The effects of maternal protein restriction on the growth of the rat fetus and its amino acid supply

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Maternal protein deficiency causes fetal growth retardation which has been associated with the programming of adult disease. The growth of the rat fetus was examined when the mothers were fed on diets containing 180, 90 and 60 g protein/kg. The numbers of fetuses were similar in animals fed on the 180 and 90 g protein/kg diets but the number was significantly reduced in the animals fed on the 60 g protein/kg diet. The fetuses carried by the mothers fed on the 90 g protein/kg diet were 7·5 % heavier than those of mothers fed on 180 g protein/kg diet on day 19 of gestation, but by day 21 the situation was reversed and the fetuses in the protein-deficient mothers were 14 % smaller. Analysis of the free amino acids in the maternal serum showed that on day 19 the diets containing 90 and 60 g protein/kg led to threonine concentrations that were reduced to 46 and 20 % of those found in animals fed on the control (180 g/kg) diet. The other essential amino acids were unchanged, except for a small decrease in the branched-chain amino acids in animals fed on the 60 g protein/kg diet. Both low-protein diets significantly increased the concentrations of glutamic acid+glutamine and glycine in the maternal serum. On day 21 the maternal serum threonine levels were still reduced by about one third in the group fed on the 90 g protein/kg diet. Dietary protein content had no effect on serum threonine concentrations in non-pregnant animals. Analysis of the total free amino acids in the fetuses on day 19 showed that feeding the mother on a low-protein diet did not change amino acid concentrations apart from a decrease in threonine concentrations to 45 and 26 % of the control values at 90 and 60 g protein/kg respectively. The results suggest that threonine is of particular importance to the protein-deficient mother and her fetuses. Possible mechanisms for the decrease in free threonine in both mother and fetuses and the consequences of the change in amino acid metabolism are discussed.

Maternal nutrition: Fetal growth: Protein restriction

In human populations strong epidemiological evidence now links low birth weight with health problems at all stages of life. Poor growth *in utero* has been linked to the development of type-2 (non-insulin dependent) diabetes mellitus and hypertension (Godfrey *et al.* 1994; Phillips *et al.* 1994; Barker, 1995). Fetal growth is a complex, dynamic process which is dependent on a continuous supply of nutrients from the mother. The early stages are so important that the growth of an animal undernourished *in utero* does not recover even when complete nutrition is restored after birth (Widdowson & McCance, 1963; Davison & Dobbing, 1966; Desai *et al.* 1996). The rat provides a convenient small animal model of this fetal programming of adult disease. The offspring of pregnant rats fed on protein-deficient diets are not only slightly smaller than controls fed on an adequate diet but also have permanent changes in the structure and function of their organs which result in reduced glucose tolerance and hypertension in adult life (Snoeck *et al.* 1990; Langley & Jackson, 1994; Langley-Evans *et al.* 1994, 1996; Desai *et al.*

1995, 1996). It is not clear how a restricted maternal protein supply perturbs fetal growth. Without an increase in intake, the dietary supply of amino acids available to mother and fetus will fall with the protein content of the diet. Embryonic cells are able to respond directly to amino acid deficiency by increasing the expression of a variety of genes whose products regulate growth, differentiation and apoptosis (Fleming *et al.* 1998). This ability to respond to amino acid deficiency, coupled with the central role of amino acids in the metabolism of the fetus and placenta suggests that there may be a direct link between amino acid supply and fetal growth. In the rat there are two phases of protein metabolism during pregnancy. In the first, anabolic phase, there is an accretion of maternal body protein which can then be used to support fetal growth during the second, catabolic phase (Naismith & Morgan, 1976; Remesar *et al.* 1987). It is not known whether this mechanism can compensate for a reduced dietary protein intake and maintain the supply of free amino acids to the fetus. In addition to protein

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synthesis, significant amounts of amino acid N are required for the *de novo* synthesis of nucleotides, further increasing the demand for those amino acids which are used as precursors, e.g. glycine and serine (Rowe & McEwen, 1983). Because of these metabolic interconversions, a low-protein diet may not necessarily cause a deficiency of an essential amino acid but may affect the supply of non-essential amino acids (Adkins *et al.* 1966; Harper, 1974). We have studied maternal and fetal amino acid concentrations in rats fed on normal and protein-deficient rations to investigate changes in the availability of amino acids to the fetus. The present paper describes the free amino acid concentrations available to the developing fetus and the resulting changes in growth.

