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Programmed obesity in intrauterine growth-restricted newborns: modulation by newborn nutrition

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Desai, Mina, Dave Gayle, Jooby Babu, and Michael G. Ross. Programmed obesity in intrauterine growth-restricted newborns: modulation by newborn nutrition. Am J Physiol Regul Integr Comp Physiol 288: R91–R96, 2005. First published August 5, 2004; doi: 10.1152/ajpregu.00340.2004.—The degree of nutrient enhancement during the newborn period may modulate programming of appetite-regulating hormones, body composition, and propensity to adult obesity in intrauterine growth-restricted (IUGR) newborns. Pregnant rats received, from day 10 to term gestation and throughout lactation, ad libitum food (AdLib) or 50% food restriction (FR) to produce IUGR newborns. AdLib vs. FR offspring were studied at day 1, and, to create two distinct groups of newborn catch-up growth (immediate, delayed) among the IUGR newborns, cross-fostering techniques were employed. The four groups of pups at 3 wk were IUGR immediate catch-up growth (FR/AdLib), IUGR delayed catch-up growth (FR/FR), control (AdLib/AdLib), and lactation FR control (AdLib/FR). From 3 wk to 9 mo, all offspring had AdLib rat chow. Maternal FR during pregnancy resulted in IUGR pups (6.0 ± 0.3 vs. 7.1 ± 0.3 g, \(P < 0.01\)) with decreased leptin (0.66 ± 0.03 vs. 1.63 ± 0.12 ng/ml, \(P < 0.001\)) and increased ghrelin (0.43 ± 0.03 vs. 0.26 ± 0.02 ng/ml, \(P < 0.001\)). Maternal FR during lactation (FR/FR) further impaired IUGR offspring growth at 3 wk. However, by 9 mo, these pups attained normal body weight, percent body fat, and plasma leptin levels. Conversely, IUGR offspring nursed by AdLib dams (FR/AdLib) exhibited rapid catch-up growth at 3 wk and continued accelerated growth, resulting in increased weight, percent body fat, and plasma leptin levels. Thus the degree of newborn nutrient enhancement and timing of IUGR newborn catch-up growth may determine the programming of orexigenic hormones and offspring obesity.

appetite-regulating hormones; leptin; ghrelin; catch-up growth; maternal food restriction

There is a well-recognized epidemic of adult obesity in Western society. In the United States, current data indicate that >20% of adults are clinically obese (body mass index \(\geq 30\) kg/m\(^2\)), and an additional 30% are overweight (body mass index \(\geq 25\) kg/m\(^2\)) (15, 29). The rates of obesity have increased markedly from 1986 to the present time and give no indication of abating (24). In conjunction with the prevalence of adult obesity, childhood and adolescent obesity has increased at an alarming rate, with ~20% of children now classified as obese (6, 17). There have been numerous postulates as to the etiology of the obesity “epidemic,” with the majority attributing to the availability of a high-fat, high-caloric diet. Barker and colleagues (1, 12) hypothesized a mechanism of developmental programming of adult offspring, such that small-for-gestational age newborns developed a “thrifty phenotype” that predisposes them to increased weight gain as offspring. Paradoxically, small-for-gestational age offspring have not only an increased risk of obesity, but a notable risk of hypertension, diabetes, and cardiovascular disease as adults (2, 9, 11, 20). The potential for these adult-onset diseases has been demonstrated in humans and a variety of animal models including sheep and rats (1, 4, 7, 13, 27).

Maternal rats have been widely used as a model for creation of intrauterine growth-restricted (IUGR) newborns. Undernutrition or malnutrition of maternal rats during select periods of pregnancy has been demonstrated to result in newborn pups that are growth restricted (8, 28). When provided appropriate newborn nutrition, these growth-restricted newborn pups may demonstrate rapid catch-up growth, such that their body weights exceed those of controls by 12 wk of postnatal life (8, 16, 26). The mechanism for the rapid catch-up growth and the eventual development of obesity is unclear. However, it may include aspects of enhanced orexigenic mechanisms (appetite), reduced anorexic mechanisms, highly efficient metabolism of substrates, and/or reduced energy expenditure.

Because hypothalamic control of appetite is likely set during the fetal or neonatal period, nutrient stress and perhaps nutrient enhancement during these periods may alter appetite set points and contribute to programming of adult obesity. We postulated that the degree of newborn nutrient enhancement and timing of catch-up growth may modulate the programming of appetite-regulating hormones, body composition, and the propensity to adult obesity in IUGR newborns.

