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The IGF Axis in Baboon Pregnancy: Placental and Systemic Responses to Feeding 70% Global *Ad Libitum* Diet

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Abstract

Information on the influence of poor maternal nutrition on the regulation of responses to pregnancy, placental and fetal growth and development is critical to a better understanding of pregnancy physiology and pathophysiology. We determined normal changes and effects of controlled and monitored moderate nutrient restriction (NR) (global nutrient intake reduced to 70% of food consumed by mothers feeding *ad libitum* from 0.16 to 0.5 of gestation) in the baboon, on important hematological, biochemical, and hormonal indices of fetal growth and placental function. Serum IGF-I:IGFBP-3 ratio was lower in pregnant than control non-pregnant baboons feeding *ad libitum*. Serum concentrations of total and free IGF-I were decreased in NR mothers compared with controls ($p < 0.05$). The decrease in fetal IGF-I did not reach significance ($p = 0.057$). Serum IGF-I: IGFBP-3 ratio was decreased by NR in both mothers and fetuses. Maternal serum IGF-II was unchanged by NR. Placental IGF-I mRNA and protein abundance were similarly reduced whereas IGF-II mRNA increased in placental tissue of NR compared to control mothers. Systemic (maternal) and local (placental) IGFBP-1 and IGFBP-3 mRNA and protein abundance were unchanged by NR. Type 1 IGF receptor protein in the syncytiotrophoblast increased in NR. Type 2 IGF receptor protein was present in the stem villi core, and decreased after NR. We conclude that moderate NR in this important non-human primate model significantly disrupts the maternal and placental IGF-IGFBP axis and influences placental expression of this key system at the gene and protein level. Changes observed appear to be directed toward preserving placental growth.

Keywords

Placenta; IGF system; Maternal nutrient restriction; Baboon

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1. Introduction

Placental nutrient transport from mother to fetus is essential for placental and fetal growth [1]. The fetus regulates placental function through signals it sends to the placenta. Insulin-like growth factors (IGFs: IGF-I and -II), high affinity IGF binding proteins (IGFBPs), and related IGF receptors (IGF-1R and IGF-2R) comprise part of the complex communication network between mother, placenta, and fetus [2]. IGFs influence autocrine and paracrine regulation of cell proliferation, migration, and survival. The placental IGF-IGFBP-receptor axis is a major influence on placental function and fetal growth [3].

Most circulating IGF arises from hepatocytic synthesis, which is regulated by nutrient availability. Changes in maternal or fetal nutritional status (such as hypoxia or nutrient deprivation) impact both systemic and placental IGF actions (reviewed in ref. [4]). The placental IGF axis has been extensively studied in rats, sheep, horses, guinea pigs and non-human primates [5,6]. However, the placenta differs across species in its structure, conductive and exchange capacities, the mechanisms employed in nutrient exchange (reviewed in ref. [7]), and IGF expression and regulation [8]. Studies conducted in non-human primates improve our understanding of the mechanisms underlying human placental function. Diverse models of maternal nutrient restriction have been reported in a wide range of animals: protein restriction (33–60%) in rats, pigs [9], and rhesus monkeys [10]; micronutrient restriction in rats [11]; and global nutrient restriction (30–100%) in rats, sheep, and guinea pigs. Surprisingly, no data has been reported from non-human primates regarding effects of maternal nutrient restriction on placental IGF and systemic IGF changes. Such data are essential for understanding the mechanisms that promote abnormal fetal growth and maturation in the setting of decreased nutrient availability to the fetus.

The goal of this study was to determine the effects of carefully controlled and monitored restriction of maternal nutrition on the IGF-IGFBP-receptor axis of the pregnant baboon (*Papio* sp.). Nutrient-restricted mothers were fed 70% of the weight matched food intake consumed by a matched *ad libitum* fed control group.

2. Methods

2.1. Animals

2.1.1. Animal care and maintenance—The pregnant baboons (*Papio* species) studied belonged to a colony numbering approximately 3800 and maintained by the Southwest National Primate Research Center (SNPRC) at the Southwest Foundation for Biomedical Research (SFBR, San Antonio, TX). All animals were housed in outdoor metal and concrete gang cages, each containing 10–16 females and 1 male. Details of housing and environmental enrichment have been published elsewhere [12]. All procedures were approved by the SFBR Institutional Animal Care and Use Committee.

2.1.2. System for controlling and recording individual feeding—The feeding system used has been described in detail elsewhere [12]. Briefly, once a day prior to feeding, all baboons were run into individual feeding cages. Baboons passed along a chute, over a scale, and into an individual feeding cage. The weight of each baboon was obtained as it crossed an electronic scale system (GSE 665; GSE Scale Systems, MI). The weight recorded was the mean of 50 individual measurements over 3 s. If the standard deviation of the weight measurement was greater than 1% of the mean weight, the weight was automatically discarded, and the weighing procedure was repeated.

