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

Abdoulaye Diané[1], W. David Pierce[2]*, James C. Russell[1], C. Donald Heth[3], Donna F. Vine[1], Denis Richard[4] and Spencer D. Proctor[1]

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

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

Over the last few decades, the incidence of paediatric obesity has increased in both developing and developed countries[1,2]. 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 CVD[3] and type 2 diabetes[4]. 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 risk[5]. The evidence suggests that genetic differences may help to explain the within-population variation in BMI[6], together with specific environmental factors[7]. 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 homeostasis[9]. 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[10].

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[11]. Thus, all isoforms of the receptor are affected, leading to a total absence of functional leptin receptors[12]. 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, hyperinsulinaemia, hyperlipidaemia and atherosclerosis[8,13]. These metabolic alterations are well documented in adult rats[8]. 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[14]. 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[15,16] and genetic background[17]. 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[18]. 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[19], 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[20], resulting in obesity[21]. Beck et al.[22] 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 not completely eliminates the response to leptin[23]. 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 littermates. 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[24] 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[8]. 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 uneaten 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 weaning 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[13] and transferred to calorimetric chambers for energy expenditure assessment before being killed on day 25. Pre-weaning or suckling pups (10 d old) were killed immediately without being subjected to whole-body indirect calorimetry; using sense 5′-ATGAATGCTG-CAGTC-3′ and antisense 5′-AAGGTTCTTCCATTCAAT-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
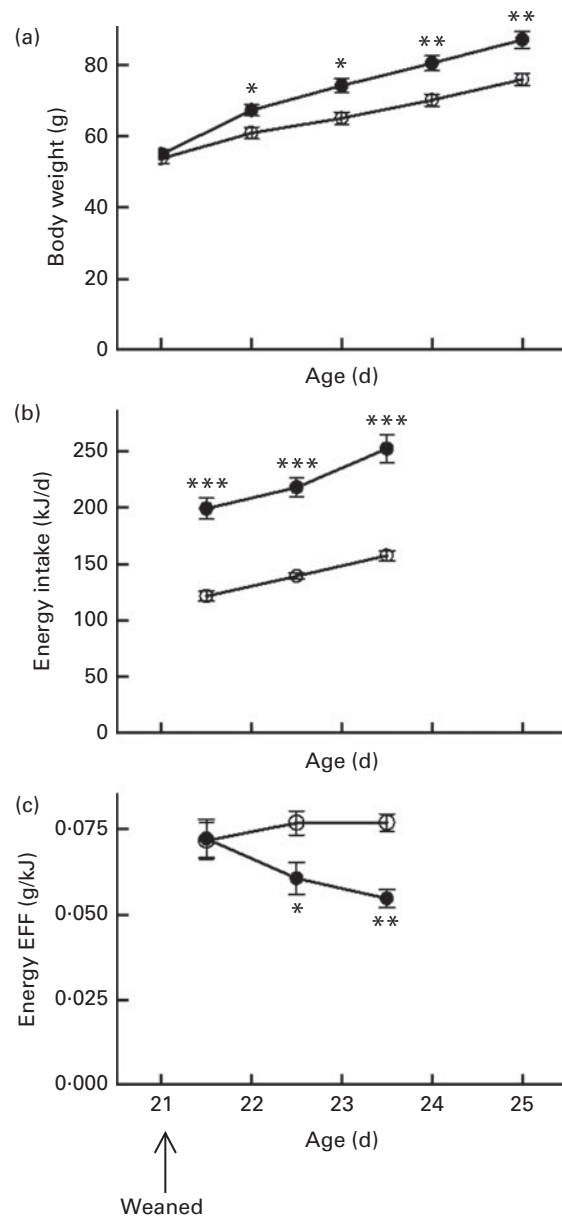

Fig. 1. Effect of genotype on the (a) body weight, (b) energy intake (food consumption expressed as kJ/d) and (c) energy efficiency (EFF, efficiency of conversion to body mass) of weanling JCR:LA-cp rats for three post-weaning days. Values were significantly different from those of the lean-prone (+/?) rats: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. –○–, Lean free-feeding; –●–, obese free-feeding.

pups in the pre-weaning 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 ($P < 0.01$; 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

