Programming of hypothalamic neuropeptide gene expression in rats by maternal dietary protein content during pregnancy and lactation

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ABSTRACT

Epidemiological studies show a link between low birthweight and increased obesity. In contrast, slow growth during the lactation period reduces obesity risk. The present study investigates the potential underlying mechanisms of these observations. Rats were established as follows: (i) control animals [offspring of control dams fed a 20% (w/v) protein diet], (ii) recuperated animals [offspring of dams fed an isocaloric low-protein (8%, w/v) diet during pregnancy and nursed by control dams], and (iii) postnatal low protein animals (offspring of control dams nursed by low-protein-fed dams). Serum and brains were collected from fed and fasted animals at weaning. Expression of hypothalamic energy balance genes was assessed using in situ hybridization. Recuperated pups were smaller at birth, but caught up with controls by day 21 and gained more weight than controls between weaning and 12 weeks of age (P < 0.05). At 21 days, they were hypoleptinaemic compared with controls in the fed state, with generally comparable hypothalamic gene expression. Postnatal low protein offspring had significantly lower body weights than controls at weaning and 12 weeks of age (P < 0.001). At 21 days, they were hypoglycaemic, hypoinsulinaemic and hypoleptinaemic. Leptin receptor gene expression in the arcuate nucleus was increased in postnatal low protein animals compared with controls. Consistent with hypoleptinaemia, hypothalamic gene expression for the orexigenic neuropeptides NPY (neuropeptide Y) and AgRP (Agouti-related peptide) was increased, and that for the anorexigenic neuropeptides POMC (pro-opiomelanocortin) and CART (cocaine- and amphetamine-regulated transcript) was decreased. These results suggest that the early nutritional environment can affect the development of energy balance circuits and consequently obesity risk.

INTRODUCTION

Over recent decades, there has been a large increase in the proportion of both adults and children that are either overweight or obese in developed countries. In the U.S., 64.5% of adults had a BMI (body mass index) greater than 25 kg/m² in 2000, increased from 55.9% in 1988–1994, whereas the proportion of adults that

Key words: developmental programming, energy balance, hypothalamic neuropeptide, lactation, obesity, pregnancy.

Abbreviations: AgRP, agouti-related peptide; ARC, arcuate nucleus; BMI, body mass index; CART, cocaine- and amphetamine-regulated transcript; DMH, dorsomedial nucleus of the hypothalamus; MC4R, melanocortin-4 receptor; NPY, neuropeptide Y; OB-Rb, long form of the leptin receptor; POMC, pro-opiomelanocortin; postnatal low protein animal, offspring of a control dam nursed by a low-protein-fed dam; PVN, paraventricular nucleus; recuperated animal, offspring of a dam fed an isocaloric low-protein diet during pregnancy and nursed by a control dam; SOCS3, suppressor of cytokine signalling 3; VMH, ventromedial nucleus of the hypothalamus.

1 Deceased.

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had a BMI over 30 kg/m² had increased from 22.9% in 1988–1994 to 30.5% in 2000 [1]. More worrying is the increase in childhood obesity, with 22 million children under the age of 5 being overweight and 10% of U.S. preschool children being obese [2]. The consequences of being overweight or obese are far reaching in terms of mortality and morbidity, as well as escalating healthcare costs. Obesity increases the risk of Type 2 diabetes, dyslipidaemia, hypertension and certain types of cancer, such as those of the breast, colon and pancreas [3].

Many of the diseases where obesity is a risk factor have been linked by numerous epidemiological studies with low birthweight [4,5]. These relationships have been found in populations worldwide, but the mechanisms underlying this link are still unclear. However, there is evidence that early nutrition plays a key role. Studies with low birthweight [4,5]. These relationships have been linked by numerous epidemiological studies with low birthweight [4,5]. These relationships have been found in populations worldwide, but the mechanisms underlying this link are still unclear. However, there is evidence that early nutrition plays a key role. Studies of adults who were in utero during the ‘Dutch Hunger Winter’ provide direct evidence in humans for the importance of maternal nutrition on offspring’s health. Men exposed to famine in early gestation had increased rates of obesity at 19 years of age, whereas those exposed during late gestation and in the first months of life had reduced rates of obesity [6]. Breast feeding provides infants with a lower plane of neonatal nutrition than formula feeding, and a meta-analysis of studies investigating the effect of breast feeding showed that breast-fed infants have a reduced risk of obesity compared with formula-fed infants [7]. A variety of protocols have been established to investigate the effects of maternal diet on obesity risk in the offspring. The offspring of severely undernourished rat dams are hyperphagic and obese [8]. Further evidence for the importance of the early postnatal period can be found in cross-fostering studies. In mice, offspring of dams fed a reduced protein diet cross-fostered in small litters with control-diet-fed dams are born small, undergo catch-up growth and are more susceptible to diet-induced obesity. In contrast, offspring of control dams cross-fostered on to a dam fed a reduced protein diet during lactation are resistant to the obesity-inducing effects of a highly palatable diet [9,10]. These studies show the long-term detrimental effects of ‘catch-up’ growth in early life, whereas slow growth during this period appears to confer a protective effect, a programmed reduction in voluntary food intake.

