

## PAPER

# Modulation of susceptibility to weight gain and insulin resistance in low birthweight rats by treatment of their mothers with leptin during pregnancy and lactation

C Stocker<sup>1</sup>, J O'Dowd<sup>1</sup>, NM Morton<sup>2</sup>, E Wargent<sup>1</sup>, MV Sennitt<sup>1</sup>, D Hislop<sup>1</sup>, S Glund<sup>1</sup>, JR Seckl<sup>2</sup>, JRS Arch<sup>1</sup> and MA Cawthorne<sup>1\*</sup>

<sup>1</sup>Clare Laboratory, University of Buckingham, Buckingham, UK; and <sup>2</sup>Endocrinology Unit, Molecular Medicine Centre, University of Edinburgh, Edinburgh, UK

**OBJECTIVES:** To investigate whether administration of leptin to rats during pregnancy and lactation affects placental 11 $\beta$ -hydroxysteroid dehydrogenase (11 $\beta$ -HSD2) activity and the susceptibility of their offspring to weight gain and insulin resistance.

**DESIGN:** Pregnant rats fed on a low-protein diet were administered leptin or saline by subcutaneous minipump from day 14 of gestation and throughout lactation. A further group was fed a normal diet and given saline. After weaning, the offspring of each group were fed on a normal diet until 6 weeks of age and then half of each group was transferred to a high-fat diet until 12 months of age.

**RESULTS:** Plasma leptin levels were raised two-fold on days 16–18 of pregnancy in the leptin-treated dams, but, despite a constant rate of infusion, at parturition they dipped to control levels before rising again. The activity of placental 11 $\beta$ -HSD2 was reduced by the low-protein diet; this reduction was prevented by treating the dams with leptin. The male offspring of the saline-treated dams gained more weight and had higher plasma leptin levels on the high fat than the chow diet, but the offspring of the leptin-treated dams did not. Fasting blood glucose and intraperitoneal glucose tolerance at 6 and 12 months of age was unaffected by the high-fat diet, but only the offspring of the leptin-treated dams achieved this control without raised insulin levels.

**CONCLUSIONS:** The rate of leptin clearance appears to increase at parturition. The administration of leptin to rats during late pregnancy and lactation makes their male offspring less susceptible to high-fat-diet-induced weight gain and insulin resistance. *International Journal of Obesity* (2004) 28, 129–136. doi:10.1038/sj.ijo.0802476

Published online 14 October 2003

**Keywords:** diabetes; insulin resistance; glucocorticoids; 11 $\beta$ -hydroxysteroid dehydrogenase; leptin clearance

## Introduction

Malnutrition during fetal life can increase the susceptibility of adults to insulin resistance and obesity. Thus, the 'thrifty phenotype hypothesis' proposes that when the fetus is nutritionally deprived, it develops an adult phenotype adapted to poor, but not plentiful nutrition.<sup>1</sup> It is based on the evidence that low birthweight is associated with an increased risk of visceral obesity and Type II diabetes in adult life.<sup>1,2</sup> The increased risk of Type II diabetes is due to both  $\beta$ -cell dysfunction and insulin resistance.<sup>3</sup> Insulin resistance and visceral obesity are also features of the metabolic syndrome (insulin resistance syndrome; Syndrome X).<sup>4</sup>

Recent reports show that low birthweight due to maternal smoking is also associated with increased obesity in offspring.<sup>5,6</sup>

Glucocorticoids have been proposed to play a role both in programming the fetus to develop the metabolic syndrome<sup>7</sup> and in causing this syndrome in the adult.<sup>8</sup> Moreover, glucocorticoid exposure during late gestation has been linked to  $\beta$ -cell dysfunction and impaired insulin secretion in adult life.<sup>9</sup> High fetal glucocorticoid levels in the small baby syndrome may result from decreased expression of type 2 11 $\beta$ -hydroxysteroid dehydrogenase (11 $\beta$ -HSD2) in the placenta. This enzyme normally protects fetal tissues from the high maternal levels of cortisol (corticosterone in rats) by catalyzing its conversion to inert cortisone (11-dehydrocorticosterone).<sup>7</sup>

The small baby syndrome has been modelled in rats by reducing the proportion of protein in their diet (see Holness *et al*<sup>10</sup>).

\*Correspondence: Dr MA Cawthorne, Clare Laboratory, University of Buckingham, Buckingham MK18 1EG, UK.

E-mail: mike.cawthorne@buckingham.ac.uk

Received 7 April 2003; revised 3 July 2003; accepted 3 August 2003

Other approaches that do not model fetal malnutrition, but do produce small pups and a similar metabolic phenotype in adults, are to administer glucocorticoids or 11 $\beta$ -HSD inhibitors to the dam.<sup>7,11</sup> Indeed, protein malnutrition also reduces placental 11 $\beta$ -HSD2 activity.<sup>12</sup>

Leptin may play a role in fetal programming. Circulating leptin levels rise during pregnancy in humans and, according to some studies, in rats.<sup>13,14</sup> Associated with this rise is the increased expression of leptin mRNA in the placenta in humans<sup>15</sup> and, according to some<sup>14</sup> although not all<sup>16</sup> studies, in rats. Whether the rise in circulating leptin levels is due to increased production in the placenta or adipose tissue, or to increased production of a binding protein that inhibits leptin metabolism is unclear.<sup>17</sup> Most of the leptin produced by the placenta appears, at least in humans, to be released into the maternal circulation.<sup>18,19</sup> This leptin may affect fetal growth by altering metabolism in the dam. In addition, some placental leptin may reach the fetal circulation<sup>18,20</sup> and influence fetal growth more directly. Thus, umbilical cord blood leptin levels and placental leptin mRNA correlate with various indices of size at birth.<sup>21,22</sup> It is also possible that leptin secreted in milk may affect metabolism in the suckling infant<sup>23</sup> and prevent later obesity.<sup>24</sup>

Leptin may affect fetal programming by altering exposure of the fetus to glucocorticoids. The administration of leptin prevents endocrine responses to fasting,<sup>25</sup> potentially suppressing the secretion of glucocorticoids *in vitro*<sup>26,27</sup> and *in vivo*.<sup>28,29</sup>

It seems quite possible that circulating leptin concentrations and, in particular, placental and embryo exposure to leptin may be reduced in mothers where there is fetal growth retardation, particularly if this is induced by diet or protein restriction. We have, therefore, investigated the effect of the administration of leptin to pregnant protein-restricted rats on insulin sensitivity,  $\beta$ -cell function and susceptibility to diet-induced weight gain in their offspring.

