Emergence of insulin resistance in juvenile baboon offspring of mothers exposed to moderate maternal nutrient reduction

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Choi J, Li C, McDonald TJ, Comuzzie A, Mattern V, Nathanielsz PW. Emergence of insulin resistance in juvenile baboon offspring of mothers exposed to moderate maternal nutrient reduction. Am J Physiol Regul Integr Comp Physiol 301: R757–R762, 2011. First published June 8, 2011; doi:10.1152/ajpregu.00051.2011.—Developmental programming of postnatal pancreatic β-cell and peripheral insulin function by maternal nutrient reduction (MNR) has been extensively investigated in rodents and sheep, but no data exist from nonhuman primate offspring of MNR mothers. We hypothesized that moderate levels of MNR would result in developmental programming of postnatal β-cell function and peripheral insulin sensitivity that lead to emergence of a prediabetic state prior to puberty. Prepregnancy phenotype of 18 nonpregnant baboons was matched. During pregnancy and lactation 12 mothers ate chow ad libitum (controls), while six ate 70% of chow consumed by controls (weight-adjusted MNR). Weaned offspring ate normal chow. At 3.5 ± 0.18 yr (mean ± SE) in an intravenous glucose tolerance test, conscious, tethered MNR juvenile offspring (2 females and 4 males) showed increased fasting glucose (P < 0.04), fasting insulin (P < 0.04), and insulin area under the curve (AUC; P < 0.01) compared with controls (8 females and 4 males). Insulin AUC also increased following an arginine challenge (P < 0.02). Baseline homeostatic model assessment insulin sensitivity was greater in MNR offspring than controls (P < 0.03). In a hyperinsulinemic, euglycemic clamp, the glucose disposal rate decreased 26% in MNR offspring. Changes observed were not sex dependent. MNR in pregnancy and lactation programs offspring metabolic responses, increasing insulin resistance and β-cell responsiveness, resulting in emergence of an overall phenotype that would predispose to later life type-2 diabetes, especially, should other dietary challenges such as a Westernized diet be experienced.

pancreas; developmental programming; nutrient restriction; diabetes

There is compelling evidence from human epidemiological studies (5, 20) and controlled animal investigations (2, 3, 19, 20, 26), indicating maternal nutrient reduction (MNR) in pregnancy and lactation programs dysfunction of β-cell secretion and peripheral insulin sensitivity (23, 25, 26). While there are many studies in altricial rodent species (25, 31, 36), studies in precocial species are limited to sheep, a species in which many studies in altricial rodent species (25, 31, 36), studies in precocial species are limited to sheep, a species in which

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MATERIALS AND METHODS

Animal care. The 18 baboons (Papio sp.) studied were born at the Southwest National Primate Research Center to mothers selected prior to pregnancy to be of similar age (11.5 ± 0.51 yr, mean ± SE) and morphometric phenotype. These nonpregnant females were housed in outdoor gang cages with a fertile male, thereby providing full social and physical activity. They were trained prior to pregnancy to feed in individual cages as described previously (29, 30). Briefly, at feeding time, all baboons passed along a chute and into individual feeding cages. Each baboon’s weight was obtained while crossing an electronic scale (model GSE 665; GSE Scale Systems, Milwaukee, WI). Water was continuously available in the feeding cages via individual waterers (Lixit, Napa, CA). Animals ate Purina Monkey Diet 5038 (Purina, St Louis, MO). From 30 days of gestation (term, 184 days) 12 females were allowed to eat Diet 5038 ad libitum (controls). The diet contains 12% energy from fat, 18% from protein, and 69% from carbohydrate. It contains 0.29% glucose and 0.32% fructose. Six
MNR females were in a group chosen randomly to be fed 70% of the feed eaten by the control females on a weight-adjusted basis from the time of diagnosis of pregnancy (~30 days gestation) for the rest of pregnancy and through lactation (15). General details of housing and environmental enrichment have been published (29, 30). All procedures were approved by the University of Texas Health Science Center and Southwest National Primate Research Center Institutional Animal Care and Use Committees.

All baboons delivered spontaneously in the group housing without any assistance. The control group contained eight female and four male offspring, and the MNR group contained two female and four male offspring. At 9 mo of age, offspring were fully weaned and were removed to juvenile group housing and fed chow ad libitum. At 3.5 ± 0.18 yr of age the 18 juveniles were transported from the Southwest National Primate Research Center to the Laboratory Animal Resources facilities at the University of Texas Health Sciences Center San Antonio, a journey that takes 20 min. All animals were jacketed while tranquilized with ketamine (10 mg/kg) on the day they arrived in the indoor facility. After 4 wk they were fitted with a training tether and 1 wk later with the tether that contained the capability to carry vascular lines and electrode wires. All the time they were in sight of at least four other animals. We have previously described these procedures in detail (14).

