Hypothalamic Neuropeptide Expression of Juvenile and Middle-Aged Rats after Early Postnatal Food Restriction

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Rats subjected to early postnatal food restriction (FR) show persistent changes in energy balance. The hypothalamus plays a major role in the regulation of energy balance. Therefore, we hypothesized that early postnatal food restriction induces developmental programming of hypothalamic gene expression of neuropeptides involved in this regulation. In the hypothalamus of juvenile and middle-aged rats that were raised in control (10 pups) or FR litters (20 pups), gene expression was investigated for neuropeptide Y (NPY), agouti-related protein (AgRP), proopiomelanocortin (POMC), and cocaine- and amphetamine-regulated transcript (CART) in the arcuate nucleus (ARC); CRH and TRH in the paraventricular nucleus; and melanin-concentrating hormone (MCH) and orexin in the lateral hypothalamic area. Early postnatal FR acutely and persistently reduced body size. Juvenile FR rats had significantly reduced CART gene expression and increased MCH expression. In middle-aged FR rats, POMC and CART mRNA levels were significantly reduced. The ratio between expression of the ARC orexigenic peptides (NPY and AgRP) and anorexigenic peptides (POMC and CART) was increased in juvenile, but not in middle-aged, FR rats. These results suggest that in neonatal rats, FR already triggers the ARC, and to a lesser extent the lateral hypothalamic area, but not the paraventricular nucleus, to increase expression of orexigenic relative to anorexigenic peptides. In addition, with enduring small body size and normalized hypothalamic gene expression, the adult FR rats appeared to have accepted this smaller body size as normal. This suggests that the body weight set-point was differently programmed in animals with early postnatal FR. (Endocrinology 149: 3617–3625, 2008)

Epidemiological studies linking low birth weight with disease in adult life (1) have led to the concept of developmental programming, which entails that differences in the environment during the plastic period of development can permanently alter physiology (2, 3). Over the years, evidence has accumulated that perinatal events can permanently alter energy balance both in humans and in animal models. For example, human fetal growth restriction is associated with a more central distribution of fat and a lower lean body mass in later life (4). In rat models of perinatal malnutrition, changes in food intake and body composition have been reported, but the direction of these changes appears to be highly dependent on the exact nature and timing of the malnutrition (5). These changes in energy balance suggest that the regulation of energy balance may be programmed.

The peripheral hormone leptin and several nuclei of the hypothalamus play an important role in this regulation of energy balance. Leptin is secreted by adipocytes in proportion to their fat content and thereby indicates the body’s energy reserves (6, 7). In the hypothalamic arcuate nucleus (ARC), the expression and release of the orexigenic neuropeptide Y (NPY) and agouti-related protein (AgRP) are inhibited by leptin, whereas those of the anorexigenic proopiomelanocortin (POMC), the precursor for α-MSH, and cocaine- and amphetamine-regulated transcript (CART) are stimulated (7, 8). NPY/AgRP and POMC/CART neurons in the ARC project to the paraventricular nucleus (PVN) and lateral hypothalamic area (LHA) among others, where levels of the anorexigenic peptides CRH and TRH and the orexigenic peptides melanin-concentrating hormone (MCH) and orexin (ORX), respectively, are modified (8).

In rats, the connections from the ARC to the PVN and LHA develop mostly in the early postnatal period (9, 10), and therefore, rats raised in large litters can be used to study developmental programming of energy balance. These rats were previously shown to have reduced growth during lactation, followed by incomplete catch-up growth after weaning (11, 12). Young rats showed reduced leptin levels and fat percentages (13), and in adult males, the fat percentage was still reduced (11). In the present set of animals, we have reported lower body weight, body length, body mass index (BMI), energy intake, and serum leptin levels until the age of 1 yr (14, 15). In addition, a subset of adult males in this group was shown to have a resting energy expenditure that was reduced but seemed appropriate for their smaller body dimensions (15).

