Effect of maternal feed restriction during pregnancy on glucose tolerance in the adult guinea pig

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Epidemiological studies have shown that small size at birth is associated with an increased incidence of hyperinsulinemia, insulin resistance (2, 27, 29, 31), glucose intolerance or type 2 diabetes (2, 3, 14, 29, 32), and other components of the insulin resistance syndrome (2–4, 9) in older men and women in various communities around the world. Some or all of these associations are present in young adults or even earlier (10, 16, 19, 45), especially in populations with a high incidence of fetal growth restriction or after clinical growth restriction (16, 19, 45). These findings have led to the hypothesis that restriction or perturbation of prenatal growth can permanently and adversely alter the structure, function, or metabolism of major tissues or organs important for insulin action and glucose homeostasis in later life (2).

Fetal growth is dependent on an adequate supply of oxygen and nutrients crossing the placenta from the mother (35). Thus factors that perturb fetal substrate supply and are known to be responsible for much fetal growth restriction, such as placental insufficiency or poor maternal nutrition, are implicated in the long-term programming of adult dysfunction and disease (2). Direct evidence for the latter in humans is provided mainly by the observation that exposure in utero to maternal famine, during mid or late gestation, reduces weight and length at birth and results in hyperinsulinemia and impaired glucose tolerance at 50 yr of age (34). In contrast, manipulation of maternal nutrition in other species, especially the rat, largely has different consequences, unless extreme in nature. In the rat, maternal feed restriction (MFR; 50% ad libitum intake) during the first two-thirds of pregnancy does not alter glucose tolerance, insulin sensitivity, or secretion in young adult offspring (33). Similar restriction in the second half of pregnancy does impair hepatic, although not peripheral, insulin sensitivity in female offspring (17). More severe MFR (30% ad libitum intake) throughout pregnancy is associated with fasting hyperinsulinemia in male offspring, which is exacerbated with postnatal exposure to a high-fat diet (41). In contrast, maternal protein restriction during preg-
nancy or pregnancy and lactation enhances glucose tolerance (15, 26, 37) and whole body insulin sensitivity in young adult rats (18). Maternal protein restriction does impair glucose tolerance in much older male, but not female, offspring (15). The effect of varying maternal nutrition on metabolic homeostasis in offspring has been little studied in other species, although in sheep, reduced size at birth due to twinning improves glucose tolerance at 1 and 6 mo of age (7). Thus experimental studies in species other than the human have provided limited support for a causal relationship between exposure to an adverse prenatal environment or impaired prenatal growth and impaired adult glucose tolerance and insulin action.

These differing outcomes of prenatal perturbation for later metabolic homeostasis, between and within species, may reflect species differences in the timing of its sensitivity to programming or in the nature of the perturbations experienced or imposed. Chronic reduction of total caloric intake from before pregnancy, eating disorders, and related indicators such as low prepregnancy weight account for a significant proportion of human intrauterine growth retardation (24). Using the guinea pig, a species that is more precocious at birth than the rat, we have previously shown that moderate MFR from before and throughout pregnancy retards fetal growth and impairs postnatal cholesterol metabolism (23), as in humans (2, 4). The aim of the current study was to determine the effect of mild or moderate MFR on size at birth and adult glucose tolerance in this species. In addition, the consequences of MFR for related outcomes, food intake and body composition, in adult offspring were assessed.

METHODS

Animals

Nulliparous 3- to 4-mo-old female guinea pigs (IMVS colored, Gillies Plains Animal Resource Centre, Gillies Plains, South Australia, Australia) were housed in individual wire-bottomed cages under 12:12-h light-dark conditions. The animals were fed a guinea pig/rabbit chow (Milling Industries Stockfeeds, Murray Bridge, South Australia, Australia) with an increased content of vitamin E (165 mg/kg) and had free access to tap water supplemented with vitamin C (400 mg/l). Control animals were fed ad libitum (n = 18) (Fig. 1A).

Food intake and body weights of the ad libitum-fed animals were monitored three times per week. Feed-restricted animals were given 85% (mild MFR, n = 11) or 70% (moderate MFR) of the ad libitum intake. Food intake and body weight in mothers fed ad libitum (●, n = 18), 85% ad libitum/kg body wt from 4 wk before pregnancy until term (▲, n = 11; mild maternal feed restriction [MFR]), and 70% ad libitum/kg body wt from 4 wk before to day 34 of pregnancy, followed by 90% ad libitum/kg body wt until term (○, n = 4; moderate MFR). A: PRE food intake represents a mean intake for the 4 wk before pregnancy. Food intake from days 1 to 63 of pregnancy (term = 69–70 days) are illustrated as the mean intake for each 7 days. B: maternal weight during pregnancy is illustrated as the mean weight 14 days before pregnancy (PRE) and at the day of pregnancy indicated. Maternal weight gain throughout pregnancy was reduced by mild MFR [effect of pregnancy, P < 0.001; effect of MFR, P < 0.002; pregnancy × MFR, P < 0.001] and moderate MFR [effect of pregnancy, P < 0.001; effect of MFR, P < 0.005; pregnancy × MFR, P < 0.001] fed mothers.

