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An imbalance in the methionine content of the maternal diet reduces postnatal growth in the rat

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Abstract

The pregnant rat fed a low-protein diet has become widely used as a model system in the study of the prenatal programming of adult metabolism and disease. When pregnant rats of the hooded Lister strain were fed semisynthetic diets containing 18% or 9% casein supplemented with 0.5% DL-methionine, there was significant postnatal mortality in the group fed the low-protein diet. In a second experiment, dams were fed diets containing 9% casein supplemented with varying concentrations of DL-methionine up to 0.4% (w/w) and compared with a group fed a diet containing 18% casein supplemented with 0.5% DL-methionine. At birth, the pups from dams fed the low-protein diets supplemented with 0.2% DL-methionine or greater were significantly smaller than those of the dams fed the diet containing 18% protein. By 25 weeks of age, the body weight of the offspring of dams fed the low-protein diet supplemented with 0.2% or 0.3% DL-methionine were approximately 10% lower than those in the control group of offspring from dams fed 18% protein supplemented with 0.5% DL-methionine. There were corresponding changes in the weights of the major organs. These data suggest that increasing the DL-methionine supplement in the low-protein diet retards the growth of the fetus and affects the mature adult body weight. In contrast to the findings of other studies that used different formulas of the low-protein diet, the glucose tolerance in the offspring was unaffected by the protein content of the maternal diet at all levels of DL-methionine supplementation. These results suggest that the changes in metabolism of the offspring result from interactions between protein, lipids, and carbohydrates in the maternal diet, rather than a consequence of postnatal growth retardation per se and highlight the importance of considering all components of the maternal diet in the programming mechanism.

1. Introduction

The pregnant rat fed a low-protein diet is widely used as an animal model to study the mechanisms that underlie the programming of adult metabolism by the prenatal environment [1]. Semisynthetic diets based on the AIN-76 formula [2,3] have been used in the majority of these experiments [4-6]. These diets are prepared with casein as the source of protein. Although casein is a highly digestible protein, it fails to meet all of the amino acid requirements of the growing rat because it contains very little cysteine. This deficiency is corrected by adding a supplement of methionine (normally a mixture of D and L isomers) to the diet, enabling the animal to synthesize additional cysteine via the transulfuration pathway. However, analysis of serum from pregnant rats fed the casein-based diets supplemented with 0.5% DL-methionine showed that concentrations of homocysteine are increased in the animals fed the diet containing 9% protein [7,8]. Because the low-protein diet contains an additional 55 mg DL-methionine per gram of casein compared to 27 mg DL-methionine per gram of casein in the high-protein diet, the increased levels of homocysteine may reflect the altered balance between methionine and other amino acids in the diet. Hyperhomocysteinemia is associated with endothelial dysfunction, a risk factor for cardiovascular disease in the adult [9,10]. Therefore some of the postnatal programming effects attributed to changes in protein metabolism in pregnant animals fed casein-based diets may be a consequence of changes in sulfur amino acid metabolism.

Previously, it has been shown that feeding the pregnant rat the protein-restricted diets described above alters the growth trajectory of the fetus [11,12]. By day 21 of gestation, there are significant asymmetries in the growth of major organs. It is not clear to what extent the change in fetal growth is a consequence of the altered balance of amino acids in the diet

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consumed by the dam. Equally, there is little information on the impact of maternal methionine intake on physiological functions of the offspring. In this study, pregnant rats were fed diets containing 9% casein with a range of methionine supplements from 0.1% w/w (equivalent to 11 mg DL-methionine per gram of casein) up to 0.4% w/w (equivalent to 44 mg DL-methionine per gram of casein) encompassing the range found in the high-protein diets. The subsequent growth, blood pressure, and glucose tolerance of the offspring were measured to evaluate the impact of the dietary treatments on postnatal development.

2. Methods

2.1. Experimental diets

The semisynthetic casein-based diets were based on those described previously [11] and their composition is shown in Table 1. Choline chloride and DL-methionine were from Sigma (Poole, Dorset, UK); the other ingredients were from Special Diet Services (Witham, Essex, UK).

