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Neonatal leptin treatment programmes leptin hypothalamic resistance and intermediary metabolic parameters in adult rats

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We previously showed that neonatal leptin treatment programmes higher body weight and food intake in adult rats. Here we investigate whether leptin treatment during lactation affects the anorectic effect of leptin on adult rats and their hypothalamic leptin receptors (OB-Rb) and whether those changes could have consequences on intermediary metabolism. When the offspring were born, pups were divided into two groups: the Lep group, injected daily with leptin (8 μg/100 g body weight, subcutaneously) for the first 10 d of lactation, and the control group, injected daily with saline. After weaning (day 21), body weight and food intake were monitored until the rats were 150 d old. Food intake was higher in the Lep group (approximately 14 %, $P<0{\cdot}05$) from day 133 onwards, and body weight was higher (approximately 10 %, $P<0{\cdot}05$) from day 69 onwards, compared with the control group. At 150 d of age, the rats were tested for food intake in response to either leptin (0·5 mg/kg body weight intraperitoneally; groups CL and LepL) or saline (groups CSal and LepSal). The CL group showed a decrease in food intake, but no response was observed in the LepL group, suggesting leptin resistance. The Lep group demonstrated a decrease in OB-Rb expression (−40 %, $P<0{\cdot}05$), hyperleptinaemia (+78 %, $P<0{\cdot}05$), hyperinsulinaemia (+100 %, $P<0{\cdot}02$), hypertriacylglycerolaemia (+17 %, $P<0{\cdot}05$) and a higher protein content in the body (+16 %, $P<0{\cdot}05$) without changes in fat mass and glycaemia. We conclude that neonatal leptin treatment programmes both hyperleptinaemia and hyperinsulinaemia in adulthood, which leads to leptin resistance by reducing the expression of the hypothalamic leptin receptor.

Leptin receptor: Rats: Lactation: Programming

Several animal experiments have shown that hormones and nutrition status, which affect development during sensitive periods early in life, permanently programme the structure and function of body tissues and systems (Walker & Courtin, 1985, Pracyck *et al.* 1992; Dorner & Plagemann, 1994; Cravo *et al.* 2002; Passos *et al.* 2002; Teixeira *et al.* 2003).

The mother's nutrition during lactation can programme the body weight of her offspring in adult life, and this can be mainly associated with a low-protein and higher-fat milk concentration (Trottier *et al.* 1998; Passos *et al.* 2000). The offspring of energy-restricted mothers show a lower body weight until weaning. After weaning, however, those animals were heavier than the offspring in the control group, without ingesting more food (Teixeira *et al.* 2002). Therefore, other factors besides food intake, especially those involved in the regulation of metabolic rate, may be responsible for those changes in body weight. We showed that malnutrition in lactating rats was associated with thyroid hyperfunction in the adult offspring (Passos *et al.* 2002). Changes in leptin action could also be programmed in this model (Passos *et al.* 2004) and theoretically affect metabolic rate.

Leptin is a protein produced by the obesity gene (*ob*) and mainly secreted by the adipocyte, regulating food ingestion and energy balance (Schwartz *et al.* 1996). This hormone regulates energy disposal in the adipose tissue by specific hypothalamic signals and affects many functions, for example body weight, food intake and body temperature, as well as metabolic rate (Campfield *et al.* 1995; Pelleymounter *et al.* 1995; Schwartz *et al.* 1996; Friedman & Halaas, 1998).

Leptin serum concentration changes from lactation through adulthood (Teixeira *et al.* 2002). It is higher in the first days of lactation, decreases thereafter, reaching its lowest level 1 week after weaning, and then progressively increases through adulthood. Malnutrition during lactation could change maternal leptin serum concentration and has been shown to change pups' leptin serum concentration (Teixeira *et al.* 2002). Both pups from protein-restricted mothers and pups from energy-restricted mothers presented, at weaning, higher serum leptin concentrations (Teixeira *et al.* 2002). As leptin is present in the milk, it is possible that this hormone is transferred to the pups through the milk (Casabiell *et al.* 1997; Houseknecht *et al.* 1997). The higher fat milk concentration in malnourished dams (Passos *et al.* 2000) could increase leptin production in the pups. This relationship has been demonstrated in man (Mantzoros *et al.* 1997) and animals (Trottier *et al.* 1998). We therefore hypothesized that this higher serum

Abbreviations: RPWAT, retroperitoneal white adipose tissue; SOCS-3, suppressor of cytokine signalling 3.

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leptin at weaning could be one of the factors that programmes the endocrine disorders observed during adulthood.