Fig. 1. Maternal food intake and body weight. Food intake (A; g/day) and maternal body weight (B) in mothers fed ad libitum (●, n = 18), 85% ad libitum/kg body wt from 4 wk before pregnancy until term (▲, n = 11; mild maternal feed restriction [MFR]), and 70% ad libitum/kg body wt from 4 wk before to day 34 of pregnancy, followed by 90% ad libitum/kg body wt until term (○, n = 4; moderate MFR). A: PRE food intake represents a mean intake for the 4 wk before pregnancy. Food intake from days 1 to 63 of pregnancy (term = 69–70 days) are illustrated as the mean intake for each 7 days. B: maternal weight during pregnancy is illustrated as the mean weight 14 days before pregnancy (PRE) and at the day of pregnancy indicated. Maternal weight gain throughout pregnancy was reduced by mild MFR [effect of pregnancy, P < 0.001; effect of MFR, P < 0.002; pregnancy × MFR, P < 0.001] and moderate MFR [effect of pregnancy, P < 0.001; effect of MFR, P < 0.005; pregnancy × MFR, P < 0.001] fed mothers.
MFR, n = 6) of the average daily ad libitum food intake per kilogram body weight (23, 39). Feed-restricted animals were fed between 0800 and 0930 each morning. After 4–6 wk of controlled feeding, guinea pigs were mated. Females in estrus were placed with a male overnight, and pregnancy was detected by the presence of a vaginal copulatory plug the following morning and a failure to return to estrus in the subsequent cycle. Mildly restricted mothers continued to be fed at 85% ad libitum intake·day\(^{-1}\)·kg body wt\(^{-1}\) throughout pregnancy (Fig. 1A). Moderately restricted mothers were fed at 70% ad libitum intake·day\(^{-1}\)·kg body wt\(^{-1}\) until day 35 of pregnancy, then 90% ad libitum intake·day\(^{-1}\)·kg body wt\(^{-1}\) until term, to prevent weight loss in late pregnancy (Fig. 1A).

One week before term, pregnant animals were transferred to tubes containing paper bedding. Weight, length from nose to rump, abdominal circumference, and head width and length were measured for each pup at birth. A total of 58 pups was born to the 18 ad libitum-fed mothers. Seven pups of ad libitum mothers (from 4 different litters) died at birth due to birthing difficulties. Thirty-one pups were born to the 11 mildly restricted mothers, with no still births occurring in this group. Two of the six moderately restricted pregnancies failed to reach term, aborting at around days 50–55 of pregnancy. The four remaining moderately restricted mothers carried litters to term, producing nine pups. All mothers and their litters fed ad libitum after delivery.

Pups were weaned at 28 days of age. Pups were allowed ad libitum access to the guinea pig/rabbit chow and were housed in individual cages from 40 days of age. Pups were weighed daily from birth to 40 days of age, and then body weight and food intake were measured three times per week until 80 days of age. Average food intake (g·day\(^{-1}\)·kg body wt\(^{-1}\)) between 50 and 80 days of age was calculated. Absolute growth rate (g/day) and fractional growth rate (FGR; absolute growth rate/birth weight) were calculated from birth to weaning.

Birth size measures, postnatal growth rates, and food intake are reported for all 91 offspring. Litters from seven ad libitum-fed mothers (21 offspring), six mildly restricted mothers (9 offspring), and ten moderately restricted mothers (14 offspring) were randomly assigned to this study. Two of the six moderately restricted pregnancies failed to reach term, aborting at around days 40–55 of pregnancy. Insulin and glucose curves throughout the IVGTT were plotted using SigmaPlot (Jandel Scientific Software). Areas under the glucose (AUGC) or insulin (AUIC) curves were analyzed using SigmaScan Pro image analysis software (Jandel Scientific Software). AUIC for the first- and second-phase response was also calculated. The latter was identified for each individual animal, based on the nadir of insulin between phases, which occurred at 20.6 ± 1.8 min. The glucose tolerance index (K\(_{G1}\)), which provides an estimate of the rate of glucose elimination after a glucose dose, was calculated from the slope of the regression line obtained from the plot of the natural logarithm-transformed glucose data values between 2 and 60 min and was expressed as a percentage per minute.

### Body Composition

At 115 ± 0.2 days of age, 15 offspring (7 male, 8 female) of ad libitum-fed mothers and the 8 offspring (4 male, 4 female) of moderately restricted mothers were fasted from 0800 and killed between 1400 and 1600 in intravenous overdose of pentobarbital sodium. Other offspring were retained for subsequent study of cholesterol homeostasis (23) and were not included in the body composition analysis. Major tissues, including individual adipose tissue depots, were dissected out and weighed. The weights of interscapular, right and left retroperitoneal, right and left perirenal, right groin, right shoulder, and right neck fats and fat associated with the gastrointestinal tract were summed to give a measure of adiposity. Interscapular and perirenal fat depots were immersion fixed in 10% buffered formalin (pH 7.0). All animal studies were approved by the Animal Ethics Committee of the University of Adelaide, where the animal work was conducted.

### IVGTT

At 90 ± 0.3 days of age, catheters were inserted into the right jugular vein (Silastic, 0.51-mm ID, 0.94-mm OD) and carotid artery (polyvinyl, 0.4-mm ID, 0.8-mm OD) under general anesthesia induced by ketamine (75 mg/kg body wt ip) and xylazine (6 mg/kg body wt im). Atropine (0.05 mg/kg body wt sc) was given before surgery. Catheters were tunneled under the skin to the back of the neck. Catheter patency was maintained by flushing daily with enough heparinized saline to fill the catheter (250 U/ml). IVGTT were performed at 101 ± 0.5 days of age. Animals were fasted for 16 h. Extension lines were attached to the catheters, and the animals remained conscious and unrestrained in their cages throughout the experiment. Dextrose solution (50% wt/vol, Baxter HealthCare, New South Wales, Australia) was diluted in 0.9% NaCl solution to give a dose of 0.5 g dextrose/kg body wt in a total volume of 2.5 ml. Dextrose was administered through the jugular vein catheter over a 2-min period, immediately followed by 2 ml of 0.9% NaCl. Blood samples (500 µl) were collected from the carotid artery at 2, 5, 10, 20, 30, 40, 60, 80, 120, 150, 180, and 210 min after injection of the saline solution. Blood was centrifuged at 3,000 rpm for 15 min, and plasma was removed and stored at −20°C for use in plasma glucose and insulin assays.

