeks of age and subsequently housed with two (SL) or three (NL) pups per cage. At 5 weeks of age six animals from SL and NL each were placed in metabolism cages and subjected to measurement of energy assimilation (48 h) followed by measurement of EE using indirect calorimetry (48 h) after which animals were killed for carcass analysis. The same was performed with rats at 8 and 12 weeks of age with the exception that energy assimilation was measured over a 3 d period and that different animals were used for measurement of EE. In week 8 and 12, animals used for measurement of EE were weight matched.

Body composition

After the indirect calorimetry, animals were killed and stored frozen (–20°C) until chemical analysis of body composition as described elsewhere (Association of Official Analytical Chemists, 1990; Proll *et al.* 1998). For this, complete carcasses were autoclaved in 30 ml HCl (50 ml/l) at 121°C for 3 h, homogenised and lyophilised. The total energy content of each carcass was measured by analysis of a subsample using a bomb calorimeter (IKA, Werke GbmH & Co. KG, Staufen, Germany; C400). The lipid content was assayed by extraction with light petroleum (40–60°C) in a Soxhlet extractor and calculated as the difference between dry carcass weight before and after lipid extraction. Lean body mass was calculated as carcass mass minus lipid mass. Protein content was determined by Kjehldahl quantification of N content.

Energy metabolism

For measurement of energy assimilation (at 5, 8 and 12 weeks of age) individual animals were placed for 2 or 3 d in metabolism cages allowing collection of faeces and urine separately. The energy contents of rat chow and faeces were determined using bomb calorimetry. The energy content of urine was calculated by measurement of N content: 1 g N was considered to correspond to 22.7 kJ (Hoffmann & Klein, 1980). Assimilated energy was calculated by subtracting the energy content of urine and faeces from the energy content of consumed food.

The mean energy assimilation was 73.6% energy intake and was not influenced by age or treatment.

EE of individual rats (at 5, 8 and 12 weeks of age) was measured over 2 d using indirect calorimetry as described by Klaus *et al.* (1998). Briefly, O₂ consumption and CO₂ production were determined every 6 min in an open-circuit respirometric system and EE was calculated according to Weir (1949). Total EE was calculated as a mean value for 24 h. Energy balance was calculated by subtracting total EE from assimilated energy, assuming a mean energy assimilation of 73.6%.

Statistics

Results are presented as mean values with their standard errors. Differences between SL and NL groups were assessed using unpaired Student's *t* test (SPSS for Windows 8.0, 1998; SPSS Inc., Chicago, IL, USA). Differences were regarded as significant at $P < 0.05$. Linear correlations were calculated using least-square regressions.

Results

In comparison with NL, male rat pups reared in SL had a significantly ($P < 0.05$) increased body weight (BW) from the second week of the sucking period up to 5 weeks, i.e. at about puberty (Fig. 1). At weaning, the body mass of SL rats was 35% greater than that of NL rats and at 5 weeks it was still greater by 25%. From 6 to 9 weeks (corresponding to late puberty) BW differences were no longer significant, but from postpuberty to early adulthood (week 10 to 12) SL rats again displayed a significantly ($P < 0.05$) higher mean BW than NL rats (Fig. 1(B)). The mean energy assimilation was 73.6% energy intake and was not influenced by age or treatment.

In accordance with the increased BW of SL rats at week 5, they also showed an increased lean body mass (LBM), absolute body fat and body protein content and increased total EE. Only total energy intake, although increased, did not reach statistical significance due to large inter-individual variations (Table 1). Relative body fat, but not relative body protein, was also increased in SL rats at week 5, indicating an increased adiposity (Fig. 2). Using the data on energy intake and EE (which were measured simultaneously), we could also estimate the energy balance or energy retention, i.e. the difference between intake and expenditure. Energy intake in this respect refers to assimilated energy, which was 73.6% total intake as described earlier. As can be seen in Table 1, both groups SL and NL had the same energy retention of about 56 kJ/d, indicating that energy balance was not different between NL and SL at 5 weeks of age.

Fig. 3 shows the relationship between total EE and BW or LBM respectively in 5-week-old rats. Results for both SL and NL groups fall on the same regression line, with BW explaining 78.2% and LBM 73.5% of the variation in total EE ($P < 0.001$ in both cases).

