Down-regulation of hypothalamic pro-opiomelanocortin (POMC) expression after weaning is associated with hyperphagia-induced obesity in JCR rats overexpressing neuropeptide Y

Abdoulaye Diane1, W. David Pierce2*, James C. Russell1, C. Donald Heth3, Donna F. Vine1, Denis Richard4 and Spencer D. Proctor1

1Metabolic and Cardiovascular Diseases Laboratory, Alberta Institute for Human Nutrition, Alberta Diabetes Institute, University of Alberta, Edmonton, Canada
2Department of Sociology, University of Alberta, H.M Tory Building, Edmonton, Canada, AB T6G 2H4
3Department of Psychology, University of Alberta, Edmonton, Canada
4Institut Universitaire de Cardiologie et de Pneumologie de l’Universite Laval, Quebec, Canada

(Submitted 23 April 2013 – Final revision received 15 August 2013 – Accepted 21 August 2013 – First published online 7 October 2013)

Abstract

We hypothesised that hypothalamic feeding-related neuropeptides are differentially expressed in obese-prone and lean-prone rats and trigger overeating-induced obesity. To test this hypothesis, in the present study, we measured energy balance and hypothalamic neuropeptide Y (NPY) and pro-opiomelanocortin (POMC) mRNA expressions in male JCR:LA-cp rats. We compared, in independent cohorts, free-feeding obese-prone (Obese-FF) and lean-prone (Lean-FF) rats at pre-weaning (10 d old), weaning (21–25 d old) and early adulthood (8–12 weeks). A group of Obese-pair-feeding (PF) rats pair-fed to the Lean-FF rats was included in the adult cohort. The body weights of 10-d-old Obese-FF and Lean-FF pups were not significantly different. However, when the pups were shifted from dams' milk to solid food (weaning), the obese-prone rats exhibited more energy intake over the days than the lean-prone rats and higher body and fat pad weights and fasting plasma glucose, leptin, insulin and lipid levels. These differences were consistent with higher energy consumption and lower energy expenditure. In the young adult cohort, the differences between the Obese-FF and Lean-FF rats became more pronounced, yielding significant age effects on most of the parameters of the metabolic syndrome, which were reduced in the Obese-PF rats. The obese-prone rats displayed higher NPY expression than the lean-prone rats at pre-weaning and weaning, and the expression levels did not differ by age. In contrast, POMC expression exhibited significant age-by-genotype differences. At pre-weaning, there was no genotype difference in POMC expression, but in the weanling cohort, obese-prone pups exhibited lower POMC expression than the lean-prone rats. This genotype difference became more pronounced at adulthood. Overall, the development of hyperphagia-induced obesity in obese-prone JCR rats is related to POMC expression down-regulation in the presence of established NPY overexpression.

Key words: Obese-prone animals: Weanling JCR rats: Homeostasis: Neuropeptides

Abbreviations: ARC, arcuate nucleus; E EFF, energy efficiency; FF, free-fed; Lean-FF, lean-prone (+/?); NPY, neuropeptide Y; Obese-FF, obese-prone (cp/cp); Obese-PF, pair-fed; POMC, pro-opiomelanocortin; WAT, white adipose tissue.

*Corresponding author: W. D. Pierce, fax +1 780 492 7196, email dpierce@ualberta.ca

Over the last few decades, the incidence of paediatric obesity has increased in both developing and developed countries1,2. Obesity is characterised by an imbalance between energy intake and energy expenditure, and in children, it is associated with acute and chronic health risks in later life, including CVD3 and type 2 diabetes4. Given the prevalence of obesity, substantial research has been directed at the underlying mechanisms in an effort to discover effective long-term treatment for excessive weight gain and associated chronic disease risk5. The evidence suggests that genetic differences may help to explain the within-population variation in BMI6, together with specific environmental factors7. In this regard, the use of GM rodent models of obesity aids the investigation of underlying mechanisms.

