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Maternal Low-Protein Diet or Hypercholesterolemia Reduces Circulating Essential Amino Acids and Leads to Intrauterine Growth Restriction

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OBJECTIVE—We have examined maternal mechanisms for adult-onset glucose intolerance, increased adiposity, and atherosclerosis using two mouse models for intrauterine growth restriction (IUGR): maternal protein restriction and hypercholesterolemia.

RESEARCH DESIGN AND METHODS—For these studies, we measured the amino acid levels in dams from two mouse models for IUGR: *1*) feeding C57BL/6J dams a protein-restricted diet and *2*) feeding C57BL/6J LDL receptor–null ($LDLR^{-/-}$) dams a high-fat (Western) diet.

RESULTS—Both protein-restricted and hypercholesterolemic dams exhibited significantly decreased concentrations of the essential amino acid phenylalanine and the essential branched chain amino acids leucine, isoleucine, and valine. The protein-restricted diet for pregnant dams resulted in litters with significant IUGR. Protein-restricted male offspring exhibited catch-up growth by 8 weeks of age and developed increased adiposity and glucose intolerance by 32 weeks of age. $LDLR^{-/-}$ pregnant dams on a Western diet also had litters with significant IUGR. Male and female $LDLR^{-/-}$ Western-diet offspring developed significantly larger atherosclerotic lesions by 90 days compared with chow-diet offspring.

CONCLUSIONS—In two mouse models of IUGR, we found reduced concentrations of essential amino acids in the experimental dams. This indicated that shared mechanisms may underlie the phenotypic effects of maternal hypercholesterolemia and maternal protein restriction on the offspring. ***Diabetes*** **58:559–566, 2009**

In humans, malnutrition during pregnancy results in babies with lower birth weight and an increased risk of neonatal mortality and morbidity (1). Low birth weight is also associated with an increased risk for certain chronic diseases, including type 2 diabetes, cardiovascular disease, and hypertension (2–4). One proposed explanation linking low birth weight to chronic diseases is the Barker "thrifty phenotype" hypothesis, which postulates that the lack of adequate nutrients in the intrauterine environment "programs" the offspring for survival in a nutrient-poor world. It follows that if the actual postnatal environment is not nutrient poor but instead nutrient rich, metabolic pathways will have been "malprogrammed," leading to adult-onset metabolic syndrome diseases, including atherosclerosis and diabetes (5). A great deal of evidence now supports the Barker hypothesis (6); therefore, current research in humans and in animal models is focused on specific mechanisms for in utero programming (4).

Many types of maternal stresses in different animal models have been used to produce intrauterine growth restriction (IUGR) (7). In the current study, we used two mouse models of IUGR, one using maternal protein restriction to examine increased adiposity and glucose intolerance end points, and one using a high-cholesterol maternal environment in $LDLR^{-/-}$ mice to examine cardiovascular end points. Previous work using the rat model has shown that maternal protein restriction results in offspring with IUGR (4), low muscle mass (8), adult-onset glucose intolerance (9), hypertension (10,11), and early aging (12,13). Maternal effects of a low-protein diet included a significant decrease in the placental protein 11 β-hydroxysteroid dehydrogenase, an enzyme that protects the fetus from maternal glucocorticoids (14). A concomitant increase in glucocorticoid-inducible enzymes was found in the fetuses of dams on a low-protein diet (15). Studies examining maternal programming for atherosclerosis have found a significant association between maternal hypercholesterolemia and increased atherosclerotic lesions in the offspring in newborn and adult rabbits (16,17), in adult mice (18), and in human fetuses (19) and children (20). Existing evidence for in utero programming from hypercholesterolemia (21) includes increased maternal oxidative stress (22) and an altered adaptive immune response to oxidized LDL (23). Although IUGR itself is associated with an increased risk for atherosclerosis in humans (24), high maternal cholesterol in humans has not been established as causative for IUGR. Using a rabbit model, however, it was shown that a moderate 0.2% cholesterol, low-fat chow gestational diet resulted in litters with IUGR (25). The decreased birth weight was associated with an excessive accumulation of lipids in the placenta, suggesting possible interference with nutrient transport to the fetus (25).

Because maternal protein restriction and hypercholesterolemia both create an abnormal maternal metabolic environment, we hypothesized that there may be a common disruption of metabolic pathways affecting the off-

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spring. To test the hypothesis, we used two mouse models for in utero conditions leading to IUGR, one of protein restriction and one of hypercholesterolemia. We then looked for commonalities in the experimental dams to identify possible pathways for the developmental origins of metabolic syndrome diseases. In both models, the dams had decreased levels of certain essential amino acids.

RESEARCH DESIGN AND METHODS

Animal husbandry. This study was approved by the UCLA Animal Research Committee and was performed in accordance with the National Institutes of Health guidelines for the use of experimental animals. FVB/J, C57BL/6J (B6), and LDLR$^{-/-}$ mice on a B6 background were purchased from the Jackson Laboratories (Bar Harbor, ME).