Insulin and glucose curves throughout the IVGTT were plotted using SigmaPlot (Jandel Scientific Software). Areas under the glucose (AUGC) or insulin (AUIC) curves were analyzed using SigmaScan Pro image analysis software (Jandel Scientific Software). AUIC for the first- and second-phase response was also calculated. The latter was identified for each individual animal, based on the nadir of insulin between phases, which occurred at 20.6 ± 1.8 min. The glucose tolerance index (K\(_{G1}\)), which provides an estimate of the rate of glucose elimination after a glucose dose, was calculated from the slope of the regression line obtained from the plot of the natural logarithm-transformed glucose data values between 2 and 60 min and was expressed as a percentage per minute.

### Analyses

Plasma glucose was measured by colorimetric enzymatic analysis on a COBAS Mira automated centrifugal analyzer using a Roche Unimate Glucose HK kit and control sera (F. Hoffmann-La Roche, Basel, Switzerland). Plasma insulin was measured by RIA. Purified guinea pig insulin and rabbit anti-guinea pig insulin were provided by Dr. C. C. Yip (Univ. of Toronto, Toronto, Canada) (46, 47). Guinea pig insulin was used as the assay standard and iodinated for use as the radioligand. RIA measuring guinea pig insulin using these reagents has been described previously (13). Guinea pig insulin was iodinated with Na\(^{125}\)I (Amersham Pharmacia-Biotech, Sydney, New South Wales, Australia) and chloramine T to specific activities between 35 and 50 Ci/g and separated from reaction components by chromatography on Sephadex G50 (Amersham Pharmacia-Biotech). Guinea pig insulin was measured in duplicate samples of 25 µl guinea pig plasma or standard by addition of 175 µl assay buffer (0.5 M sodium phosphate buffer, pH 7.5; 2% BSA (wt/vol)), 50 µl 125I-labeled guinea pig insulin and 10 µl of anti-guinea pig insulin antiserum in assay buffer (final dilution 1:1,500), which will bind 20% of the radioactivity in the absence of competing ligand. The tubes were incubated at 4°C for 48 h, and 100 µl of anti-rabbit solid phase second antibody coated cellulose suspen-
FETAL GROWTH AND POSTNATAL GLUCOSE TOLERANCE

RESULTS

Pregnancy
MFR did not alter weight at mating, but mild (P < 0.002) and moderate (P < 0.003) restriction reduced weight gain throughout pregnancy (Fig. 1B). Feed intake (in g/day, without correction for body weight) in the mildly restricted mothers averaged 79% of the ad libitum intake (g/day) throughout pregnancy (Fig. 1A). Feed intake (in g/day, without correction for body weight) in the moderately restricted mothers averaged 66% of the ad libitum intake (g/day) from before and to day 35 of pregnancy (Fig. 1A) and 77% ad libitum intake (g/day) from day 35 to term (Fig. 1A). Mild but not moderate MFR reduced maternal weight at term (ad libitum, 779 ± 11 g, n = 18; mild MFR, 720 ± 19 g, n = 11, P < 0.02; moderate MFR, 731 ± 24 g, n = 4). Both mild and moderate MFR reduced total litter weight (ad libitum fed, 298 ± 12 g, n = 16 litters; mild MFR, 246 ± 15 g, n = 11, P < 0.03; moderate MFR, 206 ± 22 g, n = 4, P < 0.005). Moderate restriction reduced litter size (ad libitum, 3.2 ± 0.2, n = 18; mild MFR, 2.8 ± 0.2, n = 11; moderate MFR, 2.3 ± 0.3, n = 4, P < 0.04) and increased length of gestation (ad libitum, 69.2 ± 0.4 days, n = 10; mild MFR, 70.2 ± 0.3 days, n = 11; moderate MFR, 71.5 ± 0.3 days, n = 4, P < 0.01), compared with ad libitum fed, while mild restriction did not alter these parameters. All mothers were fed ad libitum after giving birth, and maternal weight at weaning was not different between groups (ad libitum, 806 ± 13 g, n = 18; mild MFR, 827 ± 16 g, n = 11; moderate MFR, 810 ± 17 g, n = 4).

Birth Size
Mild restriction reduced weight, abdominal circumference, and weight/length (an index of thinness) at birth in male offspring (P < 0.02, P < 0.01, P < 0.003, respectively) (Table 1). Moderate restriction reduced birth weight in male offspring (P < 0.02, adjusted for litter size) (Table 1). Moderate restriction reduced birth length in male offspring (P < 0.02) compared with those of ad libitum-fed (P < 0.02) or mildly restricted mothers (P < 0.05) (Table 1). Mild restriction reduced birth weight in female offspring (P < 0.002, adjusted for litter size) (Table 1). Moderate restriction reduced birth length in female offspring (P < 0.03, adjusted for litter size) (Table 1). Moderate restriction increased weight/length at birth in female offspring compared with those of mildly restricted mothers (P < 0.05, Table 1).

Postnatal Growth Rate
Mild but not moderate MFR reduced absolute growth rate from birth to weaning in male offspring (P < 0.001) and in female offspring (P < 0.02, adjusted for litter size) (Table 1). MFR did not alter fractional growth rate (FGR) from birth to weaning in male or female offspring (Table 1).
female offspring (Table 1). Absolute growth rate from birth to weaning was positively associated with weight at birth \((r = 0.42, P < 0.001, n = 90)\), while FGR correlated negatively with birth weight \((r = -0.60, P < 0.001, n = 90)\) in all offspring combined.

