Prevention of diet-induced obesity and impaired glucose tolerance in rats following administration of leptin to their mothers

Claire J Stocker¹, Ed Wargent¹, Jacqueline O’Dowd¹, Claire Cornick¹, John R Speakman²
Jonathan R S Arch¹ and Michael A Cawthorne¹

¹Clore laboratory, University of Buckingham, Buckingham MK18 1EG, UK.
²Aberdeen Centre for Energy Regulation and Obesity, School of Biological Sciences, University of Aberdeen, Aberdeen, UK.

Running title: Prevention of obesity in offspring by giving leptin to mothers

Correspondence:
Claire Stocker, Clore laboratory, University of Buckingham, Buckingham MK18 1EG, UK
e-mail claire.stocker@buckingham.ac.uk
ABSTRACT

Absence of leptin is known to disrupt the development of energy balance regulatory mechanisms. We investigated whether administration of leptin to normally-nourished rats affects energy balance in their offspring. Leptin (2 mg/kg/d) was administered from day 14 of pregnancy and throughout lactation. Male and female offspring were fed either on chow or on high fat diets that elicited similar levels of obesity in the sexes from 6 weeks to 15 months of age. Treatment of the dams with leptin prevented diet-induced increases in the rate of weight gain, retroperitoneal fat pad weight, area under the intraperitoneal glucose tolerance curve and fasting plasma insulin concentration in female offspring. In the male offspring, the diet-induced increase in weight gain was prevented and increased fat pad weight reduced. Energy intake per rat was higher in response to the obesogenic diet in male offspring of saline-treated but not leptin-treated dams. A similar trend was seen in 3-month-old female offspring. Energy expenditure at three months of age was higher for a given body weight in female offspring of leptin-treated compared to saline-treated dams when these animals were fed on the high energy diet. A similar trend was seen for male rats fed on the high fat diet. Thus leptin levels during pregnancy and lactation can affect the development of energy balance regulatory systems in their offspring.

Additional key words: Energy balance; energy expenditure; glucose tolerance
Susceptibility to visceral obesity and other features of the metabolic syndrome can be affected by events during fetal and neonatal life. For example, famine and smoking during pregnancy, and other non-genetic influences that cause low birthweight increase the risk of visceral obesity in later life (6, 21, 29, 32, 41), especially where there is rapid ‘catch-up’ growth in early life (18). Fetal malnutrition in rodents also results in susceptibility to insulin resistance, and, especially when followed by catch-up growth, obesity (10, 13, 20, 30), which is associated with inactivity and hyperphagia (39). Even in the absence of overt obesity and hyperleptinemia there may be leptin resistance (23) or a redistribution of fat from subcutaneous to abdominal depots (7).

Plasma leptin concentrations in both the dam and the developing offspring may play a role in linking nutrition to development. Thus the absence of leptin in \textit{Lep}^{ob}/\textit{Lep}^{ob} mice and their dams results in permanent disruption of hypothalamic development (9). Moreover, we previously showed that administration of leptin from day 14 of pregnancy to rats fed on a low protein diet totally prevented diet-induced weight gain in their male offspring. Furthermore, these offspring did not respond to the high fat diet with elevated insulin levels, either in the fasted state or following a glucose load (36).

Here we have investigated whether administration of leptin to pregnant rats reduces susceptibility to diabetes and obesity in their offspring when the dams receive a normal diet. We have studied whether reduced energy intake or increased expenditure is involved in resistance to obesity. Moreover, since a number of studies have found gender differences in the susceptibility of rodents to obesity and diabetes (11, 17, 19, 22, 37), we have studied female as well as male offspring. The results presented are for female and male offspring that were challenged with different high fat diets. These elicited a similar level of obesity in the
two sexes, whereas when the same diet was used obesity was more pronounced in the females.
METHODS

Animals: treatments and diets

All animal procedures were conducted under the University of Buckingham, Home Office, UK project licence, under the Animals (Scientific Procedures) Act (1986), and as agreed by the University of Buckingham Ethical Review Board.