Diets. The low-protein diet (D02041002; Research Diets) contained 9% protein by weight, was isocaloric, and was formulated to match low-protein diets published previously (26). In the low-protein diet, fat content was 4.4% and carbohydrates were 77% by weight. The control protein diet (standard chow diet TD 7013; Harlan Teklad) was used to feed control protein dams, foster mothers, LDLR$^{-/-}$ chow dams, and weaned offspring from both experiments. The control protein diet contained 19% protein, 6.2% fat, and 75% carbohydrates by weight and contributed 18% kcal from fat. The Western diet (TD 88137; Harlan Teklad) contained 42% kcal calories from fat and by weight as follows: 21% fat, 17% protein, 49% carbohydrates, and 0.2% cholesterol.

Protein restriction studies. B6 females between 2 and 4.5 months of age were allowed to mate during the last 2 h of the dark cycle with males between 2 and 6 months of age. On detection of a vaginal plug at the end of this 2-h period, designated as day 0 of gestation, females were placed on either a 9% protein-restricted diet (low protein) or a control diet containing 19% protein. Pregnant females underwent cesarean section on gestational day 19. Females were deeply anesthetized with 2% isoflurane, and after cervical dislocation, cesarean section was performed under a heat lamp with aseptic technique. Pups were weighed and cross-fostered to a postpartum FVB/J mother to equalize the postpartum environment of both groups. The pups were delivered by cesarean section because our previous attempts to generate IUGR in mice via protein restriction were unsuccessful secondary to cannibalization of the pups by the mother. The range in age of the dams was from 2 to 4.5 months because the females were old enough to breed at 2 months but not so old that confounders, such as reduced litter size, may have occurred. Because the dams cannibalized many first litters, necessitating the change to cesarean section births and fostering, some of the offspring were from second litters. There were no statistical differences between the birth weights, litter sizes, or adult phenotypes between first and second litters; therefore, we combined the litters with the same in utero exposure. For low-protein birth weight comparisons, seven low-protein and seven control litters were evaluated. At 1 week of age, pups were weighed again, and the litter culled to six. Fostered pups were weaned at 4 weeks of age into cages of four animals, separated by sex and maternal environment (low-protein vs. control). The protein-restricted litters were not measured for atherosclerotic lesion size in adulthood because wild-type C57BL/6 mice do not develop lesions on a chow low-cholesterol diet (27).

Hypercholesterolemia studies. LDLR$^{-/-}$ females were placed on chow diet or high-fat, moderate-cholesterol diet (Western diet) for 6 weeks and subsequently bred with LDLR$^{-/-}$ males maintained on chow. LDLR$^{-/-}$ mice had plasma cholesterol concentrations of ~250 mg/dl on a standard chow diet, which represented the control cholesterol environment. On a Western diet, the LDLR$^{-/-}$ mice had cholesterol concentrations up to 1,000 mg/dl, which represented the experimental high-cholesterol environment. The progeny of LDLR$^{-/-}$ females on a chow diet or Western diet constituted the LDLR$^{-/-}$ control or Western offspring, respectively. Fasting plasma cholesterol concentrations were determined for both sets of LDLR$^{-/-}$ females before breeding, and the LDLR$^{-/-}$ offspring delivered vaginally were fostered at birth. Four hypercholesterolemia litters and two control litters were evaluated. The pups were weaned and separated by sex and maternal diet at 4 weeks of age. The offspring of both LDLR$^{-/-}$ control and Western-diet litters were fed a chow diet on weaning. Initially the LDLR$^{-/-}$ Western offspring exhibited a very low survival rate (~1 pup in an 8-pup litter survived) compared with LDLR$^{-/-}$ control offspring (6–7 pups survived per 8-pup litter). However, fostering the pups at birth equalized the survival rates for both groups. An important study by Napoli et al. (18) demonstrated a maternal-diet effect on lesion size at 90 days in LDLR$^{-/-}$ mice offspring. This time point was therefore chosen for the current study. After an overnight fast, LDLR$^{-/-}$ adult offspring were deeply anesthetized with 2% isoflurane and weighed, blood was collected by retro-orbital sinus puncture, tissues were harvested after cervical dislocation, and gonadal fat pads were dissected and weighed.

Maternal plasma amino acid analysis. Maternal plasma amino acid analysis was performed by high-performance liquid chromatography at Baylor University Medical Center Institute of Metabolic Diseases (http://www.baylorhealth.edu/imd/) (28). This experiment was repeated to detect possible variation in the amino acid analysis. In the first study, dams were allowed to deliver their pups and were then given anesthesia with 2% isoflurane before retro-orbital exsanguinations within 4 h of delivery. This postpregnancy time was chosen to minimize any adverse effect of exsanguinations on the fetus and to maximize the effect of various diets on mothers. In the second study, blood samples were taken from females 1–2 weeks postpartum, while maintaining the same diet they were on during pregnancy. We observed similar trends in both experiments and therefore combined our data from the two studies.

