Pre-diabetes in maternal nutrient reduced baboon offspring

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

Jaehyek Choi¹, Cun Li¹, Thomas J McDonald¹, Anthony Comuzzie², Vicki Mattern² and Peter W. Nathanielsz¹

J. Choi and Cun Li contributed equally to the paper.

¹Center for Pregnancy and Newborn Research, Dept. OB/GYN, The University of Texas Health Science Center San Antonio and ²Dept. Genetics, Southwest Foundation for Biomedical Research, Southwest National Primate Research Center, San Antonio, TX

Running head: Juvenile baboon insulin resistance following maternal nutrient reduction

Corresponding Author: Peter W. Nathanielsz, MD, PhD, ScD
Professor and Director
Center for Pregnancy and Newborn Research
The University of Texas Health Science Center San Antonio
7703 Floyd Curl Drive, MSC 7836
San Antonio, TX 78229-3900
Tel: (210) 567-5055
FAX: (210) 567-5033
Email: Nathanielsz@uthscsa.edu

Copyright © 2011 by the American Physiological Society.
Pre-diabetes in maternal nutrient reduced baboon offspring

**ABSTRACT**

**Objective:** 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 post-natal β-cell function and peripheral insulin sensitivity that lead to emergence of a pre-diabetic state prior to puberty. **Research Design and Methods:** Pre-pregnancy phenotype of 18 non-pregnant 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. **Results:** At 3.5 ± 0.18 years (mean ± SEM) in an IV glucose tolerance test, conscious tethered MNR juvenile offspring (two females and four males) showed increased fasting glucose (p < 0.04); fasting insulin (p < 0.04), and insulin area under curve (AUC; p < 0.01) compared with controls (eight females and four males). Insulin AUC also increased following an arginine challenge (p < 0.02). Baseline HOMA insulin β-cell sensitivity were greater in MNR offspring than controls (p < 0.03). In a hyperinsulinemic, euglycemic clamp, glucose disposal rate decreased 26% in MNR offspring. Changes observed were not sex dependent. **Conclusions:** 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.

**KEY WORDS:** Pancreas, developmental programming, baboon, nutrient restriction, diabetes
Introduction

There is compelling evidence from human epidemiological studies (5; 20) and controlled animal investigations (2; 3; 19; 20; 26) indicating that 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 rodents species (25; 31; 36) studies in precocial species are limited to the sheep a species in which post weaning metabolism is very different from primates (11; 27; 34). To date there are no data in nonhuman primates of similar developmental programming of impaired pancreatic development resulting from MNR to evaluate the potential for extrapolation of outcomes of the challenge of maternal – and hence fetal, nutrient reduction on human perinatal pancreatic development and predisposition to type 2 diabetes in later life.

We previously demonstrated that in female baboons carefully selected to be of similar age and phenotype at conception, consumption of 70% of the global ad libitum diet eaten by controls in pregnancy decreases number, size and insulin staining density of pancreatic islets in baboon fetuses at term (10). Additional evidence of impaired fetal organ development was observed in the fetal kidney (decreased tubular density and altered expression of key genes) (8) and fetal liver (increased liver glycogen and protein expression for the rate limiting gluconeogenic enzyme phosphoenolpyruvate carboxykinase (PEPCK) (8; 21). Changes were also observed in the IGF system in the developing fetal brain (1).

There is currently a world-wide epidemic of obesity and diabetes occurring in young children at an earlier and earlier age (13) and it has been hypothesized that maternal nutrition in pregnancy plays a role. Maternal nutrient reduction and consequent decreased fetal nutrition, is a major problem worldwide and is not restricted to the developing world. Even in the developed world, food insecurity affects a significant portion of the population. Worldwide, 852 million people experienced food insecurity in 2004 (33). In addition teenage pregnancy where the growing mother is competing with her fetus (4), pregnancy occurring in the later stages of reproductive life associated with maternal vascular disease, and placental pathology all lead to decreased flow of nutrients to the fetus. We hypothesized that moderate levels of MNR would result in developmental programming of post-natal β-cell function and peripheral insulin sensitivity that lead to emergence of a pre-diabetic state prior to puberty.
Pre-diabetes in maternal nutrient reduced baboon offspring