2.2. Animals

All experiments were performed using female rats of the Rowett hooded strain bred in the Institute's own colony. All experimental procedures were approved by the ethical review committee of the Rowett Research Institute and conducted in accordance with the UK Animals (Scientific Procedures) Act (1986).

Experiment 1: Two groups of 8 animals were fed diets containing 18% casein (w/w) or 9% casein (w/w). Both diets were supplemented with 0.5% DL-methionine (w/w), equivalent to 27 mg DL-methionine per gram of casein in the high-protein diet and 55 mg DL-methionine per gram of casein in the low-protein diet.

Experiment 2: Animals were randomly allocated into 5 groups of 8 animals and fed diets containing either 18% casein (supplemented with 0.5% w/w DL-methionine) or one of four 9% casein diets supplemented with 0.1%, 0.2%, 0.3%, or 0.4% w/w DL-methionine, equivalent to 11 mg DL-methionine per gram of casein at the lowest level increasing to 44 mg DL-methionine per gram of casein at the highest.

In both experiments the animals were fed the experimental diets ad libitum from 8 weeks of age. Two weeks later, when weighing approximately 230 to 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. Pups were delivered naturally, and on postnatal day 1 the litters were culled to 8 pups per dam with 4 males and 4 females where possible. Once they had given birth the dams were fed stock diet (CRM breeder diet, Special Diet Services) ad libitum until weaning was complete. The offspring were weaned onto stock diet (CRM breeder diet), which was fed ad libitum for the remainder of the experiment. All animals were weighed twice weekly to monitor growth. Subgroups of the male offspring were subsequently killed at 4, 16, and 22 weeks of age. The female offspring were killed at 4, 12, and 25 weeks of age. The group of offspring kept until 22 and 25 weeks of age was used for the blood pressure measurements. Glucose tolerance measurements were carried out in the male offspring at 22 weeks of age and in the female offspring at 25 weeks of age.

Table 1
Composition of the experimental diets

Component	18% casein (g/kg)	9% casein (g/kg)
Casein	180	90
Sucrose	213	243
Cellulose fiber	50	50
Cornstarch	380	440
Vitamin mix[a]	50	50
Mineral mix[b]	20	20
Maize oil	100	100
Choline chloride	2[c]	2[c]
DL-Methionine	5	1-4

[a] The vitamin mixture contained (per kilogram) the following: thiamine 200 mg, pyridoxine 200 mg, riboflavin 200 mg, *p*-aminobenzoic acid 200 mg, nicotinic acid 600 mg, calcium pantothenate 400 mg, folic acid 100 mg, biotin 100 mg, inositol 8000 mg, cholecalciferol 50 mg, all-rac-a-tocopherol 1200 mg, menadione 2 mg, retinylacetate 1200 retinol equivalents, cyanocobalamin 5 mg, choline chloride 16 g, made up to 1 kg with corn starch.

[b] The mineral mixture contained (per kg) the following: $CuSO_4 \cdot 7H_2O$ 400 mg, $FeSO_4 \cdot 7H_2O$ 5 g, $MnSO_4 \cdot 4H_2O$ 4 g, $ZnSO_4 \cdot 7H_2O$ 3.6 g, KI 40 mg, KIO_3 40 mg, NaF 120 mg, NH_4VO_3 10 mg, $NiCl_2 \cdot 6H_2O$ 80 mg, $SnCl_4 \cdot 5H_2O$ 120 mg, NaSeO 36 mg, $CrK(SO_4)$ 2.12 g, H_2O (chrome alum) 0.96 g, $CaCO_3$ 410 g, KH_2PO_4 314 g, KCl 22 g, $MgSO_4 \cdot 7H_2O$ 102 g, Na_2HPO_4 142 g, in about 1 kg [13].

[c] Total choline chloride content including 1 g derived from vitamin mix.