## Methods

### Rats

All animal procedures were conducted under the British Home Office Animals (Scientific Procedures) Act. Pregnant Wistar rats (Charles River, UK Ltd, Margate, UK) (initial weight 200–225 g) were received time-mated at day 1 of gestation (taken when vaginal plugs were detected), housed individually and maintained at 22°C on a 12:12 h light:dark cycle. A schematic of the experimental design is shown in Figure 1. Rats were fed either a diet containing 20% (w/w) protein or an isocaloric diet containing 8% (w/w) protein (Hope Farms, Woerden, Netherlands; the composition and source of the diets were as described previously<sup>30</sup>) throughout pregnancy and lactation. The deficit in energy due to protein of the low-protein diet was made up by an increase in its carbohydrate content. From day 14 of pregnancy, low-

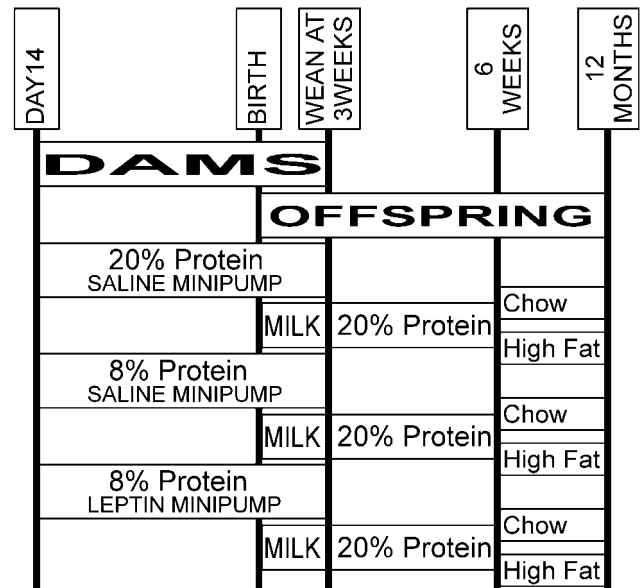


Figure 1 Schematic of the experimental protocol.

protein-fed rats were given either saline or murine leptin (2 mg/kg/day in physiological saline; PeproTech EC Ltd, London, UK) via a subcutaneously implanted Alzet™ minipump (Charles River, UK Ltd, Margate, UK) for 28 days. Normal-protein-fed rats received saline. Spontaneous delivery took place on day 22 of pregnancy after which, at 2 days, litter sizes were standardized to nine for each mother. All maternal measures and pup measurements postweaning were taken in the fed state at 10:00, with plasma levels being measured from tail blood samples. At 21 days of age, all the pups were weaned onto the 20% (w/w) protein diet until 6 weeks of age when half of the pups were transferred to a high-fat diet (Charles River, UK Ltd, Margate, UK; the composition of which (% metabolizable energy) was 20% from protein, 12% from carbohydrate and 68% from fat, as described<sup>31</sup>). Throughout the study, all the rats were allowed to eat *ad libitum* and had free access to drinking water. Further investigations were conducted on male rats that had been fasted overnight prior to commencement of procedures, at age 6 and 12 months. The results presented are from the second of two independent experiments, which gave similar results.

### Glucose tolerance test

Intraperitoneal glucose tolerance tests were conducted in rats at 6 and 12 months of age. Prior to the procedure, rats were fasted overnight and then dosed with glucose (1 g/kg, i.p.). Blood samples were drawn from the tail for glucose and insulin measurements at 0, 30, 60, 90, 120 and 180 min after glucose injection. Glucose tolerance was assessed in terms of areas under the glucose–time curves.

### Plasma analytes and pancreatic hormone measurements

Fasting plasma insulin and leptin were measured by ELISA (Crystal Chem Inc. Immunoassay, Chicago, IL, USA). Blood glucose, triglycerides (Sigma-Aldrich Company, Dorset, UK) and nonesterified fatty acids (ASC-ACOD, Wako Chemicals, Neuss, Germany) were measured colorimetrically. Fed plasma corticosterone levels were measured in the dams by enzyme-immunoassay (IDS OCTEIA Corticosterone immunoassay, Immunodiagnostic Systems, Boldon, UK). For determination of pancreatic insulin, pancreas samples were removed as soon as possible after death. After weighing, they were placed into ice-cold 180 mmol/l hydrochloric acid in 75% (v/v) ethanol (10 ml/g)<sup>32</sup> and minced vigorously. The hormones were extracted overnight at 4°C and the extract was separated from the remaining pancreatic tissue by centrifugation at 1800 × g for 20 min. The pancreatic insulin content was measured using a radioimmunoassay with rat insulin standards and an antiserum raised against rat insulin. The interassay imprecision was 4.8% and the intra-assay imprecision was 1.8%.

### Placental 11β-HSD2 activity

There are two isozymes of 11β-HSD. 11β-HSD2 is exclusively a dehydrogenase, whereas 11β-HSD1 is bidirectional with the dehydrogenase activity being most stable *in vitro*. Consequently, both were assayed in the dehydrogenase direction using their specific cofactors (NADP for HSD1 and NAD for HSD2). Placentas were homogenized in ice-cold PBS (pH 7.4) containing 0.25 M sucrose and assayed for 11β-dehydrogenase activity, as described previously,<sup>33</sup> using a protein concentration of 0.2 mg/ml in whole homogenate of placenta. After a 10-min incubation, steroids were extracted with ethyl acetate and analyzed with thin layer chromatography and high-pressure liquid chromatography against known standards.