Materials and methods

Experimental diets

The experimental diets were identical to those described by Langley-Evans *et al.* (1996), and contained 180, 90 or 60 g protein/kg. The control, 180 g protein/kg diet contained (g/kg): casein 180, sucrose 213, cellulose fibre (Solkafloc) 50, maize starch 425, vitamin mix (AIN-76) 5, mineral mix (AIN-76) 20, maize oil 100, choline chloride 2, DL-methionine 5. The low-protein diets contained only 90 and 60 g casein/kg, with compensatory increases in the amounts of sucrose and maize starch (1 : 3, w/w). Choline chloride and methionine were from Sigma (Poole, Dorset, UK), the other ingredients were from Special Diet Services (Witham, Essex, UK).

Animals

Two experiments were performed using female rats of the Rowett Hooded strain. They were fed on the experimental diets commencing at 6–7 weeks of age. At 2 weeks later, when weighing approximately 230–240 g, the animals were mated with males of the same strain. Mating was confirmed by detection of a vaginal plug and this day was denoted day 0. The female rats were maintained on the same diets throughout pregnancy. In Expt 1, three groups each of six rats were fed on one of the three diets until day 19 of pregnancy. In Expt 2, two groups each of nine rats were fed on diets containing either 180 or 90 g protein/kg until day 21 of pregnancy; two groups of four non-pregnant control rats were fed on on the same diets. In both experiments one rat was found not to be pregnant.

Adult rats were killed by exsanguination under terminal anaesthesia with halothane (Rhone Merieux Limited, Harlow, Essex, UK), the fetuses were weighed and killed by decapitation. Maternal body weight was recorded after removal of the entire uterus and contents. All experimental procedures were approved and conducted in accordance with the UK Animals (Scientific Procedures) Act, 1986.

Sample collection and analysis

Maternal blood was collected and placed on ice for a maximum of 1 h. The blood was centrifuged at 1000 *g* for 10 min at 4° and the serum was stored at −70°. The liver, kidney, heart and brain of eight fetuses, chosen from each mother at random, were rapidly dissected and weighed. Samples were rapidly frozen in liquid N_2 and subsequently stored at −70°.

Maternal serum amino acid concentrations were analysed after deproteinization with sulfosalicylic acid (final concentration 70 g/l). The fetus contained insufficient blood for amino acid analysis and therefore one whole fetus from each mother was homogenized in 3 ml 2 M-$HClO_4$ and following centrifugation the supernatant fraction was used for analysis. Norleucine (100 nmol/ml) was added to all samples to act as an external standard. Samples were analysed using a Waters Pico-Tag system (Waters Corp., Milford, MA, USA) using pre-column derivatization with phenylisothiocyanate. Derivatives were separated on a C18 column using a sodium acetate–acetonitrile gradient suitable for the analysis of physiological fluids. The amino acid derivatives were detected by u.v. absorption at 254 nm and results calculated using the Waters Millennium software package.

Liver protein estimation was performed by the method of Lowry *et al.* (1951), RNA was measured by the method of Ashford & Pain (1986) and DNA by the method of Burton (1956).

Statistics

Mean values were calculated for all the pups and placentas. Previous work with the same strain of rats has not shown any correlation between fetal number and fetal weight (Palmer *et al.* 1996). Fetal organs were dissected from up to eight fetuses chosen at random from each mother. The data in Table 2 were subjected to Student's *t* test. Data from Expt 1 (Tables 1, 3, 4 and 5) were analysed by one-way ANOVA followed by a Tukey multiple comparison test. Data were processed using the Instat statistical package (Graph Pad Software Inc., San Diego, CA, USA). Data in Table 6 were analysed by two-way ANOVA (diet *v.* pregnancy) using the Genstat 5 statistical package (Genstat 5 release 3.1, Committee of the Statistics Department, Rothampstead Experimental Station, Harpenden, Herts., UK).