The energy balance system is regulated by feedback from peripheral hormonal and metabolic signals on to central integratory circuits, which are concentrated in the hypothalamus. Within the hypothalamus, neuropeptides and their receptors are prominent. Leptin is a major energy balance signal derived from adipose tissue that relays long-term information to the brain about the size of body fat stores and shorter-term information about the state of energy flux in adipose tissue. Leptin receptors are heavily expressed in the ARC (arcuate nucleus) of the hypothalamus and co-localize with both the orexigenic [NPY (neuropeptide Y)/AgRP (agouti-related peptide)] and anorexigenic [CART (cocaine- and amphetamine-regulated transcript)/POMC (pro-opiomelanocortin)] pathways. The ARC is a site of leptin feedback into the brain. Consequently, the leptin signal and its integration into the hypothalamic network are likely to be involved in the programming of energy balance systems by early life nutrition. A number of strands of evidence support this suggestion. ob/ob mice, which lack leptin, have reduced neuronal outgrowth from the ARC. Furthermore, leptin caused neurite growth from neonatal ARC explants but not adult ones [11]. During the lactation period, it is thought that leptin is playing a developmental, rather than an energy balance, role. There is a leptin surge during this period [12], and this surge is earlier in undernourished mouse pups [13]. If control pups are given exogenous leptin, to mimic the premature leptin surge seen in the undernourished offspring, they have accelerated weight gain when placed on a high-fat diet [13]. However, in rats, if the offspring of undernourished dams are given exogenous leptin postnatally, then the detrimental metabolic phenotype seen in adulthood can be reversed [14]. Alterations in neonatal leptin concentrations induced by changes in maternal nutrition could therefore affect hypothalamic development of energy balance circuits.

The aim of the present study was therefore to investigate the effect of fetal growth restriction followed by catch-up growth or normal fetal growth followed by slowed growth during neonatal life on the anatomically resolved expression of a panel of neuropeptide and receptor genes known to be involved in energy balance regulation. An early time point was chosen allowing the elucidation of possible mechanisms rather than later-life consequences.

MATERIALS AND METHODS

Animals and experimental protocol

All procedures involving animals were carried out under the U.K. Home Office Animals (Scientific Procedures) Act, 1986.

Virgin female Wistar rats weighing 240–260 g were housed individually and maintained at 22 °C on a 12 h light/dark cycle (lights on 07.00–19.00 hours). They were mated, and day 1 of gestation was taken as the day on which vaginal plugs were expelled. Rats were then fed either a control (20% protein, w/v) diet or an isocaloric low-protein (8%, w/v) diet. These were purchased from Arie Blok and their compositions have been described previously [15]. The diet had no effect on litter size (13 ± 0.3 in the control group compared with 13 ± 0.4 in the low-protein group; P = 0.64), sex ratio (48 ± 2.3% males in the control group compared with 53 ± 4.4% males in the low-protein group; P = 0.33) or pup survival. Maternal weight and food intake were measured daily until weaning. After spontaneous birth on day 21, the control dams were either continued on the control diet with their own litter culled to eight offspring.
('control') or received and suckled four randomly selected offspring from the litter of a low-protein-fed dam ('recuperaed'). The culling of this litter to only four served to maximize the plane of nutrition received by these pups during suckling and also the rate of catch-up growth. Low-protein-fed dams that had donated their pups to a control dam received that dam's litter (both male and female pups) unculled ('postnatal low protein') (litter size, 13 ± 0.41 pups). This litter was left unculled to minimize the amount of nutrition during suckling and therefore the rate of growth. For the pups killed on postnatal day 22, weights were taken on postnatal days 3, 7, 14 and 21.

