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Organ and gestational age effects of maternal nutrient restriction on global methylation in fetal baboons

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Abstract

A sub-optimal intrauterine environment alters the trajectory of fetal development with profound effects on life-time health. Altered methylation, a proposed epigenetic mechanism responsible for these changes, has been studied in non-primate species but not nonhuman primates. We tested the hypotheses that global methylation in fetal baboon demonstrates organ specificity, gestational age specificity, and changes with maternal nutritional status. We measured global DNA methylation in fetuses of control fed (CTR) and nutrient restricted mothers fed 70% of controls (MNR) for brain, kidney, liver and heart at 0.5 and 0.9 gestation (G). We observed organ and gestation specific changes that were modified by maternal diet. Methylation in CTR fetuses was highest in frontal cortex and lowest in liver. MNR decreased methylation in 0.5G kidney and increased methylation in 0.9G kidney and frontal cortex. These results demonstrate a potential epigenetic mechanism whereby reduced maternal nutrition has long-term programming effects on fetal organ development.

Keywords

development; nonhuman primate

INTRODUCTION

Adequate maternal nutrition is essential to normal fetal development. Recently there has been much interest in the mechanisms whereby reduced maternal nutrition can alter fetal and placental development in ways that are associated with the emergence of late onset adult disease including hypertension, insulin resistance, hypertriglyceridemia, and altered pituitary adrenal function in both human and rat studies [1,2] Gestational nutritional restriction serves as a model for understanding the mechanisms mediating the long lasting effects of environmental exposures during gestation on adult disease. To date there are relatively few studies that address the mechanisms responsible for the protracted memory of the original insult. Postnatal changes in hepatic gene expression occur in rats fed a diet low in 1-carbon

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donors and this deficiency can be corrected by maternal folic acid supplementation in pregnancy [3]. A mechanism(s) involving changes in gene expression is consistent with the hypothesis that even short periods of maternal nutritional restriction as well as other exposures program gene function.

The term epigenetic refers to mechanisms, which confer long term programming of gene function without changing the DNA sequence. The epigenome consists of the chromatin and its modifications as well as a covalent modification by methylation of cytosine rings found at the dinucleotide sequence CpG [4]. The epigenome determines the accessibility of the transcription machinery. Inaccessible genes are therefore silent; whereas, accessible genes are transcribed.

We tested the hypotheses that global methylation in the fetal baboon 1) demonstrates organ specificity; 2) demonstrates gestational age specific changes and 3) is altered by maternal nutritional status in ways that are both organ and gestational age specific. We measured the global state of DNA methylation in fetuses of control fed (CTR) and fetuses of nutrient restricted dams fed 70% of *ad libitum* fed controls (MNR). We observed both organ and gestational age specific changes that were modified by maternal diet. These results demonstrate a potential epigenetic mechanism whereby reduced maternal nutrition has long term programming effects on fetal organ development. It is of particular interest that the most marked effects of nutrient restriction were observed in the fetal frontal cortex.

METHODS

Animal Care and Maintenance

All procedures were approved by the Southwest Foundation for Biomedical Research (SFBR) Institutional Animal Care and Use Committee and conducted in Association for Assessment and Accreditation of Laboratory Animal Care approved facilities. Twenty-seven pregnant baboons from two independently housed groups each consisting of a maximum of sixteen females and one male were studied. Details of housing structure and environmental enrichment provided have been published elsewhere [2,5]. Maternal morphometric measurements were made prior to pregnancy to ensure homogeneity of females in the two groups [6].