2.3. Blood pressure measurements

Systolic blood pressure was measured by recording arterial pulse in the tail (Model 229, Life Science Instruments, Woodland Hills, CA). Before analysis, rats were placed in a thermostatically controlled chamber warmed to 28°C, for a maximum of 30 minutes to dilate the tail vein. The animals were then lightly restrained by hand; a 15-mm cuff was placed over the tail and inflated to 200 mm Hg. Five separate blood pressure readings were taken, and the highest and lowest readings discarded; the average of the 3 remaining readings was recorded.

2.4. Glucose tolerance tests

Animals were fasted overnight and a baseline blood sample of approximately 150 μL was taken from the tail vein. The animals were then given a single dose (200 mg per 100 g body weight) of 30% D-glucose solution by gavage (females) or by the administration of the same dose as a flavored jelly (males). Over the following 90 minutes, six 150-μL blood samples were collected in a heparin-treated tube after venesection of the tail vein. Red cells were removed by centrifugation, and plasma glucose was estimated with a KONE selective chemistry analyzer using

the hexokinase/glucose-6-phosphate dehydrogenase method (kit number 981300, Labmedics, Manchester, UK). Plasma insulin was measured by radioimmunoassay [14]. A portion from the head of the pancreas was extracted in 0.17 mol/L HCl in 70% ethanol, and the total insulin content was measured by radioimmunoassay [15].

2.5. Carcass analysis

The offspring were killed, and the internal organs were dissected without delay. After weighing, samples of the organs were frozen in liquid nitrogen and then stored at −80°C until used for analysis. The eviscerated carcasses were minced, lyophilized, and ground to a uniform, fine powder. The fat content was determined with a Soxtec fat extraction system (Tecator, Hoganas, Sweden). Nitrogen and ash were determined by combustion in an Automated Dumas system (Foss Electric [UK], Warrington, Cheshire, UK).

2.6. Statistics

Data were analyzed by 1-way analysis of variance (ANOVA) followed by Fisher multiple comparison test (Genstat 6 statistical package, Lawes Agricultural Trust, Rothamsted Experimental Station, Harpenden, Herts, UK). Where appropriate, the dam was taken into account in the block structure.

3. Results

Experiment 1: The growth of both groups of animals was similar before and during gestation and did not differ from that expected from animals fed the stock diet. Seven of the 8 animals in the group fed the diet containing 18% protein were pregnant. The average litter size was 12.6 ± 2.2 pups per dam, and the average gestation period was 22.7 days. After culling to 8 pups per dam, a further 3 pups born to dams fed the diet containing 18% crude protein subsequently died (5.3% mortality). Two of the 8 dams fed the diet containing 9% protein supplemented with 0.5% methionine were not pregnant. In addition, 1 animal had not given birth by 24 days (10% longer than the expected period) and was removed from the study. The average gestation time for the 5 remaining animals fed the 9% protein diet was 23.5 days. Many of the neonates were lethargic, failed to suckle, and were rejected, leaving an average of 5.6 ± 3.4 viable pups per dam after 24 hours. There were further deaths in the next 24 hours, leaving a total of only 12 pups by 48 hours. When the litter was reduced to only 1 or 2 survivors, the remaining pups were also killed, and, ultimately, only 1 complete litter was weaned successfully. Postmortem analysis of the dams' reproductive tract suggested that there were on average 10.5 ± 3.5 implantation sites per dam, giving an overall mortality of approximately 75% in the first 24 hours.

Experiment 2: During a 2-week adaptation period, the weight gain was not affected by the protein content of the diet or by the balance of methionine to casein in the low-protein diets. As a result, the body weights of the dams were not significantly different at mating (Table 2). During the first 2 weeks of gestation, there were no significant differences in weight gain. Five of the 8 animals in the group fed the diets containing 18% protein supplemented with 0.5% methionine were pregnant. Six of the 8 animals in the groups fed the diet containing 9% protein supplemented with 0.1%, 0.2%, and 0.3% methionine were pregnant and produced viable litters, with litter sizes comparable to those found in the high-protein group. Seven of the 8 dams in the group fed the low-protein diet supplemented with 0.4% methionine were pregnant; however, 4 of these had small litters of between 7 and 10 pups. At birth, the pups from dams fed the low-protein diets supplemented with 0.2% methionine or more were significantly smaller than those of the dams fed the diet containing 18% protein (Table 2). The weight differential was maintained until weaning (Tables 2 and 3), although the magnitude of the effect diminished with time, and at 4 weeks of age, 1 week after the animals were weaned onto stock diet, there

