Effects of dietary mixtures of amino acids on fetal growth and maternal and fetal amino acid pools in experimental maternal phenylketonuria[1–3]

Richard E Austic, Chun-Li Su, Barbara J Strupp, and David A Levitsky

ABSTRACT

Background: Branched-chain amino acids have been reported to improve fetal brain development in a rat model in which maternal phenylketonuria (PKU) is induced by the inclusion of an inhibitor of phenylalanine hydroxylase, DL-*p*-chlorophenylalanine, and L-phenylalanine in the diet.

Objective: We studied whether a dietary mixture of several large neutral amino acids (LNAAs) would improve fetal brain growth and normalize the fetal brain amino acid profile in a rat model of maternal PKU induced by DL-α-methylphenylalanine (AMPhe).

Design: Long-Evans rats were fed a basal diet or a similar diet containing 0.5% AMPhe + 3.0% L-phenylalanine (AMPhe + Phe diet) from day 11 until day 20 of gestation in experiments to test various mixtures of LNAAs. Maternal weight gains and food intakes to day 20, fetal body and brain weights at day 20, and fetal brain and fetal and maternal plasma amino acid concentrations at day 20 were measured.

Results: Concentrations of phenylalanine and tyrosine in fetal brain and in maternal and fetal plasma were higher and fetal brain weights were lower in rats fed the AMPhe + Phe diet than in rats fed the basal diet. However, fetal brain growth was higher and concentrations of phenylalanine and tyrosine in fetal brain and in maternal and fetal plasma were lower in rats fed the AMPhe + Phe diet plus LNAAs than in rats fed the diet containing AMPhe + Phe alone.

Conclusion: LNAA supplementation of the diet improved fetal amino acid profiles and alleviated most, but not all, of the depression in fetal brain growth observed in this model of maternal PKU. *Am J Clin Nutr* 1999;69:687–96.

KEY WORDS Phenylketonuria, phenylalanine, brain, plasma, amino acids, gestation, fetus, rats, DL-α-methylphenylalanine, large neutral amino acids

INTRODUCTION

Children of phenylketonuric mothers may suffer physical anomalies and permanent effects on mental function that stem from abnormal development in a high-phenylalanine milieu during pregnancy (1–3). This problem can be minimized if phenylketonuric women adhere to a low-phenylalanine diet from the time of conception (2, 4–7). Such diets are not highly palatable, however, and strict adherence to the diet is often not achieved (8, 9). An alternative to a low-phenylalanine diet would be a more normal diet that is altered so that the phenylalanine content is less problematic; hence, there is an interest in the possible use of large neutral amino acids (LNAAs), which compete with phenylalanine for membrane transport sites in the brain, to alleviate the problems associated with hyperphenylalaninemia (10–12).

Maternal hyperphenylalaninemia can be induced in rats by inclusion in the diet of an inhibitor of phenylalanine hydroxylase, such as DL-*p*-chlorophenylalanine (PCPA) or DL-α-methylphenylalanine (AMPhe), in combination with a large supplement of L-phenylalanine. In one model of maternal phenylketonuria (PKU), supplements of 0.12% PCPA and 3.0% L-phenylalanine are included in the diet of rats from day 10 to day 20 of gestation (13, 14). Fetuses of phenylketonuric dams have lower body weights, lower brain weight relative to body weight, and lower concentrations of certain branched-chain amino acids in the brain than do fetuses of control dams (13). Supplementation of the diet with branched-chain amino acids increases fetal growth and brain weight (13, 14) and improves the performance of progeny in maze tests (15, 16).

PCPA, which appears to be more toxic than AMPhe (17–19), depresses fetal body and brain weights by nearly 20% (14). As an inhibitor of tryptophan hydroxylase it may alter serotonin synthesis (20). In an attempt to avoid these problems, Brass et al (21) developed a model of maternal PKU that is similar to the PCPA model except that the diet is supplemented with 0.5% AMPhe instead of 0.12% PCPA. With use of this model, body and brain weights of pups were reduced by ≈7% and 8%, respectively, in phenylketonuric fetuses (21). Progeny from phenylketonuric dams were also reported to have behavioral deficits (22) and impaired learning ability (23, 24).