In order to investigate a possible reprogramming of energy metabolism by early PNO independent of BW, we used weight-matched animals from SL and NL for further analysis of energy metabolism in later life (week

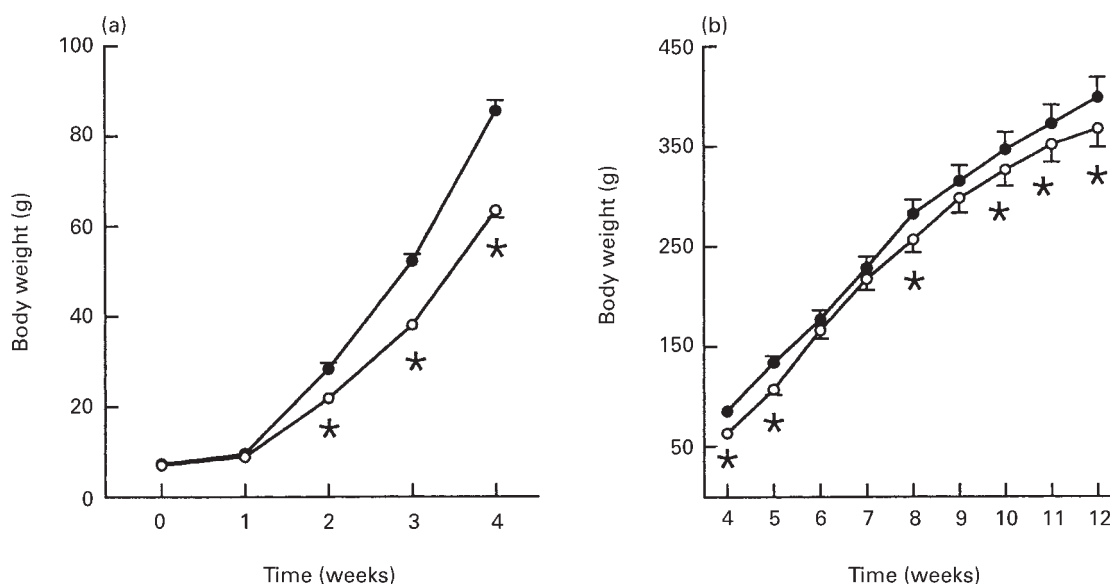


Fig. 1. Body-weight gain of rats from small litters (SL, two pups per litter; ●) compared with rats from normal litters (NL, twelve pups per litter; ○). (a), body-weight gain from birth until weaning; (b), body-weight gain after weaning. For details of diets and procedures, see p. 302. Values are means for six litters per group with standard errors shown by vertical bars (where bar is not visible, it is within the symbol size). Mean values were significantly different from those of SL group. * $P < 0.05$.

8 and 12). As can be seen in Table 2, no differences between SL and NL rats could be detected in LBM, body protein, as well as energy intake, EE and energy balance. However, body fat was increased by about 8 g (17%) in 12-week-old SL rats compared with NL rats.

Fig. 4 shows the relationship between BW and total EE for all three age groups. As in week 5, total EE also showed a strong correlation ($P < 0.001$) with BW in week 8 and 12. Regression equations are as follows: $y = 0.529x + 58.8$, $r = 0.82$ (week 8) and $y = 0.747x - 72.6$, $r = 0.89$ (week 12).

Discussion

The present study confirms that early PNO induced by reduction of litter size results in an increased BW shortly after weaning. During adolescence a 'catch up' growth in NL rats seemed to occur, resulting in similar BW in both groups from week 6 to about week 9 (Fig. 1). This

phenomenon was also evident in other studies (Bassett & Craig, 1988; Voits *et al.* 1996). However, from week 10 onwards, a slightly, but significantly ($P < 0.05$) elevated BW was apparent in SL rats, indicating a long-lasting influence of PNO on BW gain. However, in week 5, BW differences between NL and SL rats were most pronounced. This was due to an absolute gain of all body compartments, i.e. lean body mass, body protein and body fat (Table 1). In relative terms, body fat mass but not body protein was increased, indicating a preferential gain in fat mass and not muscle mass in the SL rats (Fig. 2).

Total energy intake at week 5 was slightly but not significantly higher in the SL group (Table 1), not supporting hyperphagia in SL rats, especially when considering that SL animals were about 25% heavier than NL rats. Previous studies report either hyperphagia when comparing total food intake (Oscari & McGarr, 1978; Bassett & Craig, 1988; Plagemann *et al.* 1992; Voits *et al.* 1996) or hypo-phagia when related to body mass (Miller & Personage,

