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Effect of 30 % nutrient restriction in the first half of gestation on maternal and fetal baboon serum amino acid concentrations

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Abstract
Mechanisms linking maternal nutrient restriction (MNR) to intra-uterine growth restriction (IUGR) and programming of adult disease remain to be established. The impact of controlled MNR on maternal and fetal amino acid metabolism has not been studied in non-human primates. We hypothesised that MNR in pregnant baboons decreases fetal amino acid availability by mid-gestation. We determined maternal and fetal circulating amino acid concentrations at 90 d gestation (90dG, term 184dG) in control baboons fed *ad libitum* (C, *n* 8) or 70 % of C (MNR, *n* 6). Before pregnancy, C and MNR body weights and circulating amino acids were similar. At 90dG, MNR mothers had lower body weight than C mothers ($P<0{\cdot}05$). Fetal and placental weights were similar between the groups. MNR reduced maternal blood urea N (BUN), fetal BUN and fetal BUN:creatinine. Except for histidine and lysine in the C and MNR groups and glutamine in the MNR group, circulating concentrations of all amino acids were lower at 90dG compared with pre-pregnancy. Maternal circulating amino acids at 90dG were similar in the MNR and C groups. In contrast, MNR fetal β-alanine, glycine and taurine all increased. In conclusion, maternal circulating amino acids were maintained at normal levels and fetal amino acid availability was not impaired in response to 30 % global MNR in pregnant baboons. However, MNR weight gain was reduced, suggesting adaptation in maternal–fetal resource allocation in an attempt to maintain normal fetal growth. We speculate that these adaptive mechanisms may fail later in gestation when fetal nutrient demands increase rapidly, resulting in IUGR.

Key words: **Pregnancy: Nutrition: Primates: Placenta: Growth**

Maternal undernutrition during pregnancy remains a significant challenge to fetal growth and development worldwide and constitutes a very significant problem in the USA because 48·8 million Americans lived in households experiencing food insecurity or hunger at least some time during 2010 (http://www.ers.usda.gov/Briefing/FoodSecurity/stats_graphs.htm). Furthermore, maternal undernutrition is, together with infections, the most common cause of intra-uterine growth restriction (IUGR) in developing countries[(1)] and fetal undernutrition accompanies placental insufficiency, a common aetiology of IUGR, even in more affluent societies[(2)]. In addition, maternal undernutrition may have adverse consequences for the fetus without affecting fetal growth[(3)].

An adequate supply of amino acids is essential for normal development of the placenta and fetus at all stages of pregnancy. In addition, the mother has her own needs for amino acids to accommodate the extensive energetic and tissue remodelling demands of pregnancy. Amino acids serve as building blocks for tissue protein synthesis and perform many specific cell functions such as providing antioxidant capacity and serving as substrates for hormone synthesis and cell signalling molecules. Amino acids also act as essential precursors of many molecules of physiological significance such as NO and polyamines from arginine as well as amino sugars[(4–7)]. Furthermore, amino acids also constitute important stimuli for the secretion of insulin, an important fetal growth hormone. Thus, amino availability is a key determinant of fetal growth.

The effects of maternal nutrient restriction (MNR) on maternal, placental and fetal growth have been extensively studied in rodents and sheep. Global MNR paradigms have ranged from moderate (30 % reduction) to severe (70 % reduction)[(8,9)]. All models of human pregnancy have their

Abbreviations: BUN, blood urea N; C, control; dG, d of gestation; MNR, maternal nutrient restriction; IUGR, intra-uterine growth restriction.

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strengths and weaknesses. Energetically speaking, rodents and sheep are inappropriate models for human pregnancy because the daily costs of fetal growth and milk synthesis are much higher for rodents and sheep than they are for humans. In contrast, these costs are identical for baboons and humans due to the characteristic traits of slow-growing fetuses with long gestations. However, with respect to maternal body fat stores, sheep and rats are more similar to humans than baboons, which are lean in pregnancy[10]. Our previous studies of development at 50 % of fetal primate gestation have shown significant effects of moderate MNR on structure and molecular mechanisms in several organ systems, including the brain[11], kidney[12] and liver[13]. Because a sufficient supply of amino acids is critical for normal development, we felt it important to conduct the present study on amino acid availability at the same stage of gestation where we have found organ deficits. Specifically, we tested the hypothesis that MNR in the pregnant baboon results in decreased fetal amino acid availability in mid-gestation.