Once housed in an individual cage, each animal was fed between either 07:00–09:00 h or 11:00–13:00 h. Water was continuously available in the individual feeding cage and the group

cages through individual and group litters. Animals were fed Purina Monkey Diet 5038 (Purina, St. Louis, MO), described by the vendor as “a complete life-cycle diet for all Old World Primates.” The biscuit contains stabilized vitamin C as well as all other required vitamins. Its basic composition is crude protein not less than 15%, crude fat not less than 5%, crude fiber not more than 6%, ash not more than 5%, and added minerals not more than 3%. At the start of the feeding period, each baboon was given 60 biscuits in the feeding tray of the individual cage. At the end of the 2-h feeding period, the baboons were returned to the group cage. Biscuits remaining in the tray, on the floor of the cage, and in the pan beneath the cage were counted. Food consumption of animals, weights, and health status were recorded each day. The amount of calories, protein, fat and carbohydrates received by the control group in non-pregnant stage and during period of 30–90 days of gestation was 77.4 ± 2.3 kcal/kg per day and 64.3 ± 4.6 kcal/kg per day, 46.2 ± 1.6 g/day and 37.2 ± 2.3 g/day, 33.6 ± 1.1 g/day and 27.1 ± 1.6 g/day, 181.0 ± 6.1 g/day and 145.7 ± 8.8 g/day respectively. The food consumption of NR group for the non-pregnant and pregnant stages (30–90 days of gestation) were 78.0 ± 3.0 kcal/kg per day and 45.7 ± 1.0 kcal/kg per day, 44.2 ± 2.0 g/day and 24.6 ± 0.8 g/day, 32.2 ± 1.5 g/day and 17.9 ± 0.6 g/day, 173.4 ± 7.8 g/day and 96.6 ± 3.1 g/day, respectively.

2.1.3. Study design—Fertile female baboons were selected to participate in this study on the basis of their reproductive age (8–15 years old), body weight (10–15 kg), and absence of genital and extragenital pathological signs. Initially, animals were placed into two group cages with a vasectomized male to establish a stable social group [12]. Assignment to the each group was random. At the end of the acclimation period to the group and to feeding in the individual nutritional cages (30 days), a fertile male was introduced into each breeding cage. All baboons were observed twice daily for well-being and three times per week for turgescence (swelling), color of sex skin, and signs of vaginal bleeding to enable timing of ovulation and subsequent conception.

Pregnancy was dated initially by timing of ovulation and changes in sex skin color and confirmed at 30 days gestational age (dG) (full term in this species is 180 dG) by ultrasonography. The first cohort of pregnant baboons ($n = 8$) were assigned to the control group and established the food consumption per kilogram of body weight on a daily basis from 30 to 90 dG.

Subsequently, the nutrient restriction (NR) group consisted of six pregnant animals who received 70% of the average daily amount of feed eaten (on a weight adjusted basis) by the eight animals fed *ad libitum* at the same gestational age.

2.1.4. Morphometry—The techniques used for general morphometric measurements prior to entry into the study are described elsewhere [13]. Fetal morphometric measurements were undertaken immediately after cesarean section at necropsy. Biparietal diameter, length of the dorsal-ventral abdominal axis at the level of umbilicus, and ano-genital distance were assessed with the Lange skin fold caliper (Beta Technology Inc., Cambridge, MD). Fetal length was measured from the crown to the heel of the right foot.

2.1.5. Blood sampling—Following tranquilization with ketamine HCl (10 mg/kg, i.m.), a fasting femoral venous blood sample was drawn from the non-pregnant female baboons at the time of the pre-pregnancy morphometric measurement. The inguinal site was clipped and prepared using 70% alcohol. A Precision Glide Vacutainer System (20–22 gauge needle, holder and blood tubes from Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ) was used, allowing multiple samples to be taken. One sample was drawn into a 3-mL potassium-EDTA tube (BD Vacutainer) for hematology, and another sample into a clot tube (BD Vacutainer without additives or gel separator) for assessment of serum chemistries and endocrinology. The total volume of blood taken from each animal did not exceed 15 mL. Serum and plasma

were separated by centrifugation at $10,000 \times g$ over 10 min at 4 °C, flash frozen in liquid nitrogen, and stored at -80 °C until analysis.

2.1.6. Cesarean sections—Cesarean sections were performed at 90 dG ($n = 14$) using standard techniques that have been previously described in detail [14]. Briefly, mothers were given ketamine HCl (10 mg/kg, i.m.) as described above. After intubation under isoflurane (4%, 2 L/min) administered by face mask, isoflurane (2%, 2 L/min) was used to maintain a surgical plane of anesthesia throughout surgery and umbilical cord blood sampling. Following hysterotomy, the umbilical cord was identified and elevated to the surgical opening for blood sampling. The fetus was euthanized by exsanguination while still under general anesthesia. The placenta was removed from the uterus and immediately submitted, together with fetus, for measurements, complete pathologic evaluation, and sampling. The uterus and abdominal wall were closed in layers. Post-operative analgesia was provided for 3 days (buprenorphine HCl, 0.015 mg/kg per day; Buprenex[®] Injectable, Reckitt Benckiser Health Care Ltd., Hull, UK). Ampicillin (25 mg/kg, i.m., BID) was given postoperatively for 7 days. Post-surgical management has been described in detail [14]. Animals recovered in an individual cage and were usually sitting in less than 2 h. They were observed daily until recovery by an experienced technician. Animals were released to gang cages if incisions were well healed after 2 weeks. For at least 3 months after cesarean section, females were housed with a vasectomized male to ensure that the incision was fully healed occurs before the animal became pregnant again.

