The timing of “catch-up growth” affects metabolism and appetite regulation in male rats born with intrauterine growth restriction

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1Institut National de la Recherche Agronomique, UMR 1280 Physiologie des Adaptations Nutritionnelles, Université de Nantes, Nantes, France; and 2Institut des Maladies de l’Appareil Digestif, Centre Hospital-Universitaire de Nantes, Nantes, France

Submitted 8 April 2009; accepted in final form 8 July 2009

Coupé B, Grit I, Darmaun D, Parnet P. The timing of “catch-up growth” affects metabolism and appetite regulation in male rats born with intrauterine growth restriction. Am J Physiol Regul Integr Comp Physiol 297: R813–R824, 2009. First published July 15, 2009; doi:10.1152/ajpregu.00201.2009.—Epidemiological studies demonstrated a relationship between low birth weight mainly caused by intrauterine growth restriction (IUGR) and adult metabolic disorders. The concept of metabolic programming centers on the idea that nutritional and hormonal status during the key period of development determines the long-term control of energy balance by programming future feeding behavior and energy expenditure. The present study examined the consequence of early or late “catch-up growth” after IUGR on feeding behavior and metabolic cues of male offspring of rat dams exposed to protein restriction during gestation and/or lactation. Our results suggest that early catch-up growth may be favorable for fasting metabolic parameters at weaning, as no differences were observed on plasma leptin, triglyceride, glucose, and insulin levels compared with controls. In contrast, if pups remained malnourished until weaning, low insulin concentration was detected and was accompanied by hyperphagia associated with a large increase in hypothalamic NPY and AgRP mRNA expression. At adult age, on a regular chow diet, only the meal structure was modified by fetal programming. The two IUGR groups demonstrated a reduced meal duration that enhanced the speed of food ingestion and consequently increased the rest period associated to the satiety state without changes in the hypothalamic expression of appetite neuropeptides. Our findings demonstrate that in IUGR, regardless of postnatal growth magnitude, metabolic programming occurred in utero and was responsible for both feeding behavior alteration and postprandial higher magnitude, metabolic programming occurred in utero and was responsible for both feeding behavior alteration and postprandial higher insulin level in adults. Additionally, catch-up growth immediately after early malnutrition could be a key point for the programming of postprandial hyperleptinemia.

perinatal nutrition; appetite neuropeptides; hypothalamus; feeding behavior

METABOLIC DISORDERS SUCH AS hypertension, type 2 diabetes, cardiovascular disease, and obesity are a major and growing health concern, particularly in Western countries. Although, genetic background, excessive food consumption, sedentary life style, and decreased physical activity are the main predisposing factors for these diseases, epidemiological studies demonstrated, after the 1944 Dutch famine, a relationship between a low birth weight [which is the consequence of an intrauterine growth restriction (IUGR)] and adult metabolic disorders. This led to the concept of fetal metabolic programming or nutritional imprinting (3, 24, 49). Nowadays, in Western societies, placental insufficiency, presumably through a downregulation of maternal-fetal amino acid transport, is the most common cause of IUGR (31). Experimental studies have replicated IUGR, on rodents or larger animal species, by altering maternal nutrition during gestation, by administration of glucocorticoids or by uterine artery ligature to create placental insufficiency (38). However, experimental data have revealed that low birth weight is not by itself the only cause of susceptibility to adult metabolic disorders. What happens after birth, during the early postnatal period, has a major impact as well (46).

In Western countries, small-for-gestational age babies are generally admitted to neonatology units, where every effort is made to obtain, during hospitalization, an accelerated growth, commonly called catch-up growth. In regard of the time window the catch-up growth happens, early (before the first year of life) or late (after the first year of life), it has been shown to predict the future development of metabolic disease (20, 35).

The central neural network regulating appetite is present before birth in mammals. In rodents, the differentiation of the neuronal systems of the hypothalamus responsible for the control of appetite and energy expenditure begins during the last week of gestation, with development continuing until weaning (37). Cell differentiation, migration, axon growth, and synapse formation, are processes targeted by environmental manipulation and highly dependent on the “leptin surge” which occurs between the first and second postnatal weeks (2). In neonates, leptin has a neurotrophic effect on arcuate nucleus (Arc) neurons and is suspected to promote projections from this structure to paraventricular nucleus (PVN), dorsomedial hypothalamus (DMH), and lateral hypothalamic area (LHA) (11). Interestingly, the leptin-dependent establishment of Arc pathway seems to be modulated by postnatal nutrition (17, 60). During this developmental period, leptin seems to have no or limited effect on food regulation (1, 40). After weaning and through the entire life, the Arc is the primary target for leptin’s action on food intake and energy expenditure regulation. Arc contains orexigenic neurons, which coexpress neuropeptide Y (NPY) and agouti-related protein (AgRP), and anorexigenic neurons, which coexpress proopiomelanocortin (POMC) and cocaine-and amphetamine-regulated transcript (CART), both of them bearing leptin receptors on their cell surface (39). After a meal, satiety is first initiated by a mechanical distension of gastrointestinal tract relayed to the brain by the vagus nerve and by the central action of gastrointestinal and pancreatic peptides (15). Leptin and insulin are then released by adipose tissue and endocrine pancreas, respectively, and act on Arc neuronal populations to activate POMC/CART neurons and to inhibit NPY/AgRP neurons.