Based on these findings, we developed another experimental model in which the pups were injected with murine leptin (8 μg/100 g body weight, subcutaneously) during the first 10 or last 10 d of lactation (Cravo *et al.* 2002). In that study, we observed that hyperleptinaemia in both the first and the last 10 d of lactation was associated with a higher serum leptin concentration at 150 d of age and, paradoxically, with a higher food intake and body weight. We thus hypothesized that these animals show resistance to the anorectic effect of leptin, as observed for those animals programmed by maternal malnutrition during lactation (Passos *et al.* 2004).

In the present study, we therefore aimed to evaluate whether leptin treatment in neonatal rats affected the anorexigenic effect of leptin as well as the expression of the hypothalamic long form of the leptin receptor (Ob-Rb) in the adult rat. In addition, we aimed to verify whether these changes could have consequences on intermediary metabolism.

Methods

Procedure

The use of the animals took place according to the guidelines of the Animal Care and Use Committee of the Biology Institute of the State University of Rio de Janeiro, which based its analysis on the principles described in the *Revised Guide for the Care and Use of Laboratory Animals* (Bayne, 1996). Wistar rats were kept in a room with controlled temperature (25 ± 1°C) and with artificial dark–light cycles (light period from 07.00 hours to 19.00 hours).

Within 24 h of birth, pups were divided into two groups:

1. Lep – subcutaneously injected with 50 μl recombinant mouse leptin (provided by the National Hormone and Pituitary Program, Harbor-UCLA Research and Education Institute, Los Angeles, CA, USA) at a dose of 8 μg/100 g body weight daily, based on Malendowicz *et al.* (1998), for the first 10 d of lactation;
2. The control (Con) group, which received instead the same volume of saline (0·9 % NaCl). The recombinant mouse leptin was dissolved in saline, and all the injections were made at 16.00 hours.

Within 24 h of birth, excess pups were removed so that only six male pups were kept per dam (three per experimental group), because it has been shown that this procedure maximizes lactation performance (Fishbeck & Rasmussen, 1987).

After leptin injection (2 h) two pups from each litter (Lep and Con; *n* 6) were randomly chosen, and blood samples were obtained by decapitation, trunk blood being collected to determine serum leptin. Four pups from each litter were randomly chosen and placed individually in the cage with free access to water and a normal diet until they were 150 d old.

Food intake, body weight and body composition analysis

Body weight and food intake were monitored every 4 d from birth until the 150th day of life. The amount of the diet ingested was calculated as the difference between the weight of food that remained in the food bin (Da) and the amount placed there 4 d before (Di). This was measured from weaning (21 d) until 150 d of age. These data were then used to calculate the daily food intake according to the formula: Food intake (g) = (Di – Da)/4, in which 4 corresponds to the number of days.

Body composition (fat and protein mass) was determined at 150 d by carcass analysis as reported previously (Leshner & Litwin, 1972). After being killed, the animals were eviscerated, and the carcasses were weighed, autoclaved for 1 h and homogenized in distilled water (1:1 w/v). Samples of the homogenate were stored at 4°C for analysis. Three grams of homogenate were used to determine fat mass gravimetrically (Stansbie, 1976). The samples were hydrolysed in a shaking water bath at 70°C for 2 h with 30 % KOH and ethanol. The total fat acids and free cholesterol were removed by three successive washings with petroleum ether. After drying overnight in a vacuum, all the tubes were weighed, and the results were expressed as g fat per 100 g carcass. Protein mass was determined in 1 g homogenate. The tubes were centrifuged at 2000 ***g*** for 10 min. The total protein concentrations were determined by the method of Lowry *et al.* (1951). The results were expressed as g protein per 100 g carcass.

The retroperitoneal white adipose tissue (RPWAT) was excised and immediately weighed for evaluation of central adiposity.

Leptin resistance test

Peptide. Recombinant mouse leptin (PeproTech, Inc., Rocky Hill, NJ, USA) was dissolved in saline vehicle (0·9 % w/v) and given as bolus injection at a dose of 0·5 mg/kg body weight intraperitoneally (Martin *et al.* 2000; Passos *et al.* 2004).

Feeding study. At 150 d, the rats in each experimental group (Lep, Con) were randomized to one of the following groups: leptin (LepL, CL) or saline (LepSal, CSal). The animals were deprived of food for 24 h while maintaining free access to water before the test. After the intraperitoneal injections of leptin or saline, they were returned to their home cages and provided with a supplementary amount of a standard diet. They were housed singly, and food intake was measured by weighing the food cups 2, 4, 6 and 24 h after the leptin or saline injections (Martin *et al.* 2000; Passos *et al.* 2004).