The alterations in energy balance found in these large-litter rats suggest that early undernutrition induced permanent changes in the regulation of energy balance. Moreover, these animals showed disruptions in several other processes that are regulated by the hypothalamus: a delayed onset of puberty (16), impaired testicular function (17), and changes in

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Abbreviations: AgRP, agouti-related protein; ARC, arcuate nucleus; BMI, body mass index; CART, cocaine- and amphetamine-regulated transcript; FR, food restriction; LHA, lateral hypothalamic area; MCH, melanin-concentrating hormone; NPY, neuropeptide Y; ORX, orexin; orex/anorex, ratio of orexigenic to anorexigenic expression; POMC, proopiomelanocortin; PVN, paraventricular nucleus; SDS, sd score.


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the GH axis (18). Therefore, the hypothalamus seems a likely candidate for programming. In this study, we investigated acute and long-term programming effects of early postnatal food restriction (FR) (in juvenile and middle-aged rats, respectively) on the mRNA expression levels of neuropeptides in the ARC, PVN, and LHA, using quantitative real-time PCR. In addition, the relationships between hypothalamic neuropeptide expression levels and the parameters that were reported previously (14, 15) were assessed.

**Materials and Methods**

**Experimental animals**

Primiparous timed-pregnant Wistar rats (Harlan, Horst, The Netherlands) arrived on d 14 or 15 of gestation and were housed individually under controlled lighting (12 h light, 12 h dark) and temperature (21.5 ± 0.5 °C). Animals had unlimited access to standard rat chow (Sniff R/M-H; Bio Services, Uden, The Netherlands) and tap water at all times. All procedures were approved by the Animal Experimentation Ethics Committee of the Vrije Universiteit and the VU University Medical Center in Amsterdam, The Netherlands.

Pups were born spontaneously on d 22 or 23 of gestation. The first morning after birth was designated as postnatal d 1. On d 2, pups were weaned and socially housed with two (males) or four (females) animals of the same experimental group per cage. A subset of 94 animals was used for this study.

**Body dimensions and tissue collection**

Body weight was measured regularly throughout life. Body length of manually restrained animals from the tip of the nose to the anus was measured on d 2 and on the day of killing. BMI was calculated as the ratio of body weight (grams) to body length (centimeters) squared.

During the experiment, subsets of animals were killed at different ages. Suckling pups (males and females) were killed on d 10 of life and middle-aged males and females around d 380 of life. In addition, weaning males were killed on d 25. In females, the expression levels of the CTRH gene of interest Accession no. Forward primer Reverse primer Product size (bp) PCR efficiency

<table>
<thead>
<tr>
<th>Gene of interest</th>
<th>Accession no.</th>
<th>Forward primer</th>
<th>Reverse primer</th>
<th>Product size (bp)</th>
<th>PCR efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPY</td>
<td>NM_012614</td>
<td>441–458</td>
<td>484–504</td>
<td>64</td>
<td>2.29</td>
</tr>
<tr>
<td>AgRP</td>
<td>AF206017</td>
<td>126–142</td>
<td>172–191</td>
<td>66</td>
<td>2.03</td>
</tr>
<tr>
<td>POMC</td>
<td>AF510391</td>
<td>465–484</td>
<td>512–530</td>
<td>66</td>
<td>2.12</td>
</tr>
<tr>
<td>CART</td>
<td>NM_017110</td>
<td>124–144</td>
<td>167–187</td>
<td>68</td>
<td>2.08</td>
</tr>
<tr>
<td>CRH</td>
<td>NM_031019</td>
<td>688–708</td>
<td>738–755</td>
<td>67</td>
<td>1.94</td>
</tr>
<tr>
<td>TRH</td>
<td>NM_013046</td>
<td>442–461</td>
<td>490–506</td>
<td>65</td>
<td>1.95</td>
</tr>
<tr>
<td>MCH</td>
<td>M29712</td>
<td>74–95</td>
<td>119–141</td>
<td>68</td>
<td>2.08</td>
</tr>
<tr>
<td>ORX</td>
<td>NM_013179</td>
<td>3–19</td>
<td>47–68</td>
<td>66</td>
<td>2.03</td>
</tr>
<tr>
<td>Cyclophilin</td>
<td>M19533</td>
<td>346–367</td>
<td>393–413</td>
<td>68</td>
<td>1.99</td>
</tr>
<tr>
<td>β-Actin</td>
<td>NM_031144</td>
<td>460–478</td>
<td>505–526</td>
<td>67</td>
<td>2.01</td>
</tr>
</tbody>
</table>
melting-curve analysis was carried out to confirm the formation of a single PCR product and hence the absence of primer-dimers. Threshold cycle (Ct) values were determined automatically by the Applied Biosystems software.