Table 1. Body composition and energy metabolism of rats in small or normal litters at 5 weeks of age*
(Mean values with their standard errors for six animals per group)

| | NL† | | SL† | | Statistical significance of difference between groups: <i>P</i> |
|--------------------------|-------|-----|-------|------|---|
| | Mean | SEM | Mean | SEM | |
| Body weight (g) | 106.1 | 2.7 | 135.3 | 4.2 | <0.001 |
| Lean body mass (g) | 99.8 | 2.6 | 123.4 | 4.0 | 0.001 |
| Body fat content (g) | 7.1 | 0.4 | 11.9 | 0.4 | <0.001 |
| Body protein content (g) | 20.4 | 0.6 | 24.6 | 1.1 | 0.007 |
| Energy intake (kJ/d) | 290.7 | 4.1 | 324.6 | 15.4 | 0.080‡ |
| Total EE (kJ/d) | 157.8 | 4.3 | 182.2 | 5.0 | 0.004 |
| Energy balance (kJ/d) | 56.2 | 4.4 | 56.7 | 8.5 | NS |

NL, normal litter; SL, small litter; EE, energy expenditure.

* For details of diets and procedures, see p. 302.

† NL, twelve pups per litter; SL, two pups per litter.

‡ NS.

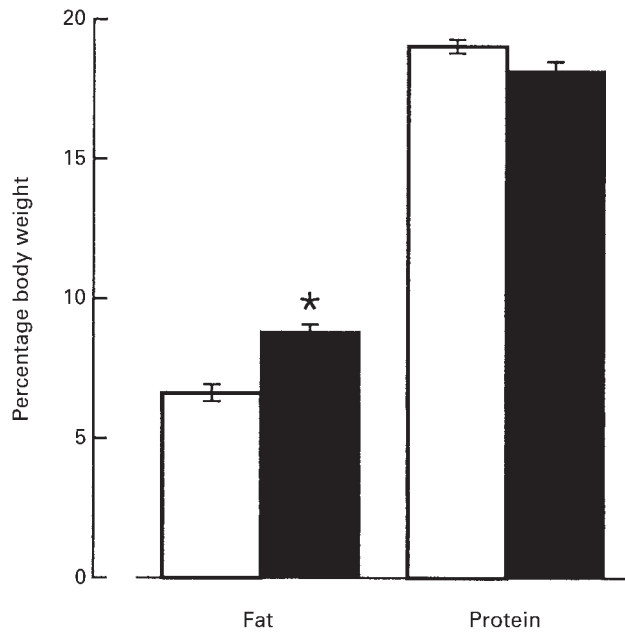


Fig. 2. Body fat and body protein content of rats from small litters (SL, two pups per litter; ■) and normal litters (NL, twelve pups per litter; □) at 5 weeks of age. For details of diets and procedures, see p. 302. Values are means for six litters per group with standard errors shown by vertical bars. Mean value was significantly different from the NL group. * $P < 0.05$.

1972; Parizková & Petrásek, 1979; Aust *et al.* 1985). However, energy balance was not determined in these studies and our present results show that even by 5 weeks of age there was no difference in energy retention between the groups. This indicates that the greater BW and adiposity in SL rats is a result of different energy budgets during the pre-weaning period only.

Total daily EE was increased in SL rats at week 5 despite increased adiposity, a phenomenon also observed in obese human subjects (Ravussin *et al.* 1982; Prentice *et al.* 1986). As in human subjects, this is due to the fact that not only body fat was increased in SL rats, but also LBM, which is the main determinant of resting EE (Ravussin *et al.* 1982; DeLany & Lovejoy, 1996). It is generally agreed that if available, LBM or fat-free mass is a better variable for normalisation than total body mass because of the low metabolic activity of adipose tissue. However, the contribution of fat mass to total EE increases with increasing fat mass (Garby *et al.* 1988). A common problem in the investigation of energy metabolism is the normalisation for differences in BW or size (Himms-Hagen, 1997). Correlation of total EE with BW or LBM (Fig. 3) showed that in the present study, BW was a better predictor of EE than LBM. Still, there was a strong linear correlation of EE with both BW and LBM. It should be noted that both regression equations have a non-zero intercept; this was also reported in studies on human subjects (Ravussin & Bogardus, 1989; DeLany & Lovejoy, 1996). This shows clearly that simply dividing total EE by BW can lead to erroneous results, i.e. an underestimation of total EE at higher BW. As is evident from Fig. 3, EE of individual SL and NL rats at week 5 fall on the same regression line when correlated with BW or LBM, indicating that