Serum leptin, insulin, glucose and triacylglycerol analysis

At 150 d of age, the animals were killed by decapitation and trunk blood was collected and centrifuged, the serum being stored at −20°C until the assay for leptin, insulin and triacylglycerol. Leptin was measured by RIA kit (Linco Research, Inc., St. Charles, MO, USA). This kit measures both rat and mouse leptin with an assay sensitivity of 0·5 ng/ml and a range of detection of 0·5–50 ng/ml. The interassay and intra-assay variations were 3·1 % and 4·2 %, respectively. Insulin was measured by RIA kit (Linco Research, Inc.), with an assay sensitivity of 0·1 ng/ml and a range of detection of 0·1–10 ng/ml. The interassay and intra-assay variations were 2·2 % and 8·9 %, respectively. Triacylglycerols were measured colorimetrically using a commercial kit (Bioclin, Belo Horizonte, Brazil). Glycaemia was measured by the

glucose-oxidase method and read in a reflectance glucometer (ACCU-CHEK Advantage; Roche Diagnostics, Mannheim, Germany).

Western blot of the hypothalamic leptin receptor

The hypothalamic tissues were homogenized on ice-cold lysis buffer (50 mM-Hepes, pH 6·4, 1 mM-$MgCl_2$, 10 mM-EDTA, Triton X-100 1 %, 1 mg/ml aprotinin, 1 mg/ml leupeptin, 1 mg/ml SBTI). The homogenates were stored at −20°C. The protein concentration was determined by the method of Bradford (1976).

The proteins (40 μg) were separated by SDS-PAGE (12 %) and transferred to a nitrocellulose membrane (Hybond ECL; Amersham Pharmacia Biotech, London, UK). Membrane was blocked for 30 min with 2 % bovine serum albumin in TBS-T buffer (20 mM-TRIS, pH 7·5, 0·5 M-NaCl, 0·1 % Tween 20), incubated with OB-R (m-18, goat polyclonal, Sc-1834, antihuman leptin receptor; Santa Cruz Biotechnology, Santa Cruz, CA, USA) and IgG primary antibody (1:1000) in TBS-T overnight, washed three times with TBS-T and incubated with secondary antibody (peroxidase-conjugated donkey IgG, 1:1000), for 1 h. After the membrane had been washed three times with TBS-T, antibody-binding was visualized using 3,3'-diaminobenzidine tetrahydrochloride (5 mg in 10 ml Tris buffer, 0·1 M, pH 7·4). The results were normalized with actin. Densitometry analyses of the immunoreactive bands were determined by Proplus image software (Infaimon, Barcelona, Spain).

Statistical analysis

The data are reported as means with standard errors. Two-way ANOVA and Newman–Keuls multiple comparison tests were used to analyse food intake in response to the acute leptin treatment. The other experimental data were analysed by Student's *t* test with the significance level set at $P<0·05$.

Results

Body composition

The pups' body weight during the first 10 d, corresponding to the period of leptin treatment, is shown in Fig. 1(a); during this time, rats in the Lep group had a lower body weight (approximately 10 %, $P<0·0001$) from the second day of leptin injection. After weaning, the Lep group had a higher body weight (approximately 10 %, $P<0·05$) from day 69 onward (Fig. 1(b)). Food intake was higher in Lep group (approximately 14 %, $P<0·05$) on days 97 and 113, and from day 133 onward (Fig 1(c)). Despite rats in the Lep group showing a higher body weight gain as adults, their fat mass and RPWAT were no different from those of controls (Fig. 2(a) and (d), respectively). However, they had a higher protein mass than the Con group, suggesting that this increase in body weight was due to the lean mass (Fig. 2(b)).

Serum leptin

Figure 3(a) shows the serum leptin concentration at 10 d of age, 2 h after the last leptin injection. The Lep group had higher serum leptin (+136 %, $P<0·05$) than the Con group, confirming the hyperleptinaemia. The serum leptin concentrations at 150 d of age are shown in Fig. 3(b). Again, the Lep group had higher serum leptin concentrations than the Con group (+78 %, $P<0·05$).

Serum insulin, glucose and triacylglycerols

The serum insulin, glucose and triacylglycerols concentrations at 150 d of age are given in Fig. 4. The Lep group had higher serum insulin (+100 %, $P<0·02$; Fig. 4(a)) and higher serum triacylglycerols concentrations (+17 %, $P<0·05$) than the Con group (Fig. 4(c)), but no changes were observed in terms of glycaemia (Fig. 4(b)).