MATERIALS AND METHODS

Maternal rat and diet. A model of rat dams that were 50% food restricted during pregnancy was utilized. Studies were approved by the Animal Research Committee of Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center and were in accordance with the American Association for Accreditation of Laboratory Care and National Institutes of Health guidelines. First-time-pregnant Sprague Dawley rats (Charles River Laboratories, Hollister, CA) were

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housed in a facility with constant temperature and humidity and a
controlled 12:12-h light-dark cycle. At 10 days of gestation, rats were
provided either an ad libitum diet of standard laboratory chow (Lab-
Diet 5001, Brentwood, MO; 23% protein, 4.5% fat, 3,030 kcal/kg of
metabolizable energy) or a 50% food-restricted diet determined by
quantification of normal intake in the ad libitum-fed rats. The respec-
tive diets were given from day 10 of pregnancy to term and through-
out the 21-day lactation period.

Maternal body weights and the food intake were recorded daily.
Rat dams gave birth normally, and, at day 1 after birth, pups were
limited to eight (4 males and 4 females) per litter to normalize rearing.

The study offspring were categorized into immediate catch-up growth
and delayed catch-up growth groups. Offspring. As stated above, control dams (n = 12) received ad
libitum (AdLib) food, whereas study dams were 50% food restricted
(FR; n = 12) to produce IUGR newborns. Thus two groups of
offspring were studied at day 1 (AdLib vs. FR). To create two groups
of newborn catch-up growth (immediate and delayed) among the
IUGR newborns, one-half of the IUGR pups born to FR mothers (n = 6)
were nursed after cross-fostering to AdLib dams (FR/AdLib), whereas the other one-half were nursed by their own FR
dams (FR/FR; n = 6). As the control group, one-half of the pups born to
AdLib mothers were nursed by AdLib dams (AdLib/AdLib). To
to control for nutrient restriction only during lactation, one-half of the
pups born to AdLib dams were cross-fostered to FR dams (AdLib/
FR). Thus there were four groups of pups at 3 wk: IUGR immediate
catch-up growth (FR/AdLib), IUGR delayed catch-up growth (FR/
FR), control (AdLib/AdLib), and a lactation FR control group (AdLib/
FR). At 3 wk of age, offspring in all four groups were housed
individually and weaned to AdLib feed.

Fig. 1. Mean body weights of male (A) and female (B) offspring from 1
day to 9 mo of age in controls (○) and FR/AdLib (●), AdLib/FR (▲), and FR/FR
pups (▼). FR/AdLib, intrauterine growth-restricted (IUGR) pups born to
food-restricted (FR) mothers and nursed after cross-fostering to ad libitum-fed
(AdLib) dams; AdLib/FR, pups born to AdLib dams and cross-fostered to FR
dams; FR/FR, IUGR pups born to FR mothers and nursed by their own FR
dams; control, pups born to AdLib mothers and nursed by AdLib dams. Insets:
body weights of males and females from 1 day to 3 wk of age. No. of animals
studied per group was 24 males and 24 females (from 6 litters) until 3 wk of
age, after which all offspring were weaned onto the ad libitum diet. Thereafter,
data are shown from 18 males and 18 females (from 6 litters). *P < 0.01: FR/AdLib vs. control offspring.

Fig. 2. Postweaning food intake of male (A) and female (B) offspring from 1
to 5 mo of age in controls (○) and FR/AdLib (●), AdLib/FR (▲), and FR/FR
pups (▼). No. of animals studied per group was 18 males and 18 females (from
6 litters). *P < 0.01: FR/AdLib vs. control offspring.

The study offspring were categorized into immediate catch-up growth
and delayed catch-up growth groups.
Body weights and food intake. Each litter from the four groups was weighed weekly, and the weight of an individual pup was calculated from it (i.e., litter weight/no. of pups). The first weight was recorded at 1 day of age, and subsequent weights were taken at 7, 14, and 21 days of age. Thereafter, the body weight and food intake were monitored weekly on an individual basis.

Body composition. At 3 wk and 9 mo of age, offspring of both sexes underwent a noninvasive dual-energy X-ray absorptiometry (DEXA) scanning, using the DXA system with software program for small animals (QDR 4500A; Hologic, Bedford, MA). In vivo scan of whole body composition was obtained, including lean and fat tissue mass, total mass, and percent body fat determinations.

Plasma hormones. Pups at 1 day of age were decapitated, and blood was collected in capillary tubes. One male and one female from each litter at ages of 3 wk and 9 mo were fasted overnight, and blood was collected via cardiac puncture in heparinized and EDTA-aprotinin (780 KIU/ml of blood) tubes for determination of plasma leptin and plasma ghrelin levels, respectively. Leptin and ghrelin concentrations were determined by RIA using a commercial kit (Rat Leptin RIA Kit, Linco Research, St. Charles, MO; and Rat Ghrelin RIA Kit, Phoenix Pharmaceuticals, Belmont, CA).