### Statistics

Glucose tolerance, plasma analyte levels and pancreatic hormone measurements were analyzed using one-way analysis of variance (ANOVA) coupled with Bonferroni's multiple comparison test. An outlier for the 11β-HSD2 results necessitated the use of a nonparametric test (Mann-Whitney *U*-test). The results are presented as means ± s.e.m.

## Results

### Effects of leptin administration on pregnant rats fed on a low-protein diet

From day 14 of pregnancy, the pregnant rats fed on a normal-protein (20% by weight) diet were given saline via a subcutaneously implanted minipump for 28 days, while those fed on the low (8%)-protein diet were divided into groups given either saline or murine leptin. Plasma leptin

levels were doubled on days 16 and 18 of pregnancy in the leptin-treated dams, but at parturition (day 21) they dipped to the levels found in the saline-treated low-protein rats before rising again from days 28 to 43 (Figure 2a). Leptin levels were no different in the low-protein and normal-protein saline-treated rats.

Leptin reduced food intake during days 16–19 of pregnancy by about 5 g/day (Figure 2b), but this was not sufficient to elicit a statistically significant reduction in body weight of the dam plus her pups (Figure 2c), in line with the findings of others in mice.<sup>34</sup> By contrast, after parturition, food intake and body weight were suppressed in both the saline- and leptin-treated low-protein diet groups, compared to the normal-protein diet group (Figure 2b, c). The major influence on body weight may have been thermogenesis, which may respond to leptin during pregnancy, but not lactation.<sup>35</sup> Differential sensitivity of energy expenditure and food intake to leptin has been reported previously.<sup>36</sup> It is unlikely that leptin caused weight loss by increasing milk production by the dams,<sup>37</sup> especially since weight gain was low in their offspring.

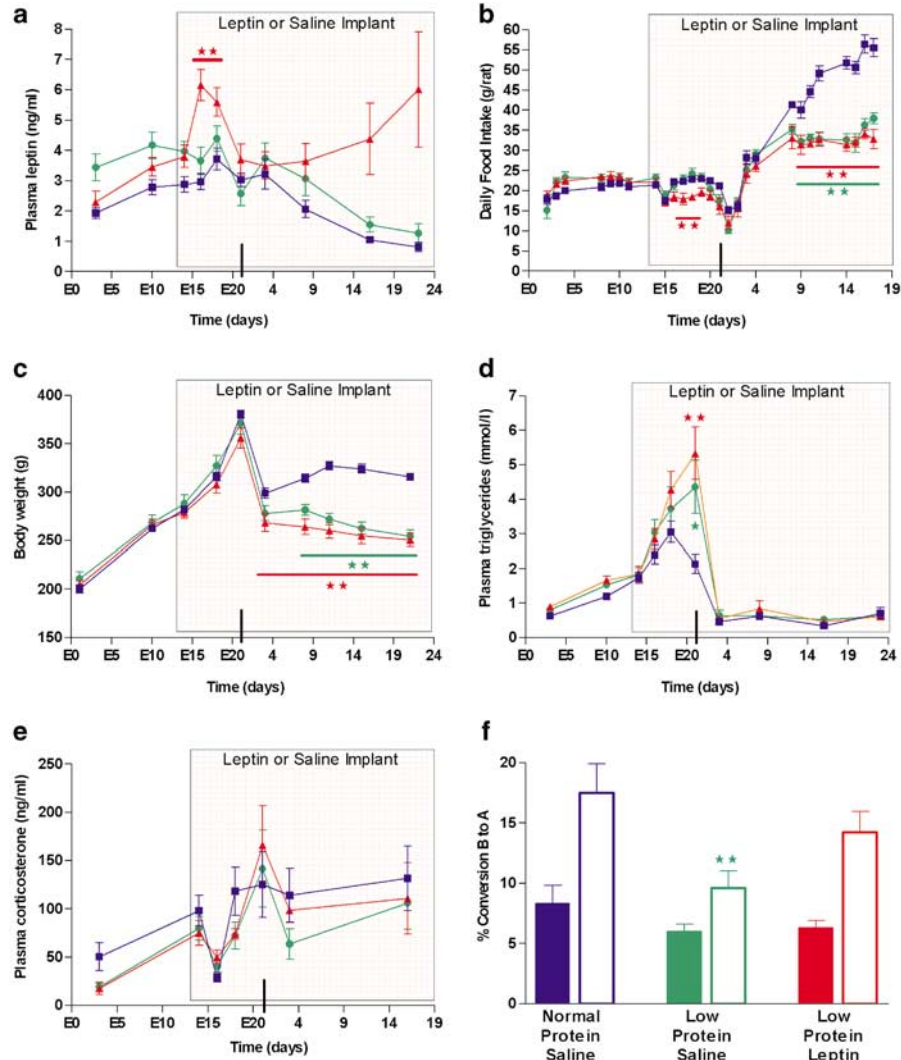
Fasting blood glucose, and plasma insulin, triglyceride and nonesterified fatty acid levels were similar in all three groups of rats up to day 18 of pregnancy (data not shown). Triglyceride levels were elevated on day 21 (preparturition) in both low-protein groups, but returned to the level in the normal-protein diet animals on postpartum day 1 (Figure 2d). The maternal plasma corticosterone levels rose during pregnancy and fell after parturition in all groups (Figure 2e).

### Placental weight and 11β-HSD activity

Placental weights were lower in both the low-protein diet groups than in the normal-protein diet group (Table 1). The activity of placental type 1 11β-HSD, which converts the inactive glucocorticoid 11-dehydrocorticosterone (cortisone in humans) to active corticosterone (cortisol in humans), was similar in all groups; that of the type 2 enzyme, which converts corticosterone to 11-dehydrocorticosterone, was significantly reduced in the low-protein diet, saline-treated dams, but not in the low-protein diet, leptin-treated dams compared to the normal-protein diet group (Figure 2f). The effect of leptin in the low-protein diet groups was close to statistical significance ( $P = 0.066$ ; Mann-Whitney *U*-test).