Pups were weaned at 21 days. One cohort of male animals was immediately killed (at 09.00 hours), and a separate group of male animals were starved overnight and killed on day 22 (at 09.00 hours). This enabled the study of the animals in both fed and fasted states. No more than two pups were taken from each litter, and each cohort of animals studied used separate groups of dams giving 4–8 dams used for each group of offspring studied at each time point. On the day of the experiment, animals were weighed and then killed by CO₂ inhalation and exsanguination. Blood serum was collected. Brains were measured weekly. At 12 weeks of age, this cohort of animals was studied up to 12 weeks of age after weaning on to a standard laboratory chow diet at 21 days of nutrition during suckling and therefore the rate of growth. As these experiments were not conducted at the same time, it is not possible to directly compare animals to fasting or weaning stress between the groups, it was possibly confounding effect of differential susceptibility to starvation by the end of gestation (388.9 ± 10.4 g; P < 0.01). Total maternal

Statistical analysis
Values are expressed as means ± S.E.M., except for serum insulin, which required log-transforming prior to testing as it was not normally distributed and is shown as geometric means (95 % confidence intervals). Statistical differences between means were assessed using one-way ANOVA with maternal diet as the independent variable, followed by Duncan’s post-hoc tests where appropriate. Differences between means were considered significant at values of P < 0.05.

RESULTS
Maternal weight and food intake
Low-protein-fed dams were smaller than control-diet-fed dams by the end of gestation (388.9 ± 10.4 g compared with 426.0 ± 4.8 g; P < 0.01). Total maternal
Growth curves of male rats during lactation

Serum and plasma characteristics of male pups at absolute and relative weights of fat pads and brain

After cross-fostering, there were three experimental groups of dams. During lactation, the weight of the low-protein-fed dams decreased further, so by the end of lactation they were 30% smaller than control dams (254.3 ± 5.1 g compared with 355.6 ± 5.7 g; P < 0.001), whereas the control dams nursing either control or low-protein-fed pups remained smaller throughout lactation and weighed 56% of control pups by day 7 (P < 0.001). Postnatal low protein animals had lower glucose concentrations compared with controls (P < 0.001 for fasted rats; Table 2). There was a significant overall effect of maternal diet manipulation (P < 0.001) on serum leptin concentrations. Leptin concentrations were lower in postnatal low protein rats compared with controls (P < 0.001). However, in the fasted state, recuperated male animals had higher serum leptin concentrations compared with controls (P < 0.05; Table 2). Maternal diet also altered insulin concentrations (P < 0.001). Insulin concentrations were lower in postnatal low protein rats

Figure 1 Growth curves of male rats during lactation

Values are expressed as means ± S.E.M. (n = 10 per group), and were examined using one-way ANOVA with appropriate Duncan’s post-hoc tests. ***P < 0.001 compared with control animals of the same age. Black squares, control (C); grey squares, recuperated (R); and white squares, postnatal low protein (PLP).

Growth of pups until weaning

The growth of pups until weaning is shown in Figure 1. At birth, pups whose mothers were fed the low-protein diet were significantly smaller than the control pups (P < 0.001). This is a mild intervention and did not significantly affect litter size (13.0 ± 0.3 pups in controls and 13.0 ± 0.4 pups in the low-protein-fed dams; P = 0.64) or the sex ratio of the offspring (48 ± 2.3% males in controls and 53 ± 4.4% males in the low-protein-fed dams). Recuperated pups caught up with control pups by day 14, but postnatal low protein pups were smaller than control pups by day 7 (P < 0.001). Postnatal low protein pups remained smaller throughout lactation and weighed less than 50% of control pups on day 21 (P < 0.001).

Organ weights of fasted 22-day-old offspring

The weight of the brain (P < 0.01), and retroperitoneal (P < 0.001) and mesenteric (P < 0.001) fat pads were all significantly lower in the postnatal low protein animals compared with controls (Table 1). However, the decrease in brain and fat pad weights were not in proportion with the reduction in body weight (Table 1). The growth of the brain (P < 0.001) was relatively spared, whereas the reduction in fat masses (both P < 0.001) were greater than overall body weight (Table 1).