2.2. Measurements of total IGF-I, total IGF-II, total IGFBP-3, free IGF-I, total IGFBP-1, growth hormone (GH), prolactin (PRL), estradiol (E2) and progesterone (P4)

Growth factor and hormone levels (IGF-I, IGFBP-3, GH, PRL, E2 and P4) in baboon serum were measured on an Immulite 2000 instrument using human kits (Diagnostic Products Corporation, Los Angeles, CA), after validating their applicability for baboon serum by demonstration of parallelism. In addition, five pooled serum samples from this study were measured in at least triplicate, both within the same assay [to give intra-assay coefficients of variation (CV) of less than 8.5%] and in separate assays [to provide inter-assay CV less than 11.7%] for all assays. When levels were out of range, samples were diluted with the assay buffer provided with the kits and measured again. Total IGF-II, total IGFBP-1, and free IGF-I concentrations in maternal serum and amniotic fluid were determined by enzyme-linked immunosorbent assay (Diagnostic Systems Laboratories, Inc., Webster, TX). This free IGF-I assay uses ethanol extraction to eliminate the IGF bound to IGFBPs. All kits were based on human protein. The cross-reactivities between the IGF-I and IGF-II assays and among the specific IGFBP ELISAs were less than 3%. Samples were measured in duplicate. Intra-assay CV was below 7%, and inter-assay CV was below 7.9%.

2.3. Hematology

Hematological data were obtained from a Coulter MAXM autoloader instrument (Beckman Coulter, Inc., Fullerton, CA) as previously described [15]. EDTA blood smears were prepared and stained with a modified Wright stain (Accustain, Sigma Diagnostics, St. Louis, MO) and reviewed manually. The following parameters were measured automatically: white blood cell (WBC) and red blood cell (RBC) counts, hemoglobin concentration, hemato-crit, mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), RBC distribution width (RDW), platelet count, mean platelet volume (MPV), and differential counts of neutrophils, lymphocytes, monocytes, eosinophils, and basophils. Samples were stored at 22 °C and analyzed within 1 h of collection. When necessary, data for WBC counts were reviewed manually to correct for nucleated red blood cells (NRBC).

2.4. Blood chemistry

We assessed circulating levels of glucose, blood urea nitrogen (BUN), creatinine, total protein, albumin, globulin, albumin/globulin ratio (A/G ratio), cholesterol, serum glutamic-pyruvic transaminase (SGPT), serum glutamic-oxaloacetic transaminase (SGOT), alkaline phosphatase, sodium, potassium, chloride, carbon dioxide, and anion gap. Blood for serum analysis was collected without anticoagulant. Clotted blood was centrifuged within 1 h of collection and the serum removed. Hemolyzed samples were not evaluated. Blood biochemistry was performed using a Beckman Synchron CX5CE (Beckman Coulter, Inc., Fullerton, CA) [15].

2.5. In situ hybridization of IGF-I, IGF-II, IGFBP-1 and IGFBP-3

Placental paraffin sections (5 μ m thick) from control and maternal NR groups were analyzed for IGF-I, IGF-II, IGFBP-1, and IGFBP-3 mRNA expression by *in situ* hybridization using 35 S-labelled antisense riboprobes as described elsewhere [16]. Briefly, tissue sections were deparaffinized, rehydrated, and treated with proteinase-K. Following prehybridization, tissue sections were hybridized overnight at 42 °C–45 °C with 35 S-labelled anti-sense riboprobes specific for each probe. Excess probe was washed and the tissue sections were treated with RNase to cleave unhybridized probe. Tissue sections then underwent washes in decreasing salt buffers (1.0 $\times$ SSC to 0.1 $\times$ SSC) at specific temperatures for each probe. Following dehydration, slides were exposed to X-ray film overnight at –80 °C to determine the signal intensity that provided guidance for the length of exposure of the nuclear track emulsion. Slides were then coated with nuclear track emulsion and exposed at 4 °C under dessicating conditions. Slides were developed, fixed, counter-stained with hematoxylin and eosin and cover-slipped under Permount® (Fisher Scientific). Tissue sections were viewed under both dark and bright fields, and appropriate photomicrographs were obtained at 20 $\times$ and 40 $\times$ magnifications using ImagePro Plus 4.5 software. Quantification was performed on one randomly chosen slide from each animal and we took six pictures at 2, 4, 6, 8, 10, and 12 o'clock on the slide. Each picture covered 2,586,000 square microns. The percentage of positive staining was recorded relative to the total area interrogated. Appropriate sense control was used for each anti-sense probe.

2.6. Immunohistochemistry for IGF-I, IGF-II, IGFBP-1, IGFBP-3, IGF-1R and IGF-2R

Paraffin tissue sections were deparaffinized in xylene, rehydrated in descending grades of alcohol (100%, 70%, and 45% in water), immersed in citrate buffer (0.01 M citrate buffer, pH 6.0), and heated to boiling for 10–15 min. After cooling for 15 min, sections were rinsed serially (7 times, 6 min each) in potassium phosphate-buffered saline (KPBS: 0.04 M K_2HPO_4 , 0.01 M KH_2PO_4 , 0.154 M NaCl, pH 7.4), then washed for 10 min in a solution of 1.5% H_2O_2 /methanol and for 5 min in KPBS. Sections were placed in diluted (10%) normal serum for 20 min and covered with primary antibody overnight at 4 °C. After overnight incubation, sections were rinsed in KPBS and incubated for 1 h at 22 °C with secondary antibody (1/1000 dilution)—biotinylated anti-rabbit IgG (IGF-I and IGFBP-3) or anti-goat (IGF-II, IGF-2R and IGFBP-1) for polyclonal antibodies and anti-mouse IgG for monoclonal antibodies. The antibodies for IGF-2R (goat polyclonal, dilution 1:50), IGFBP-1 (goat polyclonal, dilution 1:100), and IGFBP-3 (rabbit polyclonal, dilution 1:250) were from Santa Cruz, IGF-II was from R&D (goat polyclonal, dilution 1:500), IGF-1R (mouse monoclonal, dilution 1:50) and IGF-I (rabbit polyclonal, dilution 1:50) from Lab Vision. Preabsorbed negative control was performed with each antibody.