MATERIALS AND METHODS

Animal care

The eighteen baboons (Papio species) 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 years, mean ± SEM) and morphometric phenotype. These non-pregnant 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 (GSE 665; GSE Scale Systems, Milwaukee, MI). Water was continuously available in the feeding cages via individual waterers (Lixit, Napa, CA). Animals ate Purina Monkey Diet 5038 (Purina, St Louis, MO, USA). 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 (approx 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 8 female and 4 male offspring and the MNR group 2 female and 4 male offspring. At nine months of age offspring were fully weaned and were removed to juvenile group housing and fed chow ad libitum. At 3.5 ± 0.18 years of age the eighteen 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 twenty minutes. All animals were jacketed while tranquilized with ketamine (10mg.kg⁻¹) on the day they arrived in the indoor facility. After four weeks they were fitted with a training tether and one week 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 described these procedures in detail (14).
Pre-diabetes in maternal nutrient reduced baboon offspring

Surgery for catheter instrumentation

Food was withheld overnight prior to surgery. Prior to surgery baboons received ketamine (10 mg kg⁻¹) and glycopyrrolate (125 ug kg⁻¹) and 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 back were shaved. The animal was transferred to the surgical suite, intubated and maintained on isoflurane anesthesia (2%). Using standard sterile technique, an incision was made over the femoral blood vessels and side branches of the artery and vein were exposed and cleared of surrounding tissue. Two catheters were inserted into the femoral vein and one catheter into the femoral artery and directed centrally to lie in the inferior vena cava and dorsal aorta. Throughout the study catheter patency was maintained with heparin saline (10 IU ml⁻¹). After catheter insertion, a trochar was tunneled under the skin from the incision site to the middle of the back. The catheters were passed through the trochar to exit the skin which was then closed with 2-0 sorbcryl suture. Post-surgical analgesia was provided by buprenorphine 0.01 mg kg⁻¹ for three days. Ampicillin 20 mg kg⁻¹ was provided i.v for five days. The jacket and catheters were attached to the swivel system allowing free movement. Catheters were maintained patent by heparin saline infusion (10 IU ml⁻¹) and flushed two times a day. One week was allowed for recovery from surgery before undertaking any studies. All studies occurred in individual cages with animals fully conscious without pharmacological agents.

Intravenous glucose tolerance test (IVGTT)

At 0800 after an overnight fast of 16 hours, 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⁻¹, 20% Dextrose, Hospira Inc. Lake Forest, IL, US over 30 seconds). Blood samples (1.5 ml) were collected at: 1, 2, 3, 4, 5, 6, 8, 10, 15, 20, 30, 40, 50, 60 min. At 90 min 5g of L-arginine (# A6969, Sigma-Aldrich. St. Louis, MO, US, 30 ml of distilled water,) was given as a single bolus through the femoral vein catheter and blood sampled at 92, 94, 96, 98, 100, 110, 120 and 130 min. Sample tubes were immediately quenched in iced water and centrifuged at 4C (4000 rpm min⁻¹). Both EDTA plasma and serum were obtained and stored at -80 C until assayed for glucose, insulin and c-peptide. Area under the curve for insulin and glucose during the IVGTT were calculated using the trapezoidal rule.
Hyperinsulinemic euglycemic insulin clamp was conducted in the conscious baboon as previously described in detail (6). One week was allowed to elapse following the IVGTT. Briefly, after an overnight fast glucose and insulin were infused into the femoral vein and blood samples removed from the femoral artery. At time zero, insulin was infused at 60 mU.m⁻².min⁻¹ to raise plasma insulin concentration by approximately 100 μU.ml⁻¹. After the start of the insulin infusion, a 20% glucose infusion was begun and plasma glucose concentration measured every 5 min to adjust the glucose infusion rate to maintain plasma glucose of around 90 mg.dl⁻¹ and plasma insulin and c-peptide measured every 15 min. At the end of the study two samples were taken at 115 and 120 min to ensure equilibrium.