Several studies on rodents suggest that a defective programming of these hypothalamic circuits could begin in utero and continue in early postnatal life throughout the suckling period, leading to a disturbed organization and, consequently, long-
lastingly dysfunction in adulthood (10, 12, 45). Earlier studies have mostly described alterations in numbers of NPY neurons, as well as increased NPY gene expression in fetal and neonatal tissues, supporting the notion that NPY system is a key target of perinatal developmental programming (45, 47, 56). Moreover, anorexigenic pathways seem also to be targeted by nutritional programming since caloric restriction of the mother diet during gestation leads to reduction of POMC mRNA expression in arcuate nucleus and peptide content of the paraventricular nucleus of the hypothalamus of weaned rats and adult rats (13, 17). However, few studies have sought a link between activation of this system during the early postnatal period and upon adulthood. Additional experimental studies demonstrated that protein or caloric restriction during gestation and lactation induces, later on, hyperphagia, a preference for high-fat food and leads to glucose intolerance and increased body fat (5, 18, 57).

We hypothesized that perinatal maternal protein restriction would induce developmental programming that might durably modify key circulating factors acting on hypothalamus with consequences on hypothalamic gene expression in adult offspring in the postprandial period after a regular chow meal. The programmed modifications may result in alterations in long-term energy homeostasis and food intake in adulthood. The present study, therefore, examined the male offspring of rat dams exposed to protein restriction during gestation and/or lactation to determine if the short-term (upon weaning) and late effects (at 8 mo of age) of perinatal protein restriction on body weight and food intake, 2) variations in metabolic parameters (glucose, insulin, leptin, triglyceride), and 3) expression of hypothalamic peptides of appetite regulatory system after fasting and in postprandial conditions. The aim of this study is to reveal physiological anomalies associated with feeding behavior and better define the consequences of an early or a late catch-up growth on animals studied around weaning and at adulthood.

This study sheds new light on the way a low-protein maternal diet disrupts, over the long term, the function of the hypothalamus to regulate food intake, body weight, and energy expenditure along with peripheral controlling factors.

METHODS

Animals and Diet

Female and male Sprague-Dawley rats (Janvier, Le Genest Saint Isle, France), 8 wk old, were maintained under controlled conditions (22°C, 12:12-h dark-light cycle) with free access to regular food, containing 16 g protein per 100 g of pellets (A04, SAFE, Augy, France) and tap water. After 10 days of habituation, female rats were mated overnight with a male, and copulation was verified the next morning by the presence of spermatozoa in vaginal smears. At conception, 26 pregnant dams were housed individually and were fed either a normal protein diet exposed to protein restriction during gestation and/or lactation to determine if the short-term (upon weaning) and late effects (at 8 mo of age) of perinatal protein restriction on body weight and food intake, 2) variations in metabolic parameters (glucose, insulin, leptin, triglyceride), and 3) expression of hypothalamic peptides of appetite regulatory system after fasting and in postprandial conditions. The aim of this study is to reveal physiological anomalies associated with feeding behavior and better define the consequences of an early or a late catch-up growth on animals studied around weaning and at adulthood.

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At delivery, pups born from restricted mothers or from normally fed mothers were adopted randomly by six control foster mothers (C) for 12 of them and six restricted mothers (R) to create three experimental groups CC (n = 14), RC (n = 19), and RR (n = 18), in which the first letter refers to maternal dietary intake during gestation and the second letter refers to maternal dietary intake during lactation. At birth (postnatal day 0, PND0), rat pups were weighed, and the litters were equalized to eight male pups per litter. After weaning (PND22), all pups were housed individually and fed a control protein diet containing 20% protein. Then, from the age of 40 days, rats were fed a standard laboratory chow (A04; SAFE) until the end of the experiment. All experiments were performed in accordance with the European Communities Council Directive of November, 24, 1986 (86/609/EEC) regarding the care and use of animals for experimental procedures.