Materials and methods

Animals

Normally cycling female baboons (*Papio* sp., *n* 14) from 8 to 15 years of age were maintained at the Southwest National Primate Research Center and housed in two group cages, each containing one male. Housing conditions and animal care were as described previously[14]. All animals were fed *ad libitum* until the start of the study. Commencing at 30 d of gestation (dG), eight pregnant baboons were selected at random and allowed to continue feeding *ad libitum* as controls (C) to 90dG while six additional pregnant baboons consumed 70 % of the C diet, i.e. 30 % MNR, on a per kg body-weight basis. All procedures using animals in the present study were performed using the Guide for the Care and Use of Animals and were approved by the Internal Animal Care and Use Committee of the Texas Biomedical Research Institute and were conducted in Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)-approved facilities.

Feed and feeding

Table 1 shows the detailed composition of the feed (Purina Monkey Diet 5038) and the amount consumed daily by the C group throughout the study. Once per d before feeding, all baboons from a single group cage passed along a chute into individual feeding cages. The baboons were fed from 07.00–09.00 or 11.00–13.00 hours. At the start of the feeding period, each C baboon was given sixty biscuits accessed through a 6·4 cm diameter opening into the feed containers. C food consumption was expressed as the average consumption per animal per kg body weight for each week of pregnancy. Restricted animals were given 70 % of the average C consumption/kg body weight for the same week of gestation. The floor of individual cages was made of galvanised mesh to minimise the loss of biscuits. The sides of the feeding cages were solid metal so animals could not access the food of animals in adjacent cages. Water was continuously available both in the feeding cages and in group housing. At the end of the 2 h feeding session the animals were run back into their own group cage after which the biscuits remaining in the tray and on the floor of the cage and in the pan were counted. No treats or supplements were provided to either group in the period 30–90dG.

Table 1. Composition of Purina Monkey Diet 5038 and control group average consumption from 30 to 90 d of gestation*

Nutrients	% of Ration	Mean amount consumed (g/kg body weight per d)	
		Mean	SEM
Protein	15·7	2·45	0·062
Arg	0·81	0·13	0·003
Cystine	0·23	0·04	0·001
Gly	0·66	0·12	0·003
His	0·39	0·06	0·002
Ile	0·70	0·13	0·003
Leu	1·44	0·24	0·006
Lys	0·75	0·12	0·003
Met	0·43	0·07	0·002
Phe	0·74	0·13	0·003
Tyr	0·47	0·07	0·002
Thr	0·56	0·10	0·002
Trp	0·18	0·03	0·001
Val	0·76	0·13	0·003
Ser	0·80	0·12	0·003
Asp + Asn	1·48	0·22	0·006
Glu + Gln	3·79	0·59	0·015
Ala	0·95	0·15	0·004
Pro	1·43	0·22	0·006
Taurine (Tau)	< 0·01	< 0·002	
Fat (diethyl ether extract)	5·0	0·79	0·020
Fat (acid hydrolysis)	6·0	0·93	0·023
Cholesterol	75 ppm	75 ppm	
Linoleic acid	1·66	0·23	0·006
Linolenic acid	0·10	0·01	0·000
Arachidonic acid	< 0·01	< 0·002	
n-3 Fatty acids	0·13	0·02	0·000
Total SFA	1·54	0·27	0·007
Total MUFA	1·68	0·26	0·007
Fibre (crude)	4·5	0·63	0·016
Neutral-detergent fibre	16·8	2·64	0·066
Acid-detergent fibre	5·80	0·90	0·023
N-free extract (by difference)	59·3	9·60	0·241
Starch	42·40	6·52	0·164
Glucose	0·29	0·04	0·001
Fructose	0·32	0·05	0·001
Sucrose	2·24	0·34	0·009
Lactose	1·68	0·22	0·006

ppm, Parts per million.
* The full composition of Monkey Diet 5038 can be found at http://labdiet.com/pdf/5037-5038.pdf

Blood sampling

All blood samples were taken before feeding. Maternal blood samples were obtained from non-pregnant and pregnant animals by femoral venepuncture. At caesarean section, maternal uterine vein and fetal umbilical vein blood samples were collected into heparinised tubes for amino acid analysis and no-additive tubes for all other analytes as described previously[15].