HOMA-β cell function percentage

HOMA-β cell function percentage was calculated by 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 inter assay CV of 4.6%. Insulin was analyzed by chemiluminescence in a Luminex 1000 using the Endocrine Multiplex Immunoassay (Linco Research, Inc., St. Louis, MO) with an inter assay CV of 7.7%. C-peptide was analyzed by ELIZA (Millipore, St. Charles, MI; #EZHCP-20K) with an inter assay CV of 5.0%. All samples were analyzed in duplicate and all samples for each analyte were assayed in the same assay.
Pre-diabetes in maternal nutrient reduced baboon offspring

Statistical analysis

Area under the curve 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 a two-way analysis of variance (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 skewness, fasting insulin, insulin AUC 0-60, Insulin AUC, 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, North Carolina) was used throughout. Data are presented as Mean ± SEM.

RESULTS

Initial statistical analyses

We found no evidence of heterogeneity of the treatment effect by sex, body weight or age and no significant sex effect and 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 CTR 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 by the mothers decreases well before nine months 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 \pm 0.03 kg) did not differ from males (0.98 \pm 0.08 kg; p > 0.05.). At 3 months of age male MNR offspring weighed less than control males (Figure 1). At the time of the studies, female CTR offspring weighed more than female MNR offspring (10.3 \pm 0.6 vs. 8.45 \pm 0.59 kg; p < 0.03) while weights did not differ in the two groups of males (10.75 \pm 1.14 vs. 9.96 \pm 0.67 kg).

Intravenous glucose tolerance test

Fasting plasma insulin (p<0.04) and glucose (p<0.05) were both elevated in MNR compared with control offspring while fasting C Peptide was not different between the two groups (Figure 2).

Figure 3 shows the area under curve during the first 60 minutes 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 area under curve was not different in the two groups (Fig 3C). However, the increase in insulin (77%) in MNR compared with CTR 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 area under curve 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 \( \beta \)-cell function increased in offspring of MNR (6.57 \pm 2.08) compared with control mothers (2.74 \pm 2.74; 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 4A, B and C). Glucose disposal rate was significantly reduced in the MNR offspring (p < 0.03; Fig 4 D).

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 10\textsuperscript{th} 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. 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 pre-eclampsia 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 percent of U.S. households (17.4 million
people) were food insecure at some time during 2009 and 9.0 percent of U.S. households (10.6
million people) had low food security while 5.7 percent 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 this 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 post-natal 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 post-natal over-nutrition associated with western obesogenic, diabetogenic diets which 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 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 gluconeogenic capacity of the fetal liver by increasing phosphoenolpyruvate carboxykinase (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) 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 which was confirmed by the decreased glucose disposal rate in the MNR offspring. Insulin secretion in response to amino acids has been used as a non-glucose challenge to determine β-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...
Pre-diabetes in maternal nutrient reduced baboon offspring

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 post-natal 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 year 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. while in our 3.5 year old animals the glucose disposal rate averaged 29.1 mg.kg⁻¹.min. There are several potential explanations for this difference. Although BMI is a somewhat inadequate metric in quadrupeds, in the study on 20 year old baboons it was about 24.5 (6) while in our juveniles it was 17.3 in controls and 16.1 in the MNR offspring. While body fat measurements are not available in either group, the BMI data would suggest that the older baboons had more fat tissue that would contribute to the lower disposal rate. Insulin resistance increases with age (24) and thus age related differences and effects of the general anesthesia are also potential explanations of the differences. Further studies need to be conducted on baboons at several ages to resolve these possibilities.

Our statistical analysis showed no difference according to offspring sex and the imbalance between the sexes in the two groups in our study is unlikely to have affected our results since the study by Chavez et al (6) showed no difference in glucose disposal rate in male and female baboons.