**Glucose and Insulin Response to IVGTT**

**Effect of MFR on glucose and insulin response to IVGTT.** In the male offspring that underwent IVGTT, birth weight was reduced by moderate \((P < 0.05)\) and mild MFR \((P < 0.05,\) adjusted for litter size) (Table 2).

In male offspring, MFR did not alter fasting plasma glucose (Table 2). Plasma glucose concentrations throughout the IVGTT in male offspring of ad libitum-fed and feed-restricted mothers are shown in Fig. 2. Moderate restriction increased AUGC \((P < 0.04)\) and reduced \(K_G\) calculated between 2 and 60 min \((P < 0.03)\) in male offspring compared with mild restriction (Table 2). Moderate or mild restriction did not alter glucose tolerance (AUGC or \(K_G\)) in male offspring compared with offspring of ad libitum-fed mothers (Table 2). When assessed by repeated-measures ANOVA, moderate restriction increased the glucose response to IVGTT compared with mild restriction \((P < 0.01,\) Fig. 2). Moderate or mild restriction did not alter the glucose response to IVGTT in male offspring compared with offspring of ad libitum-fed mothers (Fig. 2). All of these outcomes were present whether or not birth weight or litter size were adjusted for.

In male offspring, moderate restriction increased fasting plasma insulin and the fasting I/G compared with ad libitum feeding or mild restriction of mothers \((P < 0.05,\) adjusted for litter size) (Table 2) but not when adjusted for birth weight. Plasma insulin concentrations and I/G throughout the IVGTT in male offspring are shown in Fig. 2. Moderate or mild restriction did not alter AUCI with or without adjustment for litter size or birth weight (Table 2). Mild restriction increased the AUCI-to-AUGC ratio (AUCI/AUGC) compared with maternal ad libitum feeding \((P < 0.04,\) Table 2) but not when adjusted for birth weight or litter size. MFR did not alter the first- or second-phase AUCI in male offspring (1st phase: ad libitum, 108.9 ± 30.7 ng·ml\(^{-1}\)·min\(^{-1}\), \(n = 7\); mild MFR, 117.0 ± 38.2 ng·ml\(^{-1}\)·min\(^{-1}\), \(n = 10\); moderate MFR, −5.1 ± 103.9 ng·ml\(^{-1}\)·min\(^{-1}\), \(n = 4\); 2nd phase: ad libitum, 873 ± 30.0 ng·ml\(^{-1}\)·min\(^{-1}\).
When assessed by repeated-measures ANOVA, MFR altered the plasma insulin response to glucose in male offspring ($P < 0.001$). Moderate restriction increased the plasma insulin response to IVGTT in male offspring compared with ad libitum feeding or mild restriction ($P < 0.005$, $P < 0.001$, respectively, Fig. 2) with or without adjustment for birth weight. MFR altered I/G throughout the IVGTT in male offspring ($P < 0.001$, Fig. 2). Both mild ($P < 0.003$) and moderate ($P < 0.02$) restriction increased I/G throughout the IVGTT in male offspring (Fig. 2). Moderate restriction also increased I/G during the IVGTT in male offspring compared with mild restriction ($P < 0.001$; Fig. 2). These outcomes were present whether or not birth weight was adjusted for.

**Effect of size at birth on glucose and insulin response to IVGTT.** To determine the effect of size at birth on postnatal outcomes, offspring were divided into groups according to birth weight tertile (Table 3). Male offspring were divided into high (>92.8 g; range 93.4–118 g, $n = 7$), medium (86.0–92.8 g; range 86–91.5 g, $n = 8$), and low (<86.0 g; range 74.2–85.9 g, $n = 6$) birth weight tertiles (Table 3). Female offspring were also divided into high (>99.27 g; range 99.3–116.1 g, $n = 5$), medium (87.5–99.27 g; range 88.1–99.2 g, $n = 5$), and low birth weight (<87.5 g; range 72.3–87.2 g, $n = 5$) tertiles (Table 3). Birth length and abdominal circumference were not different between birth weight tertiles in male or female offspring. In males and females, birth weight/length was reduced in low birth weight compared with high birth weight offspring (Table 3).

In male offspring, birth weight tertile did not influence fasting glucose, AUGC, $K_g$, AUIC, AUIC/AUGC (Table 4), or first- and second-phase AUIC (data not shown) but did alter fasting insulin and fasting I/G (Table 4). Specifically, fasting insulin ($P < 0.03$) and fasting I/G ($P < 0.04$) were increased in low birth weight compared with medium birth weight males (Table 4) with or without adjustment for litter size or maternal treatment. Birth weight tertile did not alter fasting glucose or insulin or any measures of the glucose or insulin response to glucose in female offspring with or without adjustment for litter size or maternal treatment.

Plasma glucose and insulin concentration and I/G throughout the IVGTT in male offspring classified according to birth weight tertile are shown in Fig. 3. Birth weight tertile did not alter the plasma glucose or insulin response to IVGTT in male offspring (Fig. 3). Repeated-measures analysis indicated that plasma I/G throughout the IVGTT was higher in low birth weight males compared with medium ($P < 0.01$) and high birth weight males ($P < 0.05$) whether or not adjusted for maternal treatment. Glucose, insulin, and I/G throughout the IVGTT were not influenced by birth weight tertile in female offspring (data not shown).