Statistical analysis. Differences between control and experimental groups were compared using either unpaired t-test (1-day-old neonate), repeated measures of ANOVA (body wt and food intake), or ANOVA with Dunnett post hoc tests (body composition and plasma hormones). At ages of 1 day and 3 wk, combined data for males and females are shown, since no sex differences were evident. However, at the age of 9 mo, sex differences justified analyzing the data according to sex. Values are expressed as means ± SE.

RESULTS

Growth. At 1 day after birth, pups of FR dams weighed significantly less than pups of AdLib dams, consistent with IUGR (6.0 ± 0.3 vs. 7.1 ± 0.3 g, P < 0.01). The subsequent growth pattern showed either an immediate or a delayed catch-up growth, dependent on the postnatal maternal nutrition. Thus, despite the reduced body weight at birth, by 3 wk of age, IUGR offspring exposed to dams provided ad libitum food during lactation (FR/AdLib) demonstrated markedly increased weight gain, such that they exceeded the body weight of control pups. In contrast, IUGR pups that were exposed to maternal food restriction during lactation (FR/FR) remained significantly below the body weight of control pups at 3 wk. Similar growth patterns were demonstrated by AdLib/FR pups at 3 wk. By 9 mo of age, FR/AdLib offspring were markedly heavier, whereas the FR/FR and AdLib/FR offspring had attained body weights comparable with controls (Fig. 1).

In all groups, the male offspring had significantly higher body weights than females from the age of 4 wk, and this difference became perceptibly accentuated with advancing age (Fig. 1).

Food intake. In the period after weaning, the FR/AdLib offspring increased food intake compared with controls, and this trend of hyperphagia persisted throughout adult life. Conversely, the AdLib/FR and FR/FR offspring showed similar consumption of food as the control offspring (Fig. 2). However, when the food intake was adjusted for body weight, all three experimental groups consumed more food than controls,
which was apparent from the age of 4–8 wk, after which no differences were discernable among the groups. In general, the male offspring consumed more food than females (Fig. 2).

**Body composition.** At 3 wk of age, the FR/AdLib offspring exhibited catch-up growth with differential impact on specific tissue mass. They had significantly higher body and percent lean body mass, with comparable percent body fat, than the controls. Conversely, the FR/FR and AdLib/FR offspring showed significantly reduced body mass and percent body fat, with increased percent lean body mass (Fig. 3).

Nonetheless, by 9 mo of age, the FR/AdLib offspring (male and female) were markedly heavier, with excess percent body fat, although the percent lean body mass was now significantly reduced. In contrast, the AdLib/FR and FR/FR offspring had attained a body composition comparable with the control offspring, illustrating a catch-up growth albeit a delayed one (Fig. 4). In all groups, adult male offspring had higher body mass than female offspring, although the lean body mass and fat when adjusted for body mass was analogous. The exception was the FR/AdLib female adult offspring, which had even higher percent body fat at 9 mo of age (Fig. 4).

**Plasma hormones.** The influence of maternal food restriction on the appetite-regulating hormones in the offspring was investigated. One-day-old neonates of FR dams had significantly reduced plasma leptin (0.66 ± 0.03 vs. 1.63 ± 0.12 ng/ml, *P* < 0.001) and increased plasma ghrelin levels (0.43 ± 0.03 vs. 0.26 ± 0.02 ng/ml, *P* < 0.001) compared with controls.

However, the plasma leptin levels in the FR/AdLib offspring, despite being reduced at birth, were significantly greater at 3 wk. Furthermore, this increment was also evident at 9 mo of age. Conversely, the ghrelin levels were similar to the control offspring at both 3 wk and 9 mo of age. (Fig. 5).

In contrast, the FR/FR and AdLib/FR offspring had plasma leptin levels that were similar to the controls, whereas the plasma ghrelin levels were significantly elevated at 3 wk of age. This trend was still evident at 9 mo of age (Fig. 5).

In general, the male adult offspring had significantly higher plasma leptin and lower plasma ghrelin levels compared with female offspring.

**DISCUSSION**

The three novel findings of this study are of primary importance in understanding the development of obesity in intrauterine growth-restricted babies. First, the rate and timing of postnatal catch-up growth play critical and significant roles. Second, this appears to be associated with altered orexigenic and anorexigenic regulatory mechanisms. Third, the resulting phenotype has markedly different muscle vs. fat mass. These results further emphasize both the plasticity and the potential of the critical appetite-regulating hormones in the pathogenesis of programming-induced obesity.