Pregnant Wistar rats (Charles River, UK Ltd, Margate, UK) (initial weight 200-225g) were received time-mated at day 1 of gestation (taken as when vaginal plugs were detected), housed individually and maintained at 22 °C on a 12:12 h light: dark cycle. They were fed on a diet containing 20% (w/w) protein that contained 14.0 kJ/g metabolisable energy throughout pregnancy and lactation. From day 14 of pregnancy they were given either saline or rat leptin (2 mg/kg/d in physiological saline; PeproTech EC Ltd, London, UK) via a subcutaneously implanted Alzet™ minipump (Charles River, UK Ltd, Margate, UK) for 28 days. Spontaneous delivery took place on day 22 of pregnancy after which, at two days old, litter sizes were standardised to nine for each mother. All maternal measures and pup measurements post-weaning were taken in the fed state at 10:00 h, with plasma concentrations being measured from tail blood samples. At 21 days of age, all the pups were housed in groups of three or four and weaned onto the 20% (w/w) protein diet until 6 weeks of age when half of the female (n = 6 to 8 per treatment group) and one third (n = 6 to 8) of the male pups were transferred to a ‘high energy’ (HE) diet (19.26 kJ/g metabolisable energy) that contained by energy 45% fat, 35% carbohydrate (of which half was sucrose) and 20% protein (Research Diets, New Brunswick, USA; D12451). Another third of the male pups were transferred to a high fat (HF) diet (UAR, Villemoisson, France) that contained by energy 20% protein, 12% carbohydrate and 68% fat. Results for male offspring fed on the HE diet are not presented because offspring of the leptin-treated dams did not become significantly

5
obese. Throughout the study all the rats were allowed to eat *ad libitum* and had free access to drinking water.

Milk was obtained on day 5 of lactation by milking the dam following separation from the offspring and administration of oxytocin. Samples of milk were weighed to obtain the wet mass and then dried to constant weight (14 days) at 60 °C. Energy contents of the solids were then determined on three replicates of the pooled samples by adiabatic microbomb calorimetry (Gallenkamp).

*Energy balance*

The daily food intake of the dams was measured during pregnancy and lactation, excluding the days immediately prior to parturition. The food intake of the offspring was measured on three consecutive days over 24 hours when they were 3, 5, 9, 12 and 15 months old.

The body composition of the pups (anaesthetised using halothane and nitrous oxide) was measured at 3 to 4 weeks of age using dual-energy X-ray absorptiometry (Lunar PIXImus 2 mouse densitometer and version 1.46 software, GE Medical Bedford, UK).

Energy expenditure was measured in offspring by open-circuit indirect calorimetry beginning at 10:30 to 11:00 h for 24 h, or over 6 h following administration of the β₃-adrenergic agonist BRL-37344 (1 mg/ kg body weight, i.p.) (4). The respiratory chambers were the rats’ home cages covered with a Perspex lid that had 8mm holes at each send for entry and exit of air. The system employed 12 chambers. Air was sucked through each box by a pump (Model C5BS, Gast Manufacturing Co. Ltd., High Wycombe, UK), dried with silica gel, passed to a mass flow meter (EZ-FLOW mass flow meter, Bronkhorst (UK) Ltd., Cambridge, UK), then
to a flow regulator (Fine Control Needle Valve FCVSL, Roxspur Measurement and Control, Sheffield, UK). Each airline then passed to a sampling unit (Instrument Design Technologies, GlaxoSmithKline, Harlow, UK), which sampled air from a different line every 90 sec. Air was pumped from the flow selector via another flow regulator to the oxygen analyser (Servomex 1440, Servomex Group Ltd., Crowborough, UK) and at the end of this time the percentage oxygen content was measured. A T-junction between the flow selector and the pump to the oxygen analyser allowed air to escape via a flow indicator (gas flow meter Platon 0-1000 cm³/min, Roxspur Measurement and Control, Sheffield, UK) to ensure that there was a positive pressure between the flow selector and the pump and that room air was not drawn into the system. The volume of the cages was 23 litres and the flow rate was 0.8 litres/min. Such a system has a calculated half-life for responding to a step change in energy expenditure of 23.5 min. It is therefore not suitable for instant measurement of energy expenditure but with the animals undisturbed in their home cages, it is ideal for measurement of 24-hour energy expenditure (2). Energy expenditure was calculated by customised software using the equation of Weir (3).