Animal models of obesity based on mutations in leptin signalling, either at the peptide level (ob/ob mouse) or at the receptor level (db/db mouse and fa/fa and cp/cp rats)8, allow investigations into the mechanisms regulating energy balance. Leptin, encoded by the ob gene, is an important mediator of feeding behaviour and energy homeostasis9. Leptin acts on the hypothalamic regions involved in energy homeostasis, through the leptin receptor. The effects of
leptin on energy balance involve the orexigenic neuropeptide Y (NPY) and agouti-related protein as well as anorexigenic neuropeptides such as pro-opiomelanocortin (POMC) and cocaine–amphetamine-regulated transcript (POMC).

The JCR:LA-cp rat has the autosomal recessive corpulence (cp) gene, a nonsense mutation in the ObR gene resulting in a premature stop codon in the extracellular domain of the leptin receptor gene. Thus, all isoforms of the receptor are affected, leading to a total absence of functional leptin receptors. Rats that are heterozygous for the cp trait (+/−) are lean-prone, while those that are homozygous (cp/cp) display characteristics observed in human obesity – including hyperphagia, hyperinsulinemia, hyperlipidaemia and atherosclerosis. These metabolic alterations are well documented in adult rats. However, the early onset of these dysfunctions has not been described in the JCR strain.

Epidemiological studies have shown that obesity and associated metabolic complications in adult humans originate, at least in part, early in postnatal life. Consistently, studies in rodents have indicated that prenatal and neonatal nutritional environments contribute to adult obesity and related metabolic dysfunctions, which can be further influenced by the post-weaning dietary environment and genetic background. However, the underlying mechanisms remain unknown.

Nutritional environment during early development has been shown to alter the expression of genes critical to the regulation of energy homeostasis. One possibility is that feeding-related neuropeptide genes are altered in obese-prone animals, resulting in the early onset of homeostatic imbalance and predisposition to the development of obesity later in life. Furthermore, studies in rodents indicate that the appetite-regulatory network in the hypothalamus, mostly in the arcuate nucleus (ARC), does not fully develop until the third postnatal week, corresponding to the weaning period. Disruption of leptin signalling would be expected to up-regulate NPY expression, which in turn inhibits POMC expression in the ARC, resulting in obesity. Beck et al. found that, at both post-weaning and adult ages, Zucker (fa/fa) rats display higher NPY and lower POMC expressions in the ARC compared with their lean counterparts, while no difference is observed between the two genotypes during the pre-weaning or suckling period. However, Zucker (fa/fa) rats have a missense mutation in the leptin receptor gene, which diminishes but does not completely eliminates the response to leptin. As has been noted, JCR rats have a nonsense mutation in the leptin receptor, and it is unclear whether the alterations in NPY and POMC expressions are established before or after weaning in these rats.

In the present study, we hypothesised that in the ARC NPY mRNA levels would be elevated and POMC mRNA levels reduced before weaning in JCR obese-prone pups compared with those in lean-prone littersmates. These differences in pre-weaning pups would be exacerbated by weaning and then maintained until adulthood. We also hypothesised that pair-feeding-induced energy restriction would affect feeding-related neuropeptides.

Materials and methods

Animals

Separate cohorts of pre-weaning (10 d old; twelve obese-prone cp/cp and twelve lean-prone +/−), weaning (3 weeks old; eight obese-prone cp/cp and eight lean-prone +/−) and young adult (8 weeks old; sixteen obese cp/cp and eight lean +/−) male JCR:LA-cp rats were obtained from the breeding colony at the University of Alberta. The rats were housed individually in polycarbonate cages (47 cm × 27 cm × 20 cm) with sterile wood chip bedding and placed under a reversed 12 h light–12 h dark cycle (lights on: 18:00–06:00 hours). Throughout the experiments, the rats had free access to water and were fed as outlined in the experimental procedures section. All food was laboratory chow (LabDiet™ 5010, PMI Nutrition International, Inc.; metabolisable energy = 14·31 kJ/g (3·42 kcal/g)). Body weight (g) and food intake (g) measurements were taken daily. The care and use of animals were in accordance with the Guidelines of the Canadian Council of Animal Care and subject to prior review and approval by the Animal Care and Use Committee: Health Sciences, University of Alberta.