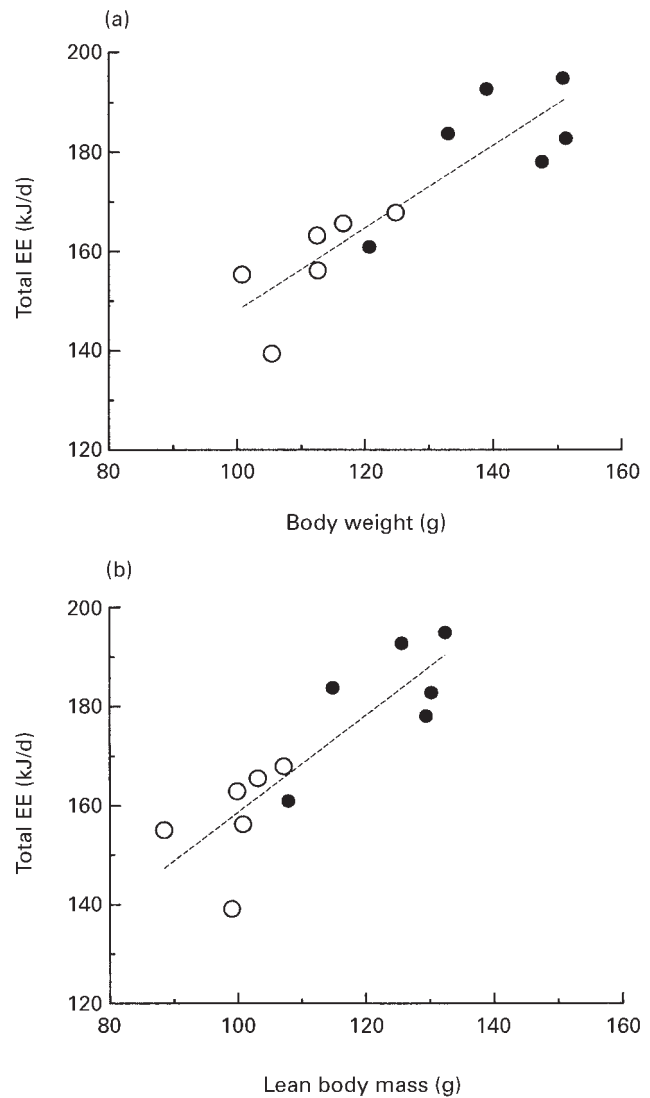


Fig. 3. Relationship between body weight (a) and lean body mass (b) and total energy expenditure (EE) in small litters (SL, two pups per litter; ●) and normal litters (NL, twelve pups per litter; ○). For details of diets and procedures, see p. 302. ----, Least-square regressions described by: (a) $y = 0.833x + 64.8$ ($r = 0.782$, $P < 0.001$), (b) $y = 0.979x + 60.7$ ($r = 0.735$, $P < 0.001$).

there were no systematic differences in EE between SL and NL rats. This is also evident in the other age groups: both at 8 and 12 weeks, BW explained 68 and 75% respectively, of individual EE (Fig. 4). It is also apparent from Fig. 4 that within each age group, the relationship between BW and total EE is different from the overall correlation.

A further complication in the comparison of SL and NL rats during adolescence and early adulthood arises from the fact that in rats between approximately 25 and 90 d of age, relative body fat content increases and energy intake and EE decrease, even if corrected for BW or body protein (Iossa *et al.* 1999). Thus, it can not be excluded that the increased adiposity observed at week 5 was merely a consequence of advanced growth and maturation induced by PNO. In order to avoid both this problem and the difficulties of data normalisation discussed earlier, we investigated

Table 2. Body composition, energy intake and total daily energy expenditure of weight-matched rats in small or normal litters at 8 and 12 weeks*

(Mean values with their standard errors for six litters per group)

| | Week 8 | | | | Statistical significance of difference between groups: <i>P</i> | Week 12 | | | | Statistical significance of difference between groups: <i>P</i> |
|--------------------------|--------|------|-------|------|---|---------|------|-------|------|---|
| | NL† | | SL† | | | NL† | | SL† | | |
| | Mean | SEM | Mean | SEM | | Mean | SEM | Mean | SEM | |
| Body weight (g) | 290.5 | 7.4 | 290.4 | 11.5 | NS | 398.6 | 9.4 | 395.7 | 8.6 | NS |
| Lean body mass (g) | 240.9 | 14.5 | 242.8 | 20.8 | NS | 322.0 | 26.1 | 312.4 | 20.3 | NS |
| Body fat content (g) | 23.1 | 5.2 | 23.9 | 5.3 | NS | 45.6 | 4.9 | 53.5 | 6.4 | 0.04 |
| Body protein content (g) | 54.1 | 4.0 | 54.2 | 4.7 | NS | 75.0 | 6.8 | 72.3 | 4.1 | NS |
| Energy intake (kJ/d) | 418.3 | 13.7 | 426.0 | 19.7 | NS | 407.2 | 14.8 | 418.7 | 35.7 | NS |
| Total EE (kJ/d) | 213.3 | 3.3 | 211.3 | 8.1 | NS | 224.3 | 7.4 | 224.0 | 8.2 | NS |
| Energy balance (kJ/d) | 94.6 | 8.6 | 102.2 | 11.2 | NS | 75.4 | 7.8 | 84.2 | 20.5 | NS |

NL, normal litter; SL, small litter; EE, energy expenditure.