The sections were then handled as described by the manufacturer: 0.3% solution A (avidin) with 0.3% solution B (biotin) (Vectastain ABC Kit; Vector Laboratories, Burlingame, CA) incubate 1 h at room temperature. Sections were then incubated with DAB (3,3'-diaminobenzidine tetrahydrochloride)/nickel substrate in 0.175 M sodium acetate buffer for

10–20 min and counterstained with 0.2% methyl green. After serial dehydration in 50%, 70%, 95%, and 100% ethanol and Histoclear (3 times, 2–5 min each), sections were mounted in Histomount (National Diagnostics, USA). Images were acquired digitally and analyzed with a 5.1 mega-pixel SPOT (Diagnostics Instruments, Inc., Sterling Heights, MI) color cooled CCD camera (2650 × 1920 pixels) and ImagePro Plus 4.5 software. Quantification was performed on one randomly chosen slide from each animal, and we took three pictures at 3, 6, and 9 o'clock on the slide. Each picture covered 64,900 square microns. The percentage of positive staining was recorded relative to the total area interrogated.

2.7. Statistical analysis

Hormone values were log-transformed before statistical analysis. All parameters compared between control and maternal NR treatment groups were evaluated using Student's two-way unpaired *t*-test. Variables measured in non-pregnant, control-fed and maternal NR animals at 90 dG were compared using ANOVA and Dunnett's test. All data are presented as mean ± SEM. Significance was set at $p < 0.05$.

3. Results

3.1. Morphometry

3.1.1. Maternal body weight during pregnancy in NR and control groups—Control mothers ($n = 8$) weighed 13.7 ± 0.5 kg 30 days before introducing the fertile male, 13.4 ± 0.6 kg at 30 dG, and 13.7 ± 0.4 kg at 90 dG. NR mothers ($n = 6$) weighed 13.0 ± 0.2 kg and 13.0 ± 0.3 and 12.2 ± 0.3 kg at the same gestational stages, respectively. The fall in maternal weight in NR mothers at 90 dG represented a 9.1% weight loss ($p < 0.05$).

Fetal weight in the NR group at 90 dG was 95.4 ± 3.3 g ($n = 6$) compared to 100.9 ± 3.4 g ($n = 8$) for fetuses of control mothers. This reduction in fetal weight of approximately 5% was not statistically significant (Table 1).

3.2. Circulating hormone profiles in non-pregnant and pregnant baboons and effect of maternal NR

Total maternal serum IGFBP-3 level was higher in pregnant control than in non-pregnant baboons ($p < 0.05$), while total circulating IGF-I level was unchanged (Fig. 1A). As a result, the IGF-I:IGFBP-3 ratio was lower in pregnant than non-pregnant baboons ($p < 0.05$) (Fig. 1A).

Maternal serum prolactin concentrations rose ($p < 0.05$), while serum growth hormone concentration was unchanged (Fig. 1B). Levels of fetal serum total IGF-I and total IGFBP-3 were lower than maternal levels in the control group ($p < 0.05$) (Fig. 1B). Compared to maternal values, circulating fetal growth hormone level was higher, whereas circulating fetal prolactin level was lower ($p < 0.05$).

Maternal total IGF-I serum levels were lower at 90 dG after NR ($p < 0.05$ compared to control) (Fig. 1A). Fetal total IGF-I level was not significantly lower after NR (Fig. 1B; $p = 0.06$). Circulating total IGFBP-3 levels were unchanged by NR in both mother and fetus. The IGF-I:IGFBP-3 ratio was lower in serum of NR mothers and their fetuses ($p < 0.05$) than controls (Fig. 1). Maternal and fetal GH and prolactin levels (Fig. 1) and total IGF-II concentration in maternal serum did not differ between control and NR groups (18.7 ± 0.38 and 18.4 ± 0.62 µg/mL, respectively). Total IGF-II concentration in amniotic fluid at 90 dG was not different in NR (390.7 ± 20.61 ng/mL) and control animals (329.9 ± 31.0 ng/mL). Maternal serum IGFBP-1 concentration did not differ at 90 dG between control (130.3 ± 3.4 ng/mL) and NR groups (133.2 ± 2.8 ng/mL). The concentration of free IGF-I in maternal serum was

significantly lower in NR than control animals (0.11 ± 0.01 ng/mL vs. 0.18 ± 0.02 ng/mL, $p < 0.05$). The maternal estradiol and progesterone concentrations in control animals (7128.75 ± 1921.47 pg/ml and 25.57 ± 4.94 ng/ml, respectively) were essentially the same as in NR group (6649.67 ± 2805.2 pg/ml and 36.34 ± 18.5 ng/ml subsequently).