Samples were excluded 1) if more than one PCR product was formed, 2) if there was no amplification (as indicated by the software and recognized in the amplification plot), or if the resulting data point was an extreme outlier (more than three times the interquartile range) 3) for the ratio of the reference genes or 4) for the expression level of one of the genes of interest in the nucleus. If a sample failed to reach these criteria, it was excluded from the analysis for all peptides in the nucleus.

Expression levels of hypothalamic neuropeptides were first normalized to that of the reference genes cyclophilin and β-actin (36) and then either to that of control males of the same age or to that of animals of 380 d of the same group and sex.

**Data analysis**

The results were analyzed using SPSS for Windows, version 12. All data were checked for a normal distribution and subjected to logarithmic transformation if necessary for analysis in ANOVA. The data are expressed as mean ± SEM. Effects of group and sex were analyzed using univariate ANOVA, and the effect of age was tested by Bonferroni post hoc tests. Pearson’s bivariate correlation analysis was used to analyze correlations. P values < 0.05 were considered to be statistically significant.

Because of the small number of pups for each biological and foster dam, it was not feasible to test for possible interference of effects of the dams with group effects on the body dimensions and gene expression levels of the pups. However, in a larger study (14), the effects of the dams could not be analyzed together using ANOVA with group and sex as independent variables. Provided that there is no significant interaction between group and sex, this analysis allows for the effect of group to be investigated independently of sex differences, and vice versa. On d 2 (before the random redistribution into control and FR litters), body weight, body length, and BMI were similar between the future FR and control animals.

**TABLE 2. Characteristics of the animals used in the experiment**

<table>
<thead>
<tr>
<th>Age</th>
<th>Biological litters</th>
<th>Foster litters</th>
<th>Body weight (g)</th>
<th>Body length (cm)</th>
<th>BMI (g/cm²)</th>
<th>Leptin (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>d 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>27</td>
<td>18</td>
<td>7.6 ± 0.17</td>
<td>5.4 ± 0.06</td>
<td>0.27 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>FR</td>
<td>30</td>
<td>20</td>
<td>7.5 ± 0.14</td>
<td>5.4 ± 0.05</td>
<td>0.26 ± 0.003</td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>18</td>
<td>16</td>
<td>6.9 ± 0.20</td>
<td>5.1 ± 0.05</td>
<td>0.26 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>FR</td>
<td>19</td>
<td>15</td>
<td>7.2 ± 0.19</td>
<td>5.2 ± 0.06</td>
<td>0.27 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>d 10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>8</td>
<td>4</td>
<td>21.0 ± 0.54</td>
<td>7.7 ± 0.12</td>
<td>0.36 ± 0.01</td>
</tr>
<tr>
<td>FR</td>
<td>10</td>
<td>9</td>
<td>2</td>
<td>14.4 ± 0.51</td>
<td>6.6 ± 0.11</td>
<td>0.33 ± 0.01</td>
</tr>
<tr>
<td>Females</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>9</td>
<td>4</td>
<td>19.5 ± 0.40</td>
<td>7.3 ± 0.11</td>
<td>0.37 ± 0.01</td>
</tr>
<tr>
<td>FR</td>
<td>9</td>
<td>7</td>
<td>2</td>
<td>13.2 ± 0.36</td>
<td>6.3 ± 0.09</td>
<td>0.33 ± 0.01</td>
</tr>
<tr>
<td>d 25</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>8</td>
<td>7</td>
<td>5</td>
<td>67.1 ± 1.61</td>
<td>12.5 ± 0.21</td>
<td>0.43 ± 0.01</td>
</tr>
<tr>
<td>FR</td>
<td>8</td>
<td>8</td>
<td>6</td>
<td>45.6 ± 2.60</td>
<td>11.1 ± 0.30</td>
<td>0.37 ± 0.02</td>
</tr>
<tr>
<td>d 380</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>9</td>
<td>8</td>
<td>6</td>
<td>610 ± 15.91</td>
<td>27.2 ± 0.28</td>
<td>0.83 ± 0.01</td>
</tr>
<tr>
<td>FR</td>
<td>12</td>
<td>10</td>
<td>6</td>
<td>548 ± 17.38</td>
<td>26.3 ± 0.17</td>
<td>0.79 ± 0.02</td>
</tr>
<tr>
<td>Females</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>8</td>
<td>7</td>
<td>4</td>
<td>324 ± 12.13</td>
<td>22.4 ± 0.21</td>
<td>0.65 ± 0.02</td>
</tr>
<tr>
<td>FR</td>
<td>10</td>
<td>10</td>
<td>6</td>
<td>286 ± 8.75</td>
<td>21.7 ± 0.13</td>
<td>0.61 ± 0.01</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM. No significant interactions were found between group and sex (P = 0.212–0.951).

