Fetal origins of hyperphagia, obesity, and hypertension and postnatal amplification by hypercaloric nutrition

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Fetal origins of hyperphagia, obesity, and hypertension and postnatal amplification by hypercaloric nutrition. Am J Physiol Endocrinol Metab 279: E83–E87, 2000.—Environmental factors and diet are generally believed to be accelerators of obesity and hypertension, but they are not the underlying cause. Our animal model of obesity and hypertension is based on the observation that impaired fetal growth has long-term clinical consequences that are induced by fetal programming. Using fetal undernutrition throughout pregnancy, we investigated whether the effects of fetal programming on adult obesity and hypertension are mediated by changes in insulin and leptin action and whether increased appetite may be a behavioral trigger of adult disease. Virgin Wistar rats were time mated and randomly assigned to receive food either ad libitum (AD group) or at 30% of ad libitum intake, or undernutrition (UN group). Offspring from UN mothers were significantly smaller at birth than AD offspring. At weaning, offspring were assigned to one of two diets [a control diet or a hypercaloric (30% fat) diet]. Food intake in offspring from UN mothers was significantly elevated at an early postnatal age. It increased further with advancing age and was amplified by hypercaloric nutrition. UN offspring also showed elevated systolic blood pressure and markedly increased fasting plasma insulin and leptin concentrations. This study is the first to demonstrate that profound adult hyperphagia is a consequence of fetal programming and a key contributing factor in adult pathophysiology. We hypothesize that hyperinsulinism and hyperleptinemia play a key role in the etiology of hyperphagia, obesity, and hypertension as a consequence of altered fetal development.

appetite; insulin resistance; leptin resistance; cardiovascular disease

IMPAIRED FETAL GROWTH is a major cause of perinatal morbidity and has long-term clinical consequences. Increasing epidemiological evidence links low birth weight to an increased risk of developing adult diseases, including type 2 diabetes, hypertension, and cardiovascular disease (4, 6, 14). Insulin resistance has been documented in otherwise well, prepubertal children born with intrauterine growth retardation (IUGR), suggesting that this may be one of the earliest metabolic abnormalities present in these children (11). The epidemiological associations between an adverse intrauterine environment and the later onset of adult metabolic and cardiovascular disorders led to the concept of fetal programming and the “fetal origins” hypothesis (5, 13, 16). This hypothesis proposes that an adverse intrauterine environment alters the fetal metabolic and hormonal milieu, resulting in developmental adaptations to ensure fetal survival. If these adaptive responses, designed for survival in a substrate-limited fetal environment, persist into postnatal life, it is proposed that they lead to metabolic, cardiovascular, and endocrine disorders.

The present article is the first report of hyperphagia as a consequence of fetal programming to use a model of fetal undernutrition that resembles the clinical and metabolic abnormalities found in human IUGR. The amplification of metabolic abnormalities in offspring of undernourished dams by hypercaloric nutrition suggests that postnatal environmental factors are important accelerators in the etiology of adult-onset disease. Hyperinsulinism and hyperleptinemia seen in the growth-retarded offspring may represent critical changes that lead to the development of hyperphagia, obesity, and hypertension.

MATERIALS AND METHODS

Virgin Wistar rats (age 100 ± 5 days) were time mated using a rat estrous cycle monitor to assess the stage of estrus of the animals before introducing the male. After confirmation of mating, rats were housed individually in standard rat cages containing wood shavings as bedding and with free access to water. All rats were kept in the same room with a constant temperature maintained at 25°C and a 12:12-h light-dark cycle. Animals were assigned to one of two nutritional groups (n = 15/group): 1) undernutrition (30% of an ad libitum diet) of a standard diet throughout gestation (UN group), and 2) standard diet ad libitum throughout gestation (AD group). Food intake and maternal weights were recorded daily until birth. After birth, pups were weighed and litter size was recorded. Pups from undernourished mothers were