Experimental procedures

The experiments were conducted in three independent batches of lean-prone and obese-prone rats of different ages. For the young adult (8-week-old) cohort, obese-prone (cp/cp) rats were matched for body weights and randomly assigned to the free-feeding (Obese-FF, n 8) or the pair-feeding (Obese-PF, n 8) group. Each Obese-PF rat was matched to a lean free-feeding (Lean-FF) littermate and given the same amount of food daily (LabDiet chow 5010; LabDiet). Food intake was measured by subtracting the amount of food eaten from the fixed amount given to the rat. Although spillage was minimal, when it did occur, the food lost from the cages was collected and added to the total not consumed. Subsequently, food intake was converted to energy (food (g) × 14·31 kJ/g (3·42 kcal/g)), yielding a measure of daily energy consumption. After 4 weeks of feeding, the young adult rats (now 12 weeks old) were transferred to calorimetric chambers for whole-body energy expenditure measurements. Rats belonging to the weanling cohort (21 d old) were assigned to the Obese-FF (n 8) and Lean-FF (n 8) groups on day 22 when obese-prone and lean-prone rats are distinguishable and transferred to calorimetric chambers for energy expenditure assessment before being killed on day 25. Pre-weanling or suckling pups (10 d old) were killed immediately without being subjected to whole-body indirect calorimetry; using sense 5′-ATGATGCTGCAGTC-3′ and antisense 5′-AAGGTTCCTCCATTCAAT-3′ primers, genotyping was carried out using tissue samples collected from the tip of the tail during brain tissue collection, as obese-prone and lean-prone rats are indistinguishable at this age.

Energy expenditure

Whole-body energy expenditure was measured by indirect calorimetry using a CLAMS system (Columbus Instruments)
for 24 h after a 24 h habituation period, following the manufacturer’s instructions. Light and feeding conditions were kept the same as those described above.

**Post-mortem analysis**

The rats were killed under anaesthesia with isoflurane after an overnight fast, except the pre-weanling rats, which were killed without fasting. Blood was collected by cardiac puncture. Plasma samples were stored at −80°C until analysis. The rats were immediately perfused intracardially with ice-cold isotonic saline: the brain was removed. Liver mass, lean body mass and white adipose tissue (WAT) mass, the sum of the mass of retroperitoneal, epididymal and subcutaneous depots dissected and weighed, are expressed as a percentage of total body weight.

**NPY mRNA expression in hypothalamic arcuate nucleus**

Neuropeptide (NPY and POMC) mRNA in the entire ARC were assessed by in situ hybridisation as described by Doyon et al.[25]. The ARC was chosen due to its proximity to the peripheral blood stream and strong responsiveness to peripheral cues to regulate energy balance. The brain tissues were prepared as described previously[26].

**Plasma biochemical analysis**

Total plasma cholesterol and TAG assay kits were obtained from Wako USA. Plasma glucose concentration was determined using the glucose oxidase method (Diagnostic Chemical Limited). Insulin and leptin concentrations were assessed using enzyme-linked immunosorbent kits for rodents (insulin kit, Mercodia AB, and leptin kit, LINCO Research). Plasma corticosterone concentration was measured using Milliplex Map (Millipore Corporation).

**Statistical analysis**

Results are presented as means with their standard errors and were plotted using SigmaPlot v11 (Systat Software, Inc.). We used two-way ANOVA to test age-by-genotype effects along with follow-up tests using one-way ANOVA for comparison of the three adult groups with the post hoc Bonferroni test for pairwise comparison of means. Student’s t test was used to determine statistical differences between the obese and lean groups. All tests and comparisons with \( P < 0.05 \) were considered to be statistically significant.

**Results**

**Energy intake and body weight**

Fig. 1 shows body weight (Fig. 1(a)), energy intake (Fig. 1(b)) and energy conversion efficiency (Fig. 1(c)) for the free-feeding weanling JCR rats by genotype. The body weights of the Obese-FF and Lean-FF groups were not significantly different on the day of weaning; furthermore, the body weights of pups in the pre-weanling cohort did not differ significantly by genotype at 10 d of age (Table 1). These findings indicate that lean-prone and obese-prone pups have similar body weights on being shifted to dams’ milk during the suckling period. Weanling rats shifted from dams’ milk to chow diet exhibited significantly higher body weights over days 22–25 in the Obese-FF group than in the Lean-FF group (\( P < 0.05 \); Fig. 1(a)). The energy intake of the Obese-FF group was also higher than that of the Lean-FF group over the post-weaning period (\( P < 0.001 \); Fig. 1(b)), but the rate of increase in body weight (regression slopes, Obese-FF group: 6.56