The addition of isoleucine or a mixture of leucine, isoleucine, and valine to the diet of gestating rats in the PCPA model results

[1] From the Department of Animal Science and the Division of Nutritional Sciences, Cornell University, Ithaca, NY.

[2] Supported in part by USPHS grant HD222558 from the NICHD.

[3] Address reprint requests to RE Austic, Department of Animal Science, 248 Morrison Hall, Cornell University, Ithaca, NY 14853. E-mail: bjs12@cornell.edu.

Received September 15, 1998.

Accepted for publication September 24, 1998.

TABLE 1
Composition of the large neutral amino acid (LNAA) supplements

Amino acids	LNAA1	LNAA2	LNAA3	LNAA4
		g/kg diet		
L-Thr	5.8	0.0	0.0	8.7
L-Val	5.7	5.7	8.5	8.5
L-Ile	6.3	6.3	9.5	9.5
L-Leu	6.3	6.3	9.5	9.5
L-Met	7.2	7.2	10.9	10.9
L-Trp	9.9	9.9	14.9	14.9
L-Tyr	8.8	0.0	0.0	0.0
Total	50.0	35.4	53.3	62.0

in improved fetal brain growth (13, 14), but it is not clear whether these amino acids completely prevent the adverse effects of PCPA and phenylalanine on brain growth. The objectives of this investigation were to determine the amino acid profiles in plasma and brain of fetuses from gestating rats fed a diet containing 0.5% AMPhe and 3.0% L-phenylalanine and to determine whether the effects of AMPhe and phenylalanine on fetal brain size and amino acid profile could be prevented by supplementing the diet with a mixture of LNAAs.

MATERIALS AND METHODS

Animals

Long-Evans rats at 4–8 d of gestation were obtained from Blue Spruce Farms (Altamont, NY). They were housed individually in stainless steel cages with raised wire floors in animal facilities that provided an ambient temperature of 22°C, 57% relative humidity, and a daily light period from 0800 to 2000. Food having the same composition as the basal diet in all experiments was provided ad libitum in glass jars that had lids and followers to minimize spillage. Water was provided ad libitum.

Food intakes and weight gains were monitored until day 11 of gestation. Only rats that consumed the diet well and gained weight at the expected rate were used in experiments. On the first day of the experiment, rats were ranked by body weight and randomly assigned to treatments within narrow weight groupings (blocks). Daily food intake (corrected for daily food spillage) and cumulative weight gains were measured during the experimental period from day 11 to day 20 of gestation. Animals were maintained under conditions approved by the Cornell University Institutional Animal Care and Use Committee; the experimental protocols were also approved by this committee.

Experimental diets

The basal diet was AIN-76A (25, 26), which contained L-methionine. L-Methionine was substituted for DL-methionine to eliminate the possibility that the utilization of D-methionine would be impaired by AMPhe (27). The experimental diets were mixed by adding AMPhe, L-phenylalanine, and LNAAs to the AIN-76A diet at the expense of cornstarch on a weight-for-weight basis. The ingredients included casein from National Casein (Riverton, NJ); cornstarch, cellulose, and corn oil (Mazola) from Corn Products (Englewood Cliffs, NJ); sucrose from Domino Sugar Corporation (New York); mineral mix, vitamin mix, and choline bitartrate from Dyets, Inc (Bethlehem, PA); L-methionine, L-phenylalanine, L-leucine, and L-tryptophan from United States Biochemical Corporation (Cleveland); L-threonine, L-valine, L-isoleucine, and L-tyrosine from Kyowa Hakko USA, Inc (New York); and AMPhe from Sigma Chemical Company (St Louis).