### Raised pancreatic insulin but slow growth in offspring of leptin-treated dams

The maternal treatments did not affect litter size (Table 1) but, by 7 days after their birth, pups from both the saline- and leptin-treated low-protein dams were smaller than pups from the normal-protein dams, and they remained so through the weaning period (onto the normal-protein diet at age 21–22 days) and until they were 6 weeks old (Figure 3).



**Figure 2** Metabolic parameters in dams and placenta during pregnancy and lactation. The mark on the x-axis shows parturition: (a) plasma leptin levels; (b) daily food intake; (c) body weight; (d) plasma triglycerides; (e) plasma corticosterone (each  $n = 12$  pregnant dams per group); (f) placental 11 $\beta$ -HSD2 activities expressed as conversion of corticosterone (B) to dehydrocorticosterone (A) ( $n = 6$ –8 placentas per group from mixed litters; filled bars, type 1 enzyme; open bars, type 2 enzyme) in dams fed a normal protein diet infused with saline (■), or a low-protein diet infused with either leptin (▲) or saline (●) during pregnancy and lactation. (★)  $P < 0.05$ , (★★) (bar)  $P < 0.01$  compared to normal-protein dams.

The insulin content per gram of pancreas was reduced in 2-day-old pups of saline-treated low-protein dams. Leptin prevented this reduction (Table 1).

### Sensitivity to dietary obesity and hyperinsulinemia in offspring

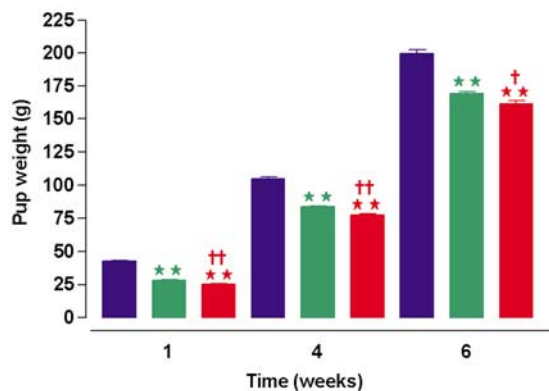
At 6 weeks of age, half the offspring of each group of dams were transferred to a high-fat diet. After 12 weeks on these diets until termination of the experiment, the body weights of the high-fat-fed male rats were raised relative to chow-fed controls, with the exception of the offspring of the leptin-treated low-protein diet dams (Figure 4a). The male offspring of the dams that had been fed on the low-protein diet tended

**Table 1** Litter size, and the development and pancreatic insulin content of the pups

	NPS	LPS	LPL
Placental weight (g)	$0.60 \pm 0.02$	$0.48 \pm 0.01^{**}$	$0.53 \pm 0.01^{**}$
Litter size	$12.68 \pm 0.57$	$13.02 \pm 1.5$	$12.34 \pm 1.26$
Pancreatic insulin day 2 ( $\mu\text{g/g}$ )	$594 \pm 107$	$338 \pm 54^*$	$550 \pm 64$

NPS = normal protein, saline; LPS = low protein, saline; LPL = low protein, leptin ( $n = 19$ –26, placental weight; 10–12, litter size; 12–25 pups from at least six litters, organ weights and insulin). Significantly different from NPS. \* $P < 0.05$ ; \*\* $P < 0.01$ .

to be shorter in length than those of the normal-protein dams, but administration of leptin to the low-protein dams did not affect length. The high-fat diet increased length only



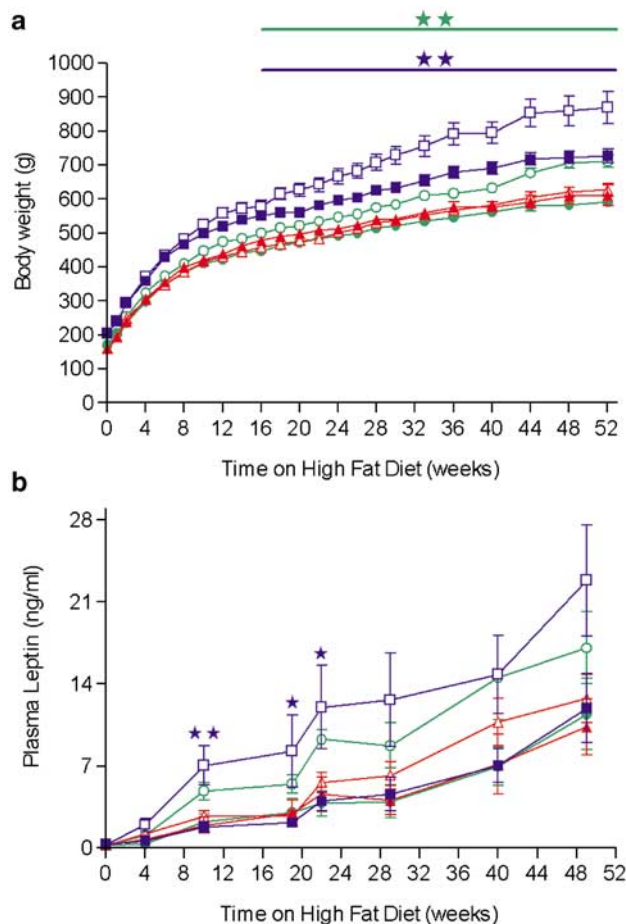
**Figure 3** Pup weights (mixed sexes) on day 7 ( $n=70-82$ ) and at 4 and 6 weeks of age ( $n=24-37$ ) in offspring of normal protein (■), low protein, saline (■), and low protein, leptin (■) dams. (★) significantly different from normal protein, saline; (†) significantly different between low-protein groups. One symbol:  $P<0.05$ ; two symbols:  $P<0.01$ .

in the offspring of the low-protein saline-treated dams (Table 2).