![Fig. 1](https://www.cambridge.org/core/core_images/fig1.png)
**Table 1. Physiological and biochemical measures in pre-weanling, weanling and adult rats by feeding groups, lean-free-fed (FF), obese-FF and obese-pair-fed (PF)**

(Mean values with their standard errors, n=8 per group)

<table>
<thead>
<tr>
<th></th>
<th>Pre-weanling rats</th>
<th>Weanling rats</th>
<th>Adult rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lean-FF</td>
<td>Obese-FF</td>
<td>Lean-FF</td>
</tr>
<tr>
<td>Initial body weight (g)</td>
<td>25·1 ±1·1</td>
<td>25·8 ±1·5</td>
<td>53·7 ±1·9</td>
</tr>
<tr>
<td>Final body weight (g)</td>
<td>–</td>
<td>–</td>
<td>76·0 ±1·7</td>
</tr>
<tr>
<td>Liver mass (%)</td>
<td>ND</td>
<td>ND</td>
<td>4·2 ±0·1</td>
</tr>
<tr>
<td>Total WAT mass (%)</td>
<td>5·5 ±0·3</td>
<td>9·5 ±0·4</td>
<td>36·8 ±0·6</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>8·1 ±1·4</td>
<td>78·3 ±1·3</td>
<td>113 ±8 ±0</td>
</tr>
<tr>
<td>Insulin (ng/ml)</td>
<td>4·3 ±0·3</td>
<td>5·8 ±0·2</td>
<td>1·2 ±0·1</td>
</tr>
<tr>
<td>TAG (mmol/l)</td>
<td>1·3 ±0·1</td>
<td>2·2 ±0·1</td>
<td>0·5 ±0·1</td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>ND</td>
<td>ND</td>
<td>2·5 ±0·1</td>
</tr>
<tr>
<td>Corticosterone (mmol/l)</td>
<td>ND</td>
<td>ND</td>
<td>630·9 ±277·9</td>
</tr>
</tbody>
</table>

ND, not determined; WAT, white adipose tissue.

*Mean values within a row with unlike superscript letters (corresponding to the results from follow-up tests using one-way ANOVA with post hoc comparisons and independent t-tests) were significantly different within each age group (P<0·05).

*The two-way ANOVA with age and genotype (lean vs. obese) as factors were used to detect main and interaction effects. Post hoc comparisons with Bonferroni correction were used to detect differences between the groups.
Plasma total cholesterol, TAG and insulin concentrations were significantly lower in the lean-prone rats than in the obese-prone rats, across the age cohorts (Table 1; \( P<0.05 \)). Pair-feeding for a period of 4 weeks significantly decreased plasma total cholesterol and TAG concentrations in young adult Obese-PF rats compared with those in the Obese-FF rats (\( P<0.05 \)); however, no effect was observed for plasma glucose and insulin concentrations (Table 1).

Interestingly, plasma corticosterone concentrations at the weanling age were significantly higher in the Lean-FF rats than in the Obese-FF rats (Table 1, \( P<0.05 \)), and this difference was reversed in young adult rats, as the Obese-FF rats had higher concentrations than the Lean-FF rats (\( P<0.05 \)).

**Energy expenditure**

Fig. 3 shows the energy expenditure of rats, either at weaning or young adult ages. Fig. 3(a) shows the RER (relative exchange ratio or RQ), an index of what macronutrients are being preferentially metabolised to produce energy. At both the ages, RER was significantly lower in the Lean-FF rats than in the Obese-FF rats, regardless of the light cycle phase (\( P<0.05 \)). RER values did not differ significantly between the Obese-FF and Obese-PF young adult rats. Weanling rats of both the genotypes expended more energy than rats in the young adult cohort (\( P<0.05 \); Fig. 3(b)). Fig. 3(b) shows that heat production was higher in the Lean-FF rats than in the Obese-FF rats during both the light and dark phases (\( P<0.05 \)) at both the ages. The Obese-FF rats exhibited significantly greater heat production than the Obese-FF rats only during the dark phase (\( P<0.05 \); Fig. 3(b)). Locomotor activity (Fig. 3(c)) differed by genotype; in the weanling cohort, the Lean-FF rats were more active than the Obese-FF rats only in the dark phase (\( P<0.05 \)), and in the young adult cohort, the Lean-FF rats were more active than the Obese-FF rats during both the phases of the diurnal cycle. In the young adult cohort, the Obese-PF group exhibited significantly greater total activity than the Obese-FF rats only in the dark phase (\( P<0.05 \), Fig. 3(b)).