Tissue and Blood Collection

Rats were rapidly euthanized between 0900 and 1100 AM by CO2 inhalation. Blood was collected in heparinized tubes (Laboratoires Léa SA, St Quentin en Yvelines, France) and centrifuged at 2,500 g for 15 min at 4°C. Plasma was frozen at −80°C. After removal of the brain from the skull, hypothalamus was dissected [according to Paxinos’s atlas coordinates: −1.0 mm to −4.5 mm from bregma and 3 mm in depth (23)] on an ice tray, snapped frozen in liquid nitrogen, and stored at −80°C. Perirenal fat pads were dissected and weighed.

Plasma Hormone and Metabolite Determinations

Heparinized plasma collected on PND22 and on adult rats was used to measure plasma glucose, triglycerides, insulin, and leptin. Glucose and triglycerides were measured by using colorimetric enzymatic reactions with specific kits (glucose and triglycerides PAP 150 kits, BioMérieux, Marcy-l’Étoile, France). Hormones were assayed with specific ELISA kits following the manufacturer’s instructions for insulin (rat/mouse insulin ELISA kit, Linco Research, St. Charles, MO) and leptin (rat leptin ELISA kit; Linco Research). Homeostatic model assessment of insulin resistance (HOMA-IR) was calculated from plasma insulin and glucose concentrations of fasted animals as HOMA-IR = plasma glucose (mmol/l) × plasma insulin (μU/ml)/22.5.

Measurement of Body Weight and Food Intake

The weight of each pup was recorded at birth and thereafter every day at 0900 AM until PND22 (weaning). After weaning and until the end of the experiment, rats were weighed every 3 days. To measure diurnal and nocturnal food consumption, food tray was weighed at regular intervals by measuring the difference between the amount of food provided at the onset of the light cycle and the amount of food remaining 12 h later and 24 h later.

Behavioral Satiety Sequence

The analysis of the behavioral sequence was performed, as described by Haldet al. (28), in animal home cage where habituation trial had been performed. To promote feeding in adult rats, food was removed for 48 h before the presentation of chow. The observation period lasted for 90 min. To analyze feeding and other behaviors, animals were videotaped, and films were scored by a trained observer who was blinded for the nutritional status of the animals. Quantified activities were feeding, drinking, grooming, and resting. The test period was divided into eighteen 5-min time bins and allowed to determine meal duration, feeding rate, grooming, and resting time, as well as the satiety period.

RNA Isolation and Real-Time RT-PCR

RNA was isolated from snap-frozen hypothalamus using the NucleoSpin RNA/protein kit (Macherey-Nagel, Hoerdt, France). Total RNA was submitted to DNase digestion following the manufacturer’s instructions, the quality was checked on agarose gels, and the quantity was estimated by the 260/280 nm UV absorbance. One microgram of total RNA was reverse-transcribed into cDNA using Random Primer and Moloney Murine Leukemia Virus (MMLV) reverse transcriptase (Promega, Madison, WI) in a total volume of 25 μl. Real-time PCR was performed on 5 μl of a 1:40 dilution of reverse transcribed reaction and 2.5 μM of both forward and reverse primers, in a final volume of 15 μl, using SYBR green PCR kit (Bio-Rad Laboratories,
Hercules, CA) in the iCycler iQ real-time PCR detection system instrument (Bio-Rad). Negative control for RT-PCR reactions were performed by omitting MMLV from the reaction mixture. The sequence of primers and the amplification size products for each gene are summarized in Table 1. mRNA expression was calculated using the $2^{-\Delta \Delta Ct}$ method after normalization with GAPDH as a housekeeping gene (36). Control group values are used as calibrator. The applicability of the CT method was first validated by determining how the amplification efficiencies of the different transcripts, including GAPDH varied with template dilution. These experiments showed that the efficiency of the PCR amplification was the same for all of the genes and that the expression of GAPDH was not influenced, either by age, or the pup growth status (data not shown).

### Statistical Analysis

Statistical analysis was performed using StatView 5.0 (SAS Institute, Cary, NC). Data were first analyzed using two-way ANOVA with age and maternal diet as the main factors. The expression of data and the differences among groups were determined by Mann-Whitney U test and represented as mean $\pm$ SE. In all tests, $P < 0.05$ was considered significant.