System for controlling and recording individual feeding

The housing system used enabled individual feeding of adult baboons maintained in their group social environment with careful monitoring and control of each animal's diet. Following a rigorous veterinary pre-study entry clinical examination, baboons were housed 16 females together with a single male in a group cage. At feeding time baboons exit the group cage and pass along a chute and over a scale into individual cages where they receive their individual diets [5]. Food intake was monitored and controlled during the stay in the individual cages. At the start of the feeding period, each CTR baboon was given 60 biscuits in the feeding tray. At the end of the 2-hour feeding period after the baboons have returned to the gang cage, the biscuits remaining in the tray and on the floor of the cage and in the pan were counted. Food was provided as Purina Monkey Diet 5038, standard biscuits. The precise diet composition and individual feeding system by which animals were either fed *ad libitum* (CTR; n=15) or nutrient restricted mothers eating 70% of the feed consumed by *ad libitum* fed animals (MNR; n=12) on a weight adjusted basis have been described in detail [5]. The MNR protocol began at 30 days gestation (dG) (0.16 G) after confirmation of pregnancy by ultrasound. Water was continuously available in the feeding cage through individual lixits and at several locations in the group housing. Food consumption of animals, their weights and health status were recorded. Baboons rapidly learned to use this system.

Food intake and weight were stable within 20 days [5]. The maintenance of the group social environment allowed observations on the group's dominance structure. All baboons were observed twice a day for appearance of injuries, stool abnormalities and body condition. Observations of turgescence (sex skin swelling) and signs of vaginal bleeding were made three times per week.

Cesarean sections were performed at 0.5G (Term 185 dG) and 0.9G under isoflurane anesthesia (2%, 2 l/min) to obtain the fetus. Techniques used and post-operative maintenance have been previously described in detail [6]. Briefly, baboons were premedicated with ketamine hydrochloride (10 mg kg⁻¹). After intubation, isoflurane (2%, 2 l min⁻¹) was administered to maintain a surgical plane of anaesthesia throughout surgery and fetal sampling. The abdomen was shaved and iodine surgical scrub followed by 90% alcohol was applied to the skin of the abdomen. A midline lower abdominal incision was made through the skin and subcutaneous layer followed by an incision through the linea alba. The uterus was gently exteriorized and a hysterotomy incision made in the main body of the uterus. Blunt dissection was used to expose the amnion for fluid sampling. The edges of the incision were carefully manipulated and swabbed to avoid blood contamination of amniotic fluid samples that were taken into a syringe and placed in metal-free vials. The umbilical cord was identified and elevated to the surgical opening for sampling. While retaining the fetus within the body of the uterus, umbilical cord venous blood was taken through a 24-gauge needle directed towards the placenta. Fetuses were exteriorized from the uterus and killed by exsanguination while under anaesthesia, an AVMA approved method.

DNA Isolation from Tissue

Tissue was homogenized in cold PBS and centrifuged for 20 min at 4400 rpm to remove particulate matter. The supernatant was transferred to a sterile tube. Cells were lysed by incubation overnight at 55°C with gentle agitation in: 100mM Tris, pH 8.0, 10mM EDTA, pH 8.0, 2% SDS, and 100 µg/ml Proteinase K. DNA was extracted twice with an equal volume of phenol and twice with an equal volume of phenol/chloroform. DNA was precipitated using 0.1x volume 3M NaOAc, pH 6.5 and 2.5x volume absolute ethanol with centrifugation at 4400 rpm for 15 min. The DNA pellet was washed with 70% ethanol and resuspended in ddH₂O [7].