**Arcuate nucleus neuropeptide mRNA expression and plasma leptin concentrations**

In Fig. 4(a) and (b), neuropeptide expression levels in the ARC are shown. In the pre-weanling cohort, the lean-prone rats displayed lower NPY expression than the obese-prone rats (\( P<0.05 \)). POMC expression was not statistically different between the genotypes. In the weanling cohort, the Lean-FF rats also exhibited lower NPY mRNA expression than the Obese-FF rats. In the weanling cohort, POMC expression levels were significantly higher in the lean-prone rats than in the obese-prone rats (\( P<0.05 \)). In the young adult cohort, NPY mRNA expression levels tended to be higher in the Obese-FF rats than in the Lean-FF rats, but this difference was not statistically significant (\( P=0.11 \)). In the young adult cohort, POMC expression in the Obese-FF and Obese-PF rats was significantly lower than that in the Lean-FF rats. Pair-feeding for a period of 4 weeks significantly increased

percentages of fat mass and lean body mass than the weanling rats. However, the weanling cohort had lower percentages of fat mass (22.1 (SE 0.17)) and lean mass (34.62 (SE 0.55)) than the young adult rats (12.54 (SE 0.37) and 39.87 (SE 0.48)). Thus, the percentage of fat mass decreased between the pre-weaning and weanling groups, but increased in the young adult cohort.
NPY expression in the obese-prone rats (Obese-FF vs. Obese-PF; *P*, *0.001). There was no difference in POMC expression between the Obese-PF and Obese-FF rats. In the lean-prone rats as well as in the obese-prone rats, NPY expression did not differ significantly by age, but POMC expression exhibited an age-dependent increase in the lean-prone rats (*P*, *0.05), while it displayed an age-dependent decrease in the obese-prone rats (*P*, *0.02).

Plasma leptin concentrations were higher in the obese-prone rats regardless of age. In the pre-weanling cohort, plasma leptin concentrations in the obese-prone pups were five times greater than those in the lean-prone pups (*P*<0.01; Fig. 4(c)). This genotype difference increased substantially with age. Plasma leptin concentrations in the obese-prone rats were twenty-eight times and eighty times higher than those in the lean-prone rats at weaning and adulthood, respectively (*P*<0.001; Fig. 4(c)). Finally, the Obese-PF rats had significantly lower plasma leptin concentrations than the Obese-FF rats (*P*<0.005).

**Discussion**

The present study shows, for the first time, that in the ARC POMC expression differs by age and genotype, while NPY expression is only genotype dependent in animals with

---

Fig. 3. Effect of feeding groups (lean-prone (+/?)-free-fed (Lean-FF, □), obese-prone (cp/cp)-free-fed (Obese-FF, □) and obese-prone (cp/cp)-pair-fed (□)) on RER, heat production (prod) and relative activity in the light and dark phases for weanling and 12-week-old JCR:LA-cp rats. Values were significantly different from those of Lean-FF rats: *P*, *0.05; **P*, *0.01; ***P*, *0.001. † Values were significantly different from those of the Obese-FF rats (*P*, *0.05).