Table 2
Maternal and fetal growth

Diet	18% casein		9% casein		9% casein		9% casein		9% casein	
Methionine supplement (%)	0.5		0.1		0.2		0.3		0.4	
n	5		6		6		6		7	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Weight at start (day −31) (g)	208.6	4.7	203.8	6.5	205.8	5.0	201.4	4.3	204.0	4.6
Weight at mating (g)	230.9	4.4	221.1	7.1	227.5	8.0	221.1	5.2	228.7	5.6
Weight at day 7 of gestation (g)	259.3	4.3	247.6	8.3	255.6	6.5	243.2	4.5	254.7	8.0
Weight at day 16 of gestation (g)	324.4	5.1	310.5	10.0	322.0	8.3	307.4	3.6	316.6	10.1
Weight at day 21 of gestation (g)	365.0	5.2	341.2	11.1	350.3	10.0	344.0	3.8	348.7	12.0
Dam weight on postnatal day 3 (g)	298.5	6.3	280.3	9.8	287.4	8.8	282.1	2.2	283.2	9.1
Average litter size	13.6	0.9	12.3	1.1	13.2	0.7	13.7	0.9	10.9	1.3
Mean pup weight on day 1 (g)*	5.8	0.1^{a}	5.3	0.2^{ab}	5.0	0.2^{b}	4.9	0.2^{b}	5.1	0.3^{b}
Mean pup weight on day 6 (g)*	12.5	0.1^{a}	12.5	0.4^{a}	10.1	0.5^{c}	10.3	0.5^{bc}	11.3	0.3^{b}
Mean pup weight on day 12 (g)*	25.1	0.4^{a}	25.4	0.7^{a}	22.2	0.5^{b}	22.0	1.0^{b}	22.8	0.8^{b}

[abc]Mean values within a row not sharing a common superscript letter were significantly different, $P < .05$ (1-way ANOVA).

* Values for mean pup weight per dam after offspring were culled to 8 pups per dam.

Table 3

A. Organ weights of the male offspring

Diet	18% casein		9% casein		9% casein		9% casein		9% casein	
Methionine supplement (%)	0.5		0.1		0.2		0.3		0.4	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
*4 wk**										
n	10		14		12		11		12	
Body weight (g)	82.5	1.6	80.6	2.1	75.6	2.1	75.8	2.8	77.0	2.3
Liver weight (g)	4.145	0.144	4.363	0.137	4.151	0.1221	4.145	0.161	4.228	0.173
Kidney weight (g)	1.022	0.013	0.969	0.030	0.986	0.026	0.985	0.032	0.971	0.014
Heart weight (g)	0.465	0.017^{ab}	0.479	0.019^{b}	0.450	0.013^{ab}	0.416	0.013^{a}	0.462	0.016^{ab}
16 wk										
n	5		6		6		6		7	
Body weight (g)	487.4	8.5^{a}	478.3	12.3^{ab}	447.6	16.2^{bc}	440.4	13.2^{c}	472.7	6.0^{abc}
Liver weight (g)	21.120	0.909	20.917	0.897	21.200	0.584	19.207	0.856	19.943	0.510
Kidney weight (g)	3.609	0.058^{a}	3.578	0.08^{a}	3.361	0.062^{bc}	3.327	0.061^{c}	3.527	0.072^{ab}
Heart weight (g)	1.404	0.084	1.57	0.097	1.381	0.038	1.393	0.079	1.374	0.055
22 wk (fasted overnight)										
n	5		6		6		6		7	
Body weight (g)	482.2	6.0^{a}	442.3	13.0^{b}	444.5	11.0^{b}	434.8	9.7^{c}	457.4	8.7^{bc}
Liver weight (g)	17.780	0.722	17.433	1.131	16.733	0.418	15.750	0.402	16.343	0.442
Kidney weight (g)	3.408	0.075	3.381	0.148	3.323	0.132	3.328	0.064	3.457	0.101
Heart weight (g)	1.438	0.046	1.422	0.064	1.442	0.060	1.425	0.031	1.528	0.046