### RESULTS

#### Body Weight and Growth

Maternal protein restriction during gestation resulted in fetal growth restriction, reflected by a lower body weight at birth (insert Fig. 1A). The body weight of male newborn pups differs between

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**Table 1. Sequences of primers used for real-time quantitative PCR**

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Accession Number</th>
<th>Primer Sequences</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gapdh</td>
<td>NM_017008</td>
<td>F: 5'-CCGCAAGTTCAACGCACAG-3' R: 5'-ATGAAGATGATGATGATGCAG-3'</td>
<td>132 pb</td>
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<tr>
<td>Npy</td>
<td>NM_012614</td>
<td>F: 5'-TTGAGATGACCTGGCTATC-3' R: 5'-AGGAGATTGAGAGAAGCGAG-3'</td>
<td>148 pb</td>
</tr>
<tr>
<td>Agrp</td>
<td>XM_574228</td>
<td>F: 5'-AGAAGGTCATAGATCAAGCT-3' R: 5'-GTCAGAAATTGAAGAAGAG-3'</td>
<td>135 pb</td>
</tr>
<tr>
<td>Pomc</td>
<td>NM_139326</td>
<td>F: 5'-GTAGTTAAAAGGAGGATCTCAATGAG-3' R: 5'-ATGAGATTGATGATGATGCAG-3'</td>
<td>109 pb</td>
</tr>
<tr>
<td>Cart</td>
<td>NM_017110</td>
<td>F: 5'-GTCAGAAATTGAAGAAGAG-3' R: 5'-ATGAGATTGATGATGATGCAG-3'</td>
<td>142 pb</td>
</tr>
</tbody>
</table>

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**Fig. 1. Growth parameters of rat offspring. A: Body weight (g) from birth to postnatal week 36. Inset: expanded view of growth from birth to weaning. Body weight gain (%) from birth to adulthood (B) and from weaning to adulthood (C). Control mothers (C, fed a 20% protein diet) or restricted mothers (R, fed a 8% protein diet) during gestation (first letter) and/or lactation (second letter). CC group (open circles), RC group (gray circles), and RR group (closed circles). Values are expressed as means $\pm$ SE; $n = 7$ or 8 per group. P < 0.05: SCC vs. RR, #RC vs. RR, $P < 0.01$: **CC vs. RC, $SSCC$ vs. RR, ###RC vs. RR.**
the C and R groups (7.18 ± 0.39 g vs. 5.85 ± 0.19 g, respectively; P < 0.01). Body weight was modulated from birth to adulthood (8 mo old), by age [F(2/528) = 274.41, P < 0.0001] and by maternal nutrition [F(2/528) = 7.69, P < 0.0005]. An interaction between age and maternal nutrition was not observed [F(64/528) = 0.06, P > 0.9999]. Body weight remained lower for the RR group until the 5th wk and later on, differences in body weight between groups disappeared (Fig. 1A).

The timing of catch-up growth, illustrated on Fig. 1, B and C, differed between RR and RC groups, since RC pups showed an accelerated weight gain during the 1st wk of life, whereas RR pups experienced a higher rate of weight gain after weaning (P < 0.01).

**Spontaneous Food Intake**

Data analysis by two-way ANOVA for repeated measures of total food intake from weaning and until the 8th mo of life revealed a significant effect of age [F(26/470) = 223.13, P < 0.0001] and no effect of maternal nutrition [F(2/470) = 0.84, P = 0.4305]. An interaction between age and maternal nutrition was observed [F(52/470) = 1.75, P < 0.01]. Nocturnal and diurnal food intake were both modulated by age [F(26/470) = 118.90, P < 0.0001 and F(26/474) = 33.79, P < 0.0001, respectively], and by maternal nutrition [F(2/470) = 23.36, P < 0.0001 and F(2/474) = 53.20, P < 0.0001, respectively]. An interaction between age and maternal nutrition was observed for the two phases [F(52/470) = 2.31, P < 0.05 and F(52/474) = 3.09, P < 0.0001, respectively]. During the first 3 wk after weaning, RR pups showed a reduction of food intake during the diurnal period (P < 0.001) compared with CC and RC pups (Fig. 2A). However, when the amount of ingested food was normalized for body weight (Fig. 2B), a hyperphagia was evident for RR rats (P < 0.01 compared with CC and P < 0.05 compared with RC) due to an increase in food intake during the night period (P < 0.01). This increased consumption was no longer apparent in 8-mo-old animals, as shown in Fig. 2, C and D, since no difference in measured food intake was detected between the three groups.