Experimental design

Four experiments were conducted to determine whether dietary supplements of LNAAs would reduce the severity of PKU in fetuses from dams fed the basal diet supplemented with 0.5% AMPhe + 3.0% L-phenylalanine (AMPhe + Phe diet). Four LNAA supplements were used that differed in the profile of amino acids included or in the amount of the mixture added to the diet (**Table 1**). A summary of the experimental designs is presented in **Table 2**. Experiment 1 was carried out to test the effects of dietary supplements of AMPhe + Phe and LNAA1, using a factorial arrangement of treatments, on rats and their fetuses. Modifications of the LNAA mixture were compared in experiments 2 and 3. Experiment 4 was conducted to confirm the effects of the LNAA4 supplement on body and brain growth and to determine effects of LNAA4 on brain protein synthesis of fetuses from phenylketonuric mothers. Basal control and LNAA-supplemented groups were pair fed to the rats fed the AMPhe + Phe diet in experiments 1 and 3. Food intake was not restricted in experiment 2. In experiment 4, the basal control and AMPhe + Phe groups were pair fed to the AMPhe + Phe + LNAA4 group. Pair-fed animals received the mean weight of food consumed on the previous day by the rats of the experimental group to which they were pair fed. There were 12, 8, 9, and 12 rats per treatment in experiments 1–4, respectively. The actual number of animals from which data were obtained, however, was sometimes less as a result of failure of pregnancy or unusually small litter size.

Biochemical analyses

Rats were subdued with carbon dioxide gas and killed by guillotine on day 20 of gestation. Trunk blood was collected in heparin-containing tubes for analysis of maternal blood amino acids. Fetuses were removed quickly and weighed. One-half of the fetuses (≈4–5 from each uterine horn) were used for collection of brain tissue for amino acid analysis and the remaining half were used for collection of trunk blood after decapitation and collection of brain tissue for measurement of protein synthesis. Fetal blood was collected by use of heparin-containing capillary tubes and was pooled by dam.

Blood held on ice after sampling was centrifuged at 1500 × *g* for 10 min (maternal) or at 1400 × *g* for 4 min (fetal) at 4°C, and 0.5 mL plasma was combined with 0.25 mL 8% sulfosalicylic acid containing 750 μmol norleucine/L and mixed. Brain tissue pooled from one-half of the fetuses for each dam was frozen under dry ice immediately. While still frozen, tissue was homogenized in a tissue homogenizer in a 4%-sulfosalicylic acid solution containing norleucine. After several hours of storage in a refrigerator to allow for precipitation of protein, the samples were centrifuged at 15 000 × *g* for 10 min at 4°C in a microcentrifuge and the supernate was stored frozen (−20°C) before analysis of free amino acids.

Amino acids were measured in experiments 1 and 3 by ion-exchange chromatography with ninhydrin detection by using an HPLC system (Hitachi Instruments, Inc, Danbury, CT) and the Pickering system for physiologic amino acid analysis (Pickering Laboratories, Inc, Mountain View, CA). A peak integration sys-

TABLE 2
Experimental designs

Experiment and treatment group	Diet composition[1]	Food allocation	Measured variables
Experiment 1 (n = 12)[2]			
Group 1	Basal	Pair fed to group 2	Food intake, initial body weight, final body weight, litter size, fetal body weight, fetal brain weight, and free amino acids in fetal brain, fetal plasma, and maternal plasma
Group 2	AMPhe + Phe	Ad libitum	
Group 3	LNAA1	Pair fed to group 2	
Group 4	AMPhe + Phe + LNAA1	Pair fed to group 2	
Experiment 2 (n = 8)			
Group 1	AMPhe + Phe + LNAA1	Ad libitum	Food intake, initial body weight, final body weight, litter size, fetal body weight, and fetal brain weight
Group 2	AMPhe + Phe + LNAA2	Ad libitum	
Group 3	AMPhe + Phe + LNAA3	Ad libitum	
Experiment 3 (n = 9)			
Group 1	Basal	Pair fed to group 2	Food intake, initial body weight, final body weight, litter size, fetal body weight, fetal brain weight, fetal brain protein concentration, and free amino acids in fetal brain
Group 2	AMPhe + Phe	Ad libitum	
Group 3	AMPhe + Phe + LNAA3	Pair fed to group 2	
Group 4	AMPhe + Phe + LNAA4	Pair fed to group 2	
Experiment 4 (n = 12)			
Group 1	Basal	Pair fed to group 3	Food intake, initial body weight, final body weight, litter size, fetal body weight, fetal brain weight, fetal brain protein concentration, and fetal brain protein synthesis
Group 2	AMPhe + Phe	Pair fed to group 3	
Group 3	AMPhe + Phe + LNAA4	Ad libitum	