Body weights

Once per d just before feeding, all baboons from a single group cage passed along a chute and stopped at a weighing scale. The weight, in kg to three decimal places of each adult female baboon, both pre-pregnancy and during pregnancy, was obtained as she crossed an electronic scale system (GSE 665; GSE Scale Systems) at the entrance to the individual feeding cages[14].

Caesarean sections

All baboons were tranquilised with ketamine hydrochloride (10 mg/kg). After intubation, isoflurane (starting rate 2 % with oxygen: 2·0 litres/min) was administered to maintain a surgical plane of anaesthesia throughout the surgery. Conventional caesarean sections using a standard sterile technique were performed as described previously[15]. At caesarean section, fetuses and placentas were towel-dried and weighed. Postoperative analgesia was provided with buprenorphine (0·015 mg/kg per d as two doses) for 3 d post-surgery[15].

Biochemical analyses

Within 1 h of collection, the clotted blood was centrifuged and the serum removed. Biochemical determinations were made in serum for glucose, blood urea N (BUN), creatinine, total protein, albumin and globulin with the Beckman Synchron CX5CE Analyzer (Beckman Coulter, Inc.)[15].

Amino acid analyses

Heparinised plasma samples (0·1 ml) were deproteinised with 0·1 ml of 1·5 M-$HClO_4$ and neutralised with 0·05 ml of 2 M-K_2CO_3. The solution was centrifuged at 12 000 ***g*** at 4°C for 1 min and the supernatant used for analyses. Amino acids were determined by HPLC methods involving pre-column derivatisation with *o*-phthaldialdehyde, as described previously in detail[16]. All amino acids were quantified on the basis of authentic standards (Sigma Chemicals) using Millenium-32 Software (Waters)[15].

Statistical analyses

Comparisons between mothers were performed with repeated-measures two-way ANOVA and the *post hoc* Bonferroni correction; fetuses were compared using Student's non-paired *t* test. The sex distribution in the controls was two males and six females, and in the MNR group three males and three females. Sex differences between fetuses were examined based on a linear model with 2 × 2 factorial and the *post hoc* Bonferroni correction. No significant differences were found, so data from the sexes were pooled. Correlation was performed using the Pearson product–moment correlation coefficient. Amino acid data were transformed to natural logs before testing. Data are presented as means with their standard errors with the α-level set at 0·05.

Results

Body weights and food consumption

The C and MNR groups did not differ in body weight or average age before pregnancy. During the study (30–90dG), the C group maintained their body weight while MNR baboons lost approximately 6 % of their preconceptional weight ($P<0·05$; Table 2). Fetal weights, placental weights and fetal:placental weight ratios were not different between the groups (Table 2). Average food consumption from 30 to 90dG was significantly ($P<0·0005$) different between the C (15·87 (SEM 1·13) g/kg body weight per d) and MNR groups (11·32 (SEM 0·24) g/kg body weight per d).

Biochemical measurements

There was a tendency for serum glucose to be reduced in MNR group fetuses (Table 3); however, due to the variability in the data, it did not reach significance. Pregnancy reduced BUN in both C and MNR mothers compared with the pre-pregnant state ($P<0·05$; Table 3). The MNR treatment reduced both maternal and fetal BUN in comparison with the C treatment ($P<0·05$) while no differences were observed in creatinine concentrations between the treatment groups. In addition, the maternal and fetal BUN:creatinine ratio was lower in the MNR group ($P<0·05$; Table 3). Although the fetal:maternal glucose ratio appeared lower in the MNR group, this difference did not reach significance ($P=0·10$). Interestingly, MNR decreased maternal serum albumin and increased serum globulin so that the maternal albumin:globulin ratio was decreased (Table 3).