3.3. Circulating metabolic and hematologic variables in pregnant baboons after NR

The only change observed in basic hematologic variables was a small but significant rise in fetal mean cell hemoglobin concentration resulting from NR (Table 2). There was no change in maternal or fetal glucose, and the fetal:maternal glucose ratio was similar (0.66 ± 0.07 in NR pregnancies vs. 0.47 ± 0.07 in CTR pregnancies; $p = 0.06$). In NR mothers and their fetuses, serum BUN decreased ($p < 0.01$ vs. control). NR lowered the ratio of BUN to creatinine and lowered the concentration of alkaline phosphatase in fetal serum ($p < 0.01$) (Table 2).

3.4. IGF-I and IGF-II gene and protein localization and abundance in the placenta by in situ hybridization and immunohistochemistry

IGF-I mRNA was expressed in both the syncytiotrophoblast and villous core (Figs. 2A,B), while protein expression was observed mostly in the villous core (Figs. 2D,E). Both mRNA expression and protein abundance were reduced to a similar extent in placental tissue of NR animals (Figs. 2C,F). Both IGF-II protein and mRNA expression were localized in the syncytiotrophoblast (Figs. 2G,H,J,K). The level of mRNA was increased by NR ($p < 0.05$) (Figs. 2I,L).

3.5. IGFBP-1 and IGFBP-3 gene and protein localization and abundance the placenta by in situ hybridization and immunohistochemistry

IGFBP-1 mRNA was localized in villous core with a low level of expression, which was not changed by maternal NR (Figs. 3A–C). Protein expression of IGFBP-1 was detected in the villous core of both control and maternal NR placentas (Figs. 3D,E). IGFBP-3 mRNA was expressed in the villous core, and IGFBP-3 protein was observed in the syncytiotrophoblast (Figs. 3G,H) but did not increase significantly with NR (Figs. 3I,L).

3.6. Placental IGF receptors by immunohistochemistry

IGF-1R protein abundance was observed in syncytiotrophoblasts and increased in the placentae of the NR group (Figs. 4A–C). IGF-2R was expressed in the core of stem villi, and its abundance decreased in placentae from the NR group (Figs. 4D–F).

4. Discussion

Endocrine and metabolic regulation of fetal growth and development differs greatly from postnatal regulation (reviewed in ref. [7]). Global NR during pregnancy at levels similar to that in our study [17,18], and even more extreme restriction [19], has been extensively studied in sheep, pigs, and rats [17–20]. The IGF system within the placenta and several fetal organs has been shown to respond to both global and other forms of NR during pregnancy. However, to our knowledge this is the first report of data on the IGF system in the placenta in response to maternal NR of any form in a non-human primate.

4.1. Systemic changes in IGF system

4.1.1. Changes between the non-pregnant and pregnant state—We observed no change in circulating maternal total IGF-I level comparing non-parous females with females at the end of the first half of pregnancy, which is consistent with other reports in non-human primates [21] and women [22]. Circulating total IGF-I concentration has been shown to peak after week 35 of gestation in pregnant women [22]. Confirming published data for human

gestation [23], serum GH levels did not differ between non-pregnant and pregnant baboons by mid-gestation in our study. During late gestation, maternal serum concentrations of total IGF-II are 3- to 10-fold higher than IGF-I in all species studied to date, including monkey [24]. Our study confirmed these observations.

4.1.2. Maternal and fetal growth—Maternal weight gain during the first trimester in pregnant women strongly predicts subsequent fetal growth [25]. By 90 dG, NR maternal baboons had lost 9.1% total body weight. However there was no significant difference in fetal weights between control and maternal NR groups. Various NR protocols in other animal species have yielded variable outcomes for fetal organ and total body weights. In sheep, mild and moderate maternal NR (30–50%) during early gestation (the period of maximal placental growth and the time comparable to the maternal NR period in our study) induced no decrease in fetal body weight, consistent with our findings [26]. As in our study, Osgerby et al. [27] detected no differences in placental weight when ewes were exposed to 30% maternal NR during the first half of gestation. Overall, fetal growth is a very poor measure of altered fetal organ development and it is now clear that considerable functional and structural changes can take place in the placenta and fetal organs in the absence of any gross weight change. We have shown that this degree of MNR significantly affects fetal renal [28] (e.g., mTOR related gene expression) and placental (placental villous tree) development [29] at 90 dG in the absence of an overall change in fetal kidney or placental weights.

We observed a fall in fetal:maternal glucose ratio that almost reached significance and may reflect decreased placental glucose transfer. BUN and BUN:creatinine ratio were decreased, consistent with decreased protein turnover and growth. Lower alkaline phosphatase levels in fetal blood after maternal NR would be expected to reflect decreased fetal osteoblast function, a problem that could eventually lead to in-utero growth restriction. We would anticipate greater differences if the maternal NR were carried forward into the later phases of gestation when greater fetal growth occurs.

4.1.3. Normal profiles of circulating components of the baboon IGF-IGFBP axis—The IGF-IGFBP axis is a major regulator of fetal growth and metabolic activity. For example, exogenous IGF-I administered to the pregnant guinea pigs increases fetal and placental weights [30]. Maternal serum total IGF-I levels at 90 dG (the middle of the normal 180-day gestation) in our pregnant baboons were comparable to data reported by Putney et al. [21] for pregnant baboons at 100 dG and for pregnant humans before 35 weeks of gestation [22]. During late gestation, maternal serum concentrations of total IGF-II are 3- to 10-fold higher than IGF-I in all species studied to date: monkey, rats, cattle, pigs, guinea pigs, and sheep. Our study confirmed these observations.