**Glucose tolerance and plasma analytes**

Intraperitoneal glucose (1g /kg, body weight) tolerance tests were conducted after fasting overnight. Blood samples were taken from the tail for glucose and insulin measurements. Leptin was measured in 3-month-old offspring after an overnight fast as previously described (36) and tri-iodothyronine and thyroid stimulating hormone were measured using kits from GE Healthcare (Amersham, UK). The experiment was terminated and fat pad weights measured in fed animals when the offspring were 15 months old.
For measurement of mitochondrial protein concentration, a mitochondrial-enriched fraction was isolated from interscapular brown adipose tissue of the male mice using a mitochondrial isolation kit (Sigma Aldrich). Briefly, tissue was homogenized in an ice-cold isotonic extraction buffer containing 10 mM HEPES, pH 7.5, 200 mM mannitol, 70 mM sucrose, 1 mM EGTA and 2 mg/ml delipidated BSA that removes free fatty acids. Following a series of low and high speed centrifugation steps, the mitochondrial pellet was suspended in 10 mM HEPES, pH 7.4, containing 250 mM sucrose, 1 mM ATP, 0.08 mM ADP, 5 mM sodium succinate, 2 mM K2HPO4, and 1 mM dithiothreitol. The samples were then assayed for their protein concentration using Bradford reagent (Sigma Aldrich, UK).

For Western blotting, protein was extracted from frozen interscapular brown adipose tissue of the male mice in ice-cold RIPA buffer (1% octylphenoxy polyethoxyethanol, 1.5% sodium deoxycholate, 0.1% SDS in PBS) containing protease inhibitors. The protein concentration of the lysate was determined using Bradford reagent. Protein lysates (20-50 µg of protein) were resolved by electrophoresis using Tris-HCl gels in Tris/glycine SDS buffer. Proteins were transferred onto a polyvinylindene fluoride membrane overnight at 4°C in tris-glycine buffer. Equal protein loading and transfer were visualised by staining the membrane with Ponceau stain. The blot was blocked for a minimum of 1 hr with 5% Marvel-milk in TBS Tween. Membranes were then incubated with a 0.1 µg/ml rabbit anti-mouse uncoupling protein-1 monoclonal antibody (Chemicon Europe Ltd, UK). A horseradish peroxidase-conjugated secondary antibody (anti-rabbit) was used to bind to the primary antibody and peroxidase was detected using an ECL kit (Amersham Pharmacia Biotech Ltd, Amersham, UK) according to the manufacturer’s protocol. The fluorescence was captured onto photographic film (Kodak Biomax Light Film) and band density determined using Alpha Innotec software.
Statistics

Glucose tolerance, plasma analyte levels and pancreatic hormone measurements were analysed using one-way analysis of variance (ANOVA) followed by Bonferroni’s post-test for selected comparisons, except for some data in Fig. 1 where variances were significantly different by Bartlett’s test, and so the Kruskal-Wallis non-parametric and Dunn’s comparison test were used. Regression lines for energy expenditure versus body weight in 3-month-old offspring were compared using GraphPad Prism software version 3.0, provided one of the regressions was statistically significant and the slopes of the regressions were not significantly different. Thus the comparison was of energy expenditure for a normalised body weight. The experiment included similar numbers of offspring of dams fed on a low protein diet, but the data for these animals are not presented because the results are very similar to those previously reported for a different experiment (36). Results are given as means ± SE. Statistical significance for effects of leptin in the tables and figures is given as *$P<0.05$; **$P<0.01$; ***$P<0.001$. 
RESULTS

Energy balance, leptin levels and milk quality in dams

Infusion of leptin from day 14 of pregnancy markedly increased the plasma leptin concentration in the dams (Fig. 1A). The concentration of leptin in milk was also increased (control, n=6, 68±24; leptin-treated, n=8, 1124±196 pg/ml). Leptin reduced both food intake (Fig. 1B) and body weight (Fig. 1C) of the dams during both pregnancy and lactation. The energy content of the milk was not affected by administration of leptin to dams during lactation (saline: 5.42 ± 0.49; leptin: 6.53 ± 0.29 kJ/g wet weight; n = 3).