Leptin resistance test

Figure 5 shows the results of the leptin resistance test. The effectiveness of the test is demonstrated by the results for the Con group, in which leptin treatment significantly suppressed food intake ($F_{1,40} = 16·52$; $P<0·002$) at 2 h (−42 %, $P<0·05$), 4 h (−38 %, $P<0·02$) and 6 h (−36 %, $P<0·05$) after leptin injection, compared with the group that received saline (Fig. 5(a)). However, the LepL group did not show a similar decrease in any of the periods studied, suggesting a resistance to the anorectic effect of leptin. In addition, the LepL group rats showed an increase in food intake ($F_{1,40} = 23·14$, $P<0·001$) at 2 h and 4 h (Fig. 5(b)). When the Con groups (CSal, LepSal) were compared after fasting for 24 h, it was observed that the LepSal group consumed less food at 2, 4 and 6 h ($P<0·05$) after saline injection (Fig. 5(c)). Even when the food intake was normalized per 100 g body weight, the results were similar, which reinforces the results described earlier.

Western blot of the hypothalamic leptin receptor

Western blots of total protein lysates from the hypothalamus of adult rats are shown in Fig. 6. The levels of OB-Rb were reduced by 40 % ($P<0·05$) in Lep rats compared with the Con group.

Discussion

The major finding of the present study is that neonatal leptin treatment programmed a higher body weight, a higher body protein content, higher serum leptin level and leptin resistance, with reduced levels of the hypothalamic leptin receptor, OB-Rb. We previously showed that this kind of treatment programmed a higher body weight and food intake (Cravo *et al.* 2002). In agreement with other authors (Ahima *et al.* 1998; Mistry *et al.* 1999; Oates *et al.* 2000; Proulx *et al.* 2001) and with our previous findings (Cravo *et al.* 2002), leptin injected peripherally increased the serum leptin concentration during the neonatal period.

The higher body weight in the Lep group does not seem to be related to food intake since the gain of body weight occurs around the 70th day, whereas the increase of food intake occurs around day 133 of age. In addition, despite the higher body weight, the fat mass remained stable, suggesting that the proportion of protein in the carcass played an important role in the increase of the body weight. Although there are studies on the earlier effects of leptin treatment, there has been