**Hormones and Metabolism at Weaning**

Hormones and metabolic factors were measured on fasted animals on PND22 (Fig. 3, A–D). Plasma leptin did not differ between the three groups, whereas plasma insulin (RR: 0.10 ± 0.02 ng/ml vs. CC: 0.29 ± 0.05 ng/ml and RC: 0.25 ± 0.05 ng/ml; P < 0.05), plasma triglycerides (RR: 0.48 ± 0.04 g/l vs. CC: 1.35 ± 0.12 g/l and RC: 1.28 ± 0.18 g/l; P < 0.05) and plasma glucose (RR: 0.63 ± 0.04 g/l vs. CC: 1.02 ± 0.05 g/l and RC: 1.07 ± 0.06 g/l; P < 0.001 and P < 0.01, respectively) were three-, three-, and twofold lower, respectively, in RR compared with CC and RC rats. A reduced HOMA-IR was calculated (RR: 0.39 ± 0.09 vs. CC: 1.82 ± 0.38 and RC: 1.50 ± 0.37, P < 0.01 for both). All parameters were comparable between RC and CC (Fig. 3).

**Fig. 2.** Mean food intake. Mean absolute food intake between week 3 and week 5 (A) and between week 27 and week 30 (C). Mean relative food intake (per g of body weight) between week 3 and week 5 (B) and between week 27 and week 30 (D). CC group (open bars), RC group (gray bars), and RR group (solid bars). Values are expressed as means ± SE; n = 7 or 8 per group. P < 0.05 SCC vs. RR, #RC vs. RR, P < 0.01: SCC vs. RR, ## RC vs. RR.
Hypothalamic mRNA Contents of NPY, POMC, AgRP and CART mRNA in PND22 Rats

Consistent with food intake data, the relative mRNA expression of the orexigenic peptides NPY and AgRP was increased in the hypothalamus of the PND22 rats in RR group compared with controls (Fig. 4, A and B). NPY and AgRP mRNA expression was twice as high in RR rats compared with CC (NPY mRNA: RR: 1.93 ± 0.23 vs. CC: 1.02 ± 0.07, P < 0.01; AgRP mRNA: RR: 2.10 ± 0.28 vs. CC: 1.02 ± 0.10, P < 0.05). This increase is less pronounced but still present in RC group compared with controls for NPY (RC: 1.50 ± 0.16 vs. CC: 1.02 ± 0.07, P < 0.05) and AgRP mRNA (RC: 1.43 ± 0.14 vs. CC: 1.02 ± 0.10, P < 0.05) (Fig. 4, A and B).

A striking difference was found between young and adults rats in the relative expression ratio of mRNAs encoding orexigenic vs. anorexigenic peptides (Fig. 8). The ratio is in favor of orexigenic peptides mRNA expression for RC (1.20 ± 0.04) and RR (2.26 ± 0.26) rats compared with CC (1.00 ± 0.04, P < 0.01 and P < 0.001, respectively) and provides evidence for a strong, sustained drive toward an enhanced food intake during the period of catch-up growth in these two groups of pups.

Feeding Behavior

To study feeding behavior and postprandial metabolism, the three groups of rats received their regular chow after a fasting period of 48 h.

Behavioral Satiety Sequence

Feeding behavior was monitored over a period of 90 min. As shown in Fig. 5, CC, RC, and RR groups exhibited a typical behavioral satiety sequence characterized by an initial phase of eating followed by an active phase of grooming and ending with a phase of resting (Fig. 5, A–C). RR and RC animals spent
significantly less time eating than CC rats (RR: 31.82 ± 1.45 min, RC: 31.70 ± 3.25 min vs. CC: 50.50 ± 3.57 min, P < 0.05 and P < 0.05, respectively) but demonstrated a longer period of resting (RC: 32.77 ± 4.65 min, RR: 25.65 ± 3.78 min vs. CC: 10.95 ± 5.27 min, P < 0.05 and P < 0.05, respectively), which is a sign of a premature satiety state (Fig. 5G). Meal size was equivalent for the three groups, even though RR and RC pups showed shorter eating sequences, which means a higher speed of ingestion (Fig. 5D). However, when food intake was expressed per unit of body weight, food intake was significantly reduced in RC group, during the refeeding period, compared with CC rats and RR rats (P < 0.05 for both) (Fig. 5E). Because feeding time was 1.5-fold longer in control rats than in the two other experimental groups, a significantly higher speed of ingestion was calculated for RC and RR rats compared with controls (P < 0.05 for both) (Fig. 5F).

**Hormones and Metabolism**

In fasted adult rats. Although adults rats no longer differed between groups regarding body weight, the reduction in body mass index [body weight (kg)/length (m²)] persisted for the RR group at 8 mo of age [RR: 7.72 ± 0.15 vs. CC: 8.40 ± 0.14, P < 0.05 and RC: 8.29 ± 0.50, nonsignificant (NS)] and was accompanied by a lower perirenal fat mass (RR: 8.14 ± 1.36 g vs. CC: 14.38 ± 2.26 g, P < 0.05 and RC: 13.82 ± 3.15 g, NS) (or perirenal fat mass, expressed as a fraction of body weight: RR: 1.36 ± 0.12% vs. CC: 2.31 ± 0.22%, P < 0.05 and RC: 2.16 ± 0.41%, NS).