Offspring from birth to the end of the chow feeding period

Litter size was unaffected by treatment with leptin. However, leptin reduced both the birthweight and lengths of the pups (Table 1). Offspring of the leptin-treated dams were lighter than those of saline-treated dams at weaning (data not shown) and at the age of six weeks (Table 2).

Body composition was measured at three to four weeks of age in the male offspring and at four weeks of age in the female offspring (Table 2). Administration of leptin to the dams reduced body fat significantly in their male offspring and lean mass significantly in their female offspring. Plasma leptin levels were not significantly altered (Table 2).

Weight gain in offspring in response to diets

The HE diet (45% fat by energy) increased weight gain in the female offspring of the dams that had been treated with saline. Administration of leptin to the dams protected the female offspring from the effect of the HE diet on weight gain (Fig. 2A). The HE diet was relatively ineffective in producing weight gain in the male offspring of dams, even of those dams that
had been treated with saline, and the data are not shown. The HF diet (68% fat by energy) promoted weight gain and obesity in the male offspring to a similar degree to the HE diet in the female offspring, but again only in the offspring of dams that had been treated with saline and not in offspring of dams treated with leptin (Fig. 2B).

Weight gain in female offspring and to a lesser extent male offspring was reflected in terminal retroperitoneal fat pad weights (Fig. 2C) and plasma leptin concentrations (Fig 2D).

Energy intake in offspring response to diets

At 3 months of age, there was a trend for energy intake per rat to be higher on the HE diet than on the chow diet in female offspring of the saline-treated but not of the leptin-treated dams (Fig. 3A). This trend had disappeared by 5 months of age, however, and relative to body weight the offspring on the HE diet actually consumed less energy at 5 months of age, irrespective of their mother’s treatment (Fig. 3B).

In the male offspring of the saline-treated dams, energy intake per rat was significantly higher on the HF diet than on the chow diet at 3, 5, 9, 12 and 15 months of age (Fig 3C). Energy intake relative to body weight was increased by the HF diet by 37% ($P<0.01$) at 9 months of age (data not shown). No effects of the HF diet on energy intake per rat or per kg body weight were found in offspring of the leptin-treated dams (Fig 3C), the increase in intake per kg body weight at 9 months of age being only 4%.

Energy expenditure in offspring in response to diets

Treatment of the dams had no effect on energy expenditure of the offspring aged 3 or 12 months when energy expenditure was expressed per rat (Fig. 4A for 3 months; 12 months not
shown). In general, however, mean energy expenditure relative to body weight was higher in offspring of dams treated with leptin, and this effect was statistically significant in females aged 3 months and fed on the HE diet (Fig. 4B). Comparison of regression lines for energy expenditure versus body weight in 3-month-old offspring supported this conclusion. This regression was significant ($r^2=0.92; n=6; P<0.01$) for female offspring of saline-treated but not leptin-treated dams fed on the HE diet, probably because body weights varied less in the offspring of the leptin-treated dams. All the values for the offspring of the leptin-treated dams sat above the regression line for the offspring of the leptin-treated dams such that energy expenditure at the mean body weight of the leptin-treated dams was 15% higher than at the same body weight on the regression line of the saline-treated dams ($P=0.015$). A similar situation and a similar trend of increased energy expenditure in offspring of leptin-treated dams were seen in 3-month-old male offspring fed on the HF diet (11%; $n=6; P=0.063$).