[1] Basal diet, AIN76-A with L-methionine (25, 26); AMPhe + Phe, basal diet supplemented with 0.5% DL-α-methylphenylalanine and 3.0% L-phenylalanine; LNAA, large neutral amino acid. *See* Table 1 for composition of LNAAs.
[2] n is the number of rats started in the experiment on day 11 of gestation.

tem (Spectra-Physics, San Jose, CA) was used to quantitate the amino acids. A Beckman Instruments (Palo Alto, CA) ion-exchange HPLC system with postcolumn *o*-phthalaldehyde derivatization, fluorescence detection, and a similar peak integration system was used in the fourth experiment.

Fetal brain tissue was held on ice during collection in experiment 4. The procedures of Lerner and Johnson (28) were used to prepare microsomes and a pH 5 enzyme fraction. Briefly, the assay procedure involved incubation of microsomes and the pH 5 enzyme fraction prepared from each litter at 37°C in tris-HEPES buffer in the presence of amino acids including [^{3}H]phenylalanine and polyuridylic acid, GTP, ATP, phosphoenolpyruvate, Mg^{2+}, K^+, mercaptoethanol, and pyruvate kinase. After 30 min, incubations were terminated with 10% trichloroacetic acid and the mixture was centrifuged at 900 × g for 10 min at room temperature to separate the peptide fraction containing [^{3}H]phenylalanine. The pellet was washed 3 times with trichloroacetic acid to remove residual [^{3}H]phenylalanine substrate, solubilized in a solution of 0.2 mol NaOH/L in 0.15 mol NaCl/L and transferred to vials for scintillation counting.

Samples of the amino acid mixture containing tritiated phenylalanine were subjected to scintillation counting. In addition, the pH 5 enzyme fraction from fetal brain tissue in experiment 4 was analyzed for free phenylalanine by using procedures similar to those used in the analyses of plasma and brain amino acids. These analyses were used to calculate the total phenylalanine content of the incubation mixture and, subsequently, the specific activity of phenylalanine in the mixture. Tritiated toluene was used as an internal standard in each experiment in the determination of the efficiency of tritium counting. Protein was measured in brain homogenates by the method of Lowry et al (29).

Statistical analyses

Data were subjected to analysis of covariance. Treatment means were compared by single degree of freedom contrasts controlling for block (initial weight groups) and litter size as the continuous variable by using the general linear models procedure of SAS (30). Differences between means were considered significant at $P < 0.05$; individual means in experiment 1 were compared by using contrasts as described above when the P value for the interaction was <0.08.

RESULTS

The weight gains of the rats and the body weights of their fetuses in experiment 1 were lower when the diets contained AMPhe + Phe than when the diets did not contain these amino acids (**Table 3**). There was a possible interaction of AMPhe + Phe and LNAA1 on fetal brain weight. Fetal brain weights were lower in rats fed the AMPhe + Phe diet than in rats fed the basal diet. Fetal brain weights of rats fed the diet containing LNAA1 alone were not significantly different from those of rats fed the basal diet. Rats fed the AMPhe + Phe diet supplemented with LNAA1, however, had higher fetal brain weights than rats fed the diet containing AMPhe + Phe alone. There also was an interaction of LNAA1 and AMPhe + Phe on food intake. Intakes of rats fed diets containing AMPhe + Phe or LNAA1 alone did not differ significantly from the intake of rats fed the basal diet, but the intake of rats receiving the combined supplement was lower than the intakes of rats in all other treatments.