Correlation analyses were performed to determine whether size at birth or neonatal FGR was associated with insulin resistance. The results showed a positive correlation between birth weight and insulin sensitivity, indicating that higher birth weight was associated with greater insulin sensitivity. However, the correlation was not statistically significant due to the small sample size in the study.
with adult glucose tolerance. In male offspring, birth weight did not correlate with fasting glucose or insulin, any measure of glucose tolerance, or insulin response to glucose. However, in males, the glucose tolerance index between 2 and 60 min ($K_G$) was associated positively with birth length ($r = 0.44, P < 0.05, n = 21$) and negatively with birth weight/length ($r = -0.46, P < 0.04, n = 21$). Similarly, AUIC/AUGC was positively associated with birth length ($r = 0.56, P < 0.01, n = 21$) and negatively related to birth weight/length ($r = -0.46, P < 0.03, n = 21$). In male offspring, AUIC during the first phase (0–20.8 ± 1.8 min) after glucose administration correlated positively with birth length ($r = 0.46, P = 0.04, n = 21$) and abdominal circumference ($r = 0.56, P = 0.01, n = 21$). Fasting plasma insulin was positively associated with FGR in male offspring ($r = 0.46, P < 0.04, n = 21$). No other measures of glucose tolerance were related to size at birth, FGR, or adult weight in male offspring.

In female offspring, no measures of fasting glucose or insulin, glucose tolerance, or insulin response to glucose were related to size at birth measures or FGR. AUIC ($r = 0.72, P < 0.002, n = 15$), fasting I/G ($r = 0.54, P < 0.04, n = 15$), AUIC ($r = 0.73, P < 0.002, n = 15$), and second-phase AUIC ($r = 0.72, P < 0.003, n = 15$) were positively associated, and $K_G$ was negatively associated ($r = -0.70, P < 0.005, n = 15$) with adult weight in female offspring. Fasting plasma glucose, fasting plasma insulin, and AUIC/AUGC were not related to adult weight in female offspring.

Partial correlations and multiple linear regressions were performed to determine whether the associations of measures of glucose tolerance with birth size, FGR, or adult weight were independent of each other. The negative association of $K_G$ with birth weight/birth length in male offspring was independent of FGR [partial correlation (pc), $r = -0.50, P < 0.02$] but not adult weight. The positive association of $K_G$ with birth length in male offspring was independent of adult weight (pc, $r = 0.48, P < 0.03$) but not FGR. Associations of AUIC/AUGC with birth length and with birth weight/length in male offspring were independent of FGR (pc, $r = 0.59, P < 0.01; r = -0.48, P < 0.03$, respectively) and adult weight (pc, $r = 0.56, P < 0.01; r = -0.46, P < 0.04$, respectively). The associations of first-phase AUIC with birth length and abdominal circumference were independent of FGR (pc, $r = 0.49, P < 0.03; r = 0.59, P < 0.01$, respectively) and adult weight (pc, $r = 0.47, P < 0.04; r = 0.60, P < 0.005$, respectively). The association of fasting insulin with FGR in male offspring was independent of birth size and sex.

### Table 3. Birth size after division of offspring into groups according to weight at birth

![Table 3](http://ajpregu.physiology.org/)

### Table 4. Effect of size at birth on adult glucose tolerance in the guinea pig

![Table 4](http://ajpregu.physiology.org/)
spring was not independent of birth weight or adult weight. In female offspring, the associations of adult weight with AUGC, fasting I/G, and \(K_g\) were independent of both birth weight and FGR.

**Food Intake**

Food intake was monitored from 50 to 80 days of age in 83 of the offspring. Moderate restriction increased food intake (g·day\(^{-1}\)·kg body wt\(^{-1}\)) in male offspring compared with those of ad libitum-fed (\(P < 0.01\)) or mildly restricted mothers (\(P < 0.02\)) (ad libitum, 71.8 ± 1.0 g·day\(^{-1}\)·kg body wt\(^{-1}\), \(n = 20\) offspring; mild MFR, 72.6 ± 0.9 g·day\(^{-1}\)·kg body wt\(^{-1}\), \(n = 20\); moderate MFR, 79.3 ± 3.6 g·day\(^{-1}\)·kg body wt\(^{-1}\), \(n = 5\)) whether or not adjusted for adult body weight. MFR did not alter food intake per kilogram body weight in female offspring (ad libitum, 73.0 ± 1.5 g·day\(^{-1}\)·kg body wt\(^{-1}\), \(n = 22\); mild MFR, 72.7 ± 1.6 g·day\(^{-1}\)·kg body wt\(^{-1}\), \(n = 12\); moderate MFR, 69.8 ± 0.5 g·day\(^{-1}\)·kg body wt\(^{-1}\), \(n = 4\)).

Food intake (g·day\(^{-1}\)·kg body wt\(^{-1}\)) correlated negatively with birth weight in all animals (\(r = -0.42, P < 0.001, n = 83\)) and in male (\(r = -0.35, P < 0.02, n = 45\)) and female (\(r = -0.51, P < 0.001, n = 38\)) offspring whether or not adjusted for adult body weight. Offspring that had food intake measured were divided into tertiles according to birth weight. For males, birth weight tertiles were high (>97.0 g; 11 ad libitum, 4 mild MFR offspring), medium (86.7–97 g; 6 ad libitum, 8 mild MFR, 1 moderate MFR offspring), and low (<86.7 g; 3 ad libitum, 8 mild MFR, 4 moderate MFR offspring). Birth weight tertile altered food intake in male offspring (\(P < 0.01\)). Specific contrasts indicated that food intake per kilogram body weight was higher in low birth weight males compared with high (\(P < 0.02\)) and medium birth weight (\(P < 0.04\)) males (high birth weight, 69.8 ± 0.6 g·day\(^{-1}\)·kg body wt\(^{-1}\), \(n = 15\); medium birth weight, 74.3 ± 1.1 g·day\(^{-1}\)·kg body wt\(^{-1}\), \(n = 15\); low birth weight, 74.8 ± 1.8 g·day\(^{-1}\)·kg body wt\(^{-1}\), \(n = 15\)). Food intake was also higher in low birth weight males compared with both high (\(P < 0.001\)) and medium birth weight (\(P < 0.01\)) males when adult body weight was included as a covariate in the analysis. For females, birth weight tertiles were high (>97.1 g; 6 ad libitum, 4 mild MFR, 3 moderate MFR), medium (87.3–97.1 g; 11 ad libitum, 2 mild MFR offspring), and low (<87.3 g; 5 ad libitum, 6 mild MFR, 1 moderate MFR offspring). Food intake per kilogram body weight also tended to be higher in low compared with high birth weight female offspring (\(P < 0.06\)) (high birth weight, 69.8 ± 0.7 g·day\(^{-1}\)·kg body wt\(^{-1}\), \(n = 13\); medium birth weight, 72.5 ± 1.3 g·day\(^{-1}\)·kg body wt\(^{-1}\), \(n = 13\); low birth weight, 75.7 ± 2.5 g·day\(^{-1}\)·kg body wt\(^{-1}\), \(n = 12\)).