5-Methylcytosine quantification by nearest-neighbor analysis

The principal DNA sequence methylated in vertebrates is the dinucleotide sequence CpG [8]. We used a modified "nearest neighbor" assay [8,9] (Fig. 1). This assay directly measures the extent of methylation in CpG nucleotides by introducing $\alpha^{32}\text{P}$ -labeled dGTP into the DNA followed by digesting the DNA to 3' mononucleotide with a 3' mononuclease leaving the labeled $\alpha^{32}\text{P}$ on the 5' neighbors of G including ApG, TpG, GpG, CpG and 5mCpG. Levels of 5-methylcytosine were quantified for frontal cortex [0.5G: CTR, n=8, females ((f) = 5, males (m) = 3) and MNR, n = 6 (f = 3, m = 3); 0.9G: CTR, n = 6 (f = 3, m = 3) and MNR, n=6 (f=3, m=3)], heart [0.9G: CTR, n=6 (f = 3, m = 3) and MNR, n = 6 (f = 3, m = 3)], kidney [0.5G: CTR, n = 3 (m = 3) and MNR, n = 3 (m = 3); 0.9G: CTR, n = 6 (f = 3, m = 3) and MNR, n = 6 (f = 3, m = 3)], and liver [0.5G: CTR, n = 7 (f = 5, m = 3) and MNR, n=6 (f = 3, m = 3); 0.9G: CTR, n = 6 (f = 3, m = 3) and MNR, n=6 (f=3, m=3)] by nearest-neighbor analysis as described previously [9]. Genomic DNA (2µg) was incubated with MboI restriction enzyme (Fermentas #ER0812) at 37°C overnight, heat-inactivated at 65°C for 20 minutes, and recovered by ethanol precipitation. Digested DNA was end-labeled with 10µCi [α -³²P] dGTP, 0.5µl Klenow and 1.5µl 10X labeling buffer (0.5 M NaCl; 66mM Tris-HCl, pH 7.4, 66 mM MgCl₂; 10mM DTT) at 15°C for 15 minutes and stopped by addition of 2µl 0.2M EDTA. Labeled DNA was purified by Sephadex G50 spin column. DNA (8µl) was digested with 1µl Micrococcal Nuclease, 1µl spleen

phosphodiesterase and 1 µl of micrococcal nuclease buffer (250 mM Tris-HCl; 10 mM CaCl₂, pH 7.6) at 37°C overnight and separated by thin-layer chromatography in two dimensions. All samples were assayed in triplicate. The intensity of 5-methylcytosine and cytosine mononucleotide spots was measured using a PhosphorImager screen with the Image Quant image analysis program. Levels of unmethylated cytosine content were expressed as a percentage of [cytosine]/[cytosine + methylcytosine].

Statistics

Three analyses were performed on percent methylation data: effect of treatment group and gestational age, the potential difference between organ and gestational age, and effect of sex and gestational age (for tissues at gestational ages where at least 3 samples were included in each group). They were assessed using 2-way ANOVA and Student-Newman Keuls test. Significance was accepted at $p < 0.05$.

RESULTS

Effect of global reduction of maternal nutrient intake on fetal and placental growth

Table 1 shows maternal and fetal morphometric data for 0.5G and 0.9G. At 0.5G, maternal weight at cesarean section and fetal anogenital distance were significantly different and umbilical cord length and fetal hip circumference were marginally significant between CTR and MNR groups. At 0.9G, maternal weight at cesarean section, fetal BMI, fetal ponderal index and placenta weight were significantly different between CTR and MNR groups.

Table 2 shows the organ weights at 0.5G and 0.9G for the whole fetal brain, liver, and kidney and at 0.9G for heart from fetuses of the CTR and MNR dams. There were no significant differences in organ weights as a result of MNR at either age for any of the tissues.

Organ specificity and effect of gestational age on global methylation

Figure 1 shows a schematic representation of location of cytosine residues after nearest neighbor analysis (A), representative images of nearest neighbor analysis of DNA (B), and representative gels from the four tissues for both time points from a fetus of a CTR and MNR baboon (C). Global methylation data for all samples are shown in Figure 2. The effects of MNR varied among tissues both within and between gestational ages. MNR increased global methylation in frontal cortex and kidney at 0.9G. There was a trend for global methylation decrease in the 0.5G kidney ($p=0.07$). In addition, there was marked organ specificity at both stages of development (Table 3). No sex-specific effects were observed.