* For details of diets and procedures, see p 302.

† NL, twelve pups per litter; SL, two pups per litter.

weight-matched SL and NL rats in order to detect possible intrinsic changes induced by PNO. However, as is evident from Table 2 and Fig. 4, there were no indications for any changes in total EE or energy balance, even at week 12 when body fat was increased in SL rats.

There are only very scarce results in the literature about EE in SL animals in comparison with NL animals. It has been reported that SL rats show a reduced postprandial EE at 7–9 weeks of life (Aust *et al.* 1986). In the present study, we found that total daily EE was virtually the same for SL and NL rats at week 8 and 12. As postprandial thermogenesis accounts for only about 10–15% total daily EE, it is evident that even if postprandial EE was reduced in our present study, it could not lead to significant changes in total EE given the relatively large inter-individual variations in EE.

In studies of human subjects, both perinatal undernutrition and overnutrition have been implicated as risk factors

for developing obesity in later life. Breast-feeding was found to have a protective effect of childhood obesity, although breast-fed infants are fatter than formula-fed babies in the first months of life (Martorell *et al.* 2001). Our present findings, together with the great inconsistency of results from previous animal studies, support the view that early postnatal nutrition *per se* might not have a very large influence on energy metabolism and obesity in later life. Rather, an interaction with other factors such as sex or genetic background seems to be necessary. It has been shown recently that nutritional influences during pregnancy, i.e. during fetal growth, can reprogramme variables of energy metabolism and are further amplified by postnatal hyperenergetic nutrition (Vickers *et al.* 2000). Using the same rat strain as in the present study, we found that prenatal exposure to a maternal high-protein diet resulted in an increased fat mass and decreased total EE in adolescent rats, whereas a postnatal high-protein diet had only minor effects on BW (Daenzer *et al.* 2002). Together, this indicates that prenatal nutritional influences might be more important than postnatal exposure in programming of energy metabolism in later life. Nevertheless, it would be interesting to investigate the effect of PNO on energy metabolism in an animal model like the heterozygous *+lfa* rat, which apparently is more susceptible to this kind of nutritional manipulation (Schmidt *et al.* 2000).

In conclusion, the present study shows that early PNO leads to increased BW and adiposity in prepubertal male Shoe–Wistar rats, which could be due to advanced growth, but not to changes in overall energy budget. Furthermore, there is no evidence for persistent changes in overall energy metabolism in later life even if PNO can result in a slightly increased adiposity in adulthood.

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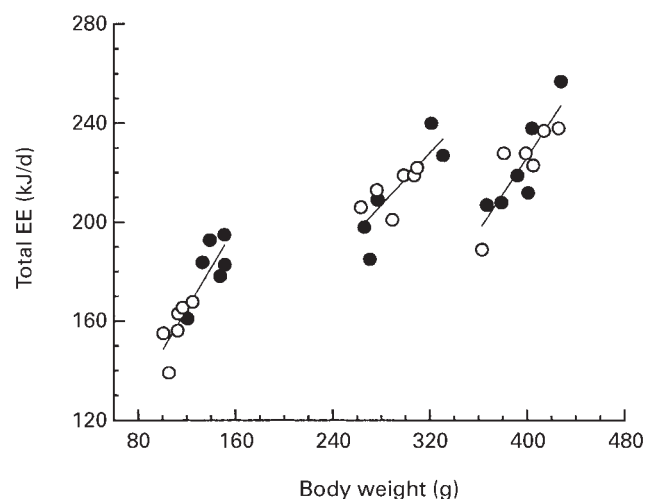


Fig. 4. Relationship between body weight and total daily energy expenditure (EE) for all age groups of rats in small litters (SL, two pups per litter; ●) and normal litters (NL, twelve pups per litter; ○). For details of diets and procedures, see p. 302. ---, Least-square regressions ($P < 0.001$) calculated separately for the three age groups.

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