Maternal obesity and fetal programming: effects of a high-carbohydrate nutritional modification in the immediate postnatal life of female rats

Malathi Srinivasan,1 Catherine Dodds,1 Husam Ghanim,2,4 Tao Gao,1 Peter J. Ross,3 Richard W. Browne,3 Paresh Dandona,2,4 and Mulchand S. Patel1

Departments of 1Biochemistry, 2Medicine, 3Biotechnical and Clinical Laboratory Sciences, School of Medicine and Biomedical Sciences, University at Buffalo, State University of New York; and 4Diabetes and Endocrinology Center of Western New York, Kaleida Health, Buffalo, New York

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Srinivasan M, Dodds C, Ghanim H, Gao T, Ross PJ, Browne RW, Dandona P, Patel MS. Maternal obesity and fetal programming: effects of a high-carbohydrate nutritional modification in the immediate postnatal life of female rats. Am J Physiol Endocrinol Metab 295: E895–E903, 2008. First published August 5, 2008; doi:10.1152/ajpendo.90460.2008.—Our earlier studies have shown that the artificial rearing of newborn rat pups [first generation high carbohydrate (1-HC)] on an HC milk formula resulted in chronic hyperinsulinemia and adult-onset obesity (HC phenotype). Offspring [second-generation HC (2-HC)] of 1-HC female rats spontaneously acquired the HC phenotype in the postweaning period. In this study, we have characterized the development of the abnormal intrauterine environment in the 1-HC female rats and the effects on fetal development under such pregnancy conditions for the offspring. 1-HC female rats demonstrated hyperphagia on laboratory chow and increased body weight gain beginning from the immediate postweaning period along with hyperinsulinemia and hyperleptinemia. During pregnancy, 1-HC female rats showed several metabolic alterations including increased body weight gain and increased plasma levels of insulin, leptin, proinflammatory markers, and lipid peroxidation products. Although there were no significant changes in the body weights or litter size of term 2-HC fetuses, the plasma levels of insulin and leptin were significantly higher compared with those of control term fetuses. Quantitation of mRNA levels by real-time RT-PCR indicated significant increases in the mRNA levels of orexigenic neuropeptides in the hypothalamus of 2-HC term fetuses. Collectively, these results indicate that the HC diet in infancy results in an adverse pregnancy condition in female rats with deleterious consequences for the offspring.

immediate postnatal period; high-carbohydrate diet; fetal adaptations

The incidence of obesity has reached epidemic proportions in the United States wherein approximately two-thirds of the adult population is classified as being either overweight or obese and only one-third of the adult population is within the normal body mass index range (27). The presence of obesity significantly increases the risk for the onset of several chronic diseases including type 2 diabetes and cardiovascular disorders (16). Hence, there is a sense of urgency for the need to unravel the etiology of the current obesity epidemic.

It is recognized that genetic changes alone do not support the observed increases in the incidence of obesity. The search for alternate explanations for the steep increase in the incidence of obesity over a relatively short span of time has implicated the contribution of environmental and behavioral influences to this outcome. In this context, it is of interest to note that epidemiological and experimental studies in animal models have underscored the relationship between early life nutritional experiences and the later development of metabolic diseases. Results from studies on a malnourished or diabetic pregnancy have shown that due to the fetal development in these adverse intrauterine environments, the offspring were predisposed for a variety of adult-onset metabolic disorders (1, 14, 19). It is recognized that the presence of obesity in the mother during pregnancy is not only a cause for morbidity and mortality in both mother and fetus but is also critically important for the programming of the fetus for an increased risk for the onset of metabolic disorders later in life (17, 30). In animal studies, it has been shown that the offspring of rats, which were obese during both gestation and lactation due to the consumption of a high-fat diet, developed obesity and metabolic syndrome in adulthood (2, 24).