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cross-fostered onto dams which received AD feeding throughout pregnancy. Litter size was adjusted to 8 pups per litter to assure adequate and standardized nutrition until weaning. After weaning, male offspring from AD and UN mothers were divided into two balanced postnatal groups to be fed either a standard diet (protein 19.9%, fat 5%, digestible energy 3,504 kcal/kg, protein energy/total energy 22.7%) or a hypercaloric diet (protein 28.5%, fat 30%, digestible energy 4,922 kcal/kg, protein energy/total energy 22.7%). The mineral and vitamin contents in the two diets were identical and in accordance with the requirements for standard rat diets. Weights and food intake of all offspring were measured daily for the first 2 wk and then every 2nd day. At day 100, systolic blood pressure measurements were recorded using tail-cuff plethysmography (19) according to the manufacturer’s instructions [a blood pressure analyzer from IITC, Life Science (Woodland Hills, CA) was used]. At least three clear systolic blood pressure recordings were taken per animal, and the coefficient of variation for repeated measurements was <5%. At day 125, rats were fasted overnight, anesthetized with halothane, and killed by decapitation. Blood was collected into heparinized vacutainers and stored on ice until centrifugation and removal of supernatant for analysis. The Animal Ethics Committee of the University of Auckland approved all animal work.

Insulin-like growth factor I (IGF-I) in rat blood plasma was measured using the IGF-binding protein-blocked RIA described previously (7, 8), and plasma insulin was measured by RIA (19). A double-antibody RIA was developed and validated for measurement of leptin in rat plasma. An antibody was raised in rabbits against a synthetic fragment (aa 30–45) of bovine leptin. The standard preparation for the RIA was rm-leptin (no. CR-6781, Crystal Chem, Houston, TX) used in concentrations ranging from 0.5 to 20 ng/ml. Samples were assayed undiluted or diluted 1:2–1:4 in assay buffer (0.05 M PBS, pH 7.4, containing 0.1 M NaCl, 0.5% BSA, 10 mM EDTA, and 0.05% NaN3). Briefly, 100 μl of primary antibody (1:2,500) were added to tubes containing 100 μl of sample or standard. After incubation for 24 h at 4°C, 100 μl of tracer (125I-labeled rm-leptin, 20,000 counts/min per tube) were added to all tubes, followed by a further incubation for 24 h at 4°C. A second antibody technique was used to separate bound from free ligand (8). Rat plasma samples showed parallel displacement to the standard curve, and recovery of unlabeled rm-leptin was 101.4 ± 2.7% (SE, n = 26). The half-maximally effective dose, or ED50, was 0.37 ng/ml, and the intra-assay coefficient of variation was 5% (all samples were measured within a single assay). Plasma glucose concentrations were measured using a YSI Glucose Analyzer (model 2300, Yellow Springs Instrument, Yellow Springs, OH). Blood plasma glycerol and free fatty acids were measured by diagnostic kits (Sigma no. 337 and Boehringer-Mannhein no. 1383175, respectively). Statistical analyses were carried out using SigmaStat (Jandel Scientific, San Rafael, CA) and StatView (version 5, SAS Institute, Cary, NC) statistical packages. Differences between groups were determined by two-way ANOVA. Plasma leptin data were also analyzed by analysis of covariance (ANCOVA), with unadjusted fat pad weight as a covariate. Data are shown as means ± SE. Statistical significance was accepted at the P < 0.05 level.

RESULTS

There was a small reduction in maternal body weights compared with day 1 of gestation in pregnant UN group females until day 15 of gestation. From day 15 of gestation, UN dams gained weight and had achieved premating weights by the time of parturition. Litter size was not significantly different between the two groups (AD 13.6 ± 0.6, UN 12.6 ± 1.1). Maternal undernutrition resulted in fetal growth retardation, reflected by significantly decreased body weight at parturition in the offspring from UN dams (AD 6.04 ± 0.46 g, UN 3.9 ± 0.38 g, P < 0.0001). From parturition until weaning at day 22, body weights remained significantly lower in the UN offspring (AD 52.5 ± 1 g, UN 33.8 ± 2 g, P < 0.001). Total body weights remained significantly lower in UN offspring for the remainder of the study. Caloric intake was calculated over three distinct periods: from weaning until puberty (22–40 days), postpuberty (60–80 days), and mature adulthood (100–125 days). UN offspring showed hyperphagia at each age period (Fig. 1). Importantly, ANOVA revealed statistical interactions between programming and diet that became significant during the postpuber-
tal period and increased even further during the adult age period. Systolic blood pressures in UN offspring at ~100 days of age were significantly ($P < 0.0001$) higher than in AD offspring on both the control and hypercaloric diets. Hypercalorically fed AD and UN offspring had significantly ($P < 0.001$, Fig. 2) elevated systolic blood pressure compared with offspring fed the control diet.