Relative to body weight, energy expenditure in the six hours after injection of BRL-37344 was higher ($P<0.01$) in HE diet-fed, female, 15-month-old offspring of dams that had been treated with leptin compared to similar offspring of dams treated with saline (data not shown). This difference (36%) was, however, not significantly higher than the difference in energy expenditure relative to body weight seen in both 3- and 12-month-old HE diet-fed rats when they were not treated with BRL-37344 (22% over 24h and almost constant over this period; see Fig 4B for 3-month-old rats). Thus the thermogenic response to BRL-37344 was not significantly different between offspring of saline- and leptin-treated dams.

The HF diet increased the amount of mitochondrial protein in the interscapular fat pad of the male offspring, whether this was expressed per fat pad or relative to fat pad weight (saline-treated dams: chow, 2.11±0.23; HF diet, 6.21±0.81 µg protein/mg tissue; $P<0.05$; leptin-
treated dams: chow, 2.25±0.21; HF diet, 7.34±1.37 µg protein/mg tissue; \( P<0.01 \)). Treatment of the dams with leptin or saline had no effect on the amount of mitochondrial protein, however. The HF diet also increased (\( P<0.001 \)) the uncoupling protein-1 protein concentrations per unit of tissue protein (saline-treated dams: chow, 2632±174; HF diet, 4258±370 arbitrary units; \( P<0.001 \); leptin-treated dams: chow, 2525±109; HF diet, 4194±207 arbitrary units, \( P<0.001 \)), but again leptin had no effect. Plasma tri-iodothyronine and thyroid stimulating hormone concentrations were unaffected by the treatment of the dams or the diet of the offspring (T3 — saline-treated dams: chow, 1.31±0.53; HF diet, 2.01±0.69 ng/ml; leptin-treated dams: chow, 1.21±0.20; HF diet, 1.96±0.73 ng/ml; TSH — saline-treated dams: chow, 14±2; HF diet, 11±2 ng/ml; leptin-treated dams: chow, 13±1; HF diet, 14±1 ng/ml)

**Glucose homeostasis in offspring in response to diets**

Treatment of the dams with leptin protected their female offspring from impaired glucose tolerance at the age of 14 months in response to the HE diet (Figs. 5A, C). The fasting plasma insulin concentration was also less affected by the HE diet in the offspring of the leptin-treated dams (Fig. 5D).

Glucose tolerance in male offspring in response to the HF diet was little affected by treatment of the dams with leptin (Figs. 5B, C), despite their protection from weight gain. Their fasting plasma insulin level was, however, slightly but not significantly less elevated if their mothers had been treated with leptin (Fig. 5D).
DISCUSSION

The remarkable finding of the present study is that administration of leptin to normally nourished rats during pregnancy and lactation resulted in both their male and female offspring being resistant to dietary obesity. The dose of leptin that we used (2 mg/kg, body weight/d) was less than that used in a number of other studies on developmental programming, where doses of up to 25 mg/kg body weight, i.p. have been used (9, 28, 31, 40, 42). Nevertheless, since the plasma leptin concentration in the dams was markedly raised, further studies using lower doses of leptin are needed in studies of developmental programming.

Increased adiposity occurs in the offspring of high fat-fed rats (5), as well as in the offspring of undernourished dams. Our results do not support the suggestion that obesity in the offspring of high fat-fed dams is a consequence of elevated plasma leptin concentrations in the dams, but do not exclude the possibility that the cause of obesity in these offspring is a premature surge in plasma leptin concentration 8 to 10 days after birth (42).

We have extended the findings of our previous study (36) in which the dams were fed on a low protein diet and male offspring were resistant to high fat diet-induced obesity. In the present study the female offspring were fed on a ‘high energy’ diet that contains a lower proportion of energy in the form of fat than does the high fat diet. The male offspring were given either the high fat or the high energy diet but the high energy diet was ineffective in producing obesity, whereas the high fat diet elicited a similar degree of obesity to that elicited by the high energy diet in the females. Therefore only data for the high fat diet are presented. The difference in diets as well as in sex may explain why treatment of the dams with leptin was more effective in female than male offspring in preventing diet-induced increases in
retroperitoneal fat pad weights, plasma leptin, fasting plasma insulin and glucose intolerance,
although enhanced weight gain was prevented in both sexes.