* P group.

b P sex.

**Results**

The 94 animals used in this study were born from 28 of the 33 dams in the complete experiment and fostered to 13 control and eight FR dams on d 2. The original litter size of the FR foster dams (12.3 pups ± 0.7) was not different from that of the control foster dams (11.9 pups ± 0.4, P > 0.650). Nor did the original litter size of FR pups (11.7 pups ± 0.3) differ from that of control pups (12.1 pups ± 0.3, P > 0.450). Of the 94 pups in this study, 83% were cross-fostered, whereas 17% (eight control and eight FR animals) remained with the same dam after the random redistribution on d 2. Other characteristics of the animals are shown in Table 2. In previous studies, we have shown that FR rats have persistently reduced body weight, BMI, fat mass, and food intake, whereas adult resting energy expenditure was normal for the reduced body size (11, 13–15).

Body dimensions were analyzed for all animals at the same age together using ANOVA with group and sex as independent variables. Provided that there is no significant interaction between group and sex, this analysis allows for the effect of group to be investigated independently of sex differences, and vice versa. On d 2 (before the random redistribution into control and FR litters), body weight, body length, and BMI were similar between the future FR and control animals.
(Table 2). In FR animals, body weight was significantly reduced from d 4 until the end of the experiment on d 380 ($P < 0.001$) (Fig. 1). At the time of killing, body weight, body length, BMI, and serum leptin levels were reduced in FR animals at all three ages (Table 2). Body weight and BMI correlated positively with leptin at all ages ($P < 0.001–0.054$, data not shown). On d 10, females were smaller than males but had a similar BMI and serum leptin levels. In adulthood, all body dimensions were significantly larger in males.

After application of the PCR criteria (see Materials and Methods), 75 ARC samples were analyzed for neuropeptide expression, whereas the analyses for the PVN and LHA were performed on 71 and 81 samples, respectively.

**ARC neuropeptide expression: effects of FR**

The levels of mRNA expression for the four ARC neuropeptides normalized to those of cyclophilin and β-actin are shown relative to those of control males in Fig. 2, A–C. Again, the absence of any significant interactions between group and sex allowed the simultaneous analysis of males and females.

On d 10, 25, and 380, the expression levels for NPY and AgRP mRNA did not differ significantly between control and FR rats. POMC mRNA expression was similar between the groups at the ages of 10 and 25 d, whereas its expression was significantly reduced in FR rats on d 380. CART mRNA expression levels were significantly reduced at all three ages tested. In addition, significant sex differences were found in expression levels on d 10 (NPY, AgRP, and POMC) and on d 380 (POMC and CART).

Although most of the ARC neuropeptides showed no significant changes in their expression level, in the young animals, there was a tendency in the FR group for increased expression of the orexigenic peptides (NPY and AgRP) and for decreased expression of the anorexigenic peptides (POMC and CART). Because the isolated ARC region of each animal contained both the NPY/AgRP neurons and the POMC/CART neurons, the ratio of orexigenic to anorexigenic expression (orex/anorex) in each animal was computed as the mean relative expression of NPY plus AgRP divided by the mean relative expression of POMC plus CART. This ratio was shifted significantly toward orexigenic expression in FR animals on d 10 and 25 (Fig. 3). On d 380, there was no significant difference between the groups, but the orex/anorex ratio was significantly elevated in middle-aged females relative to males.

**PVN neuropeptide expression: effects of FR**

On d 10, 25, and 380, the mRNA levels of both CRH and TRH did not differ significantly between control and FR rats (Fig. 2, D–F). No significant sex differences were found in the expression of these neuropeptides.