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)

| | Pre-weanling rats | | | | Weanling rats | | | | Adult rats | | | | | |
| | Lean-FF | | Obese-FF | | Lean-FF | | Obese-FF | | Lean-FF | | Obese-FF | | Obese-PF | |
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Initial body weight (g)	25.1^a	1.1	25.8^a	1.5	53.7^a	1.9	55.0^a	2.1	210.4^a	4.3	313.0^b	16.0	317.0^c	8.0
Final body weight (g)	–	–	–	–	76.0^a	1.7	87.1^b	2.4	298.1^a	4.3	509.6^b	11.1	365.1^c	6.6
Liver mass (%)	ND		ND		4.2^a	0.1	5.1^b	0.1	2.7^c	0.1	3.5^d	0.2	3.2^d	0.0
Total WAT mass (%)	5.5^a	0.3	9.5^b	0.4	1.5^a	0.1	2.9^b	0.2	2.6^b	0.1	22.5^c	0.6	19.4^d	0.4
Lean mass (%)	81.9^a	1.4	78.3^a	1.3	38.6^a	0.6	30.6^b	0.4	47.8^c	0.4	31.9^{b,d}	0.5	33.5^d	0.6
Glucose (mmol/l)	4.3^a	0.5	5.8^b	0.3	11.3^a	0.8	11.9^a	0.9	10.4^a	0.8	12.4^b	0.9	13.6^b	0.4
Insulin (ng/ml)	1.2^a	0.1	3.1^b	1.2	0.2^a	0.1	2.2^b	0.4	0.6^c	0.1	2.9^d	1.0	3.2^d	0.7
TAG (mmol/l)	1.3^a	0.1	2.2^b	0.1	0.5^a	0.1	2.3^b	0.1	0.2^c	0.0	5.6^d	0.6	2.1^{b,e}	0.3
Cholesterol (mmol/l)	ND		ND		2.5^a	0.1	3.9^b	0.2	1.9^a	0.1	3.9^c	0.1	3.4^c	0.1
Corticosterone (mmol/l)	ND		ND		630.9^a	277.9	330.7^b	21.9	583.9^a	103.9	883.7^c	222.0	944.4^c	213.7

ND, not determined; WAT, white adipose tissue.
a,b,c,d,e 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 *v.* 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.

(SE 0.8) g/d; Lean-FF group: 5.02 (SE 0.72) g/d) was not significantly different. This observation may be related to the lower energy efficiency (E EFF = body weight gain (g)/total energy (kJ)) in the Obese-FF rats (Fig. 1(c)). The E EFF was similar in the two genotypes after weaning (day 21). Thereafter, the Lean-FF rats maintained a constant E EFF, while the Obese-FF rats exhibited a significant decrease in E EFF compared with the Lean-FF rats on days 22 and 23 after weaning ($P<0.05$ and $P<0.01$, respectively).

In the young adult cohort, body weight increased significantly in the three groups during the 4-week feeding period ($P<0.001$). Body weight increased significantly in the Obese-FF rats than in the Lean-FF rats ($P<0.001$) or the Obese-PF rats ($P<0.001$; Fig. 2(a)). From 10 to 12 weeks of age, weight gain in the Obese-FF rats (30.7 (SE 7.4) g/week) was nearly double that in the Lean-FF rats (16.6 (SE 2.8) g/week) and 30 % greater than that in the Obese-PF group (20.3 (SE 4.4) g/week). The Obese-FF rats also maintained significantly higher daily energy intake compared with the Lean-FF or Obese-PF rats ($P<0.01$; Fig. 2(b)). The mean daily energy intake was constant in the Lean-FF rats over the 4-week feeding period, while that in the Obese-FF rats decreased between weeks 10 and 11 ($P<0.01$) and then remained constant. Over 4 weeks of feeding, total body weight gain in the Obese-PF rats (44.0 (SE 3.8) g) was nearly 50 % less than that in the Lean-FF group (87.3 (SE 3.1) g) ($P<0.001$). Thus, pair-feeding obese-prone rats to lean-prone rats resulted in a substantial reduction in daily body weight gain.

In the young adult cohort, the E EFF decreased during weeks 8–10 in the Obese-FF and Lean-FF rats (overall rate of change −33.5 (SE 5.9) and −36.4 (SE 5.9) mg/kJ per d (−8.0 (SE 1.4) and −8.7 (SE 1.4) mg/kcal per d), respectively, $P<0.001$). The Obese-PF rats exhibited markedly lower E EFF than the Obese-FF or Lean-FF rats ($P=0.01$) on the 1st day of pair-feeding, mirrored by a loss in body weight induced by the initiation of pair-feeding (Fig. 2(c)).