Neither the weight gain of the high-fat-fed offspring of the saline-treated dams nor the resistance to weight gain of the offspring of the leptin-treated dams can be explained in terms of energy intake, whether expressed relative to body weight (results not shown) or<sup>38</sup> to body weight<sup>0.66</sup>. For example, at the age of 50 weeks, 24 h energy intakes were: normal protein, chow  $5.1 \pm 0.1$ , normal protein, high fat  $4.7 \pm 0.2$ ; low-protein saline, chow  $4.3 \pm 0.1$ , low-protein saline, high fat  $4.3 \pm 0.1$ ; low-protein leptin, chow  $4.7 \pm 0.1$ , low-protein leptin, high fat  $4.5 \pm 0.1$  MJ/24 h/body weight<sup>0.66</sup>. Very similar results were obtained at each 2-weekly measurement of food intake. Plasma leptin levels, an indicator of body fat content, were raised by feeding a high-fat diet, again with the exception of the offspring of leptin-treated dams (Figure 4b). The offspring of the low-protein diet, saline-treated dams were no more susceptible than offspring of the normal-protein diet, saline-treated dams to weight gain or high leptin levels.

Intraperitoneal glucose tolerance tests were conducted when the rats were 6 and 12 months old. The glucose tolerance profile was similar in all groups of 6- and 12-month-old male rats irrespective of dietary treatment (data not shown) as was the overall area under the blood glucose-time profile over 180 min after the glucose load (Figure 5a). However, with the exception of the offspring of the leptin-treated mothers, rats fed on a high-fat diet were hyperinsulinemic. Thus, the integrated insulin concentration during the glucose tolerance did not differ between high-fat-fed and chow-fed offspring of leptin-treated dams, but was significantly increased in the high-fat-fed offspring of control dams that had been fed on either a normal- or a low-protein diet (Figure 5b).

Fasting glucose concentrations, measured after 4, 20, 30, 40 and 49 weeks on the high-fat diet, were similar in all groups (data not shown). However, fasting insulin levels



**Figure 4** Effect of maternal leptin treatment on the growth rate and circulating leptin in male offspring. Chow-fed/high-fat-fed offspring of dams fed a normal-protein diet infused with saline (■/□), or a low-protein diet infused with either leptin (▲/△) or saline (●/○) during pregnancy and lactation. (a) Body weights ( $n=16$ ). (★ ★) Significant ( $P<0.05$  to  $P<0.001$ ) increase in body weight in response to the high-fat diet (open symbols) compared with offspring fed on chow (closed symbols). (b) Fasting plasma leptin levels ( $n=6-8$ ). (★)  $P<0.05$ ; (★ ★)  $P<0.01$ , significant increase in plasma leptin in response to the high-fat diet.

were raised in the male offspring given the high-fat diet, with the exception of the offspring of the dams that had been treated with leptin (Figure 5c). Consequently, leptin prevented high-fat feeding from raising the insulin:glucose ratio (Figure 5d). Like body weight and leptin levels, high-fat feeding had similar effects on insulin levels—both fasted and during the glucose tolerance test—in the offspring of normal- and low-protein diet, saline-treated dams.

## Discussion

Our results demonstrate that administration of leptin from day 14 of pregnancy and throughout lactation to rats fed on a low-protein (and raised carbohydrate) diet reduces susceptibility to high-fat-diet-induced weight gain and insulin

resistance in their male adult offspring, despite low birth-weight. Leptin also prevented the reduction in islet insulin content in 2-day-old pups of low-protein diet mothers. These effects of leptin were not due to normalization of body weight: from the age of 7 days onwards, the offspring of the leptin-treated dams were the smallest of the three groups. It was recently reported<sup>39</sup> that administration of leptin to pregnant rats reduces both adipose tissue weight at adult age and skeletal growth in their offspring, but in that study leptin was given during the second trimester (days 8, 10 and 12). We found no effect of leptin on nose-to-anus length when it was given from the third trimester.

In our study, variable leptin levels were found in the dams despite a constant rate of leptin infusion (Figure 2a). Very similar results had been obtained in a preliminary experiment. This might be in part explained by a more marked reduction in endogenous leptin synthesis, following parturi-

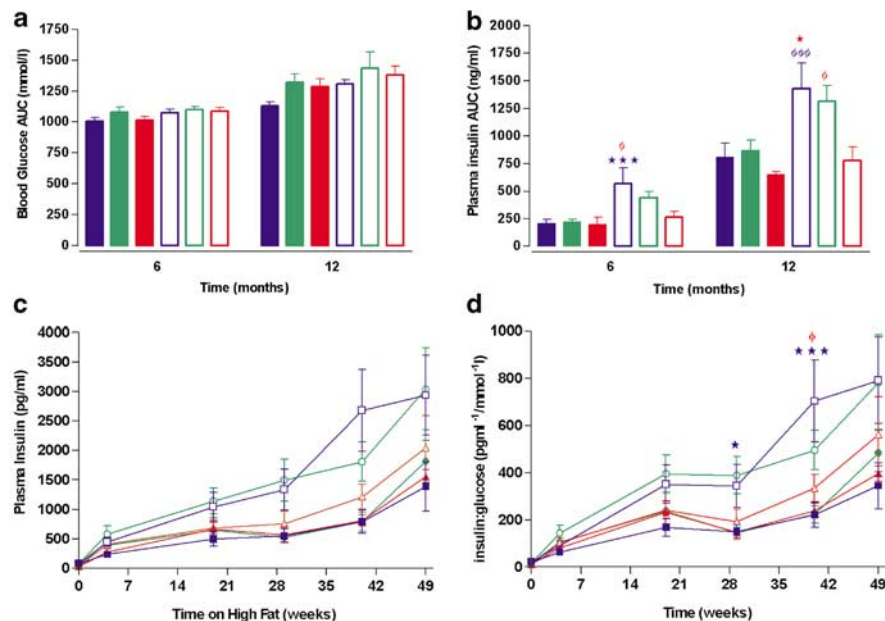
tion in leptin-treated than in control rats.<sup>16</sup> The fall in plasma leptin level was so marked, however, that it seems likely that leptin clearance was greatly increased around the time of parturition. In mice, the circulating concentration of the soluble form of the leptin receptor (Re) is markedly (40-fold) raised in pregnancy and, by binding leptin and inhibiting its metabolism,<sup>40</sup> it appears to be responsible for a rise of similar magnitude in circulating leptin levels.<sup>41</sup> Since placental cells secrete soluble leptin receptor,<sup>42</sup> it is possible that reduced production of a binding protein around the time of parturition greatly enhanced the metabolism of the infused leptin. In addition, there may be a significant clearance of leptin bound to placenta<sup>43</sup> and decidua. Thus in mice, the placenta, decidua and uterus have contents of leptin that approach those of adipose tissue, but only adipose tissue expresses leptin mRNA,<sup>44</sup> suggesting that these tissues bind leptin produced by adipose tissue. To summarize this aspect of the study: the marked fall in plasma leptin levels at parturition, despite continuous leptin infusion, suggests that alterations in the rate of leptin metabolism at this time can have profound effects on plasma leptin levels.