Amino acids

Maternal plasma concentrations of all measured amino acids, except the essential amino acids histidine and lysine in C and MNR group mothers and the non-essential amino

Table 2. Maternal and fetal/placental morphometrics from animals fed as *ad libitum* controls (C, *n* 8) or fed a 70 % C diet (maternal nutrient restriction (MNR), *n* 6) from 30 to 90 d of gestation

(Mean values with their standard errors)

	C		MNR	
	Mean	SEM	Mean	SEM
Maternal				
Pre-pregnant				
Body weight (kg)	13·7	0·5	13·0	0·2
Body length (cm)	87·6	1·2	87·7	0·8
Biparietal distance (cm)	9·7	0·6	9·7	0·3
90 d of gestation				
Body weight (kg)	13·7	0·4	12·2*†	0·3
Fetal/placental				
Fetal weight (g)	100·93	3·37	95·43	3·26
Body length (cm)	17·94	0·31	17·58	0·44
Weight:length (g/cm)	5·62	0·15	5·43	0·14
BMI (g/cm^2)	3·14	0·09	3·10	0·12
Biparietal distance (mm)	33·51	0·48	34·17	0·70
Placental weight (g)	70·36	5·09	62·93	1·48
Umbilical cord length (cm)	14·08	0·80	11·83	0·90
Fetal weight:placental weight	1·47	0·07	1·52	0·06

* Mean value was significantly different from that of the C group ($P<0·05$).
† Mean value was significantly different compared with pre-pregnant weight ($P<0·05$).

Table 3. Maternal and fetal/placental analytes from animals fed as *ad libitum* controls (C, *n* 8) or fed a 70 % C diet (maternal nutrient restriction (MNR), *n* 6) from 30 to 90 d of gestation

(Mean values with their standard errors)

	Pre-pregnant				Maternal				Fetal			
	C		MNR‡		C		MNR		C		MNR	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Glucose (mg/l)	808	64·6	837	60·5	744	96·3	858	106	471	43·5	390	74·4
BUN (mg/l)	149	10·1	135	7·6	103†	6·7	75*†	4·3	110	5·8	80*	5·2
Creatinine (mg/l)	10	0·6	10	0·6	9	0·8	9	0·7	8	0·4	8	0·4
BUN:creatinine	15·0	0·87	13·9	1·42	11·6†	1·40	8·4†	0·61	14·0	0·96	10·0*	0·98
Total protein (g/l)	71	1·6	69	1·2	67	1·9	67	1·6	26	0·6	27	0·4
Albumin (g/l)	39	0·9	39	1	31†	1·6	32†	1·4	18	0·4	19	0·4
Globulin (calculated) (g/l)	32	1·3	30	0·5	37†	1·7	35†	0·8	8	0·4	8	0·4
Albumin:globulin	1·2	0·06	1·3	0·03	0·8†	0·05	0·9†	0·04	2·5	0·14	2·3	0·15

* Mean value was significantly different from that of the C group ($P<0·05$).
† Mean value was significantly different compared with the pre-pregnant state ($P<0·05$).
‡ MNR data are from the mothers that were randomly assigned to MNR in the pregnancy group. Biochemical determinations were made in serum with the Beckman Synchron CX5CE (Beckman Coulter, Inc.).

acid glutamine in MNR mothers, were reduced at 90dG in comparison with pre-pregnant concentrations (Table 4). Maternal plasma amino acids at 90dG were similar in the MNR and C groups. MNR resulted in elevated fetal glycine, taurine and β-alanine ($P<0·05$). The fetal:maternal ratio was increased with MNR for glycine, β-alanine and arginine (Table 5). The only fetal amino acid that correlated with maternal concentrations in plasma was glutamine (r 0·95; $P<0·01$). In fetuses, no significant differences in plasma amino acid concentration between sexes were found (Table 6). Also, no differences were found between C and MNR females while very few males in the C group prevented comparisons of C *v.* MNR males.

Discussion

To our knowledge, this is the first report exploring the effects of MNR on maternal and fetal amino acid levels in non-human

Table 4. Nutritionally essential and non-essential amino acid plasma levels (μM) in female baboons before pregnancy (PP) and *ad libitum*-fed control (C, *n* 8) or globally nutrient-restricted mothers (maternal nutrient restriction (MNR), *n* 6; fed a 70 % C diet) from 30 to 90 d of gestation (dG)

(Mean values with their standard errors)