B. Organ weights of the female offspring

Diet	18% casein		9% casein		9% casein		9% casein		9% casein	
Methionine supplement (%)	0.5		0.1		0.2		0.3		0.4	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
*4 wk**										
n	10		9		12		11		14	
Body weight (g)	73.0	1.6	73.2	2.1	68.4	2.8	68.9	2.5	72.8	1.5
Liver weight (g)	3.800	0.095	4.007	0.127	3.848	0.150	3.806	0.184	3.927	0.111
Kidney weight (g)	0.927	0.019	0.945	0.031	0.902	0.040	0.921	0.032	0.929	0.018
Heart weight (g)	0.420	0.021	0.433	0.014	0.397	0.021	0.409	0.013	0.418	0.013
12 wk										
n	5		6		6		6		7	
Body weight (g)	261.6	7.3^{a}	257.5	5.7^{ab}	246.3	6.5^{ab}	237.7	9.2^{b}	246.1	6.8^{ab}
Liver weight (g)	12.045	0.198^{a}	12.002	0.557^{a}	10.576	0.345^{b}	10.009	0.619^{b}	11.263	0.396^{ab}
Kidney weight (g)	2.122	0.056^{a}	1.997	0.037^{abc}	1.916	0.045^{bc}	1.831	0.061^{c}	2.055	0.073^{ab}
Heart weight (g)	0.975	0.040^{ab}	1.041	0.050^{ab}	0.905	0.058^{b}	0.880	0.074^{b}	1.083	0.056^{a}
25 wk (fasted overnight)										
n	3		5		6		4		7	
Body weight (g)	297.0	13.3^{a}	285.3	9.4^{ab}	261.4	5.1^{b}	263.6	8.3^{b}	273.5	9.3^{ab}
Liver weight (g)	10.367	0.578^{a}	10.36	0.501^{a}	9.033	0.376^{ab}	8.5	0.389^{b}	9.614	0.406^{ab}
Kidney weight (g)	2.294	0.056^{a}	2.206	0.071^{a}	1.986	0.048^{b}	2.065	0.023^{ab}	2.063	0.095^{ab}
Heart weight (g)	1.011	0.085	1.008	0.055	0.977	0.030	0.977	0.024	1.006	0.037

[abc]Mean values within a row not sharing a common superscript letter were significantly different, $P < .05$ (1-way ANOVA).

* The data at 4 weeks of age are from 2 pups per dam and were analyzed by ANOVA blocking for dam.

were no significant differences in the weight of either male or female offspring (Table 3). The overall weight of the liver and kidneys of the male (Table 3A) and female (Table 3B) offspring was not significantly different. There were small differences in the weight of heart; however, these changes did not follow a particular pattern. Analysis of the relative organ weights showed that there were no significant differences (data not shown).

3.1. Postweaning growth of the offspring

The postweaning growth of the offspring fed the stock diet ad libitum is shown in Table 3. By 16 weeks of age, a trend was beginning to emerge in the body weights. The male offspring (Table 3A) from dams fed the diet containing 9% protein supplemented with 0.1% methionine were not significantly different from the offspring of dams fed the