Glucose tolerance tests. Glucose tolerance tests were performed as previously described (29) on the low-protein and control offspring at 126 days (4 months) and 210 days (7 months) of age. The mice were weighed, shaved on the hind limbs, and fasted overnight. The following morning, fasting glucose was measured in blood collected from saphenous vein puncture, after which 2 mg/g glucose load was administered intraperitoneally. Serial blood glucose measurements were performed at 0.5-h intervals over the next 2 h from saphenous venipunctures. The One Touch Ultra glucometer (Lifescan) was used to measure whole-blood glucose concentrations (30).

Body composition. This was performed in a rodent nuclear magnetic resonance scanner (Bruker Biospin, Billerica, MA) that was standardized to an internal control provided by the manufacturer. The mice were individually weighed on a scale and then placed in the scanner for measurement of body composition, analyzed as percent fat mass (also referred to as adiposity), percent muscle mass, and percent liquid mass. Total body fat was calculated using the scale weight of each mouse on that day.

Plasma lipid analysis. Mice were fasted overnight, and retro-orbital blood was collected under isoflurane anesthesia. Plasma total cholesterol, HDL cholesterol, unesterified cholesterol, triglyceride, and free fatty acid concentrations were determined as previously described (31).

Lesion analysis. Mice were killed at 90 days, and the heart and proximal aorta were removed, embedded in OCT compound (Miles Laboratories), and stored at −70°C. Serial 10-μm-thick cryosections from the middle portion of the left ventricle and the aortic arch were collected and mounted on poly-D-lysine–coated plates. Sections were stained with the lipid stain oil red O and hematoxylin. The lipid-stained areas were viewed under the light microscope and manually counted by a blinded observer. Scores were determined as previously described (32).

Data analysis. All values are expressed as means ± SE. A mean litter weight was used to compare birth weights in the protein restriction IUGR model. This was done to avoid a type 1 error, because the actual number of newborn pups was very large and to minimize the effect of within-litter differences. The two-way ANOVA model was used to simultaneously compare independent variables in two groups to assess the effect of sex and maternal environment on the offspring. In LDLR$^{-/-}$ litters, the litter sizes and within-litter weights did not vary significantly (6–7 pups per litter), and thus, individual pup weights were averaged instead. The P values for all group comparisons were assigned using the post hoc Fisher's protected least significant difference correction. One-way ANOVA was used when single-sex comparisons were performed. Statview version 5.0 software was used for analysis.

RESULTS

Protein-restricted mouse model for IUGR. In utero growth restriction has been associated with malnourishment during pregnancy leading to adult-onset metabolic disorders. To develop a mouse model of IUGR, we fed C57BL/6J females a low-protein diet beginning on day 0 of gestation. We then delivered pups from control and protein-restricted dams by cesarean section on gestational day 19 and cross-fostered to FVB/J foster dams on a chow diet. Mean litter birth weights of protein-restricted litters were significantly lower than controls ($P = 0.003$) (Fig. 1*A*). Low-protein and control male weights were not significantly different beginning at 8 weeks of age (Fig. 1*B*), and at 32 weeks, low-protein male offspring weights were significantly higher than controls ($P \leq 0.05$) (Fig. 1*B*). In contrast, low-protein female offspring showed significant growth restriction compared with controls until they were killed at 32 weeks of age (Fig. 1*C*).