**LHA neuropeptide expression: effects of FR**

On d 10, MCH mRNA levels were significantly increased in FR rats (Fig. 2D). This difference was not present on d 25 and 380 (Fig. 2, E and F). Expression levels of ORX were comparable between control and FR rats at all three ages tested (Fig. 2, D–F). No significant sex differences were found in the expression of these neuropeptides.

**Neuropeptide expression: ontogeny**

To investigate developmental changes, neuropeptide mRNA expression was computed relative to that of middle-aged animals, instead of control males at each age. This analysis was performed on males, because for males, an extra group was included on d 25. Because no significant interactions were found between age and group ($P = 0.205–0.956$), control and FR males were analyzed together. The absence of these interactions means that although there were group differences in expression levels, the developmental pattern was not different between the groups.

The overall effect for group (i.e., independent of age) just reached significance for CART ($P = 0.043$) but not for any of the other genes ($P = 0.265–0.841$). There was a significant effect of age for all peptides except AgRP (Fig. 4). Post hoc tests revealed significant differences between the ages. NPY mRNA levels decreased significantly between d 10 and 380. AgRP expression showed a similar pattern, but no significant differences were found. The expression levels of the anorexigenic ARC peptides POMC and CART increased significantly between d 10 and 25 and again between d 25 and 380. Expression levels for CRH and TRH in the PVN and ORX in the LHA rose significantly from d 10 to d 25 and 380, with no further increase between weaning and middle-age. MCH in the LHA, like POMC and CART, increased between d 10 and 25 and between d 25 and 380.

Overall, the developmental patterns in females resembled those in males, with the exception that females had similar levels of NPY and AgRP mRNA at both ages (data not shown).

**Neuropeptide expression: correlations with other parameters**

The expression levels of the orexigenic peptides and those of the anorexigenic peptides in each nucleus showed a strong positive correlation in all animals (Table 3). Such correlations were not...
found for the expression levels with body weight, BMI, food intake, and leptin (except for group or sex differences).

On d 10, a decrease in BMI 

score (SDS, distance from the control mean in sd) between d 2 and 10 was associated with a lower orex/anorex ratio in FR animals (r = 0.643; P = 0.018) but not in controls (r = 0.001; P = 0.997) (Fig. 5A). On d 25, there was a continuous relationship between the change in body weight SDS between d 2 and 21 and the orex/anorex ratio (r = −0.875; P < 0.001) (Fig. 5B).

Discussion

In this study, raising rats in large litters transiently affected the ratio between expression of orexigenic and anorexigenic neuropeptides in the hypothalamus. In both male and female juvenile rats that were subjected to early postnatal FR, NPY and AgRP mRNA expression levels in the ARC had increased relative to those of POMC and CART. In addition, MCH mRNA expression in the LHA was elevated in suckling food-restricted animals. In adulthood, despite still reduced body dimensions and lower POMC and CART expression, the ARC orex/anorex ratio had normalized.

Acute effects of early postnatal FR

During the lactation period, the hypothalamic circuitry that regulates energy balance in the adult rodent is not fully
possible mechanisms of body weight regulation and its programming

At all three ages tested, FR and control rats showed strong positive correlations between the expression levels of the orexigenic and anorexigenic peptides that are coexpressed within a nucleus (NPY with AgRP, POMC with CART, CRH with TRH, and MCH with ORX). This suggests that the expression of these peptides is coregulated.

On d 10, FR animals with a higher ARC orex/anorex ratio had lost fewer BMI SDS points between d 2 and 10 (and hence probably less body fat). This association was absent in control animals. Note, however, that at this age, the hypothalamic circuitry emanating from the ARC is still developing (9, 10), the orexigenic signal from the ARC did not seem to reach the PVN and LHA (present data), and FR pups have little opportunity to increase food intake during the lactation period. Therefore, this association is unlikely to be caused by increased food intake, and it must arise from another mechanism.

On d 25, and hence at the end of the FR period, all weanling males showed a negative correlation between the change in body weight SDS and the ARC orex/anorex ratio. A relative loss of body weight in the preceding weeks coincided with higher orexigenic expression. This may represent an attempt to increase energy reserves to permit catch-up growth at this time when food was already becoming available [because pups of different litter sizes have all been shown to start eating chow from d 16 (46)].