Surgery for catheter instrumentation. Prior to surgery food was withheld overnight. Baboons received ketamine (10 mg/kg) and glycopyrrolate (12.5 µg/kg) and were instrumented with femoral artery and vein catheters using procedures we have described in detail in both baboons and rhesus monkeys (14). Briefly, the inguinal area on one side and the middle of the back were shaved. The animal was transferred to the surgical suite, intubated, and maintained on isoflurane anesthesia (2%). By using standard sterile technique, an incision was made over the femoral blood vessels, and side branches of the vascular lines and electrode wires. All the time they were in sight of at least four other animals. We have previously described these procedures in detail (14).

Intravenous glucose tolerance test. At 0800 after an overnight fast of 16 h, three baseline blood samples (1.5 ml) were taken at −15, −10, and −5 min followed by intravenous administration of a bolus of glucose [300 mg/kg, 20% dextrose (Hospira, Lake Forest, IL), over 30 s]. Blood samples (1.5 ml) were collected at: 1, 2, 3, 4, 5, 6, 8, 10, 15, 20, 30, 40, 50, and 60 min. At 90 min, 5 g of L-arginine (cat. no. A6969; Sigma-Aldrich, St. Louis, MO) in 30 ml of distilled water was removed from the femoral artery. At time 0, insulin was infused at 60 mU.m⁻².min⁻¹ to raise plasma insulin concentration by ~100 µU.ml⁻¹. After the start of the insulin infusion, a 20% glucose infusion was begun. Plasma glucose concentration was measured every 5 min to adjust the glucose infusion rate to maintain a plasma glucose of ~90 mg/dl. Plasma insulin and C-peptide were measured every 15 min. At the end of the study two samples were taken at 115 and 120 min to ensure equilibrium.

Homeostatic model assessment-β cell function percentage. Homeostatic model assessment (HOMA)-β-cell function percentage was calculated by the formula 20 × fasting plasma insulin (µU/ml)/fasting plasma glucose (mmol/l) − 3.5 (17).

Blood glucose, insulin, and C-peptide assays. Blood glucose was analyzed by the glucose oxidase method on an Analox spectrophotometer (Lunenburg, MA) with an interassay coefficient of variation (CV) of 4.6%. Insulin was analyzed by chemiluminescence in a Luminex 1000 using the Endocrine Multiplex Immunoassay (Lincor Research, St. Louis, MO) with an interassay CV of 7.7%. C-peptide was analyzed by ELISA (cat. no. EZHCP-20K; Millipore, St. Charles, MI) with an interassay CV of 5.0%. All samples were analyzed in duplicate, and all samples for each analyte were assayed in the same assay.

Statistical analysis. AUC for insulin and glucose during the IVGTT were calculated using the trapezoidal rule. The relation between each of nine outcomes and treatment (treated, control) and sex (male, female) was assessed, initially with an ANOVA in terms of treatment, sex, and the treatment by sex interaction and subsequently with a one-way ANOVA in terms of treatment and a one-way ANOVA in terms of sex. In the two-way ANOVA, the main effect for treatment was tested at each level of sex, and the main effect for sex was tested at each level of treatment. The purpose of this analysis was to check the homogeneity of the treatment effect with sex. The one-way ANOVA for treatment was unadjusted for sex and is the analysis of primary importance in this study. The one-way ANOVA for sex was unadjusted for treatment and is provided as a component of our homogeneity assessment. Weight and fasting glucose were analyzed in original units and, due to skewing, fasting insulin, insulin AUC (0–60), C-peptide AUC (Arg), HOMA-β cell function, and glucose disposal rate were analyzed in log units. All statistical testing was two-sided with a significance level of 5%. SAS version 9.2 for Windows (SAS Institute, Cary, NC) was used throughout. Data are presented as means ± SE.

RESULTS

Initial statistical analyses. We found no evidence of heterogeneity of the treatment effect by sex, body weight, or age and

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<th>Table 1. Maternal and offspring morphometrics</th>
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<td>Maternal morphometrics</td>
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<td>Age at conception, yr</td>
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<td>Weight prepregnancy, kg</td>
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<td>Offspring morphometrics</td>
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Values are means ± SE. MNR, maternal nutrient reduction; BMI, body mass index. *P < 0.05; **P < 0.06.
no significant sex effect; and we conclude, therefore, that all primary analyses are valid.