DISCUSSION

Epigenetic programming occurs during development to generate the intricate patterns of gene expression characteristic of complex organisms such as humans; however in contrast to the genetic sequence, epigenetic changes are dynamic and responsive to environmental exposures especially during fetal development. The epigenome consists of the chromatin and its modifications as well as a covalent modification by methylation of cytosine rings found at the dinucleotide sequence CpG [4]. The epigenome is one of the main factors that determine the accessibility of the transcriptional machinery. Inaccessible genes are therefore silent whereas accessible genes are transcribed [10,11]. Active regions of the chromatin, which enable gene expression, are associated with hypomethylated DNA whereas hypermethylated DNA is packaged in inactive chromatin [4,12].

It is generally accepted that DNA methylation plays an important role in regulating gene expression. DNA methylation in distinct regulatory regions is believed to silence gene expression by two principal mechanisms. The first mechanism involves direct interference by a methyl residue within a transcriptional recognition element with the binding of the transcription factor thereby inhibiting activation of the gene [13,14]. A second mechanism is indirect. A certain density of DNA methylation moieties in the region of the gene attracts the binding of methylated-DNA binding proteins such as methyl CpG binding protein 2 (MeCP2) [15]. MeCP2 recruits other proteins such as SIN3A and histone modifying enzymes, which lead to formation of a “closed” chromatin configuration and silencing of gene expression [15]. While DNA methylation in certain transcriptional regulatory regions is associated with a silenced state, studies have shown that methylation of CpG islands in intergenic regions is triggered by gene expression.

While this intergenic methylation is hypothesized to be associated with cancer, it does raise the possibility that an increase in genomic methylation may not necessarily be associated with gene silencing [16]. The developmental programming hypothesis proposes that different environmental exposures at critical periods will affect the programmed changes in DNA methylation during development.

Not all CpGs are methylated in any given cell type [4] contributing to cell specific patterns of gene expression, the fundamental basis of cell type functional identity [4]. It is generally considered that DNA methylation is more stable than other epigenetic marks and thus it has extremely important diagnostic potential [17] which is yet to be taken advantage of in addressing metabolic disorders and other late onset disease. However, our data indicate that there are developmental changes in the extent of methylation.

Global changes in DNA methylation were documented early after fertilization [18] and in early development in rodents [19,20]. However, the global state of methylation of specific organs and tissues during gestation is unknown nor is it clear whether the global changes in DNA methylation reported in rodents also take place in primates. It has been reported that IUGR in rodents results in global hypomethylation (postnatal day (p) 0 and p21) and increased global acetylation in postnatal liver [21] brain and hippocampus and periventricular white matter [22] (p0 and p21) as well as changes in the overall levels of DNA methyltransferase 1 (DNMT1) and chromatin modifying enzymes in the brain and one carbon metabolism in hepatic tissue [21].

Here we tested the hypothesis that changes in global methylation occur during primate development and that a moderate level of maternal nutritional restriction during gestation affects the state of DNA methylation. We used direct measurement of methylation at CpG dinucleotides and measured the state of global DNA methylation in tissues from fetuses of *ad libitum* fed dams and fetuses of dams fed 70% of *ad libitum* fed controls from 0.16G to 0.5G and 0.16G to 0.9G. In baboon fetal liver, DNA is highly hypomethylated with methylation of 23% of CpG dinucleotides in the genome at 0.5G and 15% at 0.9G. These levels of global methylation are dramatically below previously reported values for postnatal rodent livers which were approximately 70% [21,23]. No differences were observed between CTR and MNR liver DNA samples at 0.5G or 0.9G. Kidney DNA, like liver DNA, is hypomethylated at 0.5G (36%) and 0.9G (35%). These methylation levels are markedly less than previously reported values for adult rodent kidney DNA samples. In contrast to liver, kidney DNA global methylation is effected by MNR at both 0.5G and 0.9G. Interestingly, MNR is associated with a decrease in methylation at 0.5G (17%) and with an increase in methylation at 0.9G (64%) compared with CTR samples. Frontal cortex DNA is highly methylated at 0.5G (76%) with a significant decrease at 0.9G (61%). Frontal cortex DNA results are similar to the general methylation levels observed in vertebrate rodent

tissues including brain and several rodent cell lines (nonneuroal) that have been analyzed [23]. MNR does not impact global methylation in frontal cortex at 0.5G; however, methylation significantly increases at 0.9G (76%) compared with CTR samples. Heart DNA methylation levels at 0.9G are intermediate to frontal cortex and kidney and liver with a 62% global methylation level. MNR does not significantly impact heart DNA methylation; however, there is a trend towards decreased methylation.