Since leptin levels were raised in the dams during both pregnancy and lactation, we cannot say whether leptin's actions on the offspring were exerted *in utero* or through its secretion in milk.<sup>23</sup> A direct effect on the offspring of leptin secreted in milk cannot be excluded,<sup>24</sup> but evidence for an *in*

**Table 2** Nose-to-anus length (cm) in offspring aged 9 months

	NPS	LPS	LPL
Chow	25.5 ± 0.2	24.1 ± 0.45 <sup>a</sup>	24.9 ± 0.2
High fat	26.3 ± 0.3	25.6 ± 0.3 <sup>a,b</sup>	24.9 ± 0.2 <sup>a</sup>

NPS = normal protein, saline; LPS = low protein, saline; LPL = low protein, leptin. *n* = 8 for each group. <sup>a</sup>Significantly different from NPS on same diet. <sup>b</sup>Significantly different from LPS on chow; *P* < 0.01.



**Figure 5** Glucose tolerance and fasting glucose and insulin concentrations in male offspring: (a) integrated blood glucose concentration during glucose tolerance test in 6- and 12-month-old rats; (b) integrated plasma insulin concentration during tolerance test in 6- and 12-month-old rats (*n* = 6–8); (c) fasting insulin (*n* = 6–8) during feeding of high-fat or control diet; (d) fasting insulin/fasting glucose (*n* = 6–8) during feeding of high-fat or control diet. Chow-fed/high-fat-fed offspring of dams fed a normal-protein diet infused with saline (■/□), or a low-protein diet infused with either leptin (▲/△) or saline (●/○) during pregnancy and lactation. (★) Significantly different from chow-fed rats with same maternal treatment; (ϕ) significantly different from offspring of low-protein-fed dams given leptin and fed on a high-fat diet. One symbol: *P* < 0.05; three symbols: *P* < 0.001.

*utero* action derives from leptin's prevention of the low-protein-diet-induced reduction in placental type 2 11 $\beta$ -HSD activity. The reduced activity of this enzyme has been proposed to make a significant contribution to the small baby (fetal malnutrition) and insulin resistance syndromes.<sup>7,45</sup> In our study, however, reduced activity of placental type 2 11 $\beta$ -HSD in placentas from the low-protein saline-treated dams was not associated with an increased susceptibility to weight gain and insulin resistance in their offspring. This was not surprising in the light of previous studies: dietary restriction of pregnant rats, but not a low-protein diet, has been reported to promote obesity in their offspring,<sup>46,47</sup> while insulin resistance develops slowly and may depend on the precise composition of the diet.<sup>48</sup> The different effects of dietary restriction and a low-protein diet may be because only dietary restriction lowers leptin levels in the pregnant rat. Failure to lower leptin levels may limit the value of the low-protein diet model of maternal malnutrition in humans.

Our results raise the question of the mechanism for the changes in placental type 2 11 $\beta$ -HSD activity. The low level of activity that resulted from the low-protein diet was not due to low circulating leptin levels in the dams: leptin levels were not reduced. Nevertheless, leptin did appear to prevent the reduction in type 2 11 $\beta$ -HSD activity in the low-protein diet rats. This suggests that leptin might have reduced the exposure of the fetuses to corticosterone by promoting its reduction to 11-dehydrocorticosterone. It is possible that, in low-protein diet mothers, this contributed to the subsequent reduced susceptibility to weight gain and insulin resistance. The effect of leptin in pregnant rats on a normal diet will be the subject of a future study.

Overall, our results are consistent with a role for glucocorticoids in programming the fetus to increased susceptibility to the insulin resistance and obesity in later life, but they also suggest that another factor, such as a low leptin level, is involved. Their main importance is that they suggest that it might, conversely, be possible to program offspring *in utero* or early in life to provide long-term protection from Type II diabetes and obesity.

#### Acknowledgements

We thank Anita Roberts, Joan Bushnell, Claire Cornick, Steven Wang, Kelly Smith and Lynne Ramage for their excellent technical assistance with this study.

#### References

- 1 Hales CN, Barker DJ. The thrifty phenotype hypothesis. *Br Med Bull* 2001; **60**: 5–20.
- 2 Byberg L, McKeigue PM, Zethelius B, Lithell HO. Birth weight and the insulin resistance syndrome: association of low birth weight with truncal obesity and raised plasminogen activator inhibitor-1 but not with abdominal obesity or plasma lipid disturbances. *Diabetologia* 2000; **43**: 54–60.