Critical windows of development also extend into the immediate postnatal life (suckling period), suggesting an important and independent role for this period for the induction of metabolic programming effects. Studies from our laboratory have shown that a mere change in the quality of nutrition [from fat-rich rat milk to carbohydrate-rich high-carbohydrate (HC) milk formula], without alterations in the total caloric intake in the immediate postnatal period, resulted in chronic hyperinsulinemia and adult-onset obesity (HC phenotype) in the same generation of rats [first-generation HC (1-HC) rats] (22, 37). More interestingly, our studies have shown that due to the HC dietary modification in their immediate postnatal period, 1-HC female rats spontaneously transferred the maternal phenotype to their offspring [second-generation HC (2-HC) rats] without the necessity for any dietary manipulation for them, indicating the establishment of a generational effect (38). The 1-HC female rat differs from other animal models used to investigate fetal programming effects. Unlike the malnourished dam or the high-fat diet rat model wherein the dietary treatment was enforced only during gestation and lactation, 1-HC female rats did not undergo any specific dietary manipulation during these periods since they consumed a standard rodent laboratory chow from the time of weaning (38). The mechanisms underlying the development of an adverse intrauterine environment in the 1-HC female rat are not known. Therefore, in a continued investigation of the generational effect in the HC rat model, the...
present study characterizes the metabolic aberrations that occur in the prepregnancy period and during pregnancy in the 1-HC female rat. Furthermore, the programming effects that occur in term fetuses that predispose them for the development of obesity in adulthood are also investigated.

**MATERIALS AND METHODS**

**Generation of 1-HC female rats.** The Institutional Animal Care and Use Committee approved all animal protocols. Pregnant Sprague-Dawley rats purchased from Zivic Miller (Zelienople, PA) were housed individually with access to standard rodent diet (16% protein rodent diet; Harlan Teklad, Madison, WI; percent weight distribution was 61% carbohydrate, 16% protein, 4% fat, 6% ash, 4% fiber, and 10% moisture) and water ad libitum under controlled conditions of temperature (25 ± 2°C) and a 12:12-h light-dark (6:00 AM–6:00 PM) cycle. The artificial rearing technique has been described in detail previously (21). Briefly, intrauterine cannulas were introduced into 4-day-old neonatal female rat pups under mild anesthesia, and these pups were artificially reared away from their natural dams on the HC milk formula. The caloric composition of the HC milk formula was 56% carbohydrate, 20% fat, and 24% protein compared with the rat milk composition in rat milk (and also high-fat milk formula) of 8% carbohydrate, 20% fat, and 72% protein. The plasma levels of insulin and leptin were measured by ELISA.

**Determination of metabolic parameters.** In the postweaning period, food intake was recorded on a weekly basis and body weights were determined every week and processed to separate plasma. For term fetal studies, tail blood was collected separately and frozen. During pregnancy, food intake and body weights were determined every week and the number of fetuses were determined. Trunk blood plasma concentrations of lipid peroxides were determined by HPLC after extraction with n-heptane, dissolved in mobile phase [0.1% acetic acid-3 M sodium hydroxide, neutralized with glacial acetic acid, re-extracted with n-heptane, dissolved in mobile phase (0.1% acetic acid-acetonitrile-tetrahydrofuran 41:41:18 vol/vol/vol), injected onto a 25 cm × 4.6 mm SupelcoSIL LC-18 (Supelco, Bellefonte, PA) column, and eluted isocratically with the mobile phase. The eluents were monitored from 200 to 300 nm by diode array [polyunsaturated fatty acids (PUFAs) peak at 215 nm and lipid peroxides peak at 234 nm]. Peak retention times and calibration curves were generated using pure hydroxy- and hydroperoxy-fatty acid standards (Caymen Chemical, Ann Arbor, MI). Quantitation was made after internal standard recovery correction using 5-hydroxy-eicosatetraenoic acid-methyl ester.

**Embryo transfer.** 1-HC and MF donor female rats (~75 days old) were mated with normal male rats; the presence of a vaginal plug confirmed pregnancy. The collection of embryos and their transfer to pseudo-pregnant recipient dams was performed as described previously with some modifications (20, 28). Briefly, oviducts and the uterus were removed on gestational day 4 from anesthetized (ketamine-xylazine) pregnant donor rats, and the embryos were flushed out using minimal essential medium. Embryos were collected under a microscope and maintained in the same medium in a CO₂ incubator at 37°C for less than 2 h until transferred to recipient female rats.