4.1.4. Effects of maternal NR on the maternal and fetal IGF-IGFBP axes—The effects on circulating total IGF-I levels from diverse maternal NR paradigms have been reported for several animal models. The observed decreases in circulating total IGF-I level and IGF-I:IGFBP-3 ratio for both mother and fetus in the presence of moderate maternal NR are consistent with other mammalian studies. In response to 60% global diet restriction in sheep pregnancy, Bispham et al. [17] observed lower maternal total and free IGF-I levels and normal maternal glucose, consistent with our findings in the baboon. IGFBPs stabilize the IGF-I level across the 24-h period. This stability and the exquisite responsiveness of total IGF-I release to nutrient delivery make the free IGF-I level in serum a sensitive marker of nutritional status [31]. Dietary restriction can decrease hepatic IGFBP secretion into the peripheral circulation, thereby increasing the clearance and degradation of circulating IGFs. The change in IGF-I:IGFBP-3 ratio we observed in both fetus and mother likely reflects decreased bioavailability of free IGF-I to fetal and maternal tissues. Shown here for the first time in a non-human primate model, these disturbances are accompanied by evidence of decreased protein metabolism.

Although growth hormone is a powerful regulator of prenatal IGF synthesis, fetal IGF-I production is primarily regulated by glucose (nutrient) availability to the fetus [32]. In sheep, maternal NR has also been shown to reduce fetal growth hormone production [33].

Putney et al. [21] suggested the baboon placenta does not contribute significantly to the circulating maternal IGF-I pool during pregnancy. The liver is the major site of IGF-I synthesis in mammalian adults [34,35], and hepatic production of IGF-I is sufficient to maintain the relatively large amount of IGF-I in the peripheral circulation (at least 50% of total body content) [35]. We observed that 30% maternal NR (fed 70% of food consumed by control animals) was associated with decreased free IGF-I and lower total IGF-I:IGFBP-3 ratio in the fetal circulation. We have also noted a marked fall in hepatic IGF-I mRNA and protein in this model (unpublished observations).

4.2. Changes in the placental IGF system

4.2.1. IGF-I and IGF-II—Maternal IGF-I promotes fetal growth by stimulating nutrient transport in the placenta, influencing placental blood flow and placental function. Maternal IGF-I in its bound form does not cross the placental barrier in rats; however, the placenta transports free IGF [36]. IGF-I gene expression is high in the human placenta at term, but the corresponding protein is undetectable [37,38]. In agreement with data published by Carter and Han, IGF-I mRNA expression in our study was detected in syncytiotrophoblast [5]. However, the greatest protein expression was observed in the villous core. This interesting discrepancy may reflect transport of the protein from its site of synthesis. While mRNA localization mirrors IGF-I synthesis, protein distribution reflects both synthesis and transport to active sites. The presence of IGF-I in the villous core might mirror the involvement of this molecule in the process of vascularization and angiogenesis.

One of the major roles of IGF-II is placental formation and remodeling. IGF-II is produced by the invasive extravillous trophoblast (EVT) in baboons and humans [39–41] and by decidua and trophoblast in guinea pigs [42]. In our study, both IGF-II mRNA and protein expression were detected in syncytiotrophoblast and cytotrophoblast with increased expression in placentae of the NR group. Our data are in agreement with observation of Zollers et al., who found IGF-II mRNA expression in baboon syncytiotrophoblast [6]. Increased placental expression of IGF-II has been detected with IUGR [43]. It is important to note that the placenta discriminates between IGF-II produced within the organ and that produced by the fetus [44]. In our study, the increase in IGF-II mRNA expression clearly indicates increased IGF-II synthesis. Unchanged total IGF-II in the maternal circulation under NR conditions has also been reported in sheep and may indicate that IGF-II abundance is less regulated by nutritional changes in pregnancy than IGF-I.

4.3. IGFBP-1 and IGFBP-3

IGFBP-1 in humans is produced mostly by the decidua at least in early pregnancy, and is thought to play a role in regulating trophoblast invasion [45,46]. Regulation and function of placental IGFBP-1 differs from species to species. For example, guinea pig and murine decidua do not normally express IGFBP-1 [47,48], and sheep have non-deciduate placentae in which IGFBP-3 exerts paracrine-autocrine regulation [49]. These differences have overt consequences. IUGR in humans (but not in guinea pigs, sheep, and rats) is associated with elevated placental IGFBP-1 level. Mice over-expressing placental IGFBP-1 via the AFP promoter demonstrate increased placental weight (150% of wild-type) at e16.5 [50].

IGFBP-3 proteolysis is one of the major regulators of IGF availability in pregnancy serum. IGF-I-independent mechanisms of action of IGFBP-3 are complex, predominantly intracellular, and result in apoptosis or insulin resistance. In the human placenta, IGFBP-3 localizes

immunohistochemically in the syncytium and is considered to be involved in the apoptotic process of pre-eclampsia [51]. We observed a similar location of IGFBP-3 protein in the present study.