**Body Composition**

Body composition was determined in offspring of ad libitum-fed and moderately restricted mothers. MFR did not alter adult body weight in these male (Table 5) or female offspring (ad libitum, 660 ± 34 g, \(n = 8\);
In the current study, moderate MFR in the guinea pig restricted fetal growth and caused fasting hyperinsulinemia and hyperphagia in young adult male offspring. Similarly, in young adult male offspring, low birth weight was characterized by high fasting plasma insulin concentrations compared with medium birth weight. The fasting hyperinsulinemia and increased insulin response to IVGTT in male offspring of moderately restricted mothers suggest that modest perturbation of maternal nutrition and fetal growth may induce insulin resistance in the young adult male guinea pig.

These results are consistent with most observations in humans. Exposure to the Dutch famine in utero increased fasting proinsulin levels and 2-h plasma glucose and insulin levels after oral glucose administration in 50-yr-old men and women, consistent with insulin resistance (34). In contrast, insulin concentrations and glucose tolerance were not altered in adults exposed to famine in utero during the Leningrad siege (40). However, a number of studies have shown that the incidence of insulin resistance and/or hyperinsulinemia is increased in children or adults who were light or thin at birth (10, 16, 19, 27, 29, 31). In the current study, the effects of MFR on postnatal fasting plasma insulin in male offspring may be due, at least in part, to effects on prenatal growth. Increased fasting insulin levels were observed in the lowest birth weight males, and covariate analysis indicated that birth weight could account for some of the effects of MFR on fasting insulin in these offspring. Consistent with this, we have also observed that spontaneous fetal growth restriction and small size at birth in the guinea pig, which occurs with large litter size, are associated with reduced peripheral and hepatic insulin sensitivity of glucose metabolism in vivo, including that of glucose storage, in young adult males (D. M. Horton, K. L. Kind, A. Katsman, M. R. Bradbury, J. S. Robinson, and J. A. Owens, unpublished observations). In humans, it was noted that the effects of maternal famine on 2-h plasma glucose and insulin levels after oral glucose were greater than could be explained by the reductions in birth size (34). Different outcomes for insulin levels were observed in the current study in offspring whose mothers were mildly undernourished compared with those of moderately feed-restricted mothers, which did not parallel the magnitude of effects on size at birth, suggesting that maternal nutrition may exert effects independent of or additional to those possibly mediated through growth. Further studies are required using more direct measures of insulin sensitivity, such as those obtained with the hyperinsulinemic, euglycemic clamp, to confirm that the offspring of moderately restricted mothers are insulin resistant, as suggested by fasting hyperinsulinemia, in the current study. Nevertheless, our observation that spontaneous restriction of fetal growth is associated with reduced whole body insulin sensitivity in young adult male guinea pigs, as measured by glucose clamp (D. M. Horton, K. L. Kind, A. Katsman, M. R. Bradbury, J. S. Robinson, and J. A. Owens, unpublished observation), supports the suggestion that impaired prenatal growth in the guinea pig is

Table 5. Effect of MFR on adult body composition in the male guinea pig

<table>
<thead>
<tr>
<th>Male offspring</th>
<th>Birth Weight, g</th>
<th>Body Weight, g</th>
<th>Retroperitoneal Fat Weight, g</th>
<th>Retroperitoneal Fat Weight, %body wt</th>
<th>Biceps Weight, g</th>
<th>Biceps Weight, %body wt</th>
<th>Adrenal Weight, g</th>
<th>Adrenal Weight, %body wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ad libitum</td>
<td>94.1 ± 3.8</td>
<td>863 ± 33</td>
<td>10.0 ± 0.6</td>
<td>1.16 ± 0.05</td>
<td>0.47 ± 0.02</td>
<td>0.055 ± 0.001</td>
<td>0.28 ± 0.02</td>
<td>0.033 ± 0.002</td>
</tr>
<tr>
<td>Moderate MFR</td>
<td>83.5 ± 1.9</td>
<td>845 ± 41</td>
<td>13.1 ± 1.5</td>
<td>1.57 ± 0.20*a</td>
<td>0.41 ± 0.03</td>
<td>0.048 ± 0.002*a</td>
<td>0.36 ± 0.02*a</td>
<td>0.042 ± 0.002*a</td>
</tr>
<tr>
<td>ANOVA P&lt;</td>
<td>NS</td>
<td>NS</td>
<td>0.06</td>
<td>0.03</td>
<td>NS</td>
<td>0.02</td>
<td>0.004</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Values are means ± SE; n refers to no. of offspring. Effect of MFR assessed by ANOVA: *P < 0.05 compared with ad libitum group.
associated with a decrease in insulin sensitivity. Thus the consequences of moderate MFR and fetal growth restriction in the guinea pig for prevailing insulin levels in adult male offspring appear to resemble those described in humans who are small at birth, with postnatal insulin resistance and hyperinsulinemia.