Results

The growth increments of the mother, fetus, and the major fetal organs were measured in two separate experiments, terminating on days 19 and 21 of pregnancy (total gestation time in the rat is 22·5 d). In the first experiment animals were fed on diets containing 180, 90 and 60 g protein/kg and slaughtered on day 19 of gestation (Table 1). The maternal food intakes were not significantly different on the three diets, 20·1 (SE 0·6), 21·2 (SE 0·8) and 19·8 (SE 0·7) g/d respectively during the 19 d experimental period. In the second experiment animals were fed on diets containing 180 or 90 g protein/kg and slaughtered on day 21 (Table 2). In this case the food consumption values were 19·0 (SE 0·5) g/d and 19·2 (SE 0·3) g/d respectively. The reduction in the protein content did not significantly affect the weight of the dams at slaughter on day 19 or day 21 (Tables 1 and 2). The numbers of fetuses carried by each mother were similar in rats given diets with 180 and 90 g protein/kg but were reduced by 40 % in rats given only 60 g protein/kg in the diet. There was no evidence for an increase in the number of

Table 1. Maternal, placental, fetal and fetal organ weights* on day 19 of pregnancy in rats fed on diets containing 180 (control), 90 or 60 g protein/kg†

(Mean values with their standard errors)

Dietary protein (g/kg) …	180		90		60	
	Mean	SE	Mean	SE	Mean	SE
No. of dams	6		5		5	
Maternal weight at mating (g)	241	3	240	6	234	3
Maternal weight at 19 d pregnant (g)	290	10	282	13	274	6
Fetuses/mother	14·0^a	0·6	15·0^{ab}	0·3	9·4^b	0·7
Total no. of fetuses	86		68		53	
Fetal weight (g)	1·85^a	0·04	1·99^b	0·06	1·90^a	0·04
Placental weight (g)	0·44	0·01	0·41	0·01	0·43	0·01
Placenta:fetus	0·24		0·21		0·23	
Total no. of fetal organs weighed	46		38		40	
Organ weights (mg)						
Liver	142^a	6	159^b	14	150^{ab}	10
Kidney	13·1^a	0·9	14·9^b	1·5	13·9^{ab}	1·0
Heart	9·6	0·4	9·6	0·8	9·8	0·7
Brain	102	6	108	7	102	6
Organ weights (% total weight)						
Liver	7·69	0·13	7·98	0·17	7·86	0·15
Kidneys	0·71^a	0·01	0·74^b	0·01	0·73^{ab}	0·01
Heart	0·52	0·01	0·48	0·02	0·52	0·01
Brain	5·53	0·09	5·49	0·12	5·54	0·08

^{a,b} Mean values within a row not sharing a common superscript letter were significantly different, $P < 0.05$ (one-way ANOVA).
* All the fetuses and placentas were weighed, and fetal organs were dissected from up to eight fetuses, chosen at random, from each dam.
† For details of diets and procedures, see p. 244.

resorption sites which suggested that there was either a very early failure in development or a decrease in the number of embryos which had implanted.

On day 19 the fetuses of the 90 g protein/kg group were approximately 7·5 % heavier than those of the control group. There were also significant increases in the weight of the liver (11·9 %) and kidneys (13·7 %) but not the heart or brain (Table 1). When the organ weights were expressed as a percentage of the total fetal weight, the kidneys were significantly heavier, implying that the change in fetal growth was not uniform. There was a similar but non-significant trend in the group fed on the 60 g protein/kg

Table 2. Maternal, placental, fetal and fetal organ weights† on day 21 of pregnancy in rats fed on diets containing 180 or 90 g protein/kg‡

(Mean values with their standard errors)

Dietary protein g/kg …	180		90	
	Mean	SE	Mean	SE
No. of dams	8		9	
Maternal weight at mating (g)	228	3	229	3
Maternal weight at 21 d pregnant (g)	286	6	271	5
Fetuses/mother	13·0	1·7	14·9	0·2
Total no. of fetuses	104		134	
Fetal weight (g)	4·13	0·05	3·56***	0·04
Placental weight (g)	0·59	0·01	0·53***	0·01
Placenta:fetus	0·14	0·01	0·15	0·01
Total no. of fetal organs weighed	62		72	
Organ weights (mg)				
Liver	258	5	212***	7
Kidneys	38·7	0·7	32·0***	0·4
Heart	18·8	0·4	17·2***	0·4
Brain	167	3	148***	2
Organ weight (% total weight)				
Liver	6·10	0·09	5·82*	0·08
Kidneys	0·92	0·01	0·89	0·01
Heart	0·45	0·01	0·48***	0·01
Brain	3·98	0·06	4·21*	0·07

Mean values were significantly different from those for the 180 g/kg group: * $P < 0.05$, *** $P < 0.001$ (Student's t test).
† All the fetuses and placentas were weighed, fetal organs were dissected from up to eight fetuses, chosen at random, from each dam.
‡ For details of diets and procedures, see p. 244.