4.4. IGF receptors

Our observation of IGF-1R location in the syncytiotrophoblast of placenta parallels data from human pregnancies [52]. Increased IGF-1R protein expression in our study contradicts data published for human IUGR [53]. Our data do agree with findings of Abu-Amro et al. [43] who reported that with IUGR the term placentae displayed higher levels of *IGF2* and *IGF1R* expression compared with the normal term placentae. These changes would increase the local activity of IGF-I and IGF-II, which may be compensating to maintain placental growth in the presence of decreased nutrient availability.

In conclusion, our data indicate that even moderate global dietary restriction—that is, food consumption of 70% of that consumed by *ad libitum* fed animals—significantly disrupts the circulating maternal and fetal IGF-IGFBP axis and influences placental expression of components of this key system at the gene and protein levels. Normal placental function is critical to fetal maturation. Changes in the IGF-IGFBP system in this non-human primate model appear to be directed toward promoting the local growth of the placenta, which is critical to fetal growth and development.

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Fig. 1.

A, Serum measurements in adult female baboons when non-parous (NP, $n = 12$), fed *ad libitum* (control, $n = 5$) at 90 dG, and when fed 70% of control (from 30 to 90 days gestation (dG)) and at 90 dG (NR, $n = 6$). B, Fetal serum measurements at 90 days gestation (dG) in baboons fed *ad libitum* (control, $n = 5$) and 70% of control (NR, $n = 6$) animals. Values are mean $\pm$ SE; SE is too small to show for the IGF-I/IGFBP-3 ratio (A). * $p < 0.05$ vs. control (unpaired t -test).

Fig. 2. IGF-I gene (*in situ* hybridization, first row) and protein (immunohistochemistry, second row) expression in placentae from control (A and D; $n = 6$) and 30% maternal NR (B and E; $n = 6$) groups at 90 dG. Inset (D), Negative control. Quantification for all animals (C and F). IGF-II expression (*in situ* hybridization, third row) and protein location (immunohistochemistry, fourth row) in placentae from control (G and J; $n = 6$) and 30% maternal NR (H and K; $n = 6$) groups at 90 dG. Quantification for all animals (I and L). * $p < 0.05$ vs. control. Magnification 20 $\times$.

Fig. 3.

IGFBP-1 expression (*in situ* hybridization, first row) and protein location (immunohistochemistry, second row) in placentae from control (A and D; $n = 6$) and 30% maternal NR (B and E; $n = 6$) groups at 90 dG. Quantification for all animals (C and F). IGFBP-3 expression (*in situ* hybridization, third row) and protein location (immunohistochemistry, fourth row) in placentae from control (G and J; $n = 6$) and 30% maternal NR (H and K; $n = 6$) groups at 90 dG. Quantification for all animals (I and L). * $p < 0.05$ vs. control. Magnification 20 $\times$ (A, B, G, H, J, K) and 40 $\times$ (D and E).

Fig. 4. IGF1R (first row) and IGF2R (second row) immunohistochemistry in placentae from control ($n = 6$) and 30% maternal NR ($n = 6$) groups at 90 dG. Quantification for all animals (C and F). * $p < 0.05$ vs. control. Magnification 20 $\times$.

Table 1

Morphometric characteristics of fetuses of mothers fed *ad libitum* (CTR) or fed 70% of CTR diet (NR) at 90 dGA (mean $\pm$ SEM)

	CTR <i>n</i> = 8	NR <i>n</i> = 6	<i>p</i> value
Weight (g)	100.9 $\pm$ 3.37	95.4 $\pm$ 3.27	0.276
Length (cm)	17.9 $\pm$ 0.31	17.5 $\pm$ 0.44	0.505
Body mass index (kg/m ²)	3.1 $\pm$ 0.09	3.1 $\pm$ 0.12	0.782
Biparietal distance (mm)	33.5 $\pm$ 0.48	34.2 $\pm$ 0.70	0.437
Dorsal-ventral abdominal distance (mm)	25.9 $\pm$ 3.22	29.2 $\pm$ 1.62	0.426
Femur length (mm)	35.3 $\pm$ 1.13	32.5 $\pm$ 1.12	0.117
Chest circumference (mm)	88.8 $\pm$ 2.27	87.9 $\pm$ 2.27	0.804
Waist circumference (mm)	75.5 $\pm$ 2.48	75.8 $\pm$ 2.71	0.930
Hip circumference (mm)	73.4 $\pm$ 1.70	68.3 $\pm$ 1.67	0.058
Sterno-pubis distance (mm)	67.2 $\pm$ 4.16	70.8 $\pm$ 0.83	0.471
Placenta (g)	70.4 $\pm$ 5.09	62.9 $\pm$ 1.48	0.245
Fetal membranes (g)	11.0 $\pm$ 3.06	7.5 $\pm$ 1.04	0.356
Placenta diameter (cm)	7.9 $\pm$ 0.30	7.3 $\pm$ 0.32	0.147
Umbilical cord length (cm)	14.1 $\pm$ 0.80	11.8 $\pm$ 0.90	0.089
Placental efficiency	1.5 $\pm$ 0.07	1.5 $\pm$ 0.06	0.615
Brain (g)	32.6 $\pm$ 1.75	30.1 $\pm$ 0.52	0.252
Liver (g)	3.3 $\pm$ 0.15	3.2 $\pm$ 0.20	0.687
Spleen (g)	0.6 $\pm$ 0.19	0.3 $\pm$ 0.04	0.233
Kidneys (g)	1.0 $\pm$ 0.15	0.8 $\pm$ 0.13	0.567
Lung (g)	3.5 $\pm$ 0.11	3.4 $\pm$ 0.14	0.435
Heart (g)	0.9 $\pm$ 0.27	0.6 $\pm$ 0.06	0.326
Adrenals (g)	0.4 $\pm$ 0.12	0.3 $\pm$ 0.10	0.700