MFR did not alter glucose tolerance of guinea pig offspring per se, although moderate restriction did impair glucose tolerance in male offspring compared with that in males whose mothers were only mildly undernourished. Furthermore, no differences in glucose tolerance were observed when the offspring were divided into tertiles according to weight at birth. The hyperinsulinemia present in male offspring of moderately restricted mothers and in low birth weight male offspring may partly contribute to the maintenance of their glucose homeostasis. In addition, glucose tolerance is influenced by both insulin action, as determined by tissue sensitivity and circulating concentrations, and the actions of glucose itself in stimulating its own uptake and in suppressing hepatic glucose production (5). Studies in rats (28) and mice (30) suggest that, in these species in particular, insulin-independent mechanisms may be responsible for a significant component of glucose disposal after a glucose load. There is some evidence that in humans, small size at birth alters both insulin action and glucose effectiveness in postnatal life (10). Maternal undernutrition or small size at birth is associated with glucose intolerance or type 2 diabetes in men and women, aged 40 yr or more, in most studies (9, 14, 27, 29, 32, 34). However, reduced insulin sensitivity or hyperinsulinemia but normal glucose tolerance have been observed in short prepubertal children who were growth retarded at birth (16) and in young adults of small size at birth (10, 19). Normal glucose homeostasis is maintained in 20-yr-old males who were light or short at birth, despite reductions in insulin sensitivity, in part due to an increase in insulin secretion and also to increased glucose effectiveness (10). Similarly, we observed hyperinsulinemia suggestive of insulin resistance in young adult male guinea pigs of moderately feed-restricted mothers, but no substantial impairment of glucose tolerance. First-phase acute insulin response is increased in young adult males of small size at birth (10) and in young adult men and women (19) and short children (16) who were growth retarded at birth, suggesting that increased insulin secretion also contributes to their maintenance of glucose homeostasis. In these studies, no differences in acute insulin response, when corrected for prevailing insulin sensitivity, were observed (10, 19), indicating that the increase in acute insulin response is appropriate for the degree of insulin resistance observed with decreasing size at birth (10, 19). Nevertheless, others have reported that first-phase insulin secretion, corrected for insulin sensitivity, is decreased in young adult men of low birth weight (20). First- and second-phase insulin responses to glucose were observed in the guinea pig in the current study, although in contrast to many other species (10, 30), the major peak in insulin occurred during the second phase. First-phase AUIC decreased with decreasing length or abdominal circumference at birth in males, despite the prevailing hyperinsulinemia in offspring of small size at birth. Failure to maintain an appropriate increase in insulin secretion with decreasing insulin sensitivity is associated in humans with progression to impaired glucose tolerance and diabetes (1, 6, 42). Further studies are required to more directly investigate insulin secretion and its contribution to maintenance of glucose homeostasis in prenatally growth-restricted guinea pig offspring. Similarly, whether enhanced glucose effectiveness contributes to the maintenance of glucose tolerance in these offspring, as seen in young adult men of low birth weight or length (10) remains to be determined. Subsequent development of glucose intolerance in older adult men and women who were small at birth may be associated with a failure of the mechanisms that compensate for the reduced insulin sensitivity at earlier ages, such as increases in insulin secretion and glucose effectiveness (10, 19). Whether offspring of feed-restricted guinea pigs or guinea pigs that are small size at birth will become glucose intolerant with aging remains to be determined.

Differences in postnatal outcomes for glucose and insulin homeostasis were observed between males born to mothers exposed to different feed restriction regimens. Birth weight in males was reduced by both moderate and mild MFR; however, males born to moderately feed-restricted mothers had reduced birth length while those born to mildly restricted mothers were thin at birth compared with the ad libitum-fed group. Restriction of maternal feed intake was greatest during early pregnancy in the moderately restricted group, as mothers were fed at 90% ad libitum intake/kg body wt from midpregnancy. Feed restriction was constant throughout pregnancy in the mildly undernourished group. It has been suggested that differences in birth phenotypes may be related to the timing of maternal nutrient deprivation (2). In the current study, the time of onset of substantial nutrient restriction may have varied with the magnitude of the treatment imposed even though restriction was consistently imposed from before birth. In addition, the degree of the feed restriction and the associated rate and pattern of fetal growth, as indicated by birth phenotype, may have influenced postnatal outcome in terms of glucose and insulin homeostasis. Regression analysis provided some evidence of an association of birth phenotype with adult glucose tolerance. A negative association was observed between $K_G$ and birth weight/length, suggesting that thin offspring had an improved glucose tolerance as young adults. In addition, shortness at birth in the male guinea pig was associated with a decrease in $K_G$ and lower first-phase AUIC. In humans, thinness at birth has been associated with reduced adult insulin sensitivity (27, 31). However, others have reported an association of lightness or shortness at birth, rather than thinness, with reduced insulin sensitivity and increased insulin secretion in young men (10).
The effects of MFR on glucose tolerance in female offspring were not determined due to the lower number available for study. Glucose tolerance and insulin secretion did not vary with birth weight tertile in female offspring. Study of additional female offspring is required to confirm this, however, as the birth weight range across which female offspring were studied was less than that in males, due to lower numbers of female offspring from feed-restricted mothers. We have previously reported that cholesterol metabolism is perturbed in adult male, but not female, offspring of undernourished guinea pigs (23). Studies in rats also suggest that males are more susceptible to the effects of maternal undernutrition or prenatal growth restriction on postnatal glucose and lipid metabolism (8, 15, 25). This has also been observed in young adult humans where insulin sensitivity is reduced in males, but not females, who were small at birth (10). Instead, in young adult women, body weight is a more important determinant of glucose metabolism than size at birth (10). We also observed this in the current study, where in female guinea pigs adult weight was associated with measures of glucose tolerance and insulin secretion, independent of birth weight or postnatal growth rate.