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Table 3. Composition, at day 19 of pregnancy, of livers from fetuses of rats fed on diets containing 180, 90 or 60 g protein/kg*

(Mean values, with their standard errors, of six (180 g/kg diet) or five (90 and 60 g/kg diets) values, each being the mean of two determinations (two fetuses from each dam))

Dietary protein (g/kg) …	180		90		60	
	Mean	SE	Mean	SE	Mean	SE
Fetal liver protein (mg)	13·02	0·83	14·93	0·97	13·62	1·04
Protein (mg/g of wet weight)	92·3	6·4	91·8	1·8	86·8	4·3
RNA (mg)	1·19	0·05	1·34	0·03	1·31	0·07
RNA (μg/mg protein)	8·52	0·43	8·20	0·39	8·63	0·31
DNA (mg)	0·89	0·06	1·01	0·05	1·06	0·05
DNA (μg/mg protein)	6·52	0·58	6·31	0·61	7·09	0·49
RNA : protein	0·093	0·002	0·090	0·002	0·092	0·001
DNA : RNA	0·674	0·058	0·815	0·045	0·837	0·041
DNA : protein	0·070	0·006	0·079	0·006	0·090	0·009

* For details of diets and procedures, see p. 244.

diet (Table 1). The 90 and 60 protein/kg diets did not change the weight of the placentas and therefore the ratio placenta : fetus was reduced because of the increase in fetal weight.

By day 21 the fetuses from the mothers fed on the 90 g protein/kg diet were 14 % lighter than those of the control group (Table 2). Between days 19 and 21 the weight of the control fetuses had increased so that it was more than double that at day 19 (+123 %). In comparison, fetuses from mothers fed on 90 g protein/kg diet had increased in weight by 78 %. Table 2 shows that by day 21 the weights of all of the organs were significantly reduced, the liver by 18 %, the kidneys by 17 %, the heart by 8·5 % and the brain by 11 % in the group fed on 90 g protein/kg when compared with the control group. However, when these data were expressed as a proportion of the total fetal weight, the liver was a significantly smaller part of the whole fetus whereas the heart and brain were larger. The placentas were

also significantly smaller in the group fed on the 90 g protein/kg diet. Reductions in the placental and fetal weights resulted in an unchanged placental : fetus ratio.

Table 3 shows the tissue composition of the fetal livers on day 19. The protein content of the maternal diet did not affect the tissue protein content as a percentage of the wet weight. There were small increases in total RNA and DNA contents ($P = 0·107$ and $P = 0·091$ respectively) in the animals fed on the low-protein diets. The DNA content was increased by 13 % in fetuses from mothers fed on the 90 g protein/kg diet and by 19 % in those fed on the 60 g protein/kg diet.

The free amino acid concentrations in the maternal serum on day 19 of pregnancy are shown in Table 4. The low-protein diets increased the maternal serum concentrations of glutamic acid + glutamine and glycine, but decreased the concentration of the branched-chain amino acids (valine, isoleucine and leucine) and most markedly threonine

Table 4. Amino acid concentrations (nmol/ml), at day 19 of pregnancy, in the serum of rat dams fed on diets containing 180, 90 or 60 g protein/kg*

(Mean values with their standard errors)