diet containing 18% protein. The offspring of dams fed 9% casein diets containing 0.2% and 0.3% methionine were significantly smaller; however, the offspring of dams fed the diet containing 0.4% methionine were not significantly different. By 22 weeks of age, the male offspring of dams fed the 9% casein diets were all significantly smaller than the offspring of dams fed the 18% casein diet. Although the data suggested a trend toward reduced adult body weight when the ratio of methionine to casein is increased in the low-protein diet, this was reversed in the offspring of dams receiving the highest supplement of 0.4% w/w methionine. However, some of the dams in this group gave birth to litters of less than 10 pups. These small litters, which were only found in the dams that were fed the 9% protein diet supplemented with 0.4% methionine, appear to have improved fetal growth, possibly because they are better able to deal with excess methionine or because the smaller litter ameliorates the effect of reduced protein. Also the pups had begun to suckle by the time they were first weighed. During this 24-hour period, the pups in the smaller litters may well have consumed more milk, giving them a higher body weight. We believe that these 2 effects combine to mask prenatal growth restriction. If these dams and their offspring were omitted from the analysis, then the data show a progressive reduction in birth weight and postnatal bodyweight as the methionine supplement of the low-protein diet was increased.

The maternal diet had no effect on the weight of the liver and heart. There was no effect either on the weight of the kidneys in the offspring of dams fed the 9% casein diet containing 0.1% methionine; however, the kidneys were significantly smaller in the offspring of dams fed low-protein diets supplemented with 0.2% and 0.3% methionine. The animals killed at 22 weeks had slightly lower body weights, possibly as a result of being fasted for the glucose tolerance test. This is particularly noticeable in the liver where fasting reduces the glycogen content. In this group, all of the offspring from dams fed the low-protein diets were significantly smaller; however, there was still an additional effect of increasing the methionine supplement to the diet. The body and organ weights of the female offspring are shown in Table 3B. The pattern is essentially similar to the male offspring, and by 12 weeks of age the body weight of the offspring of dams fed the diet containing 9% protein supplemented with 0.2% and 0.3% methionine was significantly lower than that of the other groups. As with the males, this trend was reversed in the offspring of dams fed the 9% protein diet supplemented with 0.4% methionine. The change in growth was also reflected in significant changes in the weight of the liver, kidneys, and heart of the offspring of dams fed the low-protein diet supplemented with 0.2% and 0.3% methionine. This pattern of changes persisted in the animals killed at 25 weeks of age except that there were no significant differences in the heart in these animals. Analysis of the remaining carcass of the female offspring at 25 weeks of age showed that there were no significant differences in either the lean tissue growth or fat accretion in the offspring regardless of the protein or methionine content of the maternal diet (data not shown).

3.2. Blood pressure and glucose tolerance in the offspring

The blood pressure of the male offspring was measured at 20 weeks of age, as shown in Table 4. The maternal diet did not produce any significant differences in the systolic blood pressure of the offspring. The values for the control

Table 4
Blood pressure, pancreatic insulin content, and glucose tolerance of the offspring

Diet	18% casein		9% casein		9% casein		9% casein		9% casein	
Methionine supplement (%)	0.5		0.1		0.2		0.3		0.4	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Systolic blood pressure (mm Hg)										
Males	160.6	3.5	170.7	7.0	161.7	5.4	159.4	7.4	169.1	2.7
n	5		6		6		6		7	
Pancreatic insulin content (μU/mg tissue)										
Males (22 wk)	2.76	0.40	3.22	0.13	2.53	0.19	2.44	0.35	2.75	0.21
Females (25 wk)	2.14	0.33	2.38	0.58	2.85	0.72	2.30	0.21	2.95	0.22
n	5		5		6		6		7	
OGTT (male offspring at 22 wk of age)										
Glucose AUC	851.6	21.7	806.8	39.5	858.9	24.6	945.5	48.2	910.6	25.2
Insulin AUC	6361	313	6676	969	6916	698	6003	346	6906	708
n	5		6		6		6		7	
OGTT (female offspring at 25 wk of age)										
Glucose AUC	623.6	76.0	672.3	49.1	625.5	51.1	672.8	87.3	653.9	32.2
Insulin AUC	4052	346	4355	484	4747	335	3739	536	4889	350
n	3		5		6		4		7	

[abc]Mean values within a row not sharing a common superscript letter were significantly different, $P < .05$ (1-way ANOVA).

animals are not significantly different to those reported for the same strain of rat in a previous study [16].