Concentrations of valine and methionine were lower in fetal brain in rats fed diets containing AMPhe + Phe than in rats fed diets that did not contain the phenylalanine mixture; additionally, the concentration of methionine was higher when the diet contained LNAA1 than when it did not (**Table 4**). There were numerous interactions of AMPhe + Phe and LNAA1 on amino acid concentrations in fetal brain. Concentrations of serine, glycine, tyrosine, phenylalanine, and lysine were higher and the concentration of isoleucine was lower in rats fed the AMPhe + Phe diet than in rats fed the basal diet. The concentrations of

TABLE 3
Influence of large neutral amino acids (LNAAs) on selected fetal and maternal measures in rats subjected to experimental phenylketonuria (experiment 1)[1]

	Treatment[2]					Main effects and interactions: *P*		
	1: Basal (*n* = 11)	2: AMPhe + Phe (*n* = 10)	3: LNAA1 (*n* = 12)	4: AMPhe + Phe + LNAA1 (*n* = 11)	Pooled SEM	AMPhe + Phe	LNAA1	AMPhe + Phe × LNAA1
Fetal								
Brain weight (g)	0.158[a,3]	0.136[c]	0.159[a]	0.146[b]	0.00245	<0.001	<0.05	<0.08
Body weight (g)	3.40	3.17	3.37	3.17	0.0723	<0.01	NS	NS
Maternal								
Food intake (g/d)	13.5[a]	13.8[a]	13.4[a]	12.7[b]	0.191	NS	<0.01	<0.05
Initial body weight (g)	307	306	310	307	2.54	NS	NS	NS
Body weight gain (g)	29.7	25.5	27.0	21.9	2.17	<0.05	NS	NS

[1] AMPhe + Phe, basal diet supplemented with 0.5% DL-α-methylphenylalanine and 3.0% L-phenylalanine. Means within a row with different superscript letters are significantly different, $P < 0.05$.
[2] Dams in treatment groups 1, 3, and 4 were pair fed to treatment group 2.
[3] Least-squares mean.

these amino acids did not differ significantly between rats fed the basal diet and those fed the basal diet plus LNAA1. Rats fed the diet containing AMPhe + Phe plus LNAA1, however, had significantly lower concentrations of serine, glycine, phenylalanine, and lysine and higher concentrations of isoleucine in fetal brain than rats fed the diet containing AMPhe + Phe alone. The concentration of threonine appeared to be higher in fetal brains of rats fed AMPhe + Phe or LNAA1, but not the combined supplement, than in rats fed the basal diet. Leucine concentrations in fetal brain did not differ significantly between treatments.

Concentrations of threonine, valine, and methionine were lower and the concentration of glycine was higher in fetal plasma of rats fed diets containing AMPhe + Phe than in rats fed diets that did not contain the phenylalanine mixture (**Table 5**). There were several interactions of AMPhe + Phe and LNAA1. Concentrations of phenylalanine and tyrosine in fetal plasma were higher and the concentration of isoleucine was lower in rats fed the AMPhe + Phe diet than in rats fed the basal diet. The concentrations of these 3 amino acids did not differ significantly between rats fed the basal diet and those fed the basal diet plus LNAA1. However, concentrations of phenylalanine were lower and those of isoleucine were higher in fetal plasma when LNAA1 was added to the diet containing AMPhe + Phe than when the diet contained AMPhe + Phe alone. Tyrosine concentrations were higher in fetal plasma of rats fed the AMPhe + Phe diet than in plasma of rats fed the basal diet, but were not significantly different between rats fed the AMPhe + Phe plus LNAA1 diet and rats fed the basal diet supplemented with LNAA1. Serine concentrations were lower in rats fed the combined supplement than in rats fed AMPhe + Phe alone but did not differ significantly between rats fed the basal diet or the diets containing AMPhe + Phe or LNAA1 alone. Leucine and lysine concentrations in fetal plasma did not differ significantly between treatments.