**Generation of 2-HC rats.** The hypothalamus was dissected from whole fetal brains obtained from MF and HC term fetuses as described earlier (34), and RNA was isolated using the TRIzol reagent-phenol-chloroform procedure (Invitrogen, Carlsbad, CA). Total RNA was quantified, and mRNA samples were reverse transcribed into cDNAs by using the iScript cDNA synthesis kit (Bio-Rad, Hercules, CA) according to manufacturer’s instructions. mRNA levels of neuropeptide Y (NPY), agouti-related polypeptide (AgRP), galanin (GAL), and insulin receptor (IR)-β were measured via real-time RT-PCR using the iCycler system (Bio-Rad). Primer sequences, which were designed to span at least one exon-exon junction of the target mRNA to prevent the amplification of any contaminating genomic DNA, are described in Table 1. The mRNA levels detected by SYBR Green (Bio-Rad) analysis were normalized to 18S mRNA levels (QuantumRNA Classic II 18S Internal Standard, 324 bp; Ambion, Austin, TX). PCR efficiency was examined by serially diluting the template cDNA, and the melting curve data were collected to assess PCR specificity. Each cDNA sample was run in triplicate, and a corresponding mRNA sample that had not been subjected to reverse transcription was included as a negative control in each run. Relative mRNA levels were calculated according to the critical threshold cycle comparative method (∆∆CT).

**Table 1. Primers for real-time PCR**

<table>
<thead>
<tr>
<th>Name</th>
<th>Primer Sequence</th>
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<tbody>
<tr>
<td>Neuropeptide Y, 236 bp</td>
<td>Sense, 5'-AGAGATCCAGCCCTGAGACA-3'</td>
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<tr>
<td></td>
<td>Antisense, 5'-AACGACAACAGGGAATGG-3'</td>
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<tr>
<td>Agouti-related</td>
<td>Sense, 5'-AGACCAACAGCCAGCAAGATA-3'</td>
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<tr>
<td>polypeptide, 114 bp</td>
<td>Antisense, 5'-GACGTGTCAGCGTACCACA-3'</td>
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<tr>
<td>Galanin, 369 bp</td>
<td>Sense, 5'-GCCAGGGGACACGGTTATGCTG-3'</td>
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<tr>
<td></td>
<td>Antisense, 5'-GACGTGTCATGCGTCTGCCA-3'</td>
</tr>
<tr>
<td>Insulin receptor-β, 331 bp</td>
<td>Sense, 5'-ATCTGGATCCGCCCTGAAACTGTC-3'</td>
</tr>
<tr>
<td></td>
<td>Antisense, 5'-ATCTGGATCCGCCCTGAAACTGTC-3'</td>
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</tbody>
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Statistics. For studies involving MF and HC rats at multiple time points or multiple groups of rats, one-way ANOVA followed by post hoc analysis using the Student-Newman-Keuls test was used to compare the significance of the difference of the means. For studies involving only one time point, the significance of the difference of the means between the HC and MF rats was determined by Student’s t-test. \( P < 0.05 \) was considered significant.

RESULTS

Metabolic characteristics of the 1-HC female rat in the prepregnancy period. To investigate the effects of the HC dietary modification on the metabolic characteristics in the postweaning period, food intake, body weight gain, and plasma levels of insulin and leptin were determined in 1-HC and age-matched MF control female rats (Fig. 1, A–D). From the time of weaning on postnatal day 24, 1-HC female rats consumed significantly increased quantities of the rodent laboratory chow compared with the consumption by age-matched MF female rats (Fig. 1A). The percent increase in food consumption by 1-HC female rats compared with age-matched MF female rats was 9% (week 5), 14% (week 6), 17% (week 7), 14% (week 8), 11% (week 9), 20% (week 10), and 18% (week 11). The body weights of 1-HC female rats were significantly higher from postnatal day 40 onward (Fig. 1B). The increase in body weight for 1-HC female rats compared with the body weights of age-matched MF female rats ranged from \( \sim 6\% \) for postnatal day 40 to 10% for postnatal day 90. Plasma insulin levels were also significantly higher in 1-HC female rats in the postweaning period compared with age-matched MF female rats (Fig. 1C). On postnatal days 45 and 60, an increase of \( \sim 25\% \) was observed for plasma insulin levels for 1-HC female rats, and the difference was further augmented (about 60%) on postnatal day 80. In the case of MF female control rats, a decrease in plasma insulin levels was observed on postnatal day 60, no further decrease was observed on postnatal day 80. Plasma leptin levels were markedly higher in 1-HC female rats on postnatal days 45 and 80 compared with their levels in age-matched MF female rats (Fig. 1D). For MF female rats, plasma leptin levels showed an increase (\( \sim 60\% \)) on postnatal day 60 compared with the levels on postnatal day 45; no further change was observed on postnatal day 80. In the case of 1-HC female rats, there were no significant changes in plasma leptin levels on postnatal day 60 compared with the levels on postnatal day 45; however, unlike MF female rats, plasma leptin levels continued to increase after postnatal day 60 in 1-HC female rats, reaching an \( \sim 1.8\)-fold increase over MF levels on postnatal day 80.