	C PP		C 90 dG		MNR† PP		MNR 90 dG	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Essential								
Arg	195·5	7·14	124·5*	7·58	191·8	6·52	117·8*	4·95
His	105·5	3·30	92·2	2·22	99·8	4·22	94·7	6·56
Ile	77·6	2·90	46·0*	2·40	71·5	4·4	46·0*	3·75
Leu	134·5	4·57	71·0*	3·37	126·9	4·92	64·9*	6·32
Lys	141·3	5·79	128·2	5·75	165·5	14·36	142·9	13·98
Met	32·2	0·99	20·9*	0·92	27·4	0·89	18·1*	0·85
Phe	68·7	3·12	34·1*	1·92	67·4	1·36	30·0*	0·96
Thr	96·4	3·48	71·9*	5·21	92·2	3·57	68·1*	7·08
Val	180·8	7·67	101·9*	5·18	159·0	8·15	98·8*	10·60
Total essential	1032·4	26·83	690·8*	26·64	1001·6	36·21	681·2*	47·33
Non-essential								
Ala	267·7	10·83	182·9*	12·1	280·9	24·21	210·8*	26·83
Asn	43·6	2·19	27·4*	1·19	37·3	5·22	26·2*	2·24
Asp	25·4	1·39	6·3*	0·53	26·1	0·98	5·9*	0·62
β-Ala	26·8	2·91	12·4*	0·92	25·4	2·91	13·0*	1·61
Cit	30·4	3·20	19·6*	1·51	29·5	1·39	17·8*	3·01
Gln	519·6	11·89	304·6*	15·87	445·8	55·94	355·2	25·58
Glu	127·4	6·47	47·9*	4·33	150·5	16·97	43·4*	5·96
Gly	381·6	9·30	200·6*	11·37	417·0	60·93	195·9*	9·74
Orn	28·1	1·59	12·6*	1·37	31·5	3·69	13·0*	1·15
Ser	132·4	5·37	77·6*	3·00	140·2	13·53	86·5*	7·76
Tau	138·9	3·56	88·5*	4·96	145·8	18·89	82·4*	9·20
Trp	47·5	2·69	30·2*	2·09	47·9	3·80	23·8*	1·91
Tyr	43·1	1·66	25·8*	1·80	39·2	2·92	24·3*	1·09
Total non-essential	1812·5	27·54	1036·5*	32·35	1817·00	127·41	1098·2*	45·98

* Mean value was significantly different compared with the PP state ($P<0·05$).
† MNR data are from mothers that were randomly assigned to MNR in the pregnancy group.

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Table 5. Fetal values (μM) and fetal (FET):maternal (MAT) ratios of essential and non-essential amino acid plasma levels in *ad libitum*-fed control (C, *n* 5) *v.* globally nutrient restricted (maternal nutrient restriction (MNR), *n* 6; fed a 70 % C diet) from 30 to 90 d of gestation
(Mean values with their standard errors)

	FET C		FET MNR		FET:MAT C		FET:MAT MNR	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Essential								
Arg	216·70	47·57	338·34†	41·78	1·57	0·39	2·86*	0·34
His	155·51	18·75	150·79	17·49	1·75	0·21	1·60	0·18
Ile	87·74	6·13	78·02	14·25	2·02	0·12	1·67	0·21
Leu	87·97	20·86	108·08	25·01	1·40	0·26	1·63	0·27
Lys	502·83	41·28	571·87	69·59	4·03	0·19	4·12	0·52
Met	52·27	4·92	43·12	5·62	2·57	0·2	2·38	0·32
Phe	60·35	7·13	56·29	8·29	1·82	0·15	1·89	0·27
Thr	181·02	18·71	163·56	30·52	2·37	0·22	2·36	0·30
Val	217·47	20·24	221·05	34·66	2·21	0·2	2·24	0·21
Total essential	1561·86	148·63	1731·13	231·69				
Non-essential								
Ala	447·5	32·13	571·02	79·18	2·42	0·37	2·71	0·27
Asn	74·66	5·86	62·99	9·68	2·66	0·14	2·39	0·29
Asp	17·74	3·45	17·35	3·07	2·82	0·69	3·18	0·69
β-Ala	12·68	0·85	22·88*	2·40	0·94	0·09	1·92*	0·32
Cit	34·81	3·41	51·55	21·50	1·65	0·15	3·32	1·51
Gln	814·3	79·45	732·10	113·12	2·63	0·21	2·01	0·21
Glu	116·52	22·39	166·82	53·34	2·09	0·50	4·04	1·22
Gly	405·35	29·03	1054·01*	101·07	2·24	0·21	5·49*	0·66
Orn	66·75	7·92	76·34	15·72	5·77	0·58	5·99	1·12
Ser	151·73	17·28	188·37	30·14	1·96	0·25	2·18	0·26
Tau	291·01	37·43	480·32*	56·41	3·58	0·22	6·43	1·27
Trp	43·63	1·54	33·59	5·13	2·59	0·39	2·14	0·32
Tyr	63·74	4·46	53·20	9·11	1·37	0·05	1·48	0·24
Total non-essential	2377·56	230·00	3510·53*	325·64	1·57	0·39	2·86*	0·34