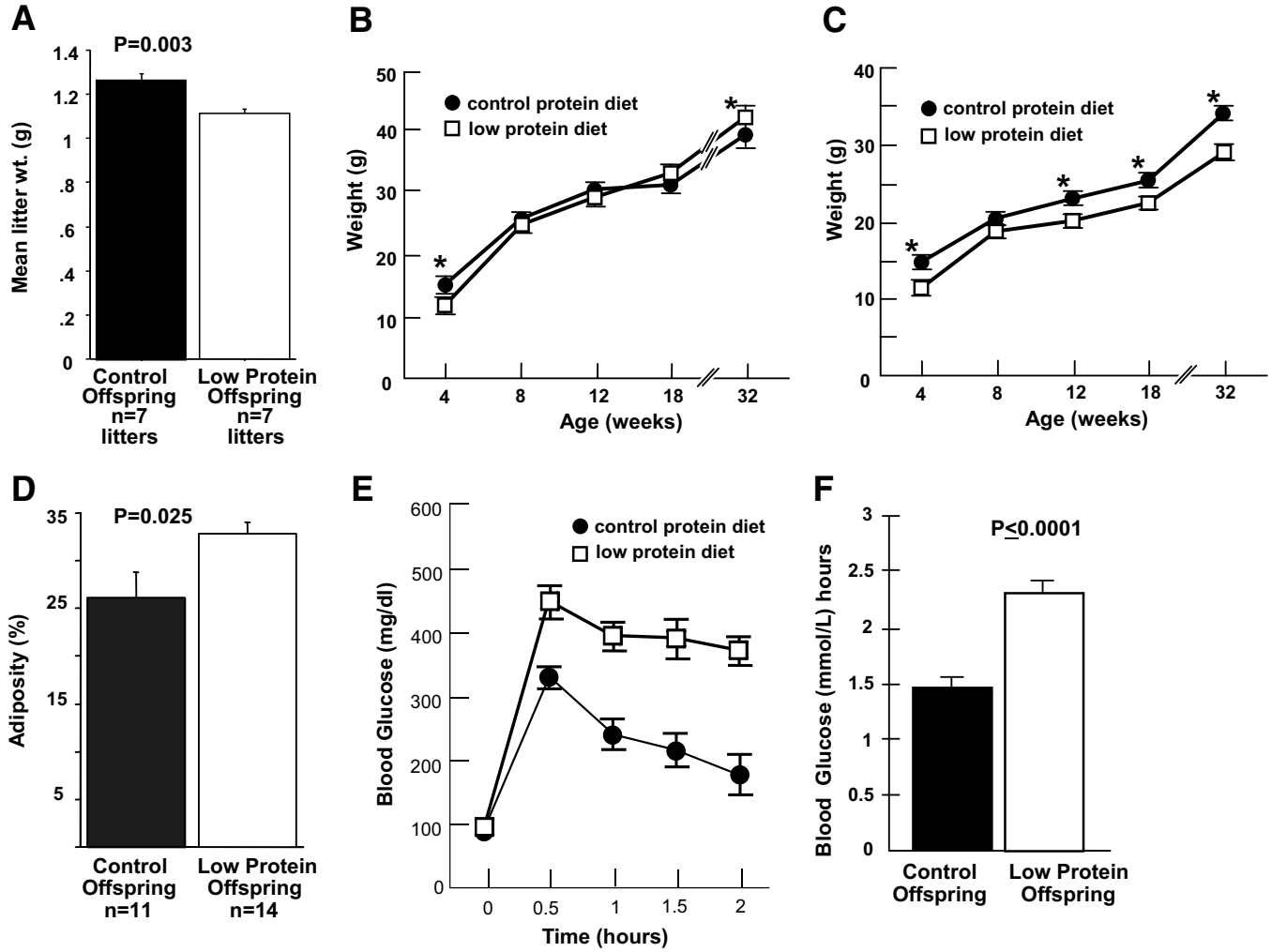

FIG. 1. Characterization of offspring from control and protein-restricted litters. *A*: Mean litter birth weights of 9% low-protein diet litters compared with 19% control protein litters, $P = 0.003$. *B*: Growth curve of male offspring from 4 to 32 weeks of age. *Weights were significantly different at the $P \leq 0.05$ level at 4 and 32 weeks. *C*: Persistent growth restriction in female protein-restricted offspring, from 4 to 32 weeks of age. *Values significantly different at the $P \leq 0.05$ level. *D*: Increased adiposity in low-protein male offspring at 32 weeks of age, $P = 0.025$. *E*: Glucose intolerance in low-protein male offspring. Blood glucose concentrations of male protein-restricted offspring after administration of a standard intraperitoneal challenge of 2 mg/g body weight (wt) glucose. $n = 12, 9, 9, 9$, and 8 for control offspring; and $n = 14, 13, 13, 10$, and 9 for the protein-restricted offspring at time 0, 0.5, 1, 1.5, and 2 h, respectively. *F*: Area under the curve (AUC) for protein-restricted male offspring after administration of a standard intraperitoneal glucose load.

Low-protein male offspring exhibited increased weight, adiposity, and glucose intolerance. Low-protein male offspring had significantly higher adiposity ($P = 0.025$) at 32 weeks of age compared with controls (Fig. 1*D*). Adiposity in low-protein female offspring at 32 weeks of age did not differ significantly from controls (data not shown). At 32 weeks (but not at 18 weeks; data not shown), low-protein male offspring demonstrated significantly higher blood glucose concentrations after a glucose challenge (Fig. 1*E*) and a significantly higher ($P < 0.0001$) area under the glucose concentration curve (Fig. 1*F*) compared with controls. Baseline glucose concentrations and response to a glucose load were not significantly different between low-protein and control female offspring at 32 weeks of age (data not shown). In both male and female offspring, there were no significant differences between low-protein and control for total cholesterol, unesterified cholesterol, triglycerides, free fatty acids, or HDL cholesterol in the plasma (data not shown).