Our findings are largely in agreement with those of studies using other models that manipulate perinatal nutrition: a transient increase in orexigenic relative to anorexigenic hypothalamic expression in juveniles (47–52), with normalized expression levels in older animals (53–55). The fact that different manipulations of perinatal nutrition produce such similar results indicates that this is an essential regulatory phenomenon. Furthermore, it is vital to recognize that this outcome still leaves the possibility of more permanent changes in other areas involved in the regulation of energy balance, such as the brainstem or higher brain areas.

Additional findings: sex differences and ontogeny

Besides the effects of early postnatal FR, we also found differences in hypothalamic gene expression between the
TABLE 3. Positive correlations between the relative mRNA levels of the orexigenic and the anorexigenic neuropeptides in each age group

<table>
<thead>
<tr>
<th></th>
<th>10 d</th>
<th>25 d</th>
<th>380 d</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Correlations</td>
<td>n</td>
</tr>
<tr>
<td>NPY and AgRP</td>
<td>28</td>
<td>$r = 0.852; P &lt; 0.001$</td>
<td>14</td>
</tr>
<tr>
<td>POMC and CART</td>
<td>28</td>
<td>$r = 0.793; P &lt; 0.001$</td>
<td>14</td>
</tr>
<tr>
<td>CRH and TRH</td>
<td>23</td>
<td>$r = 0.627; P = 0.001$</td>
<td>13</td>
</tr>
<tr>
<td>MCH and ORX</td>
<td>25</td>
<td>$r = 0.358; P = 0.079$</td>
<td>12</td>
</tr>
</tbody>
</table>

Our male and female suckling pups showed a similar ARC orex/anorex ratio and similar expression levels of PVN and LHA peptides. This is in agreement with previous reports that found no sex differences in expression in juvenile rats for NPY, AgRP, and CRH (56–58). In adulthood, we showed differential expression for POMC and CART, with lower levels in females, and a trend for lower levels of CRH. These data are in agreement with a previous study that showed similar NPY expression in both sexes (59) but in conflict with another study that did find a sex difference in ORX expression (60). For CRH, conflicting data exist with either lower or higher expression in females (58, 61–64), perhaps related to variation during the estrous cycle. Most hypothalamic gene expression studies, including the present one, seem to suggest a higher orexigenic drive in adult females.

In agreement with our present data, previous authors have also reported stable or decreasing expression of NPY and AgRP over time (65–67) and mostly increasing expression of POMC and CART (67–69). This suggests that juveniles have a stronger orexigenic drive in the ARC than adult rats. This may serve to maximize milk intake, and therefore growth, in the neonatal period (9). Our data on increasing expression levels of PVN and LHA peptides are consistent with previous reports of rising CRH, TRH, and ORX expression during the lactation period (28, 70–72). Conversely, unchanging levels of CRH, TRH, and MCH were also reported at this age (73–76). The overall increase in PVN and LHA expression levels with age that we found may be indicative of a tighter regulation of energy balance in the adult rat.

Summarizing, the reliability of our measurements is verified by the fact that we found comparable sex differences and ontogeny to those reported previously.

Implications and conclusions

In the present study, most of the individual neuropeptides were not found to be differentially expressed in large-litter pups, although the ARC orex/anorex ratio was significantly altered. It may be that FR rats use individual strategies to promote survival and catch-up growth, resulting in either the up-regulation of one or more orexigenic peptides or the down-regulation of one or more anorexigenic peptides. At any rate, the differences found in the ARC orex/anorex ratio were not solely due to the significant differences in CART mRNA expression, because omitting CART from the equation produced similar results (data not shown).