* Mean value was significantly different from that of the C group ($P<0·05$).
† Mean value was marginally significantly different from that of the C group ($P=0·085$).

primates. We show that maternal circulating amino acid concentrations were maintained at normal levels and fetal amino acid availability was not impaired in response to 30 % restriction of global maternal nutrient intake in pregnant baboons. However, weight gain in MNR mothers was significantly reduced, suggesting an adaptation in maternal–fetal resource allocation to maintain fetal growth within the normal range. We have shown previously that these adaptive mechanisms may fail later in gestation when fetal nutrient demands increase exponentially, leading to IUGR and a pre-diabetic phenotype[17]. This result is supported by studies in The Gambia during the wet season when food shortages and strenuous work put pregnant women into negative energy balance. Deliveries during this time show fetal growth faltering, but only in the last 4 weeks of gestation when fetal demand on maternal resources is at its highest[18]. However, fetal weight is a poor metric of optimal fetal development, and our previous[11–13] and present results show that the mother, placenta and fetus have begun making adaptive responses much earlier in gestation.

Pregnancy decreased the circulating levels of twenty out of twenty-two amino acids, with seven of these being essential amino acids, which is consistent with the hypoaminoacidaemia of pregnancy reported in the literature[19–21]. The lower concentrations of these amino acids in pregnancy may be explained by increased blood volume[22] and may also reflect active transport of amino acids into the fetal circulation by the placenta to be utilised for fetal growth[23]. The finding that the magnitude of the decrease differed markedly between amino acids relates to the fact that each amino acid has its own unique and tissue-specific metabolic pathway[4]. Because dietary aspartate, glutamate and glutamine are

Table 6. Amino acids in fetal plasma by sex
(Mean values and standard deviations)

	Male		Female		
Amino acid	Mean	SD	Mean	SD	P*
Gln	2·91	0·35	2·71	0·38	1
His	4·99	0·78	4·56	0·63	1
Gly	4·23	0·22	4·14	0·43	1
Thr	5·17	0·34	5·04	0·32	1
Cit	6·64	0·31	6·55	0·39	1
Arg	5·07	0·25	4·93	0·34	1
β-Ala	6·51	0·55	6·51	0·57	1
Tau	5·16	0·21	5·2	0·5	1
Ala	3·48	0·14	3·68	0·78	1
Tyr	5·68	0·26	5·44	0·62	1
Trp	2·96	0·33	2·74	0·39	1
Met	5·95	0·39	5·88	0·42	1
Val	6·36	0·2	6·05	0·42	1
Phe	4·09	0·23	3·92	0·53	1
Ile	3·62	0·17	3·56	0·54	1
Leu	3·91	0·22	3·73	0·45	1
Orn	5·38	0·2	5·33	0·34	1
Lys	4·02	0·18	4·01	0·45	1

* Mean contrast with a Bonferroni correction for multiple comparisons.

almost completely oxidised by enterocytes, these amino acids in plasma are derived primarily from endogenous synthesis from branched-chain amino acids[7]. A decrease in the availability of leucine, isoleucine and valine probably contributes to the reduced levels of the glutamate-family amino acids, which in turn would result in low levels of their metabolites, i.e. alanine, ornithine, citrulline and arginine.

The lower maternal circulating concentrations of amino acids in pregnant baboons when compared with the non-pregnant state are consistent with the findings in women[24–28]. For example, total plasma N was lower at 18–25 weeks of gestation in women in comparison with non-pregnant levels, with all of the non-essential and essential amino acids measured, except lysine, histidine and threonine, contributing to this decrease[24]. The present study shows similarities and differences with the findings reported for non-pregnant *v.* pregnant rhesus monkeys at mid-term by Kerr[29]. For example, while the ratio of total amino acids for pregnant to non-pregnant rhesus monkeys was 0·85, indicating a decrease over the first half of pregnancy, taurine, alanine, valine, lysine and arginine ratios were actually increased (range 1·03–1·65) and isoleucine was nearly unity (0·92). However, statistics were not given for the ratios[29]. In contrast, in rats, total amino acids for non-pregnant compared with pregnant animals at both 19 and 21 d of gestational age were not different in control-fed animals[30]. These inter-species differences may arise from differences in methodology or they may reflect varying placental function and/or maternal and fetal metabolic needs in different species.