The technique of nearest neighbor analysis limits itself to examining the levels of CpG methylation located at the restriction sites of MboI. While it does not present the level of methylation at every CpG site it does provide a representative image of genomic CpG methylation. An alternative method would be to examine the methylation of every cytosine nucleotide in the genome through capillary electrophoresis of nuclease P1 digested genomic DNA [24]. Other limitations of this analysis are that it does not provide information on promoter methylation. Techniques include methylated DNA immunoprecipitation (mDIP) which allow researchers to precipitate methylated DNA by a specific antibody and hybridize to a gene array, determining changes in methylation of multiple promoters [25].

Interestingly and unexpectedly the global state of CpG dinucleotide methylation follows organ-specific trajectories during the latter half of gestation; liver and kidney DNA methylation levels do not change from 0.5G to 0.9G; whereas, frontal cortex DNA methylation values decrease from mid-gestation to near-term. The values for liver and kidney DNA remain appreciably hypomethylated in late gestation compared with reported values for adult rodent tissues [23].

Maternal nutrient restriction has tissue-specific and gestational age specific effects on global methylation trajectories. At mid gestation (0.5G) MNR causes severe global hypomethylation in kidney with no significant effect on liver or frontal cortex DNA. Hypomethylation in response to placental insufficiency was previously reported in p0 and p21 livers of postnatal rats [21]. However, the data presented here shows that MNR has a complex effect on the trajectories of global DNA methylation state at late gestation. The loss of methylation, which is observed in frontal cortex late in normal gestation, is blocked by MNR; whereas in kidney, where methylation remains unchanged in normal gestation, there is marked hypermethylation. No effect is observed in liver in contrast to previous reports in rodents [21]. Thus, MNR has opposite effects on global methylation in mid and late gestation; hypomethylation during mid-gestation in kidney and hypermethylation late in gestation in kidney and frontal cortex.

Most of what we know about global methylation comes from studies of very early embryogenesis in rodents [19]. The underlying assumption has been that following the first rounds of global methylation and demethylation early in embryogenesis pre- and post-implantation the global state of methylation is stable and the main action later in embryogenesis is the sculpting of specific regulatory regions of tissue specific genes. A good example is the stage and site specific hypomethylation of the gene encoding the liver specific enzyme *phosphoenolpyruvate carboxykinase* gene in the rat during embryogenesis [26]. It is also generally accepted that global DNA methylation levels do not vary between tissues and that the main difference between tissues is in regulatory regions of the gene which account only for a small fraction of methylated CpGs in the genome. By following the developmental trajectories of global methylation of CpG dinucleotide sequences we surprisingly revealed that wide differences in global methylation exist in primate fetal tissues and that global methylation states follow tissue specific trajectories late into gestation. Thus, our data are consistent with the hypothesis that global oscillations in DNA methylation are not restricted to early embryogenesis and that they accompany latter stages of organogenesis.

Previous data in rodents suggested that the main effect of MNR was hypomethylation [21,22]. Such an effect could be explained as a consequence of a general down regulation of the DNA methylation machinery or reduced availability of S-adenosylmethionine (SAM) the methyl donor of the methylation reaction [1]. Indeed previous studies in postnatal rats indicated that nutritional restriction resulted in inhibition of DNMT1 and increased ratio of S-adenosylhomocysteine (SAH) to SAM with decreased level of methionine adenosyltransferase, the enzyme catalyzing the synthesis of SAM [21]. However, data presented here suggest a more complex and organ-specific effect of MNR than a simple inhibition of general methylation enzymes. Our data suggest that MNR interferes with and targets the programming of global DNA methylation trajectories during gestation, which results in either increased or decreased global methylation.