In fasted adult rats, plasma triglycerides were higher in RR than in RC group (P < 0.05), although leptin was low in RR (7.16 ± 1.22 ng/ml) compared with the levels of CC rats (11.90 ± 1.71 ng/ml, P < 0.05) and RC rats (10.61 ± 1.54 ng/ml, NS) (Fig. 6, A and B). Plasma insulin levels were
significantly reduced in RR (0.27 ± 0.05 ng/ml) compared with CC (0.70 ± 0.20 ng/ml, P < 0.05) and RC groups (0.70 ± 0.15 ng/ml, P < 0.05) (Fig. 6C).

Plasma glucose concentrations were significantly reduced in RC (1.18 ± 0.03 g/l) compared with control rats (1.31 ± 0.03 g/l, P < 0.05) in fasting condition (Fig. 6D). HOMA-IR was slightly, but not statistically significantly decreased in RR (2.40 ± 1.10), compared with CC rats (5.48 ± 1.55) and RC (4.89 ± 1.10).

In refed adult rats, hormone concentrations and metabolism were assessed in the postprandial period (Fig. 6). Two hours after a regular meal, the RC group demonstrated dramatically higher plasma leptin concentration (RC: 26.96 ± 2.06 ng/ml vs. CC: 11.79 ± 1.08 ng/ml and RR: 12.47 ± 2.48 ng/ml, P < 0.05 for both) (Fig. 6A), and an elevated plasma insulin concentration was observed in the RC (5.64 ± 0.25 g/l, P < 0.05) and the RR animals (4.83 ± 0.21 g/l, P < 0.05) compared with control rats (3.56 ± 0.32 g/l) (Fig. 6B). No significant differences were observed in plasma triglycerides (Fig. 6C) or glucose levels (Fig. 6D) between the three groups, even though a trend toward a higher blood glucose was observed in RC and RR rats (P = 0.08).

**Hypothalamic Appetite Neuropeptide Expression at Adulthood After a Fasting and a Refeeding Period**

Fasting state was associated with a higher CART mRNA level in RC group (1.56 ± 0.25), compared with CC rats (1.02 ± 0.11, P < 0.05) (Fig. 7D).

In the postprandial condition, a sharp decrease in the hypothalamic mRNA expression of the orexigenic neuropeptide NPY was observed in all three groups and a decline in AgRP mRNA that reached significance only for RR group (Fig. 7, A and B). No significant differences between fasting and postprandial conditions were observed for POMC expression (Fig. 7C). At the opposite, CART mRNA expression was reduced after a refeeding period (Fig. 7, C and D). CART mRNA expression increase in RC fasted rats was also observed in postprandial RC rats (0.88 ± 0.05, P < 0.05) compared with control animals (0.71 ± 0.04) (Fig. 7D).

**DISCUSSION**

Fasting condition targeted over the period of early fetal and neonatal brain development has a distinct effect on appetite regulation early in life. The current study demonstrates that maternal dietary restriction targeted over the period of early fetal and neonatal brain development has a direct effect on appetite regulation early in life.
postnatal life that is most likely driven by a strong upregulation of orexigenic peptide expression (Fig. 8). These early events also have a significant impact in adulthood although the mechanisms underlying the developmental programming of appetite and insulin and leptin homeostasis remain unclear and difficult to link to hypothalamic neuropeptides regulation.

We first confirm that protein restriction during gestation results in low birth weight (22). The growth restriction, observed at birth, persists until 6 wk, if pups are nursed by foster mothers receiving a protein-restricted diet during lactation. In contrast, offspring subjected to protein restriction in the fetal period and subsequently fostered by well-nourished dams for suckling, recover a weight similar to that of control pups during the 1st wk of life. Therefore, catch-up growth occurred before weaning for the RC pups, but it was delayed until after weaning for RR pups. It has been previously proposed that an early catch-up growth is favorable for body and organ weight development, but it increases the susceptibility to a metabolic syndrome later in life (26), and consequently reduces life span (42). Conversely, when catch-up growth takes place after weaning, it has no major impact on insulin sensitivity and life span (7, 33), although some others studies reported, on aging rats, a depressed insulin response after a glucose challenge (19, 51) linked to a “malprogramming” of the endocrine pancreas (52).

The mechanism of catch-up growth has not been fully elucidated. During the lactating period, catch-up growth is supported by the amount and quality of maternal milk, as demonstrated by Bautista et al. (4), who recently showed that milk production was higher in control nursing mothers compared with protein restricted lactating mother.