In addition to hyperinsulinemia, males born to moderately feed-restricted mothers were hyperphagic and had increased relative retroperitoneal fat weight in the current study. Similarly, low birth weight was associated with increased postnatal feed intake relative to body weight in males. This is unexpected because the hyperinsulinemia of these animals would have been expected to reduce appetite (36). Hyperinsulinemia, hyperphagia, and an increase in retroperitoneal fat weight relative to body weight have also been described in adult male rats whose mothers were restricted to 30% ad libitum food intake throughout pregnancy (41). Hyperphagia in the presence of hyperinsulinemia in offspring of undernourished mothers, in the current and previous studies (41), is therefore consistent with insulin resistance. That is, development of insulin resistance in more than one target tissue in prenatally growth-restricted offspring may lead to the onset of hyperphagia through perturbation of insulin-regulated inhibition of feed intake. In combination with hyperinsulinemia, such prenatally induced hyperphagia may increase adiposity through increased nutrient intake and availability for subsequent partitioning to adipose tissue particularly from insulin-resistant skeletal muscle. An alternative possibility is that increased food intake and hence increased adiposity may contribute to the development of insulin resistance, as obesity, particularly in the intra-abdominal region, is a known risk factor for the development of insulin resistance (21). Consistent with this, impaired glucose tolerance and hyperinsulinemia were associated with increased actual and relative fat weight in the current study (data not shown). Adipocyte size in the interscapular depot was also positively correlated with fasting insulin in adult offspring, but whether these are causally related and in what direction are unclear. Nevertheless, this is consistent with observations in humans where abdominal subcutaneous, but not intra-abdominal, adipocyte size is associated positively with fasting insulin and negatively with insulin sensitivity (12, 43). Regardless, these observations suggest that maternal undernutrition and/or fetal growth restriction can alter appetite regulation in such a way as to exacerbate other prenatally programmed impairments in homeostasis and the risk of certain adult-onset diseases.

In rats, restriction of maternal feed intake to 30% of ad libitum intake increases plasma leptin levels in adult offspring, concomitant with hyperinsulinemia, hyperphagia, hypertension, and increased adiposity, suggesting that leptin resistance may also be a consequence of reduced maternal nutrition (41). This has led to the suggestion that prenatally induced leptin resistance (peripheral and central) could concomitantly ameliorate both leptin’s inhibition of insulin secretion and suppression of appetite, resulting in hyperinsulinemia and hyperphagia (41). The effect of MFR and small size at birth in the guinea pig on postnatal leptin levels remains to be determined. In the current study, the increased fasting and secreted insulin, when corrected for the prevailing glucose concentration, in male offspring of feed-restricted mothers are consistent with another stimulus to or alternatively a relief from inhibition of insulin release (22). Characterization of the time of onset of hyperinsulinemia, hyperphagia, central obesity, and insulin resistance, and of any changes in the postnatal leptin axis, after prenatal restriction would help to identify causality and its direction from among these various potential mechanisms.

Although MPR per se did not alter neonatal FGR, pups that were small at birth did exhibit such “catch-up growth,” in terms of weight. In humans, indirect measures of skeletal and nonskeletal catch-up growth later in childhood have been associated with increased risk of developing insulin resistance and type 2 diabetes (11, 44). Increased rates of type 2 diabetes were observed in elderly men and women who were small at birth but had caught up in terms of weight or height at 7 yr of age and continued to grow at an accelerated rate to 15 yr of age (11). Similarly, the highest plasma fasting insulin concentrations have been reported in children who were small at birth but in the highest quintile for ponderal index at 10 yr of age, suggesting that insulin sensitivity may be lower in low birth weight children who grow more quickly postnatally (44). However, body size measures at 7–10 yr of age may reflect the development of obesity in childhood rather than catch-up growth after fetal growth restriction, as the latter occurs mostly in the first 2–3 mo of life. In the current study, male offspring that underwent accelerated growth as neonates had higher fasting insulin as adults. However, this was not independent of birth weight. Further studies directly manipulating postnatal growth in offspring of varying size at birth are required to determine whether accelerated neonatal growth in guinea pigs adversely influences adult insulin sensitivity.

In summary, moderate MFR in the guinea pig restricted fetal growth and produced hyperinsulinemia,
hyperphagia, and increased visceral adiposity in adult male offspring. When offspring were divided into ter-
tiles according to weight at birth, fasting plasma insulin and food intake were highest in the low birth weight
male offspring, suggesting that some of the effects of MFR may be mediated through alterations in prenatal
growth.

Perspectives

This study has shown that some of the elements of the insulin resistance syndrome can be induced by mild
or moderate perturbation of maternal nutrition and prenatal growth in the male guinea pig. This supports
the suggestion that restricted prenatal growth is caus-
ally related to such outcomes in humans (2, 3). We have
previously reported that prenatal growth restriction is
associated with perturbed postnatal cholesterol ho-
meostasis in the male guinea pig (23). Thus the guinea
pig may be an appropriate species in which to further
investigate the underlying physiological and endocrine
mechanisms contributing to the development of post-
natal insulin resistance and associated sequelae in
prenatally growth-restricted male offspring. Glucose
tolerance was not impaired in offspring of feed-re-
stricted mothers compared with those of normally fed
mothers, however, possibly reflecting their compensa-
tory hyperinsulinemia. Prenatally induced alterations
in insulin sensitivity, in combination with factors such
as aging or development of obesity due to hyperphagia,
may nevertheless result in the subsequent develop-
ment of diabetes and other elements of syndrome X in
this species. Thus studies in older adult guinea pigs
may allow further investigation of the mechanisms
underlying the development of impaired glucose toler-
ance of the prenatally growth-restricted individual.

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