What is the biological implication of a global change in DNA methylation? The simplest explanation might be that reduction in global methylation reflects a reduction in methylation of regulatory regions of numerous genes resulting in a broad activation of genes, which are otherwise methylated and silenced under hypermethylated conditions [13,14,15]. Indeed demethylating agents which induce global hypomethylation of DNA also cause activation of many genes through demethylation of methylated CGs in regulatory sequences [27,28]. Alternatively it is possible that the global state of DNA methylation plays a role in large-scale genomic organization and overall function at a genome-wide level in addition to the role that specific hypomethylated sequences play in regulation of specific genes. Indeed, global hypomethylation induced by knock out of *DNMT1* was previously shown to be associated with genome wide organizational defects [29]. Global hypomethylation is observed in pathologies such as cancer [30,31] and systemic lupus erythematosus [32,33]. It is possible that global states of DNA methylation might therefore serve as an additional level of regulation on genome function, which has both physiological and pathological roles. The regulatory circuitry which controls the global state of DNA methylation might be especially sensitive to nutritional restriction.

The results presented here point to fundamental questions that need to be addressed in future experiments. It is important to identify the nodal regulatory pathways, which control states of methylation during development and are sensitive to nutritional restriction in an organ specific manner. We must delineate the specific genes as well as global genomic functions which are affected by these changes in DNA methylation. The large effect on the state of methylation of frontal cortex genome points to the possibility that behavioral and cognitive functions are affected by nutritional restriction during gestation. This observation suggests that the concept that the fetal brain is protected from intrauterine challenges may not be universally true. Although many questions remain, our study nevertheless demonstrates for the first time that global changes in DNA methylation occur throughout gestation and organogenesis and that these are profoundly affected by maternal nutrition during gestation. This has important implications on molecular mechanisms linking early life exposures and health outcomes during adulthood.

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Figure 1. Global DNA methylation in baboon fetal tissues at two time points for CTR and MNR samples. A) Representative images of position of labeled-nucleotides after two-dimensional separation by thin-layer chromatography of the cytosine in a CpG dinucleotide. B) Diagram of the nearest neighbor analysis showing the methylated and unmethylated cytosine on the autoradiograph. C) Sample autoradiographs for each tissue at each time point for CTR and MNR samples.

Figure 2.

Tissue and gestation age specific global DNA methylation and effect of MNR on global DNA methylation: A) frontal cortex, B) liver, C) kidney, and D) heart. The y-axis indicates the percent CpG methylation and the x-axis shows CTR (black) and MNR (open) values for 0.5 gestation (G) and 0.9G samples. *P* less than 0.05: “+” indicates 0.5G CTR versus 0.9G CTR, “*” indicates CTR versus MNR at that age.

Table 1

Maternal and fetal morphometric data for control (CTR) and maternal nutrient restricted (MNR) groups.