The pancreatic insulin content of the male and female offspring at 22 and 25 weeks of age is also shown in Table 4. These data show that the prenatal treatment produces no significant difference in insulin content. This result is also reflected in the glucose tolerance test, which was carried out on the male and female offspring before they were killed at 22 and 25 weeks of age. After an overnight fast, there were no significant differences in the basal plasma insulin or glucose levels in male or female offspring in any group (data not shown). After the administration of the glucose dose, plasma glucose concentrations increased by approximately 2-fold; however, the protein content of the maternal diet did not change either the peak height or the total area under the curve (AUC). These parameters were similar in the offspring of dams fed the diet containing 18% protein and those fed 9% protein. The values for the area under the curve shown in Table 4 also show that the methionine supplement added to the maternal diet had no effect on the plasma glucose concentrations of the offspring. Plasma insulin concentrations increased by about 2-fold within 15 minutes of the oral glucose dose. The protein content of the maternal diet did not produce significant differences in either the peak height or in the total area under the curve. The data for the area under the curve in Table 4 also shows that the methionine content of the maternal diet did not have an effect on glucose-stimulated insulin release.

4. Discussion

Previous studies have shown that an inappropriate balance of methionine relative to other amino acids in the diet retards the growth of adult rats [17-19]. This study shows that when casein-based semisynthetic diets are used, the balance of methionine to casein also influences fetal growth. The growth retardation affects both birth weight and mature adult body weight. The offspring of dams fed the 9% casein diet supplemented with 0.3% methionine are approximately 10% lighter than those in the group from dams fed the 18% protein diet. In contrast, the offspring of dams fed the 9% casein diet supplemented with 0.1% methionine are only approximately 4% lighter than those in the high-protein group. The growth retardation induced by high levels of methionine supplementation appears to be additive to the effects of protein deficiency and persists throughout life. The results suggest that the final potential for the growth of lean tissues has been determined during fetal life as the mature body weights of both male and female offspring are ultimately reflected in their birth weights.

Studies of fetal growth in dams fed similar diets containing 9% protein supplemented with 0.5% methionine have shown that the growth of some fetal organs is more sensitive to the dietary treatments than others when compared to the fetuses of dams fed a diet containing 18% protein [11,12,20]. This asymmetric growth of the fetus changes the relative weights of the organs one to another and contrasts to a symmetrical growth retardation which affects all of the organs equally. This experiment shows that although there are changes in the weight of the kidneys in the adult offspring, these are proportional to the change in total body weight and there is no evidence that the disproportionate retardation of kidney growth observed in the fetal stages persists into adult life. Liver weight is also reduced during fetal development, but the data suggest that this is also proportionate to the change in overall growth and persists into adult life. In adult animals, excess dietary methionine leads to pathological changes in the liver, including the appearance of fat deposits [21]. However, the hepatic histology of the adult offspring appeared to be normal and there was no change in the triglyceride or glycogen content (data not shown). These data suggest that supplementation of the low-protein diets with up to 0.3% DL-methionine produces a progressive fetal growth retardation with symmetrical decreases in the growth of major organs. At higher levels of methionine supplementation, the pattern of growth is complicated by changes in the litter sizes.

There may be a number of reasons why we observed significant mortality in the offspring of dams fed the 9% protein supplemented with 0.5% methionine in this experiment but were unable to demonstrate an effect of maternal diet on adult blood pressures. Unlike the hooded Lister rat, there are only modest postnatal losses when animals of the Wistar strain are fed diets containing 9% protein supplemented with 0.5% w/w methionine [11]. The ability to metabolize excess methionine is particularly dependent on the glycine supply, although other factors including membrane transport activity, glycine *N*-methyl transferase activity, and the capacity of the transulfuration pathway are also important. It is interesting to note that glycine supplementation reverses the increase in blood pressure in the offspring of Wistar rats produced by feeding dams a low-protein diet supplemented with 0.5% methionine [22] and influences the vasculature of the dam [20], suggesting that this amino acid plays a critical role in the programming process. It is possible that changes in the proportions of methionine to other amino acids in the diet influence glycine demand through changes in the synthesis of sarcosine. Temporal factors are also important, as the animal has to adapt its metabolism to accommodate the different amino acid balance of the experimental diet. In this experiment, the dams were fed the experimental diets for 2 weeks before mating; however, in other studies animals have been transferred to the experimental diet immediately after mating without a period for metabolic adaptation. For example, feeding the low-protein diet only during the first 4 days after conception has been shown to increase the blood pressure of the offspring [23]. If the methionine supply exceeds the capacity for its metabolism, a transient increase in free methionine concentrations may lead to toxicity. Alternatively, the stress imposed by adaptation to the diet may elevate glucocorticoids, which have been