Fig. 4. Effect of feeding groups (lean-prone (+/?)-free-fed (Lean-FF, □), obese-prone (cp/cp)-free-fed (Obese-FF, □) and obese-prone (cp/cp)-pair-fed (□)) on the arcuate nucleus neuropeptide expression levels for neuropeptide Y (NPY) and pro-opiomelanocortin (POMC) and plasma leptin concentrations of pre-weanling, weanling and young adult JCR:LA-cp rats. Values were significantly different from those of the Lean-FF rats: *P*, *0.05; **P*, *0.01; ***P*, *0.001. Values were significantly different from those of the Obese-FF rats: † *P*, *0.05; †† † † † *P*, *0.001.
have reported that diet-induced thermogenesis, accounting for energy storage in the cp/cp animals. Glucocorticoid excess, a marker of hypothalamic–pituitary–adrenal axis activity/reactivity, has also been observed to be associated with obesity in the present study. As glucocorticoids influence the transcription of hypothalamic neuropeptides, the down-regulation of POMC expression in cp/cp animals is probably related to elevated plasma corticosterone concentrations in these animals. The higher plasma corticosterone concentrations of weanling Lean-FF rats compared with the concentrations of the Obese-FF rats is indicative of the high stress induced by maternal separation in lean-prone animals. Thus, a functional leptin pathway in the lean-prone rats is associated with the hypothalamic–pituitary–adrenal axis response to maternal separation, but without leptin regulation, obese-prone (ObR absent) rats exhibit a reduced stress response after weaning. As corticosterone concentrations were measured on day 25, one possibility is that the stress of maternal separation is related to the shift from dams’ milk to solid food. When shifted, the obese-prone rats adapted to the change in feeding more rapidly than the lean-prone rats, exhibiting higher energy intake and body weight over the first few days following weaning. This rapid adaptation of the obese-prone rats to the change in feeding would account for the low HPA axis activity in these rats when separated from their mothers.

POMC expression down-regulation is often accompanied by physiological and metabolic changes. In the weanling cohort, with free access to food, the high energy consumption of obese-prone rats was associated with body weight gain and increased plasma insulin and lipid concentrations. Furthermore, the percentage of fat mass was greater in the Obese-FF rats, while gains were associated with fat-free mass in the Lean-FF rats. Similar differences have been reported for obese-prone Fischer (F344) rats and Lou obesity-resistant rats.

A new finding was that the weanling cp/cp pups exhibited lower E EFF than the lean-prone weanling pups. The lower E EFF observed in the weanling Obese-FF pups, despite higher energy intake and body weight, may be related to the energy required to process the excess food. Notably, reduction in E EFF has been observed for rats on a high-fat diet and intermediate levels of pair-feeding, indicating that functional NPY/POMC genes are necessary for the development of obesity.

In contrast, rodents without leptin receptors, but with intact NPY and POMC genes, exhibit hyperphagia and decreased thermogenesis. However, animals lacking both leptin receptors and NPY/POMC genes do not exhibit obesity, indicating that functional NPY/POMC genes are necessary for the development of obesity. Moreover, a number of studies have reported that diet-induced thermogenesis, accounting for 5–15% of total daily energy expenditure in humans, is impaired in obese-prone subjects. Thus, overfeeding and young adult obese-prone rats together with a low thermogenic response may be a contributing factor for energy storage in the cp/cp animals.

A new finding was that the weanling cp/cp pups exhibited lower E EFF than the lean-prone weanling pups. The lower E EFF observed in the weanling Obese-FF pups, despite higher energy intake and body weight, may be related to the energy required to process the excess food. Notably, reduction in E EFF has been observed for rats on a high-fat diet and intermediate levels of pair-feeding, indicating that functional NPY/POMC genes are necessary for the development of obesity. However, animals lacking both leptin receptors and NPY/POMC genes do not exhibit obesity, indicating that functional NPY/POMC genes are necessary for the development of obesity. Moreover, a number of studies have reported that diet-induced thermogenesis, accounting for 5–15% of total daily energy expenditure in humans, is impaired in obese-prone subjects. Thus, overfeeding and young adult obese-prone rats together with a low thermogenic response may be a contributing factor for energy storage in the cp/cp animals. Glucocorticoid excess, a marker of hypothalamic–pituitary–adrenal axis activity/reactivity, has also been observed to be associated with obesity in the present study. As glucocorticoids influence the transcription of hypothalamic neuropeptides, the down-regulation of POMC expression in cp/cp animals is probably related to elevated plasma corticosterone concentrations in these animals. The higher plasma corticosterone concentrations of weanling Lean-FF rats compared with the concentrations of the Obese-FF rats is indicative of the high stress induced by maternal separation in lean-prone animals. Thus, a functional leptin pathway in the lean-prone rats is associated with the hypothalamic–pituitary–adrenal axis response to maternal separation, but without leptin regulation, obese-prone (ObR absent) rats exhibit a reduced stress response after weaning. As corticosterone concentrations were measured on day 25, one possibility is that the stress of maternal separation is related to the shift from dams’ milk to solid food. When shifted, the obese-prone rats adapted to the change in feeding more rapidly than the lean-prone rats, exhibiting higher energy intake and body weight over the first few days following weaning. This rapid adaptation of the obese-prone rats to the change in feeding would account for the low HPA axis activity in these rats when separated from their mothers.