As expected, concentrations of amino acids in both MNR and C fetuses were higher than in mothers due to the active transport of amino acids across the placenta[23]. In the present study, fetal serum concentrations of glycine, β-alanine and taurine were 2·5-, 1·8- and 1·5-fold, respectively, higher in MNR fetuses compared with C fetuses. A similar effect on fetal proline, alanine and glycine levels, together with a decrease in serine concentrations, was observed in human small-for-gestational age compared with appropriate size for gestational age fetuses. Economides *et al.*[28] suggested that elevated proline, alanine and glycine serum concentrations in the small-for-gestational age fetuses might result from reduced use in oxidation or gluconeogenesis. The significantly ($P < 0·05$) reduced BUN and BUN:creatinine in MNR mothers and fetuses (Table 3) is consistent with decreased amino acid metabolism as a strategy to conserve amino acids, while the lower albumin in pregnant *v.* pre-pregnant mothers is probably due to the blood volume expansion of pregnancy. The lack of a difference in total protein speaks to how successfully the MNR animals are compensating at mid-gestation. Finally, the increase in globulin in mothers with pregnancy is interesting and probably reflects changes in production and utilisation that are little affected by nutrient restriction at least in the first half of pregnancy.

In humans, Kalkhoff *et al.*[31] have demonstrated a strong positive relationship between plasma serine levels in mothers and birth weight of their offspring. The large increase in glycine in MNR group fetuses in the present study in comparison with C fetuses is not unique to the baboon and has been described in sheep fetuses from mothers subjected to 50 % nutrient restriction and in small-for-gestational age fetuses in human pregnancy[26,28,32]. However, rat fetuses subjected to 100 % nutrient restriction in late gestation do not have elevated glycine, suggesting that more studies are needed on this amino acid in models of pregnancy across different species[30].

In the present study, circulating concentrations of essential amino acids were not different in MNR *v.* C baboon fetuses, consistent with the maintained maternal amino acid concentrations at this stage of gestation. This finding also suggests that placental amino acid transport capacity is relatively unaltered in MNR fetuses at 90dG. However, fetuses were not growth restricted at 90dG in the present study and while amino acids may be adequate in the first half of pregnancy, based on our previous study with postnatal animals[17] and on human studies[18], we have reason to believe that fetal amino acid concentrations will be reduced with MNR later in pregnancy. In human pregnancies, small-for-gestational age fetuses typically have lower circulating concentrations of some essential amino acids and a higher ratio of non-essential:essential amino acids[24–28].

In rat fetuses of dams on 100 % food restriction on days 19 and 21 of gestation, total fetal serum amino acids were decreased by approximately 40 % while phenylalanine, tryptophan, glutamine + glutamate and lysine levels were increased in the 21 d, but not in the 19 d, fetuses. Furthermore, term dog fetuses studied at the end of a 72 h, 100 % maternal fast showed no changes in serum alanine, aspartate, glutamate or urea concentrations, but did have a 30 % decrease in glutamine[33]. These variable effects of maternal nutrient restriction on fetal amino acid concentrations are likely to be due to differences in duration and severity of the nutrient restriction and species differences as well as differences in methodologies used. This demonstrates the importance of using data obtained in the non-human primate in addition to data from other species to better understand changes in human pregnancy for translation to humans.

In summary, nutrient-restricted pregnant baboons lost their body mass in the first half of pregnancy to the advantage of the growth of their placentas and fetuses. While fetal growth was largely maintained in the first half of pregnancy, MNR can result in changes unrelated to overall body growth, such as epigenetic regulation of key metabolic pathways that will be manifest as adverse effects in postnatal life[34]. Furthermore, our recent study[17] indicates that these adaptive mechanisms can fail later in gestation, producing a metabolically significant change in postnatal phenotype, as fetuses demand an increasingly larger share of a limited nutrient supply, leading to IUGR.

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