Body composition and plasma biochemical parameters

In the pre-weanling cohort, despite the same body weight, the percentage of total WAT mass was significantly higher in the obese-prone rats than in the lean-prone rats, with no significant difference being observed in the percentage of lean body mass between the two genotypes (Table 1). In the weanling cohort, the obese-prone rats also had a higher percentage of WAT mass than the lean-prone group ($P<0.001$) and a lower percentage of lean body mass ($P<0.001$).

In the young adult cohort, the percentages of liver mass and total WAT mass were significantly higher in the Obese-FF rats than in their Lean-FF littermates ($P<0.01$); also, the Obese-FF rats had significantly lower lean body mass than the lean-prone rats ($P<0.001$) (Table 1). The Obese-PF rats had significantly lower total WAT mass ($P<0.001$) than the Obese-FF rats, but lean body mass did not differ significantly in both the groups. The two-way ANOVA of age group × genotype revealed a significant main effect of age group on the percentage of total fat mass and lean body mass ($P<0.001$), indicating that the pre-weanling rats had higher
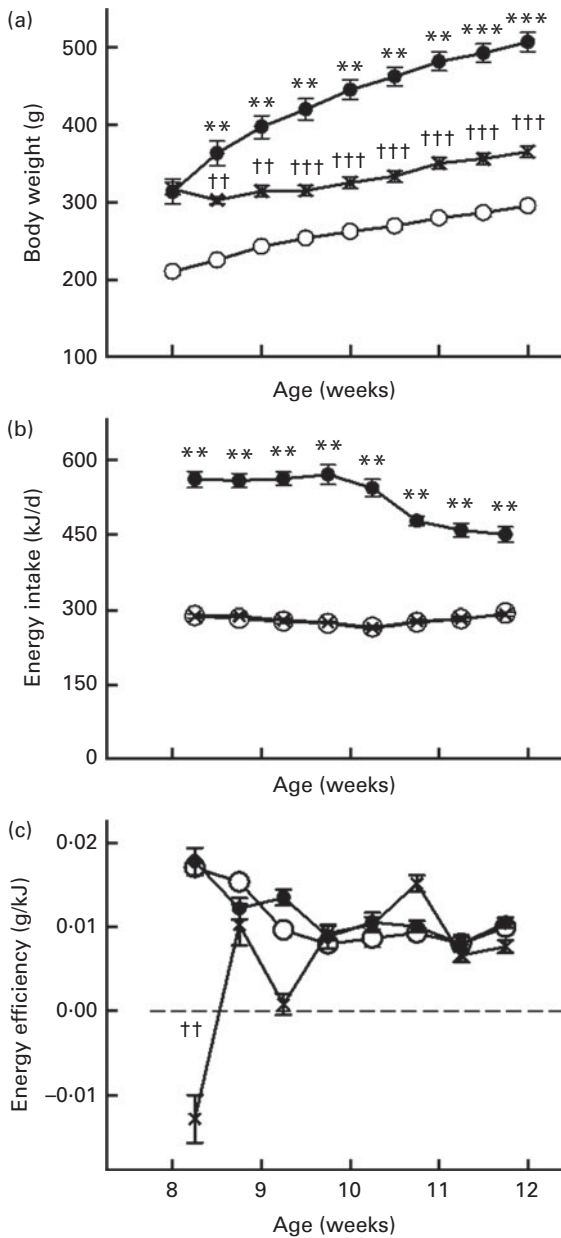
Fig. 2. Effect of feeding groups (lean-prone (+/?)-free-fed (Lean-FF, –○–), obese-prone (*cp/cp*)-free-fed (–●–) and obese-prone (*cp/cp*)-pair-fed (Obese-PF, –✳–)) on the (a) body weight, (b) energy intake (food consumption expressed as kJ/d) and (c) energy efficiency (efficiency of conversion to body mass) of JCR:LA-cp rats from 8 to 12 weeks of age (young adults). Statistical differences among the experimental groups are described in the Results section. Note that the variance in energy intake (b) of the Lean-FF and Obese-PF rats was smaller than the size of the symbols and thus not readily visible in the figure. 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-PF rats: †† $P<0.01$; ††† $P<0.001$.

percentages of fat mass and lean body mass than the weanling rats. However, the weanling cohort had lower percentages of fat mass (2·21 (SE 0·17)) and lean mass (34·62 (SE 0·53)) 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 weaning groups, but increased in the young adult cohort.