Characteristics of offspring blood/serum

There was a significant overall effect of maternal diet (P < 0.001) on blood glucose concentrations. Postnatal low protein animals had lower glucose concentrations compared with controls (P < 0.05 for fed animals, and P < 0.001 for fasted rats; Table 2). There was a significant overall effect of maternal diet manipulation (P < 0.001) on serum leptin concentrations. Leptin concentrations were lower in postnatal low protein rats compared with controls (P < 0.001 for fed animals, and P < 0.05 for fasted rats). In fed rats, recuperated animals had lower serum leptin concentrations compared with controls (P < 0.001). However, in the fasted state, recuperated male animals had higher serum leptin concentrations compared with controls (P < 0.05; Table 2). Maternal diet also altered insulin concentrations (P < 0.001). Insulin concentrations were lower in postnatal low protein rats

Table 1 Absolute and relative weights of fat pads and brain in 22-day-old male rat pups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Recuperated</th>
<th>Postnatal low protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retroperitoneal fat pad weight</td>
<td>0.75 ± 0.03</td>
<td>0.40 ± 0.02</td>
<td>0.25 ± 0.01</td>
</tr>
<tr>
<td>Mesenteric fat pad weight</td>
<td>0.60 ± 0.03</td>
<td>0.25 ± 0.01</td>
<td>0.10 ± 0.01</td>
</tr>
<tr>
<td>Brain weight</td>
<td>2.36 ± 0.14</td>
<td>1.50 ± 0.08</td>
<td>0.75 ± 0.04</td>
</tr>
<tr>
<td>Relative weight (%)</td>
<td>0.75 ± 0.03</td>
<td>0.40 ± 0.02</td>
<td>0.25 ± 0.01</td>
</tr>
<tr>
<td>Retroperitoneal fat pad weight</td>
<td>0.75 ± 0.03</td>
<td>0.40 ± 0.02</td>
<td>0.25 ± 0.01</td>
</tr>
<tr>
<td>Mesenteric fat pad weight</td>
<td>0.60 ± 0.03</td>
<td>0.25 ± 0.01</td>
<td>0.10 ± 0.01</td>
</tr>
<tr>
<td>Brain weight</td>
<td>2.36 ± 0.14</td>
<td>1.50 ± 0.08</td>
<td>0.75 ± 0.04</td>
</tr>
</tbody>
</table>

Table 2 Serum and plasma characteristics of males pups at both 21 days of age (fed state) and 22 days of age (fasted state)

Values are means ± S.E.M. (n = 8 per group), and were examined using one-way ANOVA with appropriate Duncan’s post-hoc tests. Insulin data were log-transformed prior to examination, and values are shown as geometric means (confidence intervals). *P < 0.05 and ***P < 0.001 compared with control animals.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Recuperated</th>
<th>Postnatal low protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood glucose (mmol/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fed</td>
<td>9.1 ± 0.6</td>
<td>10.0 ± 0.6</td>
<td>6.2 ± 0.8*</td>
</tr>
<tr>
<td>Fasted</td>
<td>5.0 ± 0.1</td>
<td>5.3 ± 0.1</td>
<td>4.3 ± 0.1**</td>
</tr>
<tr>
<td>Serum leptin (μg/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fed</td>
<td>0.23 ± 0.021</td>
<td>0.11 ± 0.014***</td>
<td>0.09 ± 0.013***</td>
</tr>
<tr>
<td>Fasted</td>
<td>0.06 ± 0.004</td>
<td>0.09 ± 0.009*</td>
<td>0.03 ± 0.007*</td>
</tr>
<tr>
<td>Serum insulin (pmol/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fed</td>
<td>95.8 (73.5–111.7)</td>
<td>65.7 (42.3–117.9)</td>
<td>3.9 (0.2–4.9)***</td>
</tr>
<tr>
<td>Fasted</td>
<td>20.0 (9.4–36.3)</td>
<td>10.4 (6.4–21.5)</td>
<td>7.7 (4.0–16.7)</td>
</tr>
</tbody>
</table>
Figure 2  Gene expression of OB-Rb, SOCS3 and MC4R in the indicated regions of the hypothalamus in fed 21-day-old males (A) and fasted 22-day-old males (B)

Values are expressed as a percentage of the controls and are means ± S.E.M. (n = 8 per group), and were examined using a one-way ANOVA with appropriate Duncan’s post-hoc tests. ∗P < 0.05, **P < 0.01 and ***P < 0.001 compared with control animals. Black bars, control; grey bars, recuperated; and white bars, postnatal low protein.

compared with controls (P < 0.001 for fed animals, and P < 0.05 for fasted rats; Table 2).