Dietary protein (g/kg) … *n* …	180 6		90 5		60 6	
	Mean	SE	Mean	SE	Mean	SE
Aspartic acid + asparagine	155	15	206	14	194	3
Glutamic acid + glutamine	669[a]	70	1102[b]	88	1163[b]	98
Histidine	48	4	53	6	51	7
Serine	390	31	435	32	433	41
Glycine	148[a]	11	211[b]	13	212[b]	24
Threonine	804[a]	77	369[b]	40	163[c]	12
Alanine	918	133	975	149	1149	198
Arginine	133	9	184	28	176	17
Valine	237[a]	8	175[ab]	11	160[b]	8
Isoleucine	130[a]	4	104[ab]	6	92[b]	6
Leucine	161[a]	10	123[ab]	8	103[b]	3
Methionine	106	1	107	6	119	5
Tyrosine	78	2	72	4	75	10
Phenylalanine	74	7	79	8	73	4
Tryptophan	70	11	60	12	73	8
Lysine	1130	101	1212	88	1103	60

[a,b,c] Mean values within a row not sharing a common superscript letter were significantly different, $P < 0·05$ (ANOVA).
* For details of diets and procedures, see p. 244.

Table 5. Free amino acid concentrations (nmol/g fetal weight), on day 19 of pregnancy, in fetuses from rat dams fed on diets containing 180, 90 or 60 g protein/kg*

| Dietary protein (g/kg) … | 180 | | 90 | | 60 | |
| n … | 5 | | 4 | | 2 | |
	Mean	SE	Mean	SE	Mean	SE
Aspartic acid	39	3	33	7	45	1
Glutamic acid + glutamine	201	16	171	27	181	10
Histidine	30	4	37	4	30	7
Serine	501	17	529	60	586	43
Glycine	827	56	836	82	1026	62
Threonine	678[a]	52	302[b]	26	175[c]	23
Alanine	1223	57	1368	195	1390	68
Arginine	57	6	65	3	72	2
Proline	276	12	269	38	292	16
Valine	229	9	212	16	221	4
Isoleucine	99	6	86	7	91	1
Leucine	221	9	209	20	209	18
Methionine	31	7	28	6	51	7
Tryptophan	107	28	97	38	74	12
Phenylalanine	158	18	175	21	209	18
Lysine	1053	112	1091	75	1117	14

[a,b,c] Mean values within a row not sharing a common superscript letter were significantly different, $P < 0.05$ (ANOVA).
* For details of diets and procedures, see p. 244.

(Table 4). Because of the difficulties associated with obtaining sufficient fetal blood for the amino acid analysis, the total free pools of the fetus were measured (Table 5). Changes in the concentration of glutamic acid+glutamine, glycine and the branched-chain amino acids were confined to the maternal serum and not observed in the fetus, whilst the change in threonine occurred in both the maternal serum and the fetal free pool. The concentrations of threonine in the maternal serum were reduced to 46 and 20 % of the control value and in the fetus to 45 and 26 % of the control value at 90 and 60 g dietary protein/kg respectively. With the exception of threonine, there were no significant diet-induced changes in the concentrations of any other amino acids in the fetus (Table 5).

The data in Table 6 compare the serum amino acid profiles of non-pregnant rats with those found on day 21 of pregnancy. At both 180 and 90 g/kg levels of dietary protein there was an increase in the concentration of lysine in pregnancy (90 % and 54 % respectively). Branched-chain amino acids were decreased whilst glycine was increased as a result of pregnancy. Again, as on day 19 the amino acid most affected on day 21 was threonine which showed a significant decrease due to pregnancy and a significant interaction between pregnancy and diet (Table 6). This decline in free threonine was also observed in the fetus where the concentration fell from 676 (SE 74) nmol/g in the group fed on 180 g protein/kg to 468 (SE 46) nmol/g in the group fed on 90 g protein/kg (both n 8; $P > 0.05$). A comparison of the values from the animals fed on the 180 g protein/kg diet on day 21 with those found on day 19 (Table 4) shows that the majority of amino acids were unchanged, the only exception was threonine where the levels were nearly halved.

Discussion

The interaction between maternal nutrition and the growth of the fetus is poorly understood. The present study shows that a reduction in the protein content of the maternal diet does not produce a generalized reduction in the amino acid supply via the serum. Free lysine concentrations in the mother are approximately doubled as a result of pregnancy, yet this increase still occurs when the protein content of the diet is reduced to 90 or even 60 g/kg. It is probable that any dietary deficit is made up by the mobilization of maternal proteins, maintaining both fetal and maternal lysine concentrations. The more extreme reduction in dietary protein to 60 g/kg does decrease the fetal number, thus reducing the total fetal load. Whilst the concentrations of most essential amino acids are maintained, there are highly significant decreases in free threonine in both mother and fetuses when the pregnant animals are fed on low-protein diets. The changes in threonine concentrations occur only in the pregnant animals; feeding the 90 g protein/kg diet to non-pregnant female rats had no effect on serum threonine, showing that this level of protein is not deficient with regard to threonine requirements of the non-pregnant animal.