- 3 Phillips DI, Barker DJ, Hales CN, Hirst S, Osmond C. Thinness at birth and insulin resistance in adult life. *Diabetologia* 1994; **37**: 150–154.
- 4 Hopkins PN, Hunt SC, Wu LL, Williams GH, Williams RR. Hypertension, dyslipidemia, and insulin resistance: links in a chain or spokes on a wheel? *Curr Opin Lipidol* 1996; **7**: 241–253.
- 5 Power C, Jefferis BJ. Fetal environment and subsequent obesity: a study of maternal smoking. *Int J Epidemiol* 2002; **31**: 413–419.
- 6 von Kries R, Toschke AM, Koletzko B, Slikker Jr W. Maternal smoking during pregnancy and childhood obesity. *Am J Epidemiol* 2002; **156**: 954–961.
- 7 Seckl JR, Cleasby M, Nyirenda MJ. Glucocorticoids, 11beta-hydroxysteroid dehydrogenase, and fetal programming. *Kidney Int* 2000; **57**: 1412–1417.
- 8 Bjorntorp P, Rosmond R. Obesity and cortisol. *Nutrition* 2000; **16**: 924–936.
- 9 Holness MJ, Sugden MC. Dexamethasone during late gestation exacerbates peripheral insulin resistance and selectively targets glucose-sensitive functions in beta cell and liver. *Endocrinology* 2001; **142**: 3742–3748.
- 10 Holness MJ, Langdown ML, Sugden MC. Early-life programming of susceptibility to dysregulation of glucose metabolism and the development of Type 2 diabetes mellitus. *Biochem J* 2000; **349** (Part 3): 657–665.
- 11 Lindsay RS, Lindsay RM, Waddell BJ, Seckl JR. Prenatal glucocorticoid exposure leads to offspring hyperglycaemia in the rat: studies with the 11 beta-hydroxysteroid dehydrogenase inhibitor carbenoxolone. *Diabetologia* 1996; **39**: 1299–1305.
- 12 Langley-Evans SC, Phillips GJ, Benediktsson R, Gardener DS, Edwards CR, Jackson AA, Seckl JR. Protein intake in pregnancy, placental glucocorticoid metabolism and the programming of hypertension in the rat. *Placenta* 1996; **17**: 169–172.
- 13 Schubring C, Englaro P, Siebler T, Blum WF, Demirakca T, Kratzsch J, Kiess W. Longitudinal analysis of maternal serum leptin levels during pregnancy, at birth and up to six weeks after birth: relation to body mass index, skinfolds, sex steroids and umbilical cord blood leptin levels. *Horm Res* 1998; **50**: 276–283.
- 14 Amico JA, Thomas A, Crowley RS, Burmeister LA. Concentrations of leptin in the serum of pregnant, lactating, and cycling rats and of leptin messenger ribonucleic acid in rat placental tissue. *Life Sci* 1998; **63**: 1387–1395.
- 15 Hassink SG, de Lancey E, Sheslow DV, Smith-Kirwin SM, O'Connor DM, Considine RV, Opentanova I, Dostal K, Spear ML, Leef K, Ash M, Spitzer AR, Funanage VL. Placental leptin: an important new growth factor in intrauterine and neonatal development? *Pediatrics* 1997; **100**: E1–E6.
- 16 Kawai M, Yamaguchi M, Murakami T, Shima K, Murata Y, Kishi K. The placenta is not the main source of leptin production in pregnant rat: gestational profile of leptin in plasma and adipose tissues. *Biochem Biophys Res Commun* 1997; **240**: 798–802.
- 17 Reitman ML, Bi S, Marcus-Samuels B, Gavrilova O. Leptin and its role in pregnancy and fetal development—an overview. *Biochem Soc Trans* 2001; **29**: 68–72.
- 18 Hoggard N, Crabtree J, Allstaff S, Abramovich DR, Haggarty P. Leptin secretion to both the maternal and fetal circulation in the ex vivo perfused human term placenta. *Placenta* 2001; **22**: 347–352.
- 19 Lepercq J, Challier JC, Guerre-Millo M, Cauzac M, Vidal H, Hauguel-de Mouzon S. Prenatal leptin production: evidence that fetal adipose tissue produces leptin. *J Clin Endocrinol Metab* 2001; **86**: 2409–2413.
- 20 Yura S, Sagawa N, Mise H, Mori T, Masuzaki H, Ogawa Y, Nakao K. A positive umbilical venous-arterial difference of leptin level and its rapid decline after birth. *Am J Obstet Gynecol* 1998; **178**: 926–930.
- 21 Ong KK, Ahmed ML, Sherriff A, Woods KA, Watts A, Golding J, Dunger DB. Cord blood leptin is associated with size at birth and predicts infancy weight gain in humans. ALSPAC Study Team.