Effect of hypercholesterolemic fetal environment on adult offspring. To study the effect of IUGR and maternal hypercholesterolemia, pregnant LDLR$^{-/-}$ dams were fed either a Western diet (high fat with moderate cholesterol) or a control (chow) diet. Plasma total cholesterol concentrations in LDLR$^{-/-}$ dams on a Western diet were significantly higher ($P < 0.0001$) than those on a chow diet (Fig. 2*A*). Other plasma lipids, including unesterified cholesterol, triglycerides, free fatty acids, and HDL cholesterol were not significantly different (data not shown). Offspring of the LDLR$^{-/-}$ dams on a Western diet exhibited significant IUGR at birth compared with control ($P = 0.02$) (Fig. 2*B*).

At 90 days of age, Western-diet progeny had significantly decreased weight ($P \leq 0.05$) and length ($P = 0.0004$) compared with controls (Fig. 2*C* and *D*). Western diet progeny had significantly lower ($P = 0.04$) gonadal fat pad–to–body weight ratios than controls, and males had significantly higher ratios than females ($P = 0.0001$) (Fig. 2*E*). Western-diet LDLR$^{-/-}$ progeny had significantly larger atherosclerotic lesions than controls ($P = 0.002$) (Fig. 2*F*). A representative section of the aorta with the lesions [*1*) entire section and *2*) one lesion] observed at 90 days of age is shown in Fig. 2*G*. Males and females showed no significant difference in