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-PF 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-weaning 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
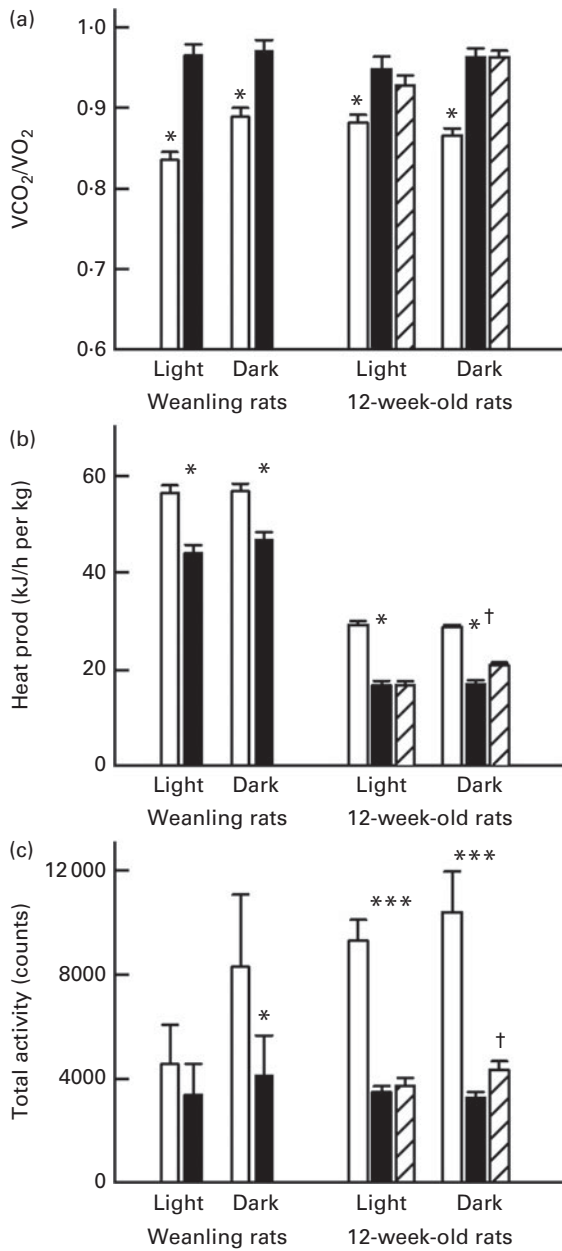
Fig. 3. Effect of feeding groups (lean-prone (+/?)-free-fed (Lean-FF, □), obese-prone (cp/cp)-free-fed (Obese-FF, ■) and obese-prone (cp/cp)-pair-fed (▨)) on RER, heat production (prod) and relative activity in the light and dark phases for weanling and 12-week-old JCR:LA-cp rats. Values were significantly 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 ($P<0.05$).

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 ($P<0.05$).

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
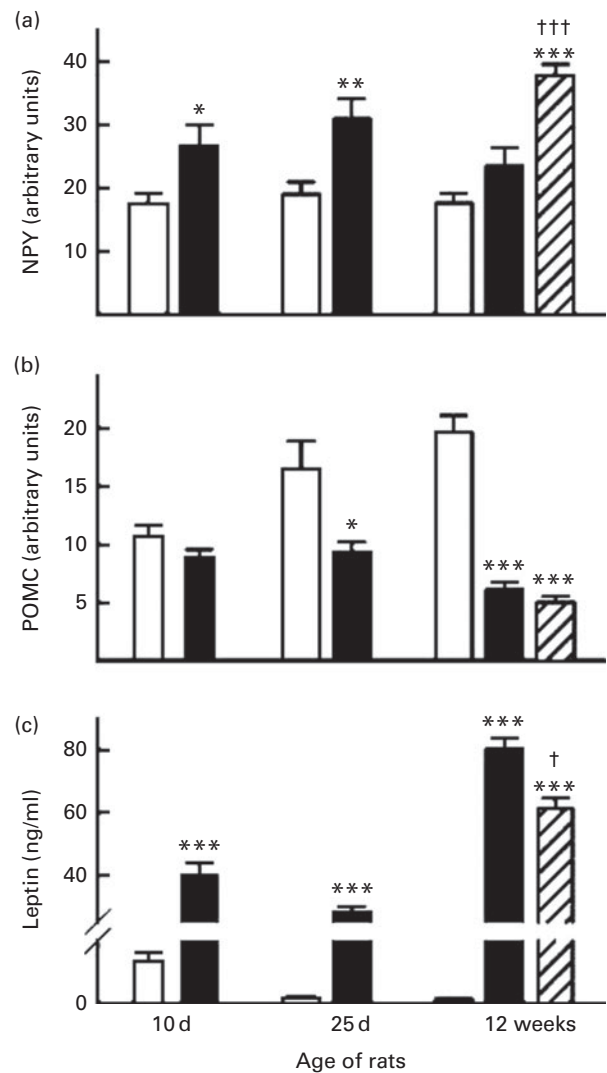