Fig. 1. A: food intake of first-generation high-carbohydrate (1-HC) and age-matched mother-fed (MF) female rats from time of weaning on postnatal day 24. Food intake is represented as the quantity of food consumed (grams) per week. B: body weights of 1-HC and age-matched MF female rats in the postweaning period. Body weights were recorded once every 10 days. Plasma levels of insulin (C) and leptin (D) on postnatal days 45, 60, and 80 of 1-HC and age-matched MF female rats are shown. Values are means \( \pm SE \) (\( n = 6 \)). The significance of the difference of the means for MF and HC rats at various time points was analyzed using 1-way ANOVA followed by post hoc analyses using the Student-Newman-Keuls test. Only \( P \) values for comparison between MF and HC rats for each time point are indicated in the figure. \( *P < 0.05 \) was considered significant.
Metabolic characteristics of the pregnant 1-HC female rat during pregnancy. To determine the effects of the intake of the HC milk formula for 3 wk only, in the immediate postnatal period on the pregnancy environment in the 1-HC female rat, food intake, body weight gain, and plasma levels of insulin and leptin were determined during gestation (Fig. 2, A–D). During weeks 1 and 2 of gestation, the body weight gains were similar for 1-HC and age-matched MF pregnant rats. During week 3, pregnant 1-HC female rats gained significantly more body weight (Fig. 2A). Pregnant 1-HC female rats consumed significantly more food during the first and third week of gestation (Fig. 2B). Plasma insulin levels were ~40% higher in 1-HC female rats on gestational days 7 and 14 compared with age-matched MF female rats (Fig. 2C). On gestational day 21, plasma insulin levels decreased markedly in MF pregnant rats, whereas in 1-HC pregnant rats only a marginal decrease was observed, resulting in a dramatic difference (~2-fold) in plasma insulin levels between the two groups of rats (Fig. 2C). The decrease in plasma insulin levels in both groups of rats on gestational day 21 might be due to the possible reduction in food intake and hormonal changes before the initiation of parturition. Plasma leptin levels were also significantly elevated in pregnant 1-HC female rats compared with pregnant MF female rats; increases of 98%, 121%, and 75% in plasma leptin levels were observed in 1-HC female rats on gestational days 7, 14, and 21, respectively (Fig. 2D).

Since 1-HC pregnant rats were markedly heavier compared with age-matched MF female rats during pregnancy, it was of interest to determine whether an augmented inflammatory and oxidative stress response was evident during this period in these rats. Figure 3, A–F, indicates that an augmented proinflammatory response and oxidative stress characterized the 1-HC pregnancy. A large difference was observed in the plasma levels of proinflammatory markers such as IL-6 (~300% increase; Fig. 3A) and IL-12 (~126% increase; Fig. 3B) in 1-HC pregnant rats on gestational day 21. Significant increases were observed in the levels of MIF-α (~28% increase; Fig. 3C), MCP-1 (~43% increase; Fig. 3D), and the angiogenic protein VEGF (~53% increase; Fig. 3E). An approximately twofold increase was observed for plasma levels of the linoleic acid peroxidation products, 13- and 9-hydroxy octadecadienoic acid (HODE), and 13- and 9-hydroperoxy octadienioic acid in 1-HC pregnant rats on gestational day 21 (Fig. 3F).

To determine the effects of the adverse intrauterine environment of the 1-HC female rats on its offspring, litter size, body weight of fetuses, and plasma levels of insulin and leptin were determined on gestational day 21. There were no significant changes in the number of fetuses per litter and body weight of fetuses on gestational day 21 between the MF and HC groups of rats (Fig. 4, A and B). However, plasma levels of insulin (~82%) and leptin (~150%) were markedly higher in 2-HC term fetuses compared with MF term fetuses (Fig. 4, C and D).