Hypothalamic gene expression

Receptors and SOCS3

As shown in Figure 2, there was a significant overall effect of maternal diet on OB-Rb gene expression in the ARC of ad-libitum-fed rats (P < 0.001) and overnight-fasted rats (P < 0.01). In each case, ARC OB-Rb gene expression was elevated in postnatal low protein rats compared with controls, but this difference was more substantial in fed rats than in fasted ones. In the VMH, there were no effects on OB-Rb gene expression in ad-libitum-fed rats, but there was a significant overall effect of maternal diet (P < 0.001) in overnight-fasted animals. OB-Rb gene expression was high in the VMH of postnatal low protein animals compared with control animals in the fasted state.

There was a significant overall effect of maternal diet (P < 0.001) on SOCS3 (Socs3) gene expression in the ARC of ad-libitum-fed animals. Both recuperated and postnatal low protein animals had reduced SOCS3 expression compared with control animals in the fed state. There was also an effect of maternal diet (P < 0.01) on SOCS3 expression in overnight-fasted animals; however, the effect of maternal diet was only observed in postnatal low protein animals, which had lower SOCS3 gene expression compared with control animals.

There was an overall effect of maternal diet (P < 0.01) on MC4R gene expression in the PVN in ad-libitum-fed animals, with postnatal low protein animals having lower MC4R gene expression; however this effect was not observed in fasted animals.

Orexigenic peptides

As shown in Figure 3, there was a significant effect of maternal diet (P < 0.001) on NPY gene expression in the ARC of both fed (P < 0.001) and fasted (P < 0.05) rats. In each case, ARC NPY gene expression was elevated in postnatal low protein rats compared with controls. This difference was again more marked in the fed rats than in the fasted animals. In the DMH, there was no effect of maternal diet on NPY gene expression in either fed or fasted conditions. There was a significant effect of maternal diet on AgRP gene expression in the ARC of both fed (P < 0.001) and fasted (P < 0.01) rats. In each case, ARC AgRP gene expression was elevated in postnatal low protein rats compared with controls. This difference was again more marked in the fed than in the fasted rat.
Table 3  Body weight and energy intakes at 12 weeks of age in male rats

Values are means ± S.E.M. (n = 10 per group), and were examined using a one-way ANOVA with appropriate Duncan’s post-hoc tests. *P < 0.05 and ***P < 0.001 compared with control animals. †P = 0.06.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Recuperated</th>
<th>Postnatal low protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight gain from weaning to 12 weeks (g)</td>
<td>322.2 ± 6.6</td>
<td>347.3 ± 8.4*</td>
<td>282.3 ± 5.9***</td>
</tr>
<tr>
<td>Body weight at 12 weeks (g)</td>
<td>403.3 ± 7.5</td>
<td>425.8 ± 9.4*</td>
<td>322.0 ± 5.1***</td>
</tr>
<tr>
<td>Cumulative energy intake from weaning to 12 weeks (kJ)</td>
<td>16641 ± 224</td>
<td>17237 ± 467*</td>
<td>13853 ± 258***</td>
</tr>
<tr>
<td>Absolute weight (g)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epididymal fat pad</td>
<td>5.33 ± 0.32</td>
<td>6.20 ± 0.44†</td>
<td>3.08 ± 0.18***</td>
</tr>
<tr>
<td>Retroperitoneal fat pad</td>
<td>6.56 ± 0.42</td>
<td>7.81 ± 0.54</td>
<td>3.27 ± 0.35**</td>
</tr>
<tr>
<td>Relative weight (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epididymal fat pad</td>
<td>1.34 ± 0.08</td>
<td>1.48 ± 0.07†</td>
<td>0.95 ± 0.06***</td>
</tr>
<tr>
<td>Retroperitoneal fat pad</td>
<td>1.65 ± 0.11</td>
<td>1.88 ± 0.10†</td>
<td>1.01 ± 0.11***</td>
</tr>
</tbody>
</table>

Figure 4  Gene expression of POMC and CART in the ARC of the hypothalamus in fed 21-day-old males (A) and fasted 22-day-old males (B)

Values are expressed as a percentage of the controls and are means ± S.E.M. (n = 8 per group), and were examined using a one-way ANOVA with appropriate Duncan’s post-hoc tests. **P < 0.01 and ***P < 0.001 compared with control animals. Black bars, control; grey bars, recuperated; and white bars, postnatal low protein.