All organ and tissue weight data presented at the time animals were killed (125 days of age) are expressed as a percentage of body weight unless otherwise stated. Body weights of UN offspring on either diet were significantly ($P < 0.001$) lower than those of AD offspring, and body weights were significantly ($P < 0.05$) increased on the hypercaloric diet (Table 1). Nose-to-anus lengths were significantly ($P < 0.005$) shorter in UN offspring and were not affected by hypercaloric feeding (Table 1). UN offspring had larger retroperitoneal fat pads relative to body weight than AD offspring ($P < 0.05$). Hypercaloric nutrition significantly ($P < 0.001$) increased retroperitoneal fat pad weights in both groups of animals (Table 1). UN offspring had significantly ($P < 0.005$) smaller kidneys than AD offspring (Table 1). Heart and spleen weights were not significantly different between UN and AD offspring and were not affected by diet. UN offspring had significantly ($P < 0.005$) smaller livers than AD offspring, and liver weight relative to body weight was decreased ($P < 0.05$) by hypercaloric nutrition (Table 1).

Plasma IGF-I concentrations were not different between UN and AD offspring and were not affected by diet (Table 2). UN offspring had significantly ($P < 0.001$) higher fasting insulin concentrations, which were markedly increased by hypercaloric nutrition ($P < 0.005$, Fig. 2). UN offspring had significantly ($P < 0.005$) higher fasting leptin concentrations. UN offspring fed a hypercaloric diet had markedly ($P < 0.005$) elevated plasma leptin concentrations, and a significant ($P < 0.05$) programming × diet interaction was observed (Fig. 3). When unadjusted fat pad weight was used as a covariate in ANCOVA analysis, there was no significant difference between AD and UN animals, and plasma leptin was therefore proportional to retroperitoneal fat content. Fasting glucose concentrations were not different between AD and UN offspring, and no significant effect of diet was observed (Table 2). There was no difference in plasma glycerol concentrations between UN and AD offspring, although hypercaloric nutrition significantly ($P < 0.05$) elevated plasma glycerol in both UN and AD offspring. Programming or diet did not affect the plasma free fatty acid concentrations (data not shown).

**DISCUSSION**

Two novel observations are of particular importance for our understanding of the mechanisms and the physiological basis of human biology and disease. First, fetal undernutrition induced inappropriate hyperphagia in adult life. Second, postnatal hypercaloric nutrition amplified the metabolic abnormalities induced by fetal programming.
fetal undernutrition, which included hyperinsulinism, hyperleptinemia, hypertension, and obesity. Although several rodent models of hyperphagia have been reported, our study is the first to show the development of profound adult hyperphagia as a consequence of fetal programming. The mechanism by which hyperphagia is induced by fetal programming is not clear, but the hyperleptinemia and increased fat pad mass seen in offspring from UN mothers suggest a state of leptin resistance. Elevated plasma concentrations of leptin, as seen in offspring from UN mothers, would normally decrease appetite (9, 10). However, amplification of the hyperphagia occurred in this group with a hypercaloric diet, and this was paralleled by a highly significant programming × diet interaction in plasma leptin concentrations. These data suggest that the increased appetite simply did not reflect appetite-driven catch-up growth and likely reflected inappropriate hyperphagia as a consequence of fetal programming. Although increased plasma insulin levels are normally associated with reduction in appetite (17), the hyperinsulinism seen in the UN offspring is likely to reflect insulin resistance and reduced insulin action, as seen in children born with IUGR (11). Thus reduced insulin action may further contribute to inappropriate stimulation of appetite. Offspring from UN mothers that were fed on a hypercaloric diet showed the highest plasma insulin concentrations and developed marked hyperphagia. Thus we propose that fetal undernutrition induces impaired neuroendocrine regulation in which hyperleptinemia, hyperinsulinism, and insulin and leptin resistance may lead to hyperphagia, obesity, and hypertension during adult life.