Figure 5 shows the body weights of offspring on postnatal day 80 from the embryo transfer experiment. When MF embryos were transplanted into pseudo-pregnant 1-HC female rats, the body weights of the male offspring on postnatal day 80 were significantly greater than the body weights of the natural male offspring (without embryo transfer) or the male offspring of a MF-to-MF embryo transfer. The body weights of

![Fig. 2](http://ajpendo.physiology.org/)

**Fig. 2.** Body weight gain (A) and food intake (B) during the 3 wk of gestation and plasma levels of insulin (C) and leptin (D) on gestational days 7, 14, and 21 in pregnant MF and 1-HC female rats. Values are means ± SE (n = 6). The significance of the difference of the means for the MF and HC rats at various time points was analyzed using 1-way ANOVA followed by post hoc analyses using the Student-Newman-Keuls test. Only P values for comparison between MF and HC rats for each time point are indicated in the figure. *P < 0.05 was considered significant.
The male offspring of the MF-to-HC embryo transfer did not differ from those of controls (both natural and MF-to-MF transfer) on postnatal days 30 and 50 (data not shown). This is in agreement with earlier published observations from our laboratory showing that the body weights of 2-HC male offspring demonstrated significant changes beginning from postnatal day 60 (38). Interestingly, when HC embryos were transplanted into pseudo-pregnant MF female rats, the body weights of the male offspring (HC to MF) were similar to those observed for MF male rats (both natural and from MF-to-MF transfer).

Since 2-HC offspring become distinctly obese in adulthood, it was of interest to determine whether a predisposition to the later onset of obesity was established during fetal development. For this purpose, mRNA levels of NPY, AgRP, GAL, and IR-β genes involved in body weight homeostasis were determined by real-time PCR analyses of total RNA extracted from the hypothalamus from term fetal brains. Interestingly, these studies indicated significantly higher mRNA levels of the orexigenic neuropeptides in term fetal 2-HC brains (Fig. 6). mRNA levels of NPY, AgRP, and GAL showed an approximately two-, three-, and twofold increase, respectively, in term fetal 2-HC brains compared with their levels in term fetal MF brains, mRNA levels of the IR-β were significantly decreased (~35%) in term fetal 2-HC brains (Fig. 6).

**DISCUSSION**

The two significant observations of this study are that an abnormal metabolic profile develops in the 1-HC female rat beginning from the immediate postweaning period (a consequence of the metabolic programming effects initiated in the immediate postnatal life in response to the HC milk formula), which is severely amplified during pregnancy, and that fetal development in the altered intrauterine environment in the 1-HC female rats causes fetal hyperinsulinemia, hyperleptinemia, and fetal brain programming.

*Altered intrauterine environment in the 1-HC female rat.* In the 1-HC female rat, the immediate responses to the HC dietary experience during the suckling period generate a sequel of metabolic effects in the postweaning period, which is predisposed for the altered 1-HC intrauterine milieu during pregnancy. Our paradigm for the development of the aberrant metabolic profile of the 1-HC dam is as follows: suckling period (on the HC milk formula): early onset of hyperinsulinemia/hyperleptinemia and alterations in hypothalamic energy
circuitry → postweaning period (on standard laboratory chow): hyperphagia, increased body weight gain, hyperinsulinemia, and hyperleptinemia → pregnancy: increased adiposity, low-grade inflammation/oxidative stress, and adverse 1-HC intra-uterine milieu.

The results from this study show that the artificial rearing of newborn female rats on a HC milk formula for 3 wk in their immediate postnatal life resulted in hyperphagia starting in the immediate postweaning period (Fig. 1A). In a recent study, our laboratory reported that mRNA levels of the orexigenic neuropeptides NPY, AgRP, and GAL were markedly increased in the hypothalami of 12-day-old 1-HC rats (34). Concomitantly, there was a diminution in anorexigenic signaling as evidenced by reductions in the mRNA levels of proopiatemelanocortin and insulin and leptin receptor genes in the hypothalami of these rats (34). These results suggest the establishment of a predisposition for hyperphagia during the period of the HC

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**Fig. 4.** Litter size (A), body weight (B), and plasma levels of insulin (C) and leptin (D) in second-generation HC (2-HC) and MF term fetuses. Values are means ± SE (n = 6). Student’s t-test was used to compare the significance of the difference of the means between MF and HC rats. *P < 0.05 was considered significant.