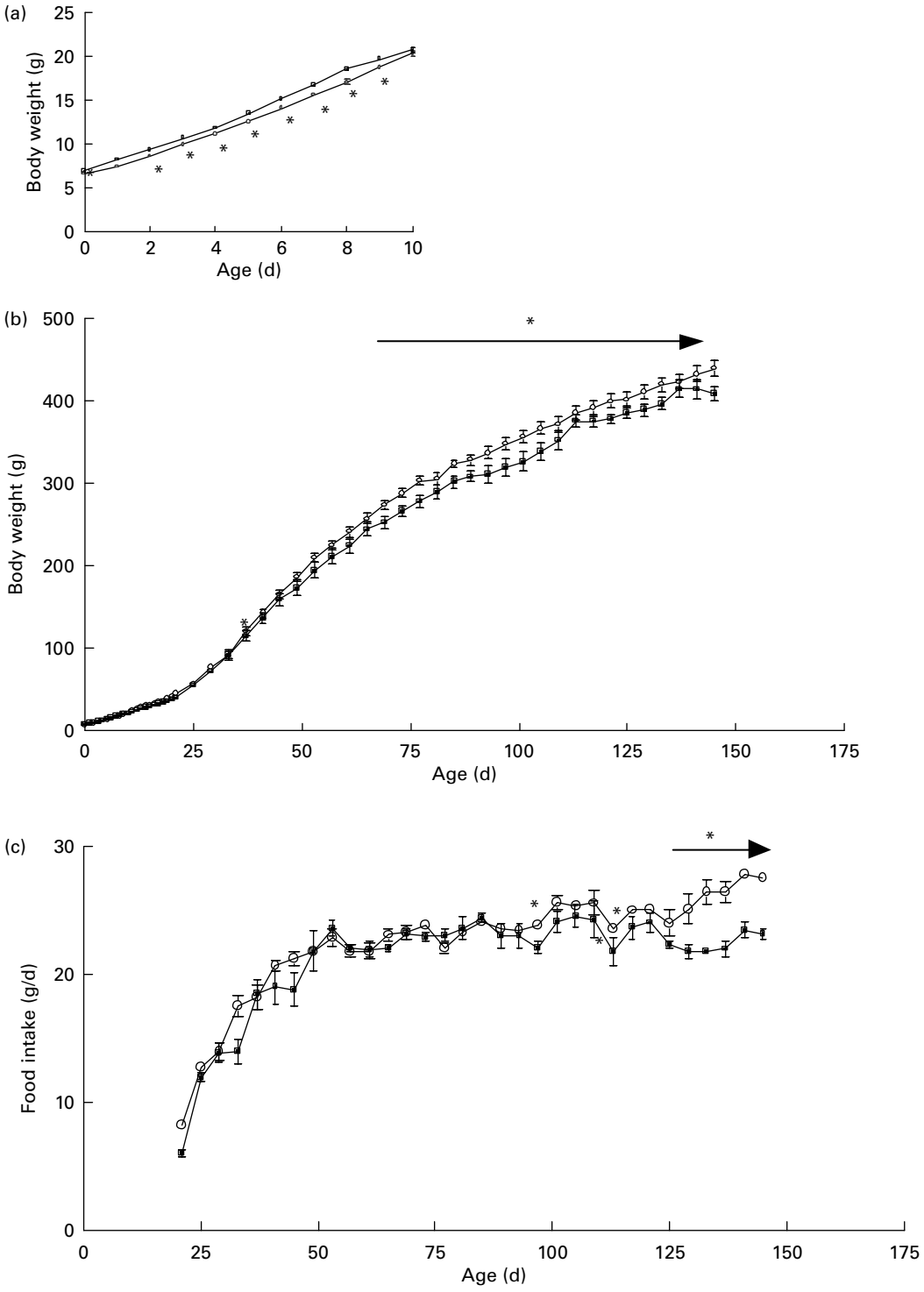

Fig. 1. Body weight from birth until 10 d of age (a), body weight (b) and food intake (c) from weaning to adulthood of pups that were given daily injections of leptin for the first 10 d of lactation (O), and controls (■), which received the same volume of saline. Values are given as the mean with their standard errors for twelve animals per group. Mean values were significantly different between the treated and control groups: $^{*}P < 0{\cdot}05$. For details of procedures, see p. 831.

no report on the long-term consequences of this treatment on body composition, mainly on the body's protein content.

Leptin and leptin receptor expression were both detected in skeletal muscle (Wang *et al.* 1998; Steinberg & Dyck, 2000; Maroni *et al.* 2003), but there are few data on the effect of leptin on skeletal muscle. The only study on the effect of leptin on protein synthesis in skeletal muscle showed that it indirectly decreased amino acid incorporation into rat skeletal muscle (Carbo *et al.* 2000). If the leptin resistance observed by us were also peripheral, it could explain the higher body protein content. On the other hand, some studies have shown a direct effect of leptin on muscle, increasing glucose and

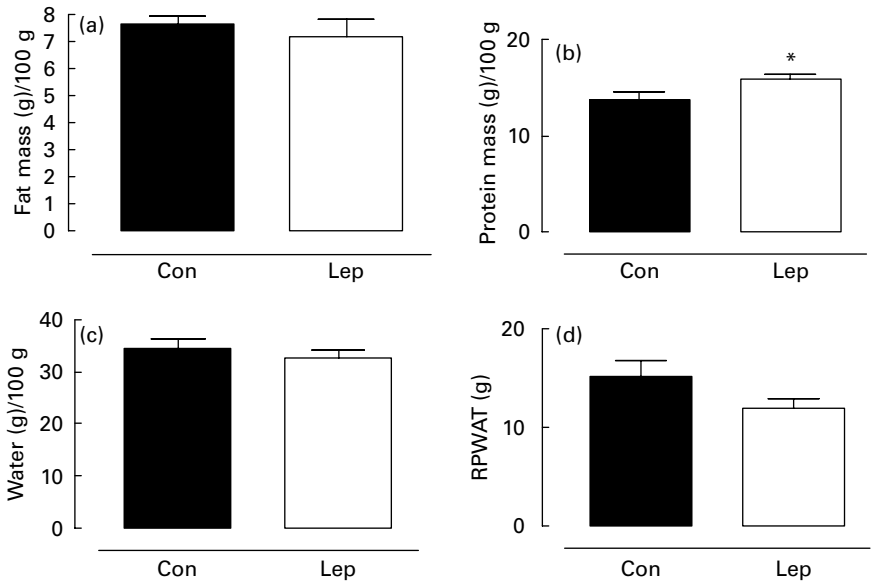

Fig. 2. Fat mass (a), protein mass (b), water content (c) and retroperitoneal white adipose tissue (RPWAT; (d)) of rats at 150 d of age that were given daily injections of leptin for the first 10 d of lactation (Lep) (□) and the control (Con) group (■), which received the same volume of saline. Values are given as the mean with their standard errors for twelve animals per group. Mean values were significantly different between the Lep and Con groups: $^*P < 0{\cdot}05$. For details of procedures, see p. 831 of proofs.

fatty acid metabolism (Steinberg & Dyck, 2000; Ceddia *et al.* 2001). Leptin was found to stimulate growth hormone release by a stimulation of growth hormone-releasing hormone (Chan *et al.* 1996, Tannenbaum *et al.* 1998). It is known that growth hormone increases amino acid uptake into muscles, increases protein synthesis and decreases protein catabolism (Casanueva & Dieguez, 1998). It is therefore probable that, in the present study, leptin stimulated muscle protein synthesis through the action of growth hormone. To unravel this apparently conflicting interpretation, it is fundamental to study the leptin signalling pathway in muscles in this model.