The reason for feeding the dams on a low protein diet in our previous study (36) was that we
hypothesised that this diet would lower the plasma leptin level and that low exposure of the
fetus to leptin might sensitise it to diet-induced obesity. Thus, there are other examples of the
consequences of leanness being prevented by administration of leptin, although leanness
itself is not prevented (1). In our recent unpublished work, we have found, as we originally
predicted, a reduced plasma leptin concentration in dams fed on the low protein diet, but in
the published study (36), the plasma leptin concentration was not detectably low in these
dams, suggesting that leptin might be able to benefit the offspring by altering normal
development. This was confirmed in the present study.

Our results are in some respects similar to those of Nilsson et al (28). They reported that
injections of leptin into pregnant rats on days 8, 10 and 12 of gestation reduced adipose tissue
weight in 10-week-old male and 12-week-old female offspring. Body weight was also
reduced in the adult females. The offspring in these experiments were all fed on a low fat
diet, however. We did not detect any significant differences in fat pad weights or plasma
leptin levels at 15 months of age when the offspring were fed on chow: it was only when they
were fed on the HE or HF diets that such differences were apparent. Nilsson et al also
reported that their offspring showed reduced skeletal growth, and the females but not the
males had a reduced body weight. This is consistent with the reduced birth length in our
mixed sex offspring and reduced body weight in the female but not the male offspring fed on
the chow diet.
An unresolved question is whether leptin administered to the dam reaches the fetus and directly affects it development, or whether its effect is indirect. It might be argued that leptin’s effect was an indirect consequence of its negative effect on energy balance in the dams, but the effect on energy balance was small despite the high leptin dose, suggesting that energy balance is resistant to leptin not only during pregnancy (27) but also during lactation (16). We previously presented evidence that leptin might reduce exposure of the fetus to corticosterone by increasing the activity of placental 11β-hydroxysteroid dehydrogenase type 2. However, leptin crosses the placenta, especially in late gestation in rats, and might also affect the fetus directly (35). We have found (unpublished data) an elevated plasma leptin level in fetuses of dams that were injected with leptin on day 18 of pregnancy. Moreover, transplacental passage of leptin is decreased by glucocorticoids (35) and so the increased activity of 11β-hydroxysteroid dehydrogenase type 2 might increase exposure of the fetus to leptin as well as decreasing exposure to corticosterone. Recent reports have highlighted direct effects of leptin on hypothalamic development. Specifically, the absence of leptin in Lepob/Lepob mice disrupts the development of neuronal projection pathways from the arcuate to other hypothalamic nuclei (8, 9). Treatment of neonates with leptin rescued the development of these pathways (9). Our study raises the possibility that administration of leptin to pregnant rats permanently enhances the development of fetal neuronal projection pathways from the arcuate to other hypothalamic nuclei in a manner that protects the offspring from dietary obesity. Whether leptin acted directly via hypothalamic leptin receptors in Lepob/Lepob mice (9) or in the offspring of our experiments has not been established.