- Avon Longitudinal Study of Pregnancy and Childhood. *J Clin Endocrinol Metab* 1999; **84**: 1145–1148.
- 22 Ben X, Qin Y, Wu S, Zhang W, Cai W. Placental leptin correlates with intrauterine fetal growth and development. *Chin Med J (Engl)* 2001; **114**: 636–639.
- 23 Houseknecht KL, McGuire MK, Portocarrero CP, McGuire MA, Beerman K. Leptin is present in human milk and is related to maternal plasma leptin concentration and adiposity. *Biochem Biophys Res Commun* 1997; **240**: 742–747.
- 24 Locke R. Preventing obesity: the breast milk-leptin connection. *Acta Paediatr* 2002; **91**: 891–894.
- 25 Ahima RS, Prabakaran D, Mantzoros C, Qu D, Lowell B, Maratos-Flier E, Flier JS. Role of leptin in the neuroendocrine response to fasting. *Nature* 1996; **382**: 250–252.
- 26 Pralong FP, Roduit R, Waeber G, Castillo E, Mosimann F, Thorens B, Gaillard RC. Leptin inhibits directly glucocorticoid secretion by normal human and rat adrenal gland. *Endocrinology* 1998; **139**: 4264–4268.
- 27 Glasow A, Haidan A, Hilbers U, Breidert M, Gillespie J, Scherbaum WA, Chrousos GP, Bornstein SR. Expression of Ob receptor in normal human adrenals: differential regulation of adrenocortical and adrenomedullary function by leptin. *J Clin Endocrinol Metab* 1998; **83**: 4459–4466.
- 28 Proulx K, Clavel S, Nault G, Richard D, Walker CD. High neonatal leptin exposure enhances brain GR expression and feedback efficacy on the adrenocortical axis of developing rats. *Endocrinology* 2001; **142**: 4607–4616.
- 29 Heiman ML, Ahima RS, Craft LS, Schoner B, Stephens TW, Flier JS. Leptin inhibition of the hypothalamic-pituitary-adrenal axis in response to stress. *Endocrinology* 1997; **138**: 3859–3863.
- 30 Snoeck A, Remacle C, Reusens B, Hoet JJ. Effect of a low protein diet during pregnancy on the fetal rat endocrine pancreas. *Biol Neonate* 1990; **57**: 107–118.
- 31 Pearson SL, Cawthorne MA, Clapham JC, Dunmore SJ, Holmes SD, Moore GB, Smith SA, Tadayyon M. The thiazolidinedione insulin sensitiser, BRL 49653, increases the expression of PPAR-gamma and aP2 in adipose tissue of high-fat-fed rats. *Biochem Biophys Res Commun* 1996; **229**: 752–757.
- 32 Eriksson U, Andersson A, Efendic S, Elde R, Hellerstrom C. Diabetes in pregnancy: effects on the foetal and newborn rat with particular regard to body weight, serum insulin concentration and pancreatic contents of insulin, glucagon and somatostatin. *Acta Endocrinol (Copenh)* 1980; **94**: 354–364.
- 33 Jamieson PM, Chapman KE, Edwards CR, Seckl JR. 11 beta-hydroxysteroid dehydrogenase is an exclusive 11 beta-reductase in primary cultures of rat hepatocytes: effect of physicochemical and hormonal manipulations. *Endocrinology* 1995; **136**: 4754–4761.
- 34 Yamashita H, Shao J, Ishizuka T, Klepcyk PJ, Muhlenkamp P, Qiao L, Hoggard N, Friedman JE. Leptin administration prevents spontaneous gestational diabetes in heterozygous Lepr(db/+) mice: effects on placental leptin and fetal growth. *Endocrinology* 2001; **142**: 2888–2897.
- 35 Garcia MD, Casanueva FF, Dieguez C, Senaris RM. Gestational profile of leptin messenger ribonucleic acid (mRNA) content in the placenta and adipose tissue in the rat, and regulation of the mRNA levels of the leptin receptor subtypes in the hypothalamus during pregnancy and lactation. *Biol Reprod* 2000; **62**: 698–703.
- 36 Scarpace PJ, Matheny M, Zhang Y, Shek EW, Prima V, Zolotukhin S, Tumer N. Leptin-induced leptin resistance reveals separate roles for the anorexic and thermogenic responses in weight maintenance. *Endocrinology* 2002; **143**: 3026–3035.
- 37 Johnstone LE, Higuchi T. Food intake and leptin during pregnancy and lactation. *Prog Brain Res* 2001; **133**: 215–227.
- 38 Heusner AA. Body size and energy metabolism. *Annu Rev Nutr* 1985; **5**: 267–293.
- 39 Nilsson C, Swolin-Eide D, Ohlsson C, Eriksson E, Ho HP, Bjorntorp P, Holmang A. Reductions in adipose tissue and skeletal growth in rat adult offspring after prenatal leptin exposure. *J Endocrinol* 2003; **176**: 13–21.
- 40 Huang L, Wang Z, Li C. Modulation of circulating leptin levels by its soluble receptor. *J Biol Chem* 2001; **276**: 6343–6349.
- 41 Gavrilova O, Barr V, Marcus-Samuels B, Reitman M. Hyperleptinemia of pregnancy associated with the appearance of a circulating form of the leptin receptor. *J Biol Chem* 1997; **272**: 30546–30551.
- 42 Yamaguchi M, Murakami T, Yasui Y, Otani S, Kawai M, Kishi K, Kurachi H, Shima K, Aono T, Murata Y. Mouse placental cells secrete soluble leptin receptor (sOB-R): cAMP inhibits sOB-R production. *Biochem Biophys Res Commun* 1998; **252**: 363–367.
- 43 Schulz S, Hackel C, Weise W. Hormonal regulation of neonatal weight: placental leptin and leptin receptors. *BJOG* 2000; **107**: 1486–1491.
- 44 Tomimatsu T, Yamaguchi M, Murakami T, Ogura K, Sakata M, Mitsuda N, Kanzaki T, Kurachi H, Irahara M, Miyake A, Shima K, Aono T, Murata Y. Increase of mouse leptin production by adipose tissue after midpregnancy: gestational profile of serum leptin concentration. *Biochem Biophys Res Commun* 1997; **240**: 213–215.
- 45 Bjorntorp P, Rosmond R. Neuroendocrine abnormalities in visceral obesity. *Int J Obes Relat Metab Disord* 2000; **24** (Suppl 2): S80–S85.
- 46 Anguita RM, Sigulem DM, Sawaya AL. Intrauterine food restriction is associated with obesity in young rats. *J Nutr* 1993; **123**: 1421–1428.
- 47 Vickers MH, Breier BH, Cutfield WS, Hofman PL, Gluckman PD. Fetal origins of hyperphagia, obesity, and hypertension and postnatal amplification by hypercaloric nutrition. *Am J Physiol Endocrinol Metab* 2000; **279**: E83–7.
- 48 Petry CJ, Ozanne SE, Hales CN. Programming of intermediary metabolism. *Mol Cell Endocrinol* 2001; **185**: 81–91.