shown to program blood pressure in the rat [24]. Maternal protein restriction during pregnancy also selectively attenuates placental [11] β-HSD2 activity, which acts as a barrier to protect the developing fetus from maternal glucocorticoids [25]. The impact of changes in the proportions of methionine to other amino acids on the activity of this key enzyme is unknown. Because blood pressure is the product of a number of different factors, subtle differences in the protocol, genetic differences between different rat stains, and the influence of postnatal diet [26] may all contribute to the apparent variability between laboratories.

More striking are the lack of diet-related changes in insulin after a glucose challenge to the offspring. Previous reports have suggested that there are small changes in glucose metabolism in the offspring of dams fed high- and low-protein diets, similar to those used in this study [27]. However, we have been unable to show any significant effect of maternal dietary protein on blood glucose concentrations during the oral glucose tolerance test (OGTT). The concentrations at any given time are similar regardless of the protein or methionine content of the maternal diet. In contrast, the same experiment carried out on the offspring of dams fed diets prepared to a different formula (Hope Farms, Woerden, Netherlands) showed that the protein content of the maternal diet significantly altered glucose-stimulated insulin release, leading to a highly significant increase in the area under the curve for insulin [28,29]. Laboratories using the Hope Farms diet formulation have reported that the insulin content of the pancreas is reduced in the offspring of dams fed a diet containing 8% protein [30], whereas in this experiment we have been unable to detect any changes in the pancreatic insulin content (Table 4). The offspring of dams fed the high- or low-protein Hope Farms diets have significantly different adult body weights because of changes in the accumulation of body fat [29]. In contrast, the protein content of the maternal diet had no significant effect on the body composition of the offspring in this experiment (data not shown). These data suggest that there are profound differences in the effects of the 2 different formulas of protein-restricted diet on the development and subsequent function of the insulin axis in the offspring.

These studies along with previous studies which showed differential effects on the blood pressure of the offspring of dams fed either the University of Southampton (UoS) diet used in this study and the Hope Farms diet used in other studies [31] illustrate the critical importance of the diet formula. Semisynthetic animal diets are a useful tool in research requiring control of discrete nutrients. However, because the animal is continuously provided with a fixed mixture of amino acids, carbohydrates, and fatty acids they lead to compromises in metabolism when compared to animals consuming a mixture of different proteins, carbohydrates, and lipids. These results suggest that much of the interlaboratory variability in studies of fetal programming in rodents may be attributed to the diets used. The Hope Farms diet is high in dextrose and low in complex carbohydrate, whereas the UoS diet is prepared with sucrose and starch. The diets also differ in the composition of the fats and in particular the proportions of polyunsaturated fatty acids. The UoS diet is prepared with corn oil, which has a lower 18 n3 fatty acid content than soya oil used in the Hope Farms diet formula. There are a number of reports that feeding diets containing 18 n3 fatty acids during pregnancy and lactation in the rat programs glucose-stimulated insulin release [32-34]. In these studies, the animals show no differences in the plasma glucose profile during a glucose tolerance test; however, the profile of insulin release shows changes similar to those observed in experiments with low-protein diets prepared with soya oil. All of these observations suggest that the programming of fetal growth and development is not the result of a simple growth restriction but is the product of more fundamental metabolic changes in mother or fetus resulting from interactions between several diet components.

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