Our animal model closely resembles the clinical and metabolic abnormalities seen in humans born with low birth weight. Epidemiological data suggest that children born with IUGR also have an increased risk of developing obesity. This was most clearly shown in the Dutch Famine Study, in which poor nutrition in the first trimester of pregnancy resulted in increased rates of obesity in 19-yr-old males (15). Although the programming effects due to fetal undernutrition were anticipated in our study, it was surprising to see marked amplification of hyperphagia, hyperinsulinism, hyperleptinemia, and hypertension in offspring from UN mothers with the postnatal hypercaloric diet. The mechanism underlying the amplification of hyperphagia by the hypercaloric diet remains to be determined. However, diet-induced obesity will exacerbate insulin resistance with compensatory hyperinsulinism; thus the present study suggests that environmental factors, such as a hypercaloric diet, can amplify the metabolic and cardiovascular abnormalities in programmed offspring. The amplification of the effects of fetal programming may be a result of hyperinsulinism. Whereas insulin sensitivity by definition reflects insulin action on glucose and carbohydrate metabolism, it is now established that not all insulin-receptive tissues become insulin resistant (1, 2). Thus secondary hyperinsulinism can have stimulating and undesirable effects, such as chronic renal sodium retention and increased sympathetic nervous system activity, whereas resistance in other tissues, such as the endothelium, can result in impaired vasodilation (3, 18). Although it is likely that insulin resistance or hyperinsulinism is involved in the blood pressure abnormalities seen in offspring from undernourished mothers, other factors may also contribute. In particular, the kidneys in the growth-retarded animals were smaller, suggesting that there may be a renal component to the hypertension (12).

In summary, this study in a model of fetal undernutrition confirms the clinical and metabolic abnormalities found in both low birth-weight humans and other animal models of IUGR. However, this is the first report of hyperphagia in an animal model of IUGR, and the first study to investigate the effects of a hypercaloric diet on postnatal sequelae of fetal programming. The amplification of the metabolic and cardiovascular abnormalities by hypercaloric nutrition seen in these growth-retarded animals suggests that postnatal environmental factors are important in the etiology of adult-onset disease. We propose that leptin and insulin

![Fig. 3. Plasma leptin concentrations at 125 days of age in AD and UN offspring fed either a control or a hypercaloric diet. Data are means ± SE and were analyzed by two-way ANOVA. Effect of fetal programming P < 0.001, effect of hypercaloric diet P < 0.0005, and programming × diet interaction P < 0.05.](http://ajpendo.physiology.org/)

Table 2. Plasma IGF-I, glucose, and glycerol

<table>
<thead>
<tr>
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<th>IGF-I, ng/ml</th>
<th>Glucose, mmol/l</th>
<th>Glycerol, mmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>AD</td>
<td>506 ± 19</td>
<td>10.4 ± 1.0</td>
<td>0.212 ± 0.013</td>
</tr>
<tr>
<td>UN</td>
<td>449 ± 20</td>
<td>9.3 ± 0.5</td>
<td>0.232 ± 0.018</td>
</tr>
<tr>
<td>Hypercaloric</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>AD</td>
<td>503 ± 27</td>
<td>9.0 ± 0.4</td>
<td>0.248 ± 0.017</td>
</tr>
<tr>
<td>UN</td>
<td>481 ± 32</td>
<td>8.2 ± 0.3</td>
<td>0.291 ± 0.028</td>
</tr>
<tr>
<td>Programming effect</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Diet effect</td>
<td>NS</td>
<td>NS</td>
<td>P &lt; 0.05</td>
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<tr>
<td>Programming × diet interaction</td>
<td>NS</td>
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Data are means ± SE. Analysis is by 2-way ANOVA. IGF-I, insulin-like growth factor I.
resistance, and the resulting hyperleptinemia and hyperinsulinism seen in the growth-retarded animals, are critical in the development of hyperphagia, obesity, and hypertension. The findings of the present study extend and reinforce the theory of fetal programming, the phenomenon that the intrauterine environment can have significant long-term health sequelae in offspring. They also suggest that health care funding may be better spent on preventing health problems during pregnancy than in waiting until metabolic and cardiovascular disorders manifest, years or even decades later.

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