**Fig. 5.** The effect of embryo transfer on the body weights of male progeny on postnatal day 80. Results are means ± SE (n = 6). The significance of the difference of the means among the various groups of rats was analyzed using 1-way ANOVA followed by post hoc analyses using the Student-Newman-Keuls test. *P < 0.05 was considered significant.

**Fig. 6.** mRNA levels of neuropeptides and insulin receptor (IR)-β in the brains from 2-HC and MF term fetuses. Values are means ± SE (n = 6). Student’s t-test was used to compare the significance of the difference of the means between MF and HC rats. *P < 0.05 was considered significant. NPY, neuropeptide Y; AgRP, agouti-related polypeptide; GAL, galanin.
dietary modulation (immediate response). 1-HC female rats demonstrated significant increases in body weight gains in the postweaning period, which could be a consequence of the observed hyperphagia in these rats.

As in the case of plasma insulin levels, plasma leptin levels were markedly increased in the nonpregnant 1-HC female rats. The large increase in plasma leptin levels on postnatal day 80 is suggestive of increased adipose mass and leptin resistance in 1-HC female rats. Earlier it was shown that the weight of the epididymal adipose tissue was markedly increased in 1-HC male adult rats (22). Leptin has been implicated in the regulation of insulin secretion by islets (25). The presence of hyperinsulinemia in 1-HC female rats in the face of hyperleptinemia indicates leptin resistance at the level of pancreatic islets. Hyperleptinemia in adult 1-HC female rats also indicates hypothalamic leptin resistance resulting in hyperphagia and increased body weight gain in the postweaning period. In an earlier study, decreased mRNA and protein levels of the leptin receptor were observed in the hypothalami of adult male 1-HC rats (34).

The severe degree of hyperinsulinemia observed in the term pregnant 1-HC rat suggests that insulin resistance associated with a normal pregnancy is further aggravated in 1-HC female rats. This could be a direct consequence of the presence of obesity in these rats since many studies in animal models have shown that obesity is characterized by insulin resistance (26, 35). Since random plasma glucose levels in the fed condition were not different between the two groups of rats (33), it appears that the compensatory hyperinsulinemia was sufficient to maintain normal plasma glucose levels. Whether 1-HC pregnant rats display glucose intolerance in response to a glucose load was not determined in this study. Similar to our observations on the 1-HC pregnant rat, increased levels of plasma insulin have been reported in the HF diet-induced rat model for obesity during pregnancy without changes in random circulating blood glucose levels (7).

Additional complications of the 1-HC pregnancy included marked increases in circulating levels of several proinflammatory factors (IL-6, IL-12, MIF-α, MCP-1, and VEGF; Fig. 4). It has been shown that obesity is characterized by an abnormal production of many of these factors resulting in a state of chronic mild inflammation, the progression of which leads to insulin resistance, impaired glucose tolerance, and even diabetes (3). Inflammatory proteins such as C-reactive protein, tumor necrosis factor-α, IL-6, MIF-α, MCP-1, and IL-18 have been shown to be increased in the obese (9, 12, 15). Elevated concentrations of IL-6 and C-reactive protein were observed in obese pregnant women (30). The damaging effects of an abnormal inflammatory profile during pregnancy are indicated by observations from a study showing that the offspring of rats injected with IL-6 throughout pregnancy had a greater body fat mass and reduced insulin sensitivity (8). Furthermore, it has been shown that increased maternal levels of IL-6 were predictive of the increased growth and adiposity in the offspring (29). The increased inflammatory response observed in pregnant 1-HC female rats could be one of the causes for the fetal programming of the 2-HC offspring for adult-onset disorders.

The plasma levels of lipid peroxidation products were markedly increased in pregnant 1-HC rats (Fig. 4). Lipid peroxidation is a prominent manifestation of oxidative stress, and abnormal lipid peroxidation has been shown to be involved in the development of several chronic diseases (31). Lipid peroxides (markers of oxidative stress) are specific oxidative products of reactive oxygen species that attack PUFA (5). Linoleic acid (octadecadienoic acid) is the major PUFA in biological systems, and its peroxidation products predominate and 9- and 13-HODE have been reported to be excellent markers of lipid peroxidation (32). The plasma levels of lipid peroxides are markedly higher in obese individuals and are significantly reduced after a dietary restriction regimen (10).