Our present data show that the growth of the fetuses had already changed by day 19 of gestation but paradoxically, growth had increased in the protein-deficient group. Langley-Evans et al. (1996) showed a similar early increase in the growth of the fetuses in pregnant rats of the Wistar strain fed on an identical diet. The data in Table 3 are not conclusive but may suggest that the small increase in the total DNA content reflects an increase in the number of cells. It is probable that this is also the case for other fetal organs and accounts for the increase in total fetal weight. However, the results clearly show that the highly significant fall in the threonine concentrations does not reduce fetal growth before day 19, indicating that the total amino acid supply, and in particular threonine, is not limiting for protein synthesis. Between days 19 and 21 the growth of the fetuses in the groups fed on the low-protein diets was

Table 6. Amino acid concentrations (nmol/ml) in the serum of non-pregnant rats and pregnant rats (at 21 d of pregnancy) fed on diets containing 180 g protein/kg†
(Mean values with their standard errors)

Dietary protein (g/kg) …	Non-pregnant				Pregnant				Statistical significance of effect of treatments‡		
	180		90		180		90				
n …	4		4		8		9				
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Pregnancy	Diet	Interaction
Aspartic acid + asparagine	129	19	162	27	195	10	209	8	–	–	–
Glutamic acid + glutamine	656	120	750	137	798	41	939	38	–	–	–
Histidine	49	16	30	1	37	1	36	2	–	–	–
Serine	332	63	420	99	349	22	410	21	–	–	–
Glycine	230	58	261	40	292	33	402	25	*	–	–
Threonine	660	111	994	164	407	28	262	20	***	–	**
Alanine	587	208	839	158	592	57	651	57	–	–	–
Arginine	135	39	163	30	168	8	198	9	–	–	–
Valine	219	62	241	38	146	6	129	6	*	–	–
Isoleucine	104	29	122	24	77	5	73	4	*	–	–
Leucine	134	35	162	36	120	7	116	8	*	–	–
Methionine	100	24	120	16	87	4	88	6	–	–	–
Tyrosine	76	13	88	13	75	6	76	4	–	–	–
Phenylalanine	62	12	71	12	72	4	78	3	–	–	–
Tryptophan	91	27	72	10	76	4	70	6	–	–	–
Lysine	574	126	752	116	1092	60	1159	50	***	–	–

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.
† For details of diets and procedures, see p. 244.
‡ Data were subjected to two-way ANOVA.

slower and it is possible that the availability of threonine influences growth in these latter stages. Threonine was the only amino acid which demonstrated a diet-induced decrease in both maternal serum and the fetal free amino acid pool. It should be stressed that the total pool of free amino acids in one entire fetus was measured. Despite the fact that the serum pool probably represents only 5 % of the total free amino acids in the body, the similarity between the maternal and fetal threonine values, at each dietary protein level, and the magnitude of the fall in threonine is striking.

There are two principal metabolic fates for threonine: direct deamination by the enzyme threonine–serine dehydratase (*EC* 4.2.1.16) to yield 2-ketobutyric acid, or conversion by the enzyme L-threonine 3-dehydrogenase (*EC* 1.1.1.103) to 2-amino-3-ketobutyrate which is then cleaved to yield glycine + acetyl-CoA. The control of partitioning between these two pathways is not well understood. Threonine dehydratase is induced in rats by both high- and low-protein diets but the mechanism involved has not been established (Kang-Lee & Harper, 1978; Bird & Nunn, 1983). In the present study, threonine concentrations decreased by about 50 % during pregnancy and by 75 % (to 25 % of normal) when the pregnant animal was fed on a low-protein diet. On the other hand pregnancy caused a 25 % increase in free glycine concentrations in the animals fed on the 180 g protein/kg diet, and an increase of nearly 2-fold in the rats on low-protein intakes. The fall in circulating threonine concentrations may, therefore, be due to its metabolism to produce glycine. In the growing pig Ballevre *et al.* (1990) showed that up to 66 % of threonine was oxidized and 76–80 % of this oxidation was through glycine formation. Although usually considered as non-essential, glycine may become essential during