In the present study, exposure to maternal food restriction in utero resulted in offspring with reduced body weight, decreased plasma leptin, and increased plasma ghrelin at 1 day of

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**Fig. 4.** Body mass (A), %lean body mass (B), and %body fat (C) in 9-mo-old offspring. Because sex differences were evident, data for male (solid bars) and female (shaded bars) are shown separately. No. of animals studied/group was 18 males and 18 females (6 litters) at 9 mo of age. *P < 0.001 vs. control offspring.
age. It appears that the appetite-regulating hormones in the growth-restricted pups are altered in a direction expected under conditions of “starvation.” The phenomena of low birth weight as a result of nutritional perturbations in utero have been established in numerous studies (1, 7, 28), and its potential risk for adult diseases has been extensively demonstrated in humans and animal models (1, 3, 11, 13). Leptin and ghrelin are involved in the hypothalamic regulation of energy homeostasis. The decreased plasma leptin among growth-restricted newborns is consistent with previously reported studies (5, 10). However, this represents the first report of increased plasma ghrelin among growth-restricted pups. Ghrelin is a significant appetite stimulator secreted by the stomach. These results suggest that elevated plasma ghrelin may potentiate or drive the appetite so as to facilitate rapid weight gain, and indeed these offspring exhibit hyperphagia in the immediate postweaning period.

Whether this nutritionally compromised environment persisted or was alleviated in the postnatal period ultimately determined the phenotype of the offspring. For instance, if the growth-restricted newborns were subjected to further insult, where maternal food restriction persisted through the nursing period as well (i.e., FR/FR), it resulted in a delayed catch-up growth in the presence of continued elevated ghrelin levels. This reduction in the rate of postnatal catch-up growth was also observed in the control offspring reared by mothers 50% food restricted during lactation (i.e., AdLib/FR). The rate of catch-up growth was significantly reduced, as these offspring remained significantly smaller than controls at 3 wk of age. However, by 9 mo of age, the catch-up growth had reached control levels. In addition, these offspring consumed more food per body weight during the early postweaning period. As shown in Fig. 1, the delayed catch-up growth pups demonstrated a similar rate of growth, although its onset shifted by ~3 wk. These results indicate that the mechanism of programming is less likely to be dependent on the rate of catch-up growth and more likely involves the period of time at which the catch-up growth occurs. At 9 mo of age, the delayed catch-up growth group had comparable body mass, lean body mass, and percent body fat. These offspring showed evidence of elevated plasma ghrelin levels, suggesting that a continued appetite orexigenic drive may potentiate further catch-up growth. Whether this growth rate would ultimately exceed that of controls at more advanced adult ages is unknown. However, these offspring exhibited normal levels of plasma leptin, indicating a markedly different phenotype from those offspring that experienced only prenatal exposure to maternal food restriction (i.e., FR/AdLib).

The FR/AdLib offspring demonstrated a rapid catch-up growth and increased food intake during the early postweaning period. More importantly, these offspring at 9 mo of age had higher body mass with increased body fat and reduced percent...
lean body mass. Whereas they demonstrated reduced plasma leptin at birth, the plasma leptin levels were markedly increased at 3 wk and 9 mo, suggesting that a leptin resistance was contributing toward an inhibition of anorexia. Similar leptin resistance has been demonstrated in obese humans (23, 25) as well as in offspring of rat dams undernourished in pregnancy (26). These results suggest that rapid catch-up growth may in part be related to elevated ghrelin levels, which may result in leptin resistance, with increased body fat and reduced lean mass potentially leading to obesity and other metabolic disorders in later life.

Several maternal conditions have been linked to obesity in both human and rodent offspring. Paradoxically, both maternal nutrition deprivation (2, 16, 21, 26) and maternal obesity (19) produce obese offspring. The association of impaired fetal development and obesity has been clearly demonstrated in the Dutch Famine Study, where poor maternal nutrition resulted in increased rates of obesity in adult males (20). In the present study, however, maternal food restriction during the second one-half of gestation was associated with both male and female offspring obesity, although the impact was more profound among females. As expected, males were heavier than females, with similar percent lean body and fat mass. In contrast to humans (14, 22), but consistent with studies on rat (18), leptin concentrations were generally higher in males than in female offspring. In addition, this is the first study that notes sex difference in the levels of plasma ghrelin, with males exhibiting significantly lower levels.

Because rats represent an altricial species, the period of lactation has been correlated with a third trimester of human gestation. Thus these results would suggest that nutritional interventions may be of importance during the last trimester of human pregnancy. Although one may not be able to extrapolate from rats to humans, these results suggest the potential for modifying rates of catch-up growth so as to reduce the programming of orexigenic and anorexic mechanisms.

In conclusion, these results demonstrate that offspring obesity may result from prenatal and/or newborn programming of orexigenic and anorexigenic regulatory mechanisms, and that this programming may be dependent on the timing of nutrient reduction and nutrient availability. These results suggest that the prevention of immediate rapid newborn catch-up growth (e.g., nutrient manipulation) may reduce the risk of childhood and/or adult onset obesity.

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