The results from the embryo transfer experiment emphasize the importance of the intrauterine environment of the 1-HC female rat for the spontaneous transfer of the maternal phenotype to its offspring. Although MF embryos transferred to the 1-HC intrauterine environment developed obesity in adulthood, HC embryos developing in the MF intrauterine environment did not develop this abnormality. MF embryos developing in the 1-HC intrauterine environment did not attain the body weight of naturally developing 2-HC offspring on postnatal day 80. A possible explanation for this observation could be that in this study MF embryos developed for 4 days in the MF intrauterine environment before being transferred to the 1-HC intrauterine environment. It is possible that the complete process of embryonic development beginning from the preconceptual period needs to occur in the 1-HC female rat for the full extent of expression of the HC phenotype. In an earlier study, our laboratory reported that pair-feeding of 1-HC female rats to age-matched MF female rats resulted in the normalization of the pregnancy conditions (normal body weight gains and plasma insulin levels) in 1-HC female rats and that 2-HC offspring that developed in the normalized 1-HC intrauterine environment did not acquire the HC phenotype (33). This observation, along with the results from the embryo transfer experiment performed in this study, strongly supports the hypothesis that it is only the intrauterine environment and not genetic susceptibility or epigenetic modifications that contribute to the generational effect observed in this model. In contrast, studies by Gill-Randall et al. (18) have shown that embryos from the diabetic Goto-Kakizaki rat developing in the normoglycemic Wistar intrauterine environment developed diabetes, showing that genetic predisposition to diabetes is not alleviated by a euglycemic intrauterine environment. In another study, embryos from intrauterine growth-retarded F1 generation females, when transferred to a normal intrauterine environment, still developed the maternal phenotype, indicating the dominant effect of epigenetic modifications (36). Future studies should determine whether in the third generation of HC rats genetic propensity (established probably due to epigenetic alterations) would gain dominance over the intrauterine environment by transferring embryos from 2-HC rats to MF females.

Fetal programming. In an earlier study, we showed that islets from 2-HC fetuses demonstrated a hypersecretory capacity, indicating that the propensity for chronic hyperinsulinemia in 2-HC rats was programmed in utero. The present study confirms an earlier observation from this laboratory (33) on the increased levels of plasma insulin in term 2-HC fetuses. In addition to increased levels of plasma insulin, 2-HC term fetuses also demonstrated hyperleptinemia. It is of interest to note that during early periods in life both insulin and leptin function as tropic factors and have been shown to impact neuronal development during early periods of life. The lack of
leptin, as observed in the ob/ob mouse, has been shown to underlie the development of obesity in these mice (4). Abnormal levels of insulin in the fetal or early postnatal periods have been shown to induce alterations in the hypothalamic appetite-regulating mechanisms, resulting in adult-onset obesity (11). The results from the present study also indicate that the mRNA levels of NPY, AgRP, and GAL are increased in fetal brains, whereas the mRNA levels of IR-β are reduced, suggesting that aberrations in appetite regulation are evident in term 2-HC fetuses and that these early malprogramming effects underlie the development of obesity later in life. The observed changes in the mRNA levels in fetal 2-HC brains could be a direct consequence of the abnormal hormonal profile in 2-HC fetuses.

The 1-HC rat model provides the opportunity to identity metabolic aberrations that occur in the prepregnancy period that predispose for the development of abnormalities associated with the presence of obesity during pregnancy. In humans, as observed in the 1-HC female rat, the development of obesity is often the result of a sequence of metabolic dysfunctions occurring over a period of time. As indicated by this study, metabolic and hormonal aberrations are evident in the prepregnancy period in the 1-HC female rat, and such abnormalities predispose for the development of the adverse intrauterine environment in the 1-HC female rat, resulting in adult-onset obesity in the 2-HC offspring.

The deleterious effects of an obese pregnancy on the offspring is indicated from a population-based study in Finland (17) showing a positive relationship between maternal body mass index and death rate from coronary heart disease in male offspring. A higher incidence of type 2 diabetes has been reported in the offspring of mothers who were above average weight during pregnancy (13). The observation that more than one-third of women of childbearing age in the United States report in the offspring of mothers who were above average weight during pregnancy (13). The observation that more than one-third of women of childbearing age in the United States will be overweight or obese during pregnancy (23) does not portend well for the good health of their offspring. The observation from our studies showing that malprogramming effects predisposing for adult-onset obesity and possibly associated disorders are induced during fetal development underscores the importance of optimal pregnancy conditions.

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