Moreover, the availability of appropriate amounts of nutrients early during lactation could lead to a catch-up growth by normalization of the somatotropic axis which is altered after fetal growth restriction (29). Indeed, a recent study in overfed mice, during postnatal period, showed a similar IGF-1 level
compared with 10-day-old control mice, whereas restricted mice showed reduced IGF-1 levels (34).

The results of the present study suggest that early catch-up growth may be favorable for metabolic parameters at weaning, since no differences were observed between RC and control rats on plasma leptin, triglyceride, glucose, and insulin levels under fasting conditions. In contrast, and in agreement with other authors, we found that if pups remained malnourished until weaning (as was the case in RR group), low insulin concentration was detected at that period (19). It was suggested that lower insulin secretion is a consequence of a reduction in pancreatic endocrine cell mass (9). Indeed, the reduction of dietary protein intake, in utero, alters the vascularization of the pancreas, and the latter is the cause or the consequence of the reduced β-cell mass (8). This has been already documented in protein-malnourished offspring (16), which later developed insulin resistance between 15 to 17 mo (27, 44).

In the RR pups we observed a late catch-up growth that occurred only after weaning, around 35 to 45 days of age. This was accompanied by hyperphagia, associated with a large increase in NPY and AgRP mRNA and a lower expression of anorexigenic peptides at weaning, compared with adulthood. This is in accord with previous work from Remmers et al. (50) and suggests a strong drive to food intake to sustain the high, physiological velocity of growth occurring in suckling period. Leptin levels were identical in our three groups despite NPY and AgRP mRNA being overexpressed in the RR group. This finding supports the conclusions of Ahima and Hileman (1), who postulated that during early development, leptin had no anorexigenic effect. The action of leptin to regulate the expression of appetite regulating hypothalamic neuropeptides has been under debate in a number of publications. Proulx et al. (48) demonstrated that a leptin injection at PND10 increases POMC mRNA expression but does not affect food intake (48) and that leptin alters metabolic rates before acquisition of its anorectic effect in developing neonatal mice (38). However, it is likely that, during this period, the regulation of neuropeptide expression may occur through the action of insulin. Accordingly, in the present study, low insulin level paralleled high NPY and AgRP expression in the RR pups.

Plagemann et al. (45) proposed that a disruption of orexigenic pathway persisting through adulthood could contribute to increase metabolic risk, and other authors further hypothesized that the developmental programming of adult obesity may be
mediated by appetite programming. In keeping with this hypothesis, alterations in food intake were observed in the present work. We observed an increase in food intake in RR rats in the first few weeks following weaning. Although by the time pups had reached adulthood, food intake was normalized in the three groups of rats. Hyperphagia at adulthood was previously observed but only after a drastic prenatal diet restriction (FR30), followed by a hypercaloric diet (57). It seems that the hyperphagic phenotype of protein-restricted offspring appeared only when food palatability increased, and not when regular chow was offered. This could be related to food preferences observed in 12-wk-old male rats which chose a high-fat diet rather than a high carbohydrate diet (5).

In our experiments, night and day food intakes were identical when the three groups of rats were put on regular chow diet, so a “macroscopic” circadian perturbation of feeding behavior could be excluded. In contrast, in prenatal maternal food-restricted diet model (FR30), 4-mo-old rats ingested more food during the day and less at night (13). Circadian perturbations were also observed sporadically in offspring of dams exposed to a low-protein diet at a different time of night and/or day (41), which suggests that a more precise and automated recording of food intake, during at least 48 h, would be warranted to draw any firm conclusion.

In the current study, we showed that more than the quantity of food ingested, the structure of meal itself was modified by nutritional programming. During a refeeding period, meal duration was reduced in RR and RC rats without affecting meal size, but the speed of food ingestion increased in both groups. Consistent with our data, a hyperphagic sequence has also been observed in adult rats during the first step of a meal, after a fast of 24 h (32). Accordingly, Ji and Friedman observed an increase in satiety signal without recovered liver energy status and concluded that early first satiety signals were coming from the gastrointestinal tract (32). Indeed, satiety is first initiated by two effects on gastrointestinal tract: the distension (vagal afferent projecting to the solitary tract nucleus) and the peptide release (15). The rapid satiety taking place in IUGR rats is interesting and could be due to a large array of physiological events such as alterations of mechanoreceptors of the stomach, accelerated gastric emptying, alteration of stomach, or gut peptide release (ghrelin, CCK, PYY), which could be considered in future work.