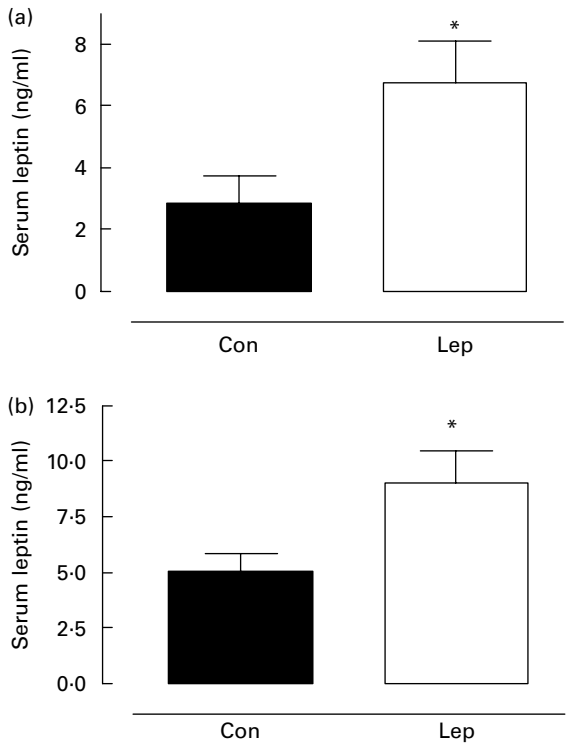

Fig. 3. Serum leptin concentration of rats at 10 (a) and 150 d of age (b) that were given daily injections of leptin for the first 10 d of lactation (Lep) (□) and the control (Con) group (■), which received the same volume of saline. Values are given as the means with their standard errors for six (a) and twelve (b) animals per group. Mean values were significantly different between the Lep and Con groups: $^*P < 0{\cdot}05$. For details of procedures, see p. 831 of proofs.

In the present study, the Lep group demonstrated no change in serum glucose concentration, suggesting that these animals presented insulin resistance, since the levels of insulin were two-fold higher. Insulin is well known for its anabolic effects, and hyperinsulinaemia in the Lep group could also explain the increase in total body protein of these animals. However, as insulin has no effect on the adipose tissue of these animals, since they did not differ in total fat mass from controls, it is possible that a selective insulin resistance existed. This event of selective insulin resistance was demonstrated by Kim *et al.* (2000), but these authors showed that selective resistance in muscle promoted the redistribution of substrates to adipose tissue, contributing to a higher adiposity. In our model, if the selective resistance occurred in the adipose tissue, we could expect the redistribution of substrates going to the muscle.

Leptin decreases insulin sensitivity in rat adipose tissue (Muller *et al.* 1997). Chronic exposure to leptin has been hypothesized to promote glucose and fatty acid degradation, preventing the accumulation of triacylglycerols and consequently leading to the development of insulin resistance (Unger *et al.* 1999). Therefore, the insulin resistance in adipose tissue could be, in part, due to the high levels of leptin. In addition, this insulin resistance in adipose tissue would also explain the higher serum triacylglycerol levels found in the Lep group.

The usual anorexigenic response to an acute dose of peripheral leptin is not observed in those adult animals treated with leptin during early lactation; in contrast, leptin significantly