Maternal morphometrics. Maternal weight was not different in the two groups prior to pregnancy. However, MNR mothers weighed less than control mothers at delivery and weaning as a result of the decreased food availability in pregnancy and lactation (Table 1). Weaning is a very gradual process in the baboon as the offspring eat more and more solid adult food in the group cage. The proportion of food provided to offspring in the milk produced by the mothers decreases well before 9 mo when the juveniles are completely weaned and separated from the mothers. Thus mothers put on weight in the total period between delivery and weaning. This weight increase between delivery, and weaning was similar in the two groups.

Offspring morphometric characteristics and growth. There was no difference in the duration of gestation. Birth weight of female control offspring (0.82 ± 0.03 kg) did not differ from males (0.98 ± 0.08 kg; P > 0.05). At 3 mo of age male MNR offspring weighed less than control males (Fig. 1). At the time of the studies, female control offspring weighed more than female MNR offspring (10.3 ± 0.6 vs. 8.45 ± 0.59 kg; P < 0.03), while weights did not differ in the two groups of males (10.75 ± 1.14 vs. 9.96 ± 0.67 kg).

Intravenous glucose tolerance test. Fasting plasma insulin (P < 0.04) and glucose (P < 0.05) were both elevated in offspring of MNR compared with control offspring, while fasting C-peptide was not different between the two groups (Fig. 2).

Figure 3 shows the AUC during the first 60 min of the IVGTT for the three variables studied: glucose, insulin, and C-peptide. While the glucose changes in response to the IVGTT were not different in the two groups (Fig. 3A), MNR offspring secreted more insulin to maintain the same levels of glucose as control offspring (P < 0.01; Fig. 3B). The C-peptide AUC was not different in the two groups (Fig. 3C). However, the increase in insulin (77%) in MNR compared with control offspring was similar to the rise in C-peptide (71%). Following the arginine challenge, there was no increase in glucose in either group (data not shown), while the insulin AUC was higher in MNR offspring than controls (Fig. 3D; P < 0.03) and the rise in AUC for C-peptide did not reach significance (Fig. 3E). HOMA β-cell function increased in offspring of MNR (6.57 ± 2.08) compared with control mothers (2.74 ± 0.43; P < 0.03).

Hyperinsulinemic euglycemic clamp. There were no differences between the blood glucose, insulin, or C-peptide concentrations achieved in the two groups at any time during the clamp (Fig. 4, A–C). Glucose disposal rate was significantly reduced in the MNR offspring (P < 0.03; Fig. 4D).

DISCUSSION

Decreased delivery of nutrients to the fetus subsequent to MNR is a challenge faced by many fetuses during human development when marked, decreased nutrient delivery to the fetus leads to intrauterine growth restriction (IUGR). IUGR can be defined in many ways, most commonly when birth weight is less than the 10th percentile. However, there is growing evidence that even moderate degrees of decreased fetal growth stimulate compensatory responses in the fetus leading to altered body composition at birth. Some of these changes may be adaptive and promote fetal survival, such as the redistribution of blood during fetal hypoxia that serves to maintain supplies of oxygen to vital organs such as the brain, heart, and adrenals (7), while others may represent impaired development of fetal organs in ways that may program later life problems.

It has become clear that birth weight is only a proxy for altered body composition and function that can predispose to chronic disease in later life. When the placenta fails, or maternal blood supply is compromised, nutrient supply by the fetal placental supply line decreases. We have shown that overall
placental size and detailed architecture are changed in this baboon model of moderate MNR (28). In teenage pregnancy, the still growing mother is competing for nutrients with her fetus (16). Many women now delay pregnancy until well past the biologically optimal age and maternal vascular perfusion is often compromised at the end of reproductive life. Finally, uterine arterial disease accompanied by decreased placental perfusion and placental insufficiency due to placental disease as occurs in preeclampsia both decrease the flow of nutrients to the fetus. As a result of these different pregnancy characteristics and complications, the fetus will be nutrient deprived with resultant potential for developmental programming effects of MNR.

In this study, we have used a model of moderate 30% MNR similar to that used by other investigators in sheep and rodents (2, 3, 27). Human MNR occurs in developed countries in many situations not just countries affected by famine and food shortage. According to a 2009 survey by the USDA Economic Research Service, 14.7% of US households (17.4 million people) were food insecure at some time during 2009 and 9.0 percent of US households (10.6 million people) had low food security, while 5.7% of U.S. households (6.8 million people) had very low food security at some time in the year (http://www.ers.usda.gov/Briefing/FoodSecurity/stats_graphs.htm). Clearly in spite of its low profile, poor nutrition (including in pregnant women) is an important and growing problem in the USA today. The UNICEF website presents more dramatic statistics with 852 million people worldwide experiencing food insecurity in 2004 (22).