Anorexigenic peptides

As shown in Figure 4, there was a significant effect of maternal diet on POMC gene expression in the ARC of fed (P < 0.001) and fasted (P < 0.01) rats. POMC gene expression was lower in postnatal low protein rats compared with controls. The down-regulation of POMC in the postnatal low protein group was more substantial in fed than in fasted rats.

There was a significant effect of maternal diet on CART gene expression in the ARC of fed (P < 0.001) and fasted (P < 0.01) rats, with CART gene expression being lower in postnatal low protein rats compared with controls. This effect was not as substantial as that observed in fed rats.

Growth and energy intake post-weaning

There was a significant effect of maternal diet on body weight gain from weaning to 12 weeks of age (P < 0.001). Recuperated animals gained more weight than controls (P < 0.05), whereas postnatal low protein animals gained less weight (P < 0.001; Table 3). This led to recuperated animals being heavier than control animals (P < 0.05) at 12 weeks of age and postnatal low protein animals were lighter (P < 0.001; Table 3). There was a significant effect of maternal diet on cumulative energy intake during this period with the same pattern: recuperated animals consumed more energy (P < 0.05) compared with control animals, whereas postnatal low protein animals consumed less energy (P < 0.01; Table 3).

The weights of both the retroperitoneal and epididymal fat pads were affected significantly by maternal diet in adult rats (P < 0.001 for both). Postnatal low protein animals had smaller fat pads than control animals, whereas there was a tendency (P = 0.06 for both) for the recuperated animals to have heavier fat pads than control animals (Table 3). When expressed relative to body weight, there was still an overall effect of maternal diet (P < 0.001 for both fat pads), with postnatal low protein animals having lower relative fat masses (P < 0.001 for both), but the recuperated animals relative fat pad weights were not significantly different from control animals (Table 3).

DISCUSSION

Alterations in maternal dietary protein content during gestation and lactation have permanent effects on
susceptibility to weight gain and longevity in rodents [9,10,24], but the molecular mechanisms that underlie these programming events remain to be resolved. The present study examined alterations in leptin and the expression of neuropeptides and receptors implicated in energy balance control after manipulation of maternal diet protein content and the plane of nutrition received during lactation. In addition, the effect of fasting on these young animals and their energy-balance-related hypothalamic gene expression was examined.

Long-term effects on weight gain could be mediated by changes in energy intake and/or energy expenditure. In the model used in the present study, recuperated animals consumed more energy than control animals and this was accompanied by a larger increase in body weight, whereas postnatal low protein animals consumed less energy and so gained less weight in adulthood. It is therefore clear that simple alterations in early life nutrition had long-term effects on energy balance via energy intake. Currently, it is unknown whether there are similar effects on energy expenditure.

In the present study, we addressed the mechanisms by which these programmed changes in energy intake could occur by studying animals at weaning. By choosing an early time point, programmed changes that could lead to the development of the phenotype, rather than changes as a consequence of the phenotype, can be examined. At this age, the recuperated animals did not differ from the control animals in terms of body weight. We demonstrated that the serum concentration of leptin was altered by manipulation of maternal protein intake. Leptin during the lactation period is a candidate for programming of the appetite system [11,25,26]. Postnatal low protein animals had substantially reduced blood leptin concentration compared with control animals under both fed and fasted conditions. This reduction is likely to be a consequence of the dramatically reduced adiposity in this group. Recuperated animals had lower leptin concentrations than controls in the fed state, despite similar body weights, suggesting a possible alteration in the control of leptin secretion. This could lead to an increased drive to eat and could increase their susceptibility to obesity, as seen by their higher cumulative energy intake and body weights from weaning to 12 weeks of age. In addition, there was a tendency for increased fat pad mass in the recuperated animals at 12 weeks of age when compared with control animals, although this was in proportion with the increased body weight. After an overnight fast, the leptin concentration in recuperated animals did not show the expected large fall seen in both control and postnatal low protein animals. This resulted in recuperated animals having an elevated leptin concentration compared with the control animals in the fasted state. Recuperated animals at this age therefore appear to have a defect in their ability to alter their plasma leptin concentrations in response to feeding status.