Table 2

Hematologic and clinical chemistry values in control vs. maternal nutrient restricted (NR) baboons at 90 dG (values are mean $\pm$ SEM)

	Fetus		Mother	
	Control (<i>n</i> = 5–7)	NR (= 3–6)	Control (<i>n</i> = 8)	NR (<i>n</i> = 6)
<i>Hematology</i>				
WBC ($\times 10^9/L$)	1.4 $\pm$ 0.2	2.4 $\pm$ 1.3	11.0 $\pm$ 1.1	9.1 $\pm$ 0.7
RBC ($\times 10^{12}/L$)	2.7 $\pm$ 0.1	2.7 $\pm$ 0.1	4.8 $\pm$ 0.1	5.0 $\pm$ 0.1
Hemoglobin (g/dL)	11.1 $\pm$ 0.2	11.8 $\pm$ 0.1	12.6 $\pm$ 0.3	12.9 $\pm$ 0.4
Hematocrit (%)	34.6 $\pm$ 0.6	36.3 $\pm$ 0.6	37.9 $\pm$ 1.3	38.6 $\pm$ 1.1
MCV (fL)	128.2 $\pm$ 1.4	132.6 $\pm$ 1.4	78.7 $\pm$ 1.0	77.9 $\pm$ 0.6
MCH (pg)	41.0 $\pm$ 0.3	42.8 $\pm$ 0.7 ^a	26.2 $\pm$ 0.3	26.0 $\pm$ 0.4
MCHC (g/dL)	32.0 $\pm$ 0.2	32.3 $\pm$ 0.3	33.3 $\pm$ 0.3	33.4 $\pm$ 0.4
RDW (%)	17.0 $\pm$ 0.3	17.6 $\pm$ 0.5	11.5 $\pm$ 0.2	11.1 $\pm$ 0.2
Platelet ($\times 10^9/L$)	381.0 $\pm$ 19.7	387.3 $\pm$ 19.6	231.8 $\pm$ 22.0	257.7 $\pm$ 31.3
MPV (fL)	8.3 $\pm$ 0.4	8.6 $\pm$ 0.4	9.5 $\pm$ 0.3	9.5 $\pm$ 0.4
Neutrophils (%)	9.0 $\pm$ 4.1	4.7 $\pm$ 2.7	58.1 $\pm$ 3.9	56.2 $\pm$ 6.1
Lymphocyte (%)	89.8 $\pm$ 4.2	94.7 $\pm$ 2.4	36.5 $\pm$ 3.2	37.3 $\pm$ 8.0
Monocyte (%)	0.8 $\pm$ 0.5	0.7 $\pm$ 0.7	2.7 $\pm$ 0.5	2.3 $\pm$ 0.6
Eosinophil (%)	0.7 $\pm$ 0.7	0.0 $\pm$ 0.0	1.9 $\pm$ 0.7	0.7 $\pm$ 0.2
Basophil (%)	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.4 $\pm$ 0.1	0.1 $\pm$ 0.1
<i>Clinical Chemistry</i>				
Glucose (mg/dL)	47.1 $\pm$ 4.4	39.0 $\pm$ 7.4	74.4 $\pm$ 9.6	85.8 $\pm$ 10.6
BUN (mg/dL)	11.0 $\pm$ 0.6	8.0 $\pm$ 0.5 ^a	10.3 $\pm$ 0.7	7.5 $\pm$ 0.4 ^a
Creatinine (mg/dL)	0.8 $\pm$ 0.0	0.8 $\pm$ 0.0	0.9 $\pm$ 0.1	0.9 $\pm$ 0.1
Total protein (g/dL)	2.6 $\pm$ 0.1	2.7 $\pm$ 0.0	6.7 $\pm$ 0.2	6.7 $\pm$ 0.2
Albumin (g/dL)	1.8 $\pm$ 0.0	1.9 $\pm$ 0.0	3.1 $\pm$ 0.2	3.2 $\pm$ 0.1
Globulin (calc) (g/dL)	0.8 $\pm$ 0.0	0.8 $\pm$ 0.0	3.7 $\pm$ 0.2	3.5 $\pm$ 0.1
A/G ratio (calc)	2.5 $\pm$ 0.1	2.4 $\pm$ 0.2	0.8 $\pm$ 0.1	0.9 $\pm$ 0.0
Cholesterol (mg/dL)	65.6 $\pm$ 4.1	58.5 $\pm$ 3.4	51.6 $\pm$ 5.6	42.2 $\pm$ 1.7
Triglycerides (mg/dL)	36.4 $\pm$ 4.9	28.8 $\pm$ 5.5	28.9 $\pm$ 3.4	30.0 $\pm$ 6.1
Alkaline phosphatase (mg/dL)	116.8 $\pm$ 4.4	126.5 $\pm$ 11.6	460.3 $\pm$ 37.2	346.5 $\pm$ 33.3 ^a

^a $p < 0.05$ vs. control by two-sided test.