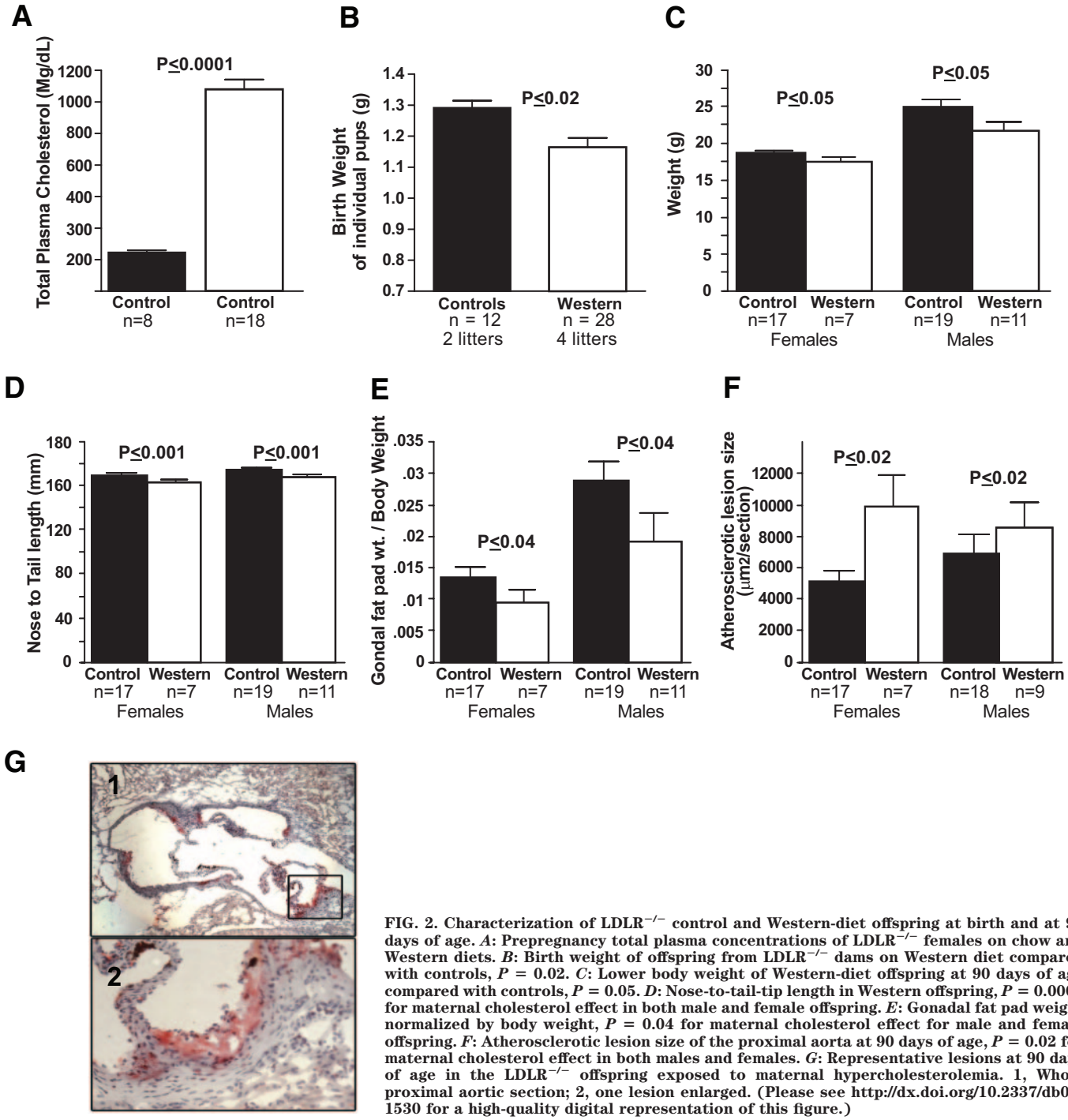

FIG. 2. Characterization of LDLR$^{-/-}$ control and Western-diet offspring at birth and at 90 days of age. *A*: Prepregnancy total plasma concentrations of LDLR$^{-/-}$ females on chow and Western diets. *B*: Birth weight of offspring from LDLR$^{-/-}$ dams on Western diet compared with controls, $P = 0.02$. *C*: Lower body weight of Western-diet offspring at 90 days of age compared with controls, $P = 0.05$. *D*: Nose-to-tail-tip length in Western offspring, $P = 0.0004$ for maternal cholesterol effect in both male and female offspring. *E*: Gonadal fat pad weight normalized by body weight, $P = 0.04$ for maternal cholesterol effect for male and female offspring. *F*: Atherosclerotic lesion size of the proximal aorta at 90 days of age, $P = 0.02$ for maternal cholesterol effect in both males and females. *G*: Representative lesions at 90 days of age in the LDLR$^{-/-}$ offspring exposed to maternal hypercholesterolemia. 1, Whole proximal aortic section; 2, one lesion enlarged. (Please see http://dx.doi.org/10.2337/db07-1530 for a high-quality digital representation of this figure.)

lesion size. The morphology and cellular composition of lesions (macrophages vs. smooth muscle cells) was not different between groups (data not shown). No significant differences were found in offspring plasma lipids including free fatty acids, triglycerides, total cholesterol, unesterified cholesterol, and HDL cholesterol (data not shown).

Decreased maternal plasma amino acid levels in both low-protein and hypercholesterolemic dams. As previously observed in other species (33–35), the levels of several essential nonbranched and branched chain amino acids and urea in plasma were reduced in low-protein dams compared with control dams (Fig. 3*A*). A representative chromatogram is shown in Fig. 3*B*. Additional representative chromatograms are provided in supplemental material (available in an online appendix at http://dx.doi.org/10.2337/db07-1530). Amino acid concentrations may vary at different time points, and thus, we repeated these studies in a separate experiment. We observed similar trends in both experiments, and therefore combined our data from the two studies. Particularly noteworthy in the low-protein versus control dams were reduced concentrations of phenylalanine ($P \leq 0.01$) and the branched chain amino acids leucine ($P = 0.03$), isoleucine, and valine ($P = 0.015$). Lysine, which is also essential, was higher in the low-protein dams than in the control dams ($P \leq 0.05$). The LDLR$^{-/-}$ dams on a Western diet exhibited significantly reduced concentrations of phenylalanine