POMC expression down-regulation is often accompanied by physiological and metabolic changes. In the weanling cohort, with free access to food, the high energy consumption of obese-prone rats was associated with body weight gain and increased plasma insulin and lipid concentrations. Furthermore, the percentage of fat mass was greater in the Obese-FF rats, while gains were associated with fat-free mass in the Lean-FF rats. Similar differences have been reported for obese-prone Fischer (F344) rats and Lou obesity-resistant rats.

A new finding was that the weanling cp/cp pups exhibited lower E EFF than the lean-prone weanling pups. The lower E EFF observed in the weanling Obese-FF pups, despite higher energy intake and body weight, may be related to the energy required to process the excess food. Notably, reduction in E EFF has been observed for rats on a high-fat diet and interpreted as a way to resist the development of obesity, occurring mostly during the growth period of 0–30 days.

With regard to pair-feeding, an imposed energy restriction, the present results indicate that the young adult cp/cp rats had significantly reduced plasma lipid concentrations. Long-term severe food restriction (12 g/d) reduces the plasma TAG concentrations of cp/cp rats (largely in the VLDL fraction) and prevents end-stage CVD. In the present study, we showed that a modest energy restriction also significantly reduces
plasma cholesterol (15%) and TAG (62%) concentrations of Obese-PF rats compared with those of the Obese-FF rats. Low plasma lipid concentrations in the Obese-PF rats were associated with increased NPY and reduced POMC expressions, which are known to trigger food intake. However, due to the pair-feeding procedure that makes food availability intermittent, the catabolic pathway is also activated to produce energy, explaining the reduction in WAT mass and TAG concentrations in the Obese-PF rats. The high NPY expression in the Obese-PF rats is also consistent with the increase in the locomotion of these rats, attesting the role of NPY in the regulation of appetitive ingestive behaviour such as locomotion or food-related travel when food is scarce. One possibility is that pair-feeding activated the catabolic pathway, explaining the low WAT mass and TAG concentrations in the Obese-PF rats.

Overall, we have shown that in the ARC NPY mRNA expression is up-regulated in pre-weaning obese-prone suckling rats. This NPY overexpression in obese-prone suckling rats is similar to that in weaning and young adult rats with well-established features of the metabolic syndrome. A difference in POMC expression between lean-prone and obese-prone rats occurs after weaning and it is age dependent. These findings suggest that overeating-induced obesity and associated metabolic syndrome in rats with no functional leptin receptors may be the consequence of the down-regulation of POMC expression in the ARC in the presence of established NPY overexpression, suggesting that POMC plays a crucial role in energy homeostasis.

Acknowledgements

The present study was funded by a grant from the National Sciences and Engineering Research Council of Canada (NSERC) to W. D. P. and C. D. H.; the Alberta Livestock Industry Development Fund and NSERC supported the research of S. D. P.

The Alberta Livestock Industry Development Fund and NSERC had no role in the design and analysis of the study or in the writing of this article.

The authors’ contributions were as follows: A. D., W. D. P. and S. D. P. were responsible for the conception and design of the research; A. D. conducted the experiments; A. D., D. R. and W. D. P. analysed the data and interpreted the results of the experiments; A. D. and J. C. R. prepared the figures; A. D. drafted the manuscript; D. F. V., C. D. H., J. C. R., W. D. P. and S. D. P. edited and revised the manuscript; D. F. V., C. D. H., J. C. R., D. R., W. D. P. and S. D. P. approved the final version of the manuscript.

None of the authors has any conflicts of interest, financial or otherwise, to declare.

References


British Journal of Nutrition