pregnancy if the demands of the fetuses exceed the capacity for synthesis. This observation is not unique to rats, since glycine is considered to be potentially limiting during pregnancy in human subjects (Jackson *et al.* 1997). In recent studies of human populations Lenton *et al.* (1988) have demonstrated a significant inverse relationship between 5-L-oxo-proline, a marker of glycine insufficiency, and head circumference at birth. Glycine and its downstream metabolites, particularly methyl tetrahydrofolate, are important precursors for the synthesis of nucleoside bases and for the methylation of DNA. As well as nucleic acids, glycine is important in the synthesis of many compounds required for growth, both structural and functional e.g. collagen, haem, bile salts and glutathione (Jackson, 1991).

Threonine and glycine are also important in the metabolism of methionine and homocysteine. An identical diet, with additional methionine added to supplement the deficit in casein, was used by Langley-Evans *et al.* (1996) to demonstrate a link between the protein content of the diet and the induction of hypertension in the offspring. A constant methionine supplement (5 g/kg) was used regardless of the protein content. Therefore in the animals fed on the low-protein diets the supply of methionine remained constant and formed a higher proportion of the total amino acid supply. However there was no corresponding increase in circulating methionine concentrations, despite the fact that this excess methionine cannot be used for protein synthesis, suggesting that it was being metabolized. Girard-Globa *et al.* (1972) showed that feeding male weanling rats on a diet supplemented with 20 g methionine/kg caused a drop in free threonine concentrations which was very similar to the change observed in the present experiments. The metabolism of methionine also transiently changed levels of the

non-protein amino acid taurine suggesting that two alternative pathways are responsible for methionine oxidation. Initially methionine is metabolized via cystathionine and taurine until threonine–serine dehydratase has been induced as part of an alternative oxidative pathway (Girard-Globa *et al.* 1972). The decrease in free threonine may simply be a consequence of its oxidation by threonine–serine dehydratase induced by excess methionine. A change in taurine concentration has been observed in the serum of pregnant rats fed on low-protein diets and linked to changes in the ability of the pancreatic islets to release insulin (Reusens *et al.* 1995; Cherif *et al.* 1996). Because of the metabolic changes that occur when the mother is fed the deficient diet, the time allowed for adaptation before mating may be important in determining the final outcome.

There is also circumstantial evidence to suggest that changes in S-amino acid metabolism are responsible for the changes in fetal growth and survival. The fetus lacks cystathionine β-synthase (*EC* 4.2.1.22) and has no means to eliminate methionine and homocysteine. Methionine synthase (*EC* 2.1.1.13) progressively declines during gestation in a number of species (Gaull *et al.* 1973) while S-adenosyl homocysteine hydrolase (*EC* 3.3.1.1) remains relatively constant (VanAerts *et al.* 1995). The addition of homocysteine to embryo cultures *in vitro* leads increased growth because homocysteine is converted to S-adenosyl methionine by the fetus (VanAerts *et al.* 1994). An excess of methionine in the maternal diet may therefore be responsible for the early overgrowth of the fetuses from mothers fed on the 90 g protein/kg diet because of its metabolism to homocysteine. There is clinical evidence that elevated homocysteine levels are associated with early pregnancy loss in human subjects (Wouters *et al.* 1993) and a further increase of homocysteine to toxic levels in the animals fed on the 60 g protein/kg diet may be responsible for the reduction in the fetal number.

In conclusion, changes in the threonine–methionine–homocysteine group of amino acids early during pregnancy may be the nutritional trigger leading to the adverse effects of protein-deficiency. Methionine–homocysteine metabolism has already been shown to play a crucial role in the aetiology of neural-tube defects where disruption of folate metabolism leads to increases in homocysteine levels (Bakker & Brandjes, 1997). These results suggest that this group of amino acids may also be involved in more subtle changes in fetal development leading to insulin resistance and hypertension later in life.

Acknowledgements

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