To our knowledge our study is the first attempt in an experimental primate model to determine the emergence of developmental programming of β-cell function and peripheral insulin resistance as a result of poor maternal nutrition. While there are extensive investigations on developmental programming of pancreatic function and peripheral insulin resistance in altricial, polytocous species, the only data in a precocial species come from sheep, which are ruminants, and have very different nutrition from primates after weaning (27, 34). We developed our model to determine the extent to which the sheep and rodent findings could be extrapolated to primates. Our results show that offspring of pregnant baboons who are moderately poorly nourished during development are also programmed for emergence of impaired postnatal glucose homeostasis. Importantly, the baboon offspring in this study had eaten a very normal primate chow since weaning and had not been exposed to any of the challenges related to postnatal overnutrition associated with Western obesogenic, diabetogenic diets that could have impaired their glucose metabolism further.

We have shown that this level of MNR significantly decreases fetal islet size and staining for insulin, IGF-I (15), and IGF-II at term (10) and is accompanied by low circulating levels of fetal IGF-1, a major pancreatic growth factor. We have also shown that this degree of MNR increases the gluco-

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**Fig. 3. Intravenous glucose tolerance test (IVGTT) data.** Changes in variables expressed as area under the curve (AUC) during the first 60 min of the IVGTT for glucose mg·dl⁻¹·min (A), insulin μU·ml⁻¹·min (B), and C-peptide ng·ml⁻¹·min (C), and during the arginine challenge for insulin μU·ml⁻¹·min (D), and C-peptide ng·ml⁻¹·min (E). Offspring of control mothers (CTR; n = 12) and of mothers fed 70% of controls through pregnancy and lactation (MNR; n = 6). Data are means ± SE; ‡P < 0.01; #P < 0.03.

**Fig. 4. Hyperinsulinemic euglycemic clamp data.** Final glucose level (A), final insulin level (B), final C-peptide level (C), and glucose disposal rate (RD: D). CTR; n = 12, MNR; n = 6. Data are means ± SE; #P < 0.03.
neogenic capacity of the fetal liver by increasing PEPCK protein expression in the liver (21). PEPCK is the rate-limiting enzyme for gluconeogenesis and persistence of an increase in its activity would also tend to raise plasma glucose in offspring. We therefore hypothesized that insulin function and glucose homeostasis would be compromised in offspring of MNR mothers. Since general anesthetics have been shown to affect β-cell function and peripheral insulin resistance (32) and to reduce peripheral fatty acid release (12), we conducted our studies in conscious baboons on a swivel and tether system (14).

The raised fasting plasma glucose and insulin and increased insulin secretion during the IVGTT indicate an increase in peripheral insulin resistance that was confirmed by the decreased glucose disposal rate in the MNR offspring. Insulin secretion in response to amino acids has been used as a nonglucose challenge to determine β-cell mass in humans (35) and baboons (18). Our observations of greatly reduced islet size and number in this model at the fetal stage, as mentioned above, would suggest an alternative explanation, namely that at this stage of development the increased activity required to maintain normoglycemia by the fewer islets present in the MNR offspring has increased the sensitivity and or capacity of individual islets. We propose to evaluate that hypothesis by future in vitro studies of the postnatal β-cell in islets exposed to poor nutrition during development.

The only other study to use the hyperinsulinemic euglycemic clamp in baboons was conducted in 20-yr-old males and females (6). In these aging animals, glucose disposal rate was not different between males and females, averaging 6.2 mg·kg \(^{-1}\)·min \(^{-1}\), while in our 3.5-yr-old animals the glucose disposal rate averaged 29.1 mg·kg \(^{-1}\)·min \(^{-1}\). There are several potential explanations for this difference. Although body mass index is a somewhat inadequate metric in quadrupeds, in the potential explanations for this difference. Although body mass index is a somewhat inadequate metric in quadrupeds, in the overall metabolic phenotype of baboons to our 3.5-yr-old baboons, the MNR offspring has increased sensitivity and or capacity of individual islets. We propose to evaluate this hypothesis by future in vitro studies of the postnatal β-cell in islets exposed to poor nutrition during development.

In conclusion, our study shows that exposure of the developing primate fetus and newborn to moderately reduced nutrient availability during pregnancy and lactation results in developmental programming of both β-cell function and peripheral insulin sensitivity prior to puberty.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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