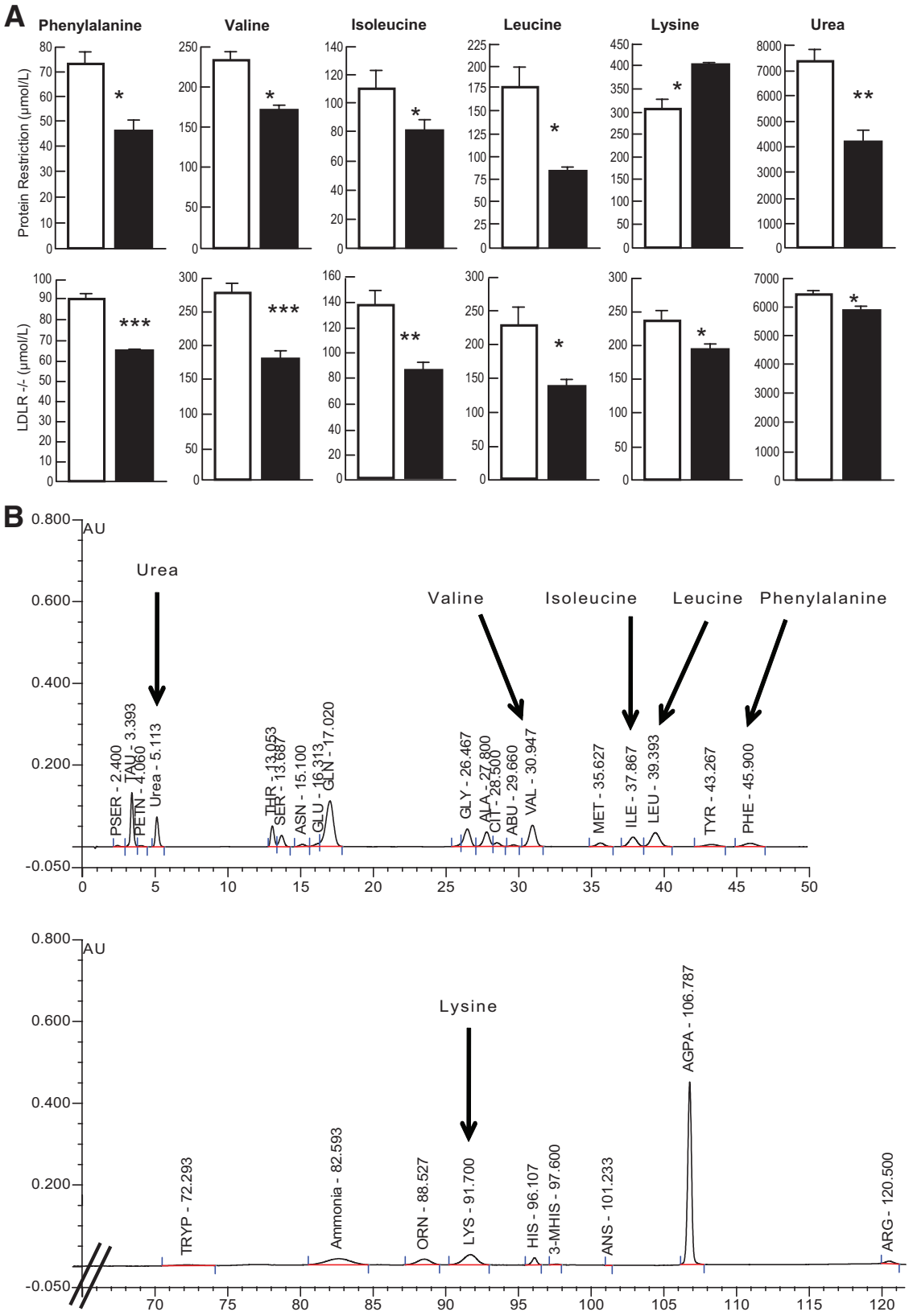

FIG. 3. Hypoaminoacidemia in protein-restricted and hypercholesterolemic dams. *A*: Plasma urea and amino acid concentrations in dams on a 23% protein control diet ($n = 8$; □) and 9% low-protein diet ($n = 3$; ■) and in LDLR$^{-/-}$ dams on a Western diet ($n = 11$; ■) and control chow diet ($n = 9$; □). * P < 0.05. ** P < 0.005. *** P < 0.0005. *B*: Representative chromatogram of the high-performance liquid chromatography peaks for one plasma sample.

($P = 0.00015$) and lysine ($P = 0.028$), in addition to the branched chain amino acids valine ($P \leq 0.0005$), isoleucine ($P = 0.0085$), and leucine ($P \leq 0.01$) when compared with control. No other amino acids, either essential (threonine, tryptophan, and histidine) or nonessential, were significantly different between experimental and control

dams (data not shown). Urea was significantly reduced in both Western and low-protein–diet dams.

DISCUSSION

To test the hypothesis that common in utero mechanisms may participate in the development of IUGR and associated metabolic syndrome traits in adulthood, we developed and characterized two mouse models for IUGR: one of protein restriction and one of high cholesterol. Our low-protein model mimicked the well-established rat low-protein IUGR model (36) and complemented a previously described protein-restricted IUGR mouse model characterized for lifespan and adiposity (37). In our study, the IUGR male offspring of protein-restricted dams became glucose intolerant with increased adiposity compared with controls. IUGR low-protein females did not differ significantly from controls in glucose tolerance and maintained a lower growth curve throughout. In the second arm of the experiment, we used genetically altered LDLR$^{-/-}$ mice that are susceptible to atherosclerosis to induce features caused by IUGR that may contribute to atherosclerosis. The offspring of LDLR$^{-/-}$ dams fed a Western diet in our study had lower birth weights and more severe atherosclerotic lesions than controls at 90 days of age. Of particular significance, both protein-restricted and hypercholesterolemic dams had decreased plasma concentrations of several essential amino acids.

The IUGR models in our study differed in a few key areas. First, a sex difference in weight gain and glucose intolerance was seen in the low-protein model. Males, but not females, caught up in weight to the control offspring by the eighth week of age and developed glucose intolerance by 32 weeks of age. This sex difference has been found in other mouse models, including a recent investigation of heterozygous GLUT3-null mice (30). Also, in a transgenic mouse model of the hypothalamic-expressed regulator for food intake, neuropeptide Y receptor 1, a sex effect was seen. NPYR1 transgenic males, but not females, gained excess weight on a high-fat diet and lost weight with the administration of leptin (38). Thus, the sex difference that we saw in weight gain, adiposity, and glucose tolerance in the low-protein group is consistent with other findings and indicates the likely participation of the endocrine system in the etiology of these traits.