Differences in long-term susceptibility to weight gain may be a consequence of changes in central leptin sensitivity. The mRNA of the signalling form of the leptin receptor (OB-Rb) was more highly expressed in the ARC of postnatal low protein animals compared with control animals in both the fed and fasting conditions. This, combined with the lower expression of the negative regulator of leptin signalling SOCS3, may increase leptin sensitivity in these animals, which may contribute to their long-term reduction in weight gain compared with control animals. It has been shown previously that low leptin concentrations can lead to the up-regulation of leptin receptors in the ARC [27]. The fed recuperated animals had low concentrations of circulating leptin compared with the control animals; however, this hypoleptinaemia was not accompanied by an increase in the expression of the leptin receptor in the ARC. This contrasts with the postnatal low protein animals, and suggests there is a difference in the response to low leptin levels between these two groups of animals. High levels of leptin are presumed to signal positive energy balance and counteract this state through two pathways. The expression of anorexigenic genes, such as CART and POMC, are increased in response to leptin, whereas the expression of orexigenic genes, such as NPY and AgRP, are decreased [28]. In the fed state, expression of the mRNAs for the orexigenic peptides NPY and AgRP in the ARC was higher in the postnatal low protein animals and expression of the mRNAs for the anorexigenic peptides CART and POMC was lower compared with control animals. These changes are appropriate for the low serum leptin concentrations in this group. This suggests that these animals register the negative energy balance they are in, i.e. their low body weight compared with developmentally appropriate controls. However, these postnatal low protein animals remain small even when fed ad libitum and do not respond appropriately to this multi-component signal. This suggests that these animals have a fault downstream of leptin signalling.

The present study reinforces the suggestion, which is growing in the literature, that fetal and early life nutrition affects energy balance systems. Small litter size animals that go on to become obese have reduced ARC gene expression of OB-Rb and increased arcuate mRNA expression of the orexigenic peptides NPY and AgRP post-weaning [29]. Neonatally overnourished rats at weaning have been shown to have alterations in the PVN of the hypothalamus with decreased numbers of CCK (cholecystokinin)-positive neurons [30] and increased numbers of galanin-positive neurons [31]. There is also evidence for a change in the response of the VMH to leptin. In neonatally normally nourished rats, leptin increases firing from VMHs, whereas in neonatally overnourished animals leptin inhibits firing [32]. Other models of altered early nutrition have shown an altered expression of energy balance
neuropeptides in the hypothalamus; maternal high-fat feeding leads to increased expression of galanin in the PVH and orexin in the lateral hypothalamus [33], whereas the offspring of food-restricted rat dams have a lower level of hypothalamic POMC expression [34]. This could cause an increased drive to eat in these offspring. Maternal obesity, as a consequence of the genetic background of the dam, during lactation of the pups has been shown to influence the expression of energy balance neuropeptides in adult offspring [35].

The present study has shown that hypothalamic mRNA expression of regulatory neuropeptides and signalling proteins of energy balance systems are altered by maternal dietary protein manipulations during pregnancy and lactation. Those animals that grew slowly during lactation have evidence of changes in expression of neuropeptides that should signal that they are in a state of negative energy balance; however, the anticipated physiological response to these signals, namely behavioural and energetic compensation, does not develop and the animals stay small during adolescence and adult life when fed ad libitum, with lower energy intakes over this period than control animals. This could be a result of the action of the neuropeptides being blocked further downstream or a complete alteration of the wiring of the system. These animals that remain lean have changes in expression of leptin signalling molecules that could indicate increased sensitivity to this important regulator of energy balance. Animals that underwent in utero growth restriction followed by catch-up growth had a similar expression of examined neuropeptides to control animals, but reduced leptin in the fed state compared with control animals. This could provide an increased drive to eat, even when fed, thus providing a possible mechanism to control animals. This could provide an increased drive to eat in these animals, but reduced leptin in the fed state compared with control animals. This could be a result of the induction of obesity by a highly palatable diet. Clin. Sci. 106, 141–145

In conclusion, the present study provides new insight into the mechanistic basis that underlies the link between low birthweight and obesity and slow growth and leanness in adulthood.

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