Leptin given to dams solely during gestation can alter body composition in offspring (28), and we showed that administration of leptin to lactating rats did not affect the energy content
of their milk. However, since treatment of neonates rescued the development of neuronal projection pathways in Lep\textsuperscript{ob}/Lep\textsuperscript{ob} mice (9), it is feasible that the markedly increased concentration of leptin in the milk of leptin-treated dams played a role in our study. This leptin can, to some degree, pass into the infant circulation (12, 25, 33), and it has been suggested that leptin in breast milk protects children from subsequent obesity (26). It does not support this hypothesis that administration of leptin to lactating, normally nourished rats increased weight gain and adiposity in their offspring (24). Leptin was administered to the dams only during the last three days of lactation in this previous study, however, and it may be that the pattern of variation in plasma leptin concentration in suckling rodents is critical (34, 42). There are contrasting reports of the effects of administering leptin to the neonates themselves. In one study, injection of leptin into neonatal rats increased their food intake and body weight when they became adults (15), this effect being more pronounced when leptin was given during the first rather than the last ten days of suckling. Moreover, administration of leptin to suckling mice from day 5.5 to 10.5 increased their sensitivity to a high fat diet given from nine weeks of age (42). By contrast, in another study, administration of leptin to neonatal offspring of undernourished rats protected them from later diet-induced obesity (40). This latter study, is consistent with the view that it was the presence of leptin in milk that was critical in our study, except that there was no demonstrable effect of leptin in offspring of normally fed dams (40), whereas the protective effect of leptin in our current study was achieved in dams that were fed normally. These comparisons are, of course, confounded by the fact that administration of leptin directly to the offspring may produce far higher concentrations of leptin in the offspring than was achieved in the present study through its transfer in milk.
We addressed the question of whether treatment of the dams with leptin protected their offspring from diet-induced obesity by reducing energy intake or increasing energy expenditure. Interpretation of such data is complicated if body weights differ between treatment groups, because larger animals are expected to have higher energy intakes and expenditures. Moreover, since intake and expenditure are not directly proportional to body weight, expression of energy turnover relative to body weight is not ideal. A better approach is to compare the regression lines of energy intake or expenditure versus body weight between groups, but this approach depends on these regressions being significant, which is more likely when body weights vary widely (3).

Treatment of the dams with leptin prevented the male offspring from increasing their energy intake (expressed per rat) on the HF diet. Although the increase in energy intake in offspring of saline-treated dams fed on the HF diet could have been secondary to increased body weight, it is notable that even when energy intake was divided by body weight – an overcorrection because the extra body weight contained a high proportion of fat – energy intake at nine months of age was increased by the HF diet in the male offspring of the saline-treated, but not the leptin-treated dams.

Energy expenditure per rat was not affected by treatment. Relative to body weight, energy expenditure was higher in females fed on the HE diet if their mothers had been treated with leptin. This may, however, reflect a greater proportion of metabolically active lean tissue in the offspring of the leptin-treated dams. It is more convincing that regression analysis showed that, for the same body weight, energy expenditure was higher in the female offspring of the leptin-treated compared to the saline-treated dams. A similar trend was seen in the males fed on the HF diet. Thus, administration of leptin to the dams appears to protect
their offspring from dietary obesity by influencing either or both of energy intake and energy expenditure. This is unsurprising because many peptides that regulate energy balance influence both energy intake and expenditure in a synergistic manner.

We investigated whether there might be an increased capacity for thermogenesis in brown adipose tissue in offspring of leptin-treated dams, but found no effect of leptin on the mitochondrial protein and uncoupling protein-1 content of the interscapular fat pad. Increases would have been expected if sympathetic activity had been increased. Consistent with these findings, there was no significant difference in the thermogenic effect of the β3-adrenoceptor agonist BRL-37344 between the HE diet-fed female offspring of saline- and leptin-treated dams. Possible mechanisms for effects of leptin treatment on energy expenditure suggested by other work include increased testosterone levels (28) and thyroid function (14, 38), but we found no effect of leptin on plasma triiodothyronine or thyroid stimulating hormone concentrations in male offspring.

In conclusion, administration of leptin to pregnant rats during the third trimester of pregnancy and lactation reduced susceptibility to diet-induced obesity, glucose intolerance and insulin resistance in their offspring, especially the females. Leptin programmed both reduced intake of the obesogenic diets and increased energy expenditure. These results show that leptin levels in dams can affect the development of energy balance regulatory systems in their offspring.
**Table 1:** Litter size, and pup weight and length

<table>
<thead>
<tr>
<th></th>
<th>Saline</th>
<th>Leptin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Litter size</td>
<td>12.1±1.1</td>
<td>13.3±0.5</td>
</tr>
<tr>
<td>Birthweight (g)</td>
<td>7.4±0.2</td>
<td>6.8±0.2*</td>
</tr>
<tr>
<td>Birth length (cm)</td>
<td>7.70±0.07</td>
<td>7.42±0.10*</td>
</tr>
</tbody>
</table>