Interestingly, the consequence of this early satiety signal is a rapid loss of activity of the RR and RC rats, which demonstrated a time of rest significantly elevated compared with CC rats. This could be linked to the reduction in locomotor activity, and the decrease in energy expenditure revealed at early and late adult age in offspring of dams exposed to caloric or protein restriction (6, 58).

The differences in feeding behavior observed in IUGR rats during the refeeding period did not directly correlate with the changes in the hypothalamic expression of neuropeptides known to be regulated by leptin and insulin. After a 2-h refeeding period, very little difference in mRNA expression of hypothalamic appetite neuropeptides was found between the three groups, except for CART mRNA expression. It has been previously shown that a leptin surge normally occurs 4 h after a meal (30) and that the effect of leptin on hypothalamic appetite-regulatory neuropeptides mRNA expression occurs 6 h after the start of the refeeding period, and implies a gradual postabsorptive regulation (55), whereas α-MSH neuron activation starts 2 h after the meal (53). This implies neuropeptides are regulated by other pathways, such as insulin, which rises ~30 min after the beginning of the food intake (30). In our study, we observed, for the three groups of rats, an insulin rise 2 h after a meal, which was associated with a decrease of orexigenic neuropeptide expression. Anorexigenic peptides of the hypothalamus did not change at that time point and are probably not involved in the first step of satiety. However, a recent study by Breton et al. (13), which differed from our work by the maternal nutritional status (food restricted to 30% of the need) and the age at which offspring has been studied, reported an alteration of POMC neurons response on free-fed animals. What will be, in that precise protocol, the consequence of the satiety state on POMC neurons, is not known.

Upon adulthood, the most commonly reported metabolic alteration observed in models of IUGR, whether induced by protein restriction, caloric restriction, or uterine artery ligation, is the occurrence of insulin resistance (for a review, see Ref. 38). There is extensive evidence in the literature to suggest that 9-mo-old offspring, born with a low birth weight due to prenatal caloric restriction, have higher fasting glucose and insulin levels than controls (19). However, in agreement with our data, when dietary protein restriction was maintained during lactation, fasting insulin level was low and remained so in adulthood (7). The alteration in the development of the pancreatic β cells is at the origin of the impaired secretion of insulin. In the present study, fasting glucose metabolism did not differ between groups, but the postprandial state revealed higher secretion of insulin that failed to normalize glucose plasmonic concentration in the adults RC and RR rats. This may account for an impaired insulin sensitivity.

Significant hyperleptinemia was also observed in RC rats 2 h after a regular chow meal. This was not associated with an exacerbated expression of the anorexigenic hypothalamic neu-

Fig. 8. Orexigenic to anorexigenic hypothalamic peptide mRNA expression ratio. CC group (open bars), RC group (gray bars), and RR group (closed bars). Values are expressed as means ± SE; n = 8 at PND22, and n = 6 or 7 per group at 8 mo of age. **P < 0.01: #CC vs. RR, **P < 0.001: $SSCC$ vs. RR, ###RC vs. RR. Two letters over a box indicates a statistically difference between PND22 and 8 mo old, P < 0.01.
ropeptides involved in appetite regulation. Hyperleptinemia was previously observed in maternal food-restricted offspring but after a period of being on a high-fat diet at adulthood (57) and always associated with a higher adipose tissue development. The hypothesis that hyperleptinemia is the consequence of higher leptin mRNA synthesis in adipose tissue needs further evaluation. Other organs, like the stomach, could participate in the leptin synthesis (25), in response to the bolus of food, since the increase takes place early after feeding.

Hyperleptinemia is often interpreted as evidence for a state of leptin resistance and could be the consequence of early nutritional programming by leptin itself. Indeed, in pups exposed to undernutrition, daily injections of leptin for the 1st wk of lactation, prevented the subsequent onset of an obese phenotype or hyperleptinemia (59).

Perspectives and Significance

These data support the hypothesis that the timing of catch-up growth affects differently the metabolism and appetite regulation in male rats born with IUGR. By testing the effect of early vs. late catch-up growth on metabolism and food behavior, we demonstrate that regardless of postnatal growth pattern, a metabolic programming occurred in utero and could be even more detrimental for the adult health. The elucidation of molecular and developmental events, taking place, in various organs as pancreas, hypothalamus, or adipose tissue, during the lactation period, should be emphasized.

GRANTS

B. Coupé was sponsored by a doctoral fellowship from Institut National de la Recherche Agronomique and Région Pays de la Loire and by a grant from La Fondation Louis Bonduelle (France). This study was supported by a grant from la Recherche Agronomique and Re ´gion Pays de la Loire and by a grant from GRANTS

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