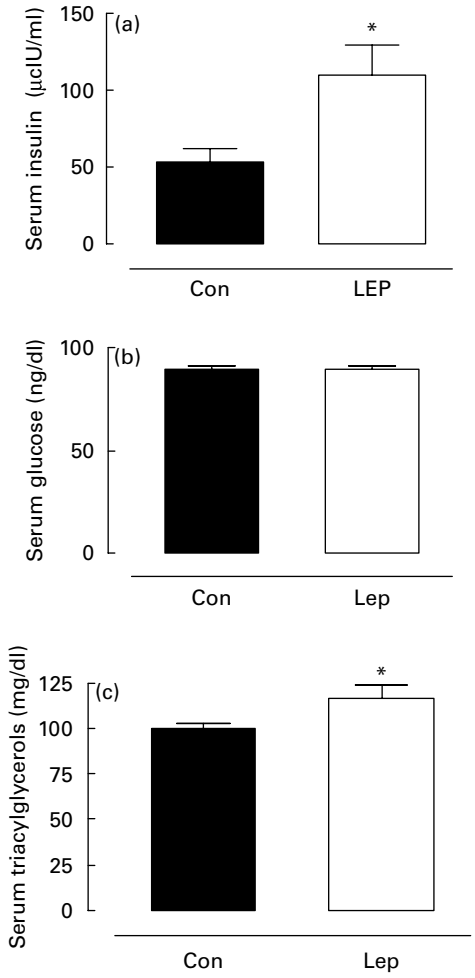

Fig. 4. Serum insulin (a), serum glucose (b) and serum triacylglycerol (c) concentrations in rats at 150 d of age that were given daily injections of leptin for the first 10 d of lactation (Lep) (□) and the control (Con) group (■), which received the same volume of saline. Values are given as the mean with their standard errors for twelve animals per group. Mean values were significantly different between the Lep and Con groups: $^*P < 0{\cdot}05$. For details of procedures, see p. 831 of proofs.

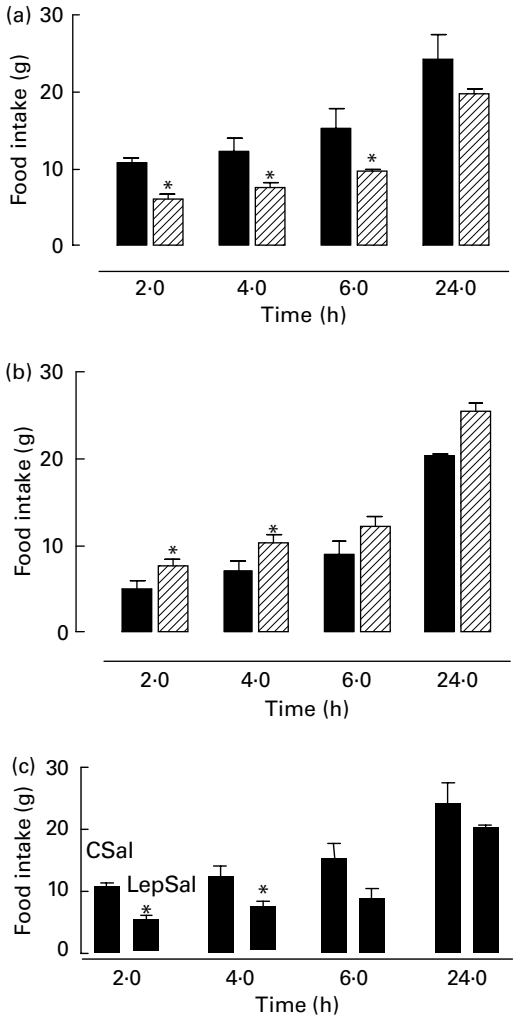

Fig. 5. The effect of the acute leptin treatment on food intake of the adult rats, which were treated with saline or leptin for the first 10 days of lactation. At 150 days, the rats of each experimental group (C and Lep) were randomized to be assigned to one of the following groups: a) ■ CSal, ▨ CL; (b) ■ LepSal, ▨ LepL and (c) correspond the comparison between the two controls (CSal and LepSal). Values represent the mean ± SEM of cumulative intake; six animals per group. $^*P < 0{\cdot}05$ compared with respective saline control group.

suppressed food intake in the Con groups, as early as 2 h, as expected (Lin *et al.* 2001; Passos *et al.* 2004). These data suggest that the leptin administered during early lactation could programme hypothalamic leptin resistance. This resistance was also shown by other authors in adult rats submitted to chronic treatment with leptin (Martin *et al.* 2000) and in response to a high-fat diet (Lin & York, 1998), both groups showing a higher serum leptin concentration, similar to the present study. The present study is thus the first to associate hyperleptinaemia during lactation with leptin resistance in adult animals.

Leptin resistance may occur for one of several reasons: leptin may fail to cross the blood–brain barrier, the hypothalamic receptors may be downregulated or there may be abnormalities in the leptin receptor signalling pathway, for example an inhibition of the JAK2–STAT3 pathway or the phosphatidylinositol-3 kinase–phosphodiesterase 3B–cAMP pathway, followed by activation of suppressor of cytokine signalling 3 (SOCS-3; Jéquier, 2002; Sahu, 2004).

The downregulation of long-form OB-Rb attenuates the function of leptin, causing impaired signal transduction (Scarpace *et al.* 2001). To test the possibility of downregulation in Lep rats, we measured the OB-Rb level by western blotting and found a lower hypothalamic expression. It is likely that leptin resistance is caused by this reduction in the leptin receptor. This hypothalamic decrease in OB-Rb expression associated with higher leptin levels and consequent leptin resistance was also shown in other models of hyperleptinaemia, such as histamine-deficient transgenic rats (Hegyi *et al.* 2004), young rats treated with a high-fat diet (Madiehe *et al.* 2000; Martin *et al.* 2000) and diet-induced obesity rats (Levin *et al.* 2003).