Now that the effects of early postnatal FR on gene expression have been described, a next step may be to investigate possible programming effects on neuropeptide protein levels and functionality. The results of a previous study which reported increased levels of NPY peptide in juvenile large-litter animals (77) suggests that protein levels may be similarly affected as gene expression. Programming of the projections between the ARC and other hypothalamic nuclei may be particularly interesting to examine. Because neonatal leptin is known to be essential for the formation of these projections (9) and leptin levels were extremely low in the suckling pups, although the ARC orex/anorex ratio was down-regulation of one or more anorexigenic peptides. At any rate, these projections (9) and leptin levels were extremely low in the suckling FR rats, the ARC projections might be expected to be permanently altered in adult FR rats due to underdevelopment in early life. In addition, studies by Davidowa and colleagues in small-litter rats suggest that hypothalamic functionality may be permanently altered in adult FR rats due to underdevelopment in early life. In addition, studies by Davidowa and colleagues in small-litter rats suggest that hypothalamic functionality may be permanently altered in adult FR rats due to underdevelopment in early life. In addition, studies by Davidowa and colleagues in small-litter rats suggest that hypothalamic functionality may be permanently altered in adult FR rats due to underdevelopment in early life. In addition, studies by Davidowa and colleagues in small-litter rats suggest that hypothalamic functionality may be permanently altered in adult FR rats due to underdevelopment in early life. In addition, studies by Davidowa and colleagues in small-litter rats suggest that hypothalamic functionality may be permanently altered in adult FR rats due to underdevelopment in early life. In addition, studies by Davidowa and colleagues in small-litter rats suggest that hypothalamic functionality may be permanently altered in adult FR rats due to underdevelopment in early life. In addition, studies by Davidowa and colleagues in small-litter rats suggest that hypothalamic functionality may be permanently altered in adult FR rats due to underdevelopment in early life. In addition, studies by Davidowa and colleagues in small-litter rats suggest that hypothalamic functionality may be permanently altered in adult FR rats due to underdevelopment in early life. In addition, studies by Davidowa and colleagues in small-litter rats suggest that hypothalamic functionality may be permanently altered in adult FR rats due to underdevelopment in early life. In addition, studies by Davidowa and colleagues in small-litter rats suggest that hypothalamic functionality may be permanently altered in adult FR rats due to underdevelopment in early life. In addition, studies by Davidowa and colleagues in small-litter rats suggest that hypothalamic functionality may be permanently altered in adult FR rats due to underdevelopment in early life. In addition, studies by Davidowa and colleagues in small-litter rats suggest that hypothalamic functionality may be permanently altered in adult FR rats due to underdevelopment in early life. In addition, studies by Davidowa and colleagues in small-litter rats suggest that hypothalamic functionality may be permanently altered in adult FR rats due to underdevelopment in early life. In addition, studies by Davidowa and colleagues in small-litter rats suggest that hypothalamic functionality may be permanently altered in adult FR rats due to underdevelopment in early life. In addition, studies by Davidowa and colleagues in small-litter rats suggest that hypothalamic functionality may be permanently altered in adult FR rats due to underdevelopment in early life. In addition, studies by Davidowa and colleagues in small-litter rats suggest that hypothalamic functionality may be permanently altered in adult FR rats due to underdevelopment in early life.
In this regard, it may also be interesting to investigate the effects of highly palatable diets in adult FR rats.

Early postnatal FR induces persistent reductions in body size. In this study, we demonstrated that this is accompanied by a transient increase in the ratio of orexigenic to anorexigenic expression in the hypothalamic ARC. This orexigenic signal hardly affected gene expression in the PVN and LHA, which is consistent with the immature connections that were previously reported at this age. Despite still reduced body dimensions, middle-aged FR rats showed normalized levels of hypothalamic neuropeptide expression. This may indicate programming of the hypothalamic regulation of energy balance. When these results are combined with the ones that were previously obtained, a phenotype arises of juvenile rats that grow very little as a result of undernutrition, that are very thin with a low fat mass, and that increase hypothalamic orexigenic expression, perhaps in an attempt to increase energy availability for growth (13, 14) (present data). In adulthood, these animals remain thin (low BMI) and lean (low fat percentage) despite considerable catch-up growth, with a low energy intake but rather normal energy expenditure (11, 14, 15). In contrast to what might be expected in such a situation, hypothalamic orexigenic expression is normal at this age (present data). In summary, the present study demonstrates that in rats, early postnatal FR can alter the programing of the hypothalamic regulation of energy balance. If energy balance regulation is similarly affected in perinatal programming of the hypothalamic regulation of energy balance, hypothalamic orexigenic expression is normal at this age (present data).

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