Fig. 4. Effect of feeding groups (lean-prone (+/?)-free-fed (Lean-FF, □), obese-prone (cp/cp)-free-fed (Obese-FF, ■) and obese-prone (cp/cp)-pair-fed (▨)) on the arcuate nucleus neuropeptide expression levels for neuropeptide Y (NPY) and pro-opiomelanocortin (POMC) and plasma leptin concentrations of 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: † $P<0.05$; ††† $P<0.001$.

NPY expression in the obese-prone rats (Obese-FF v. 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,

a nonsense mutation in the leptin receptor. In contrast, in animals with a missense mutation in the leptin receptor gene (Zucker fa/fa rats), NPY expression is both genotype- and age-dependent[22] – indicating that the onset of NPY expression up-regulation in obese-prone animals is a function of the mutant leptin receptor. At postnatal day 10, obese-prone JCR rats exhibited higher NPY expression than the lean-prone rats, but no difference in POMC expression. At this age, it is difficult to link NPY expression to feeding, as the determination of energy intake (milk consumption) in suckling rats is very difficult. The high fat mass found in the obese-prone rats, however, may be related to the metabolic and anti-thermogenic effects of NPY[27].

The difference in NPY expression by genotype remained in the weanling cohort, but we detected a difference in POMC expression, with obese-prone pups exhibiting down-regulation compared with the lean-prone rats. One possibility is that the onset of overeating and body weight gain in obese-prone animals is more related to the down-regulation of the expression of POMC, an anorexigenic peptide, than to the activation of NPY alone. Furthermore, the difference in POMC expression in obese-prone and lean-prone weanling pups coincides with the change from dams' milk to chow diet at weaning. One possibility is that the shift to solid food may combine with genotype to reduce POMC expression at weaning. The results obtained for the young adult cohort support the POMC down-regulation hypothesis of obesity; at this age, the POMC concentrations of the obese-prone rats were further suppressed compared with those of the lean-prone rats, coinciding with the onset of obesity and metabolic syndrome in the young adult obese-prone rats. It is possible that the down-regulation of *POMC* mRNA expression in the ARC is central for the development of obesity in JCR rats, a finding reported previously for tubby mice[28]. POMC is also an important anorexigenic regulator of feeding behaviour. In fact, POMC neurons are suppressed by NPY, and NPY-induced feeding behaviour is related to the suppression of POMC signalling[29]. One implication is that the down-regulation of POMC expression observed after weaning involves the suppressive effects of NPY on POMC neurons, leading to high energy intake in weanling and young adult obese-prone rats.

Leptin regulates arcuate hypothalamic neurons directly by binding to the ObR receptor long isoform (LRb), activating Janus kinase/signal transducer and activator of transcription 3 (JAK-STAT3) signalling and suppressing the activation of NPY neurons[30]. Thus, the early NPY overexpression observed in the pre-weanling obese-prone rats could be linked to the absence of functional leptin receptors. Leptin also induces the expression of uncoupling proteins-1, -2 and -3 in the mitochondria, thereby leading to increased thermogenesis[31]. In contrast, rodents without leptin receptors, but with intact *NPY* and *POMC* genes, exhibit hyperphagia and decreased thermogenesis[32]. However, animals lacking both leptin receptors and *NPY/POMC* genes do not exhibit obesity[33], 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[32],

is impaired in obese-prone subjects[34,35]. Thus, overfeeding in weanling 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[36], has also been observed to be associated with obesity in the present study. As glucocorticoids influence the transcription of hypothalamic neuropeptides[37], 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[38]. 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[20]. 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 pre-weaning pups than in the weanling pups regardless of the genotype. This age group difference may reflect metabolic and endocrine traits not fully established in neonates[39], making the regulation of energy metabolism inefficient. Under chow diet, from weaning to adulthood, gains in body weight were predominately related to fat mass 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[40].

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[41], occurring mostly during the growth period of 0–30 d.

With regard to pair-feeding, an imposed energy restriction[24], 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[8]. 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[42]. 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[43]. 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 weanling 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

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