It is well established that leptin acts at the level of the hypothalamus to reduce appetite, and there is growing evidence for an endocrine feedback system between the adipose tissue and the pancreatic β-cells via the hormones leptin and insulin, respectively. Insulin is adipogenic and increases the production of leptin by adipose tissue, whereas leptin inhibits the production of insulin in the pancreatic β-cells (Kieffer & Habener, 2000).

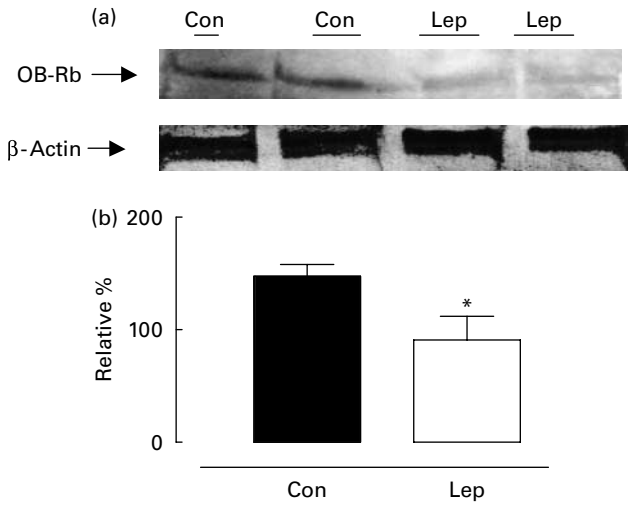

Fig. 6. (a) Western blot analysis of the hypothalamic leptin receptor (OB-Rb) in rats at 150 d of age that were given daily injections of leptin for the first 10 d of lactation (Lep), and the control group (Con), which received the same volume of saline. Also shown is the western blot analysis of β-actin. (b) Densitometric analysis of the immunoreactive bands for OB-Rb, expressed as relative (%) to the control group (saline). Data are means and their standard errors for four animals in each group. Mean values were significantly different between the Lep and Con group: $^{*}P < 0{\cdot}05$.

The suppressive effect of leptin on insulin production is mediated by the autonomic nervous system and by a direct action via leptin receptors on the β-cells (Kieffer & Habener, 2000). The hyperleptinaemia in the presence of hyperinsulinism in programmed offspring suggests the development of leptin resistance at the level of the pancreatic β-cells.

Long before the discovery of the hypothalamic action of leptin, insulin was proposed as a regulator of food intake and adipose mass (Woods *et al.* 1979). The neurones of the arcuate nucleus express both insulin (Marks *et al.* 1990) and leptin (Schwartz *et al.* 1996) receptors. Both hormones inhibit neuropeptide Y/Agouti-related protein neurones and stimulate proopiomelanocortin neurones. The cellular and physiological responses to insulin and leptin in the hypothalamus are similar (Niswender *et al.* 2004). Leptin activates both the JAK/STAT pathway and the insulin-like phosphatidylinositol-3 kinase pathway in a phenomenon referred to as 'cross-talk' (Niswender *et al.* 2004). Both hormones augment SOCS-3 levels, which inhibit JAK/STAT phosphorylation (Elias *et al.* 1999). The desensitization of the OB-R system may be mediated by defective signalling induced by the increase in SOCS-3 (Ahima *et al.* 2000, Seufert *et al.* 2000).

The Lep programmed group showed both higher leptin and insulin serum concentrations. However, it seems that these animals showed hypothalamic resistance to both hormones, since they had a higher food intake than the Con group. In this paper, we describe not only resistance to leptin, but paradoxically, an increase in food intake when the rats received exogenous leptin after 24 h fasting. Lep animals compared with the Con group, when fasted for 24 h, showed a lower food intake at 2, 4 and 6 h after saline injection. This could have happened because, during fasting, there is an acute decrease in insulinaemia and leptinaemia, reversing the leptin and insulin resistance, and allowing an anorexigenic effect of those hormones. When the rats received exogenous leptin, the higher hormone level achieved again induced leptin resistance**.**

Based on these findings, we hypothesize that leptin treatment during lactation increases both serum leptin and insulin in adulthood, which leads to leptin resistance by reducing the expression of the hypothalamic leptin receptor. In addition, we suggest that there is also a peripheral leptin resistance in muscle and pancreatic β-cells, as well as selective insulin resistance in the adipose tissue.

Acknowledgements

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