In his 2009 Banting lecture De Fronzo writes “It now is recognized that the β-cell failure (associated with Type 2 diabetes) occurs much earlier and is more severe than previously thought.” (9). We would agree. In addition our data indicates that, in this model of developmental programming, increased peripheral resistance to insulin, indicated by the decreased glucose disposal, is an early event. Our data support the view that the increased demand placed on the pancreas for insulin in situations in which pancreatic development has been compromised during development would predispose to early pancreatic failure and onset of type 2 diabetes.

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.
Acknowledgements

We would like to thank Karen Moore and Susan Jenkins for their assistance with the manuscript and data archiving. Joel Michalek, PhD, Professor and Vice Chair, Department of Epidemiology and Biostatistics, University of Texas Health Science Center at San Antonio, provided assistance with the statistical analysis. This work was supported by NIH: NIDDK 1R21DK085420-01 for the specific conduct of this study and 5R24RR21367-4, and HD 21350 – 19, 1C06RR011715, 1C06RR013556.

Disclosures: Authors have none to make.
Pre-diabetes in maternal nutrient reduced baboon offspring

Reference List


Pre-diabetes in maternal nutrient reduced baboon offspring


Pre-diabetes in maternal nutrient reduced baboon offspring


Pre-diabetes in maternal nutrient reduced baboon offspring


Pre-diabetes in maternal nutrient reduced baboon offspring


Pre-diabetes in maternal nutrient reduced baboon offspring


Pre-diabetes in maternal nutrient reduced baboon offspring

FIGURE LEGENDS

**Fig 1** Growth of offspring of *ad lib* fed mothers (controls, filled circles; female n = 8, male n=4) and mothers fed 70% of food consumed by controls (open circles; female n = 2, male n=4). *p < 0.05.

**Fig 2** Fasting blood values: A) glucose, B) insulin and C) C Peptide. Offspring of control mothers (CTR; n=12) and offspring of MNR mothers fed 70% of the controls through pregnancy and lactation (MNR; n=6). Data are mean ± SEM; *p < 0.05; † p < 0.04.

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

**Fig 4** Hyperinsulinemic euglycemic clamp data: A) Final glucose level; B) final insulin level; C) final C Peptide level and D) glucose disposal rate (CTR; n=12, MNR; n=6). Data are mean ± SEM ; # p<0.03

**Table 1.** Maternal and offspring morphometrics  ##p< 0.06; *p < 0.05
Figure 1.
Figure 3.
Figure 4.
Table 1.

<table>
<thead>
<tr>
<th></th>
<th>Control offspring</th>
<th>MNR offspring</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>n</strong></td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td><strong>Maternal morphometrics</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age at conception (years)</td>
<td>11.1 ± 0.69</td>
<td>12.2 ± 0.66</td>
</tr>
<tr>
<td>Weight pre-pregnancy (kg)</td>
<td>13.4 ± 0.58</td>
<td>14.0 ± 0.62</td>
</tr>
<tr>
<td>Weight at delivery (kg)</td>
<td>14.4 ± 0.61</td>
<td>12.1 ± 0.7*</td>
</tr>
<tr>
<td>Weight at weaning (kg)</td>
<td>14.8 ± 0.53</td>
<td>12.6 ± 0.50*</td>
</tr>
<tr>
<td><strong>Offspring morphometrics</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration of gestation (days)</td>
<td>182.3 ± 2.30</td>
<td>183.0 ± 2.46</td>
</tr>
<tr>
<td>Birth weight (kg)</td>
<td>0.9 ± 0.04</td>
<td>0.8 ± 0.03##</td>
</tr>
<tr>
<td>Age at study</td>
<td>3.7 ± 0.21</td>
<td>3.4 ± 0.09</td>
</tr>
<tr>
<td>Weight at study</td>
<td>10.5 ± 0.53</td>
<td>8.5 ± 0.59*</td>
</tr>
<tr>
<td>BMI at study</td>
<td>17.3 ± 0.29</td>
<td>16.1 ± 0.33*</td>
</tr>
<tr>
<td>Surface area at study</td>
<td>0.6 ± 0.02</td>
<td>0.52 ± 0.03*</td>
</tr>
</tbody>
</table>