Contrasting with the outcomes for low-protein offspring, LDLR$^{-/-}$ Western-diet progeny in our study did not catch up with controls in weight or in adiposity by the 90-day time point that was chosen for evaluation of atherosclerotic lesions. Several explanations for this might be considered, including *1*) that these IUGR mice require >3 months to catch up with controls, or *2*) that a combination of a high-fat diet and hypercholesterolemia programmed the offspring differently for metabolism and weight gain in adulthood. A high-fat maternal diet in rats was recently shown to prevent excess weight gain in adulthood (39). Based on tracking of food intake, this study provided evidence that the high-fat in utero diet likely programmed the offspring to metabolize or use energy less efficiently, preventing them from gaining excess weight in adulthood (39). Therefore, the absence of excess adiposity and weight gain in the hypercholesterolemia IUGR offspring in the current study could be due to hypothalamic programming via the high-fat maternal diet. Although the LDLR$^{-/-}$ Western diet IUGR litters may have eventually led to excess weight gain and glucose intolerance at an advanced age, other experiments have shown that the chow diet does not produce these end points in B6 LDLR$^{-/-}$ mice by 90 days of age. Instead, a high-fat diet was necessary to bring about these metabolic syndrome traits in LDLR$^{-/-}$ mice at this time point (40). We purposely used a chow diet in the current study to establish that increased atherosclerosis in the offspring was due to in utero programming. The effect of programming on offspring may extend into the second generation: A recent high-fat diet programming study using mice showed that a high-fat diet leading to obesity during gestation produced glucose intolerance in both the first and second generations of offspring (41).

Hypoaminoacidemia in our hypercholesterolemic model could possibly have resulted from reduced protein absorption, reduced amino acid availability via altered metabolism in the dams, or reduced protein consumption due to the high caloric content of the Western diet. The Western and control diets used in our study were 17.3 and 18.6% by weight protein, a difference that would be unlikely to produce the significant reduction of certain amino acids in the LDLR$^{-/-}$ Western-diet dams. Reduced food intake because of the higher percentage of calories supplied by fat in the Western (42%) versus chow (18%) diets could have led to an overall reduction of protein intake. However, if that were the case, all essential amino acids would probably have been reduced on the Western diet, which they were not. Furthermore, the essential amino acid lysine was lower in LDLR$^{-/-}$ dams on the Western diet than controls but was higher in the protein-restricted dams compared with controls. Thus, the Western and low-protein diets do not necessarily lead to a reduction of all essential amino acids, indicating other factors besides dietary intake of protein as causative for the hypoaminoacidemia in the dams. The lower urea content in the plasma of low-protein dams was expected because less protein consumption would result in less urea generated as a byproduct of protein catabolism (42). The lower urea content in the plasma of hypercholesterolemic dams was another indication of less protein accretion in these animals.

Previous studies have observed a correlation between high maternal cholesterol concentrations and enhanced lesions of atherosclerosis in the offspring in rabbits (17,43) and in LDLR$^{-/-}$ mice (18). Maternal oxidative stress in a high cholesterol environment may adversely affect the placenta and may directly or indirectly enhance atherosclerotic lesions in the offspring (44). Experiments in hypercholesterolemic rabbits have shown that administration of the antioxidant vitamin E during pregnancy resulted in reduced atherosclerotic lesions in the offspring (16,17), indicating that maternal oxidative stress likely plays a role in maternal programming for atherosclerosis. However, given the complexity of metabolic syndrome traits, it is also likely that more than one physiological pathway is involved. The placentas of women who gave birth to IUGR babies were found to have decreased placental leucine transport and decreased placental mammalian target of rapamycin (mTOR) activity (45). Similarly, the IUGR litters in our study were associated with reductions in essential maternal amino acids, including leucine; therefore, hypoaminoacidemia in the dams may have resulted in reduced levels of fetal amino acids, resulting in metabolic malprogramming of the fetal hypothalamus. mTOR, a kinase that is upregulated by leucine, regulates cell-cycle progression and growth throughout the body by sensing changes in energy status. Downstream

from the leptin receptor, mTOR is also involved in energy homeostasis and metabolism (46). Because leucine via mTOR regulation has been shown to participate in muscle formation in fetal sheep (47) and pancreatic β-cell function in a cell culture system (48), downregulation of mTOR by a decrease in leucine, both in hypercholesterolemic and protein-restricted in utero environments, could play a role in IUGR and in malprogramming of the fetal hypothalamus, leading to the previously described "thrifty phenotype."

It is becoming increasingly apparent that embryonic and fetal cells have a complex system integrating nutritional signals from their environment to maximize the potential for survival. The association of maternal malnutrition and IUGR leading to adult-onset metabolic disorders, such as obesity, type 2 diabetes, and atherosclerosis, has been demonstrated in several epidemiological studies (2,49–51). Our results mimicked population studies in humans where maternal malnutrition and the resultant low birth weight were identified as the common risk factors for adult-onset diseases. In addition, our studies demonstrated that maternal protein restriction and hypercholesterolemia were both associated with maternal hypoaminoacidemia. Therefore, maternal hypoaminoacidemia may be an important antecedent in both models of IUGR and may be an important link in the mechanisms that contribute to adult-onset glucose intolerance, obesity, and atherosclerosis.

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No potential conflicts of interest relevant to this article were reported.

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