0.5G	CTR	N	MNR	N	p value
<i>Maternal</i>					
Weight pre-conception (kg)	13.68±0.51	8	13.02±0.24	6	0.313
Weight at cesarean section (kg)	13.72±0.41	8	12.16±0.34	6	0.016*
<i>Fetal</i>					
Weight (g)	100.92±3.36	8	95.43±3.26	6	0.276
Length (cm)	17.94±0.31	8	17.58±0.44	6	0.505
BMI (kg/m ²)	3.14±0.09	8	3.10±0.12	6	0.782
Ponderal index (kg/m ³)	17.58±0.7	8	17.74±1.07	6	0.896
Biparietal diameter (cm)	3.35±0.05	8	3.42±0.07	6	0.437
Abdominal distance (cm)	2.59±0.32	8	2.92±0.16	6	0.426
Femur length (cm)	3.52±0.11	8	3.24±0.11	6	0.117
Chest circumference (cm)	8.87±0.23	8	8.79±0.23	6	0.804
Waist circumference (cm)	7.55±0.25	8	7.58±0.27	6	0.930
Hip circumference (cm)	7.34±0.17	8	6.83±0.17	6	0.058#
Sterno-pubic distance (cm)	6.72±0.42	8	7.08±0.08	6	0.471
Anogenital distance (cm)	0.06±0.01	5	0.03±0.01	3	0.044*
Placenta weight (g)	70.36±5.09	8	62.93±1.48	6	0.245
Umbilical cord length (cm)	14.08±0.8	8	11.83±0.90	6	0.089#
0.9G	CTR	N	MNR	N	p value
<i>Maternal</i>					
Weight pre-conception (kg)	15.00±0.82	7	14.93±0.39	6	0.944
Weight at cesarean section (kg)	16.63±0.69	7	14.11±0.77	6	0.032*
<i>Fetal</i>					
Weight(g)	744.43±31.59	7	668.50±33.41	6	0.128
Length (cm)	36.36±0.76	7	37.50±1.02	6	0.379
BMI (kg/m ²)	5.64±0.20	7	4.77±0.20	6	0.010*

0.5G	CTR	N	MNR	N	p value
Ponderal index (kg/m ³)	15.58±0.77	7	12.80±0.81	6	0.030*
Biparietal diameter (cm)	5.51±0.18	7	5.67±0.08	6	0.477
Abdominal distance (cm)	5.09±0.32	7	5.53±0.26	6	0.310
Femur length (cm)	7.61±0.26	7	7.25±0.21	6	0.316
Chest circumference (cm)	17.57±0.43	7	16.67±0.38	6	0.148
Waist circumference (cm)	14.96±0.50	7	14.25±0.21	6	0.239
Hip circumference (cm)	14.00±0.90	7	13.33±0.59	6	0.563
Sterno-pubic distance (cm)	12.82±0.14	7	12.75±0.36	6	0.848
Anogenital distance (cm)	1.06±0.16	4	0.83±0.08	3	0.301
Placenta weight (g)	177.29±7.89	7	145.00±7.23	6	0.013*
Umbilical cord length (cm)	18.14±0.96	7	17.25±1.82	6	0.658

Means ± SEM are shown,

* indicates significance at $p \leq 0.05$,

indicates marginal significance at $0.05 \leq p \leq 0.1$.

Table 2

Organ weights in control (CTR) and maternal nutrient-restricted (MNR) baboons at 0.5G and 0.9G.

		CTR	MNR	p value
0.5G	Brain (g)	32.6 ± 1.75	30.08 ± 0.52	0.252
	Liver (g)	3.21 ± 0.16	3.17 ± 0.20	0.878
	Kidney (g)	0.97 ± 0.20	0.9 ± 0.21	0.830
	Heart (g)	0.63 ± 0.19	-	-
0.9G	Brain (g)	77.17 ± 3.07	77.69 ± 2.49	0.900
	Liver (g)	20.48 ± 0.44	17.98 ± 1.36	0.089 [#]
	Kidney (g)	3.45 ± 0.24	3.56 ± 0.27	0.775
	Heart (g)	4.43 ± 0.37	3.52 ± 0.08	0.071 [#]

Means ± SEM are shown,

[#] indicates marginal significance at $0.05 \leq p \leq 0.1$.

Table 3

Significant differences in percentage methylation between the various tissues of fetuses of control (CTR) and nutrient restricted mothers at two gestational ages. Significance was set at 0.05. Individual cells represent different tissues at either 0.5 or 0.9 gestation.

	CTR	Brain	Liver	Kidney	Heart
MNR	Brain		0.5	0.5	NS
	Liver	0.9		NS	0.5
	Kidney	0.9	0.9		0.5
	Heart	NS	0.9	0.9	