Results are means ±SE of 7 or 8 values for litter size, and 10 to 16 values for birthweight and pup length.
### Table 2: Body composition and weight in pups on chow diet

<table>
<thead>
<tr>
<th>Sex of offspring</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saline</td>
<td>Leptin</td>
</tr>
<tr>
<td>Treatment of dams</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bodyweight at 6 weeks (g)</td>
<td>202±14</td>
<td>172±18***</td>
</tr>
<tr>
<td>Body composition</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat (g)</td>
<td>7.1±1.3</td>
<td>5.2±0.6***</td>
</tr>
<tr>
<td>Lean (g)</td>
<td>37.0±1.8</td>
<td>33.3±1.6</td>
</tr>
<tr>
<td>% fat</td>
<td>16.3±1.4</td>
<td>13.7±0.8</td>
</tr>
<tr>
<td>Plasma leptin at 6 weeks (pg/ml)</td>
<td>440±52</td>
<td>413±44</td>
</tr>
</tbody>
</table>

Results are means ±SE of 8 to 13 values for body weights, 4 to 6 values for plasma leptin, and 6 to 8 values for body composition of the males at 3 to 4 weeks of age and the females at 4 weeks of age.
**Fig. 1:** Plasma leptin concentration (A), food intake (B) and body weight (C) of saline-treated (●) and leptin-treated (▲) dams. Results are means of 6 to 9 values with bars for SE. (A) Plasma leptin levels were significantly ($P<0.05$) raised at all times in the leptin-infused rats (one-way ANOVA and Bonferroni’s post test, except at times where variances were significantly different by Bartlett’s test, in which case the Kruskal-Wallis non-parametric and Dunn’s comparison test were used). (B) Leptin reduced food intake on days 15 and 17 of pregnancy. Repeated measures ANOVA suggested ($P=0.05$) an effect of leptin on days 5L to 17L. (C) Leptin reduced body weight significantly on days 7 and 21 of lactation.

**Fig. 2:** Body weights (A, females; B, males), terminal retroperitoneal fat pad weights (C) and terminal plasma leptin concentration (D) of offspring fed on chow and HE (females) or HF (males) diets. Body weights are means of 5 or 6 values with bars for SE. Fat pad weights and plasma leptin concentrations are means of 4 or 5 values. In Figs A and B, offspring fed on chow diets are shown with filled symbols and those fed on HE or HF diets with open symbols. Treatment of dams: ●, ○ saline; ▲, △ leptin. In Figs C and D, saline and leptin refers to treatment of the dams, female and male to the sex of the offspring, and C, HE and HF to feeding of the offspring on chow, high energy and high fat diets.

**Fig. 3:** Energy intake in female offspring aged 3 an 5 months expressed (A) per rat and (B) per kg body weight, and (C) in male offspring aged 3, 5, 9, 12 and 15 months expressed per rat. Results are means of 2 to 4 values with bars for SE. The dams of the offspring were treated with saline (S) or leptin (L). The offspring were fed on chow (C), high energy (HE) of high fat (H) diets.
**Fig. 4:** Energy expenditure in offspring aged 3 months expressed (A) per rat, and (B) per kg body weight. Results are means of 5 or 6 values with bars for SE. Column footers are explained in the legend to Fig. 2. Data were not obtained for 3-month-old female offspring fed on the chow diet.

**Fig. 5:** Glucose tolerance and fasting plasma insulin in male and female offspring at age 14 months. A: Glucose tolerance in females. B: glucose tolerance in males. C: Area under the glucose tolerance curves. D: Plasma insulin concentrations 30 min before administering glucose. Results are means of 4 to 8 values with bars for SE. Symbols and abbreviations are explained in the legend to Fig. 2.


36. Stocker C, O'Dowd J, Morton NM, Wargent E, Sennitt MV, Hislop D, Glund S, Seckl JR, Arch JR, and Cawthorne MA. Modulation of susceptibility to weight gain and