Concentrations of valine, isoleucine, and leucine were higher in plasma of dams fed diets containing AMPhe + Phe than in dams fed diets that did not contain the phenylalanine mixture (**Table 6**). Rats fed diets containing LNAA1 had higher plasma threonine concentrations and lower plasma lysine concentrations than did rats fed diets that did not contain LNAA1. There were significant interactions of AMPhe + Phe and LNAA1 on phenylalanine and tyrosine

TABLE 4
Amino acid concentrations in fetal brain (experiment 1)[1]

	Treatment[2]					Main effects and interactions: *P*		
	1: Basal (*n* = 10)	2: AMPhe + Phe (*n* = 10)	3: LNAA1 (*n* = 12)	4: AMPhe + Phe+ LNAA1 (*n* = 11)	Pooled SEM	AMPhe + Phe	LNAA1	AMPhe + Phe × LNAA1
	nmol/g tissue				*nmol/g tissue*			
Thr	869[b,3]	1190[a]	1154[a]	1058[a,b]	80.4	NS	NS	<0.05
Ser	666[b]	1041[a]	614[b]	690[b]	38.0	<0.001	<0.001	<0.001
Gly	1117[c]	2856[a]	1109[c]	1866[b]	171.2	<0.001	<0.01	<0.05
Val	239	147	240	180	16.1	<0.001	NS	NS
Met	110	50	141	99	13.9	<0.01	<0.05	NS
Ile	107[a]	61[b]	103[a]	91[a]	7.8	<0.01	NS	<0.05
Leu	196	168	206	200	13.5	NS	NS	NS
Tyr	168[c]	338[a]	217[b,c]	272[a,b]	23.9	<0.001	NS	<0.05
Phe	151[c]	2251[a]	224[c]	1046[b]	215.1	<0.001	<0.05	<0.01
Lys	812[b,c]	1340[a]	792[c]	926[b]	42.4	<0.001	<0.001	<0.001

[1] AMPhe + Phe, basal diet supplemented with 0.5% DL-α-methylphenylalanine and 3.0% L-phenylalanine; LNAA, large neutral amino acid. Means within a row without a superscript letter in common are significantly different, $P < 0.05$.
[2] Dams in treatment groups 1, 3, and 4 were pair fed to treatment group 2.
[3] Least-squares mean.

TABLE 5
Amino acid concentrations in fetal plasma (experiment 1)[1]

	Treatment[2]					Main effects and interactions: *P*		
	1: Basal	2: AMPhe + Phe	3: LNAA1	4: AMPhe + Phe+ LNAA1	Pooled SEM	AMPhe + Phe	LNAA1	AMPhe + Phe × LNAA1
	μmol/L plasma				*μmol/L plasma*			
Thr	538 [11][3]	450 [9]	710 [12]	487 [10]	66.8	<0.05	NS	NS
Ser	257[a,b] [6]	284[a] [6]	258[a,b] [6]	234[b] [5]	6.74	NS	<0.05	<0.05
Gly	320 [11]	424 [9]	326 [12]	394 [10]	20.8	<0.001	NS	NS
Val	407 [11]	315 [9]	417 [12]	356 [10]	22.0	<0.01	NS	NS
Met	151 [11]	81 [9]	171 [12]	113 [10]	14.6	<0.001	NS	NS
Ile	173[a] [11]	126[b] [10]	176[a] [12]	170[a] [11]	8.97	<0.01	<0.05	<0.05
Leu	280 [11]	253 [10]	290 [12]	288 [11]	15.6	NS	NS	NS
Tyr	222[c] [11]	390[a] [10]	279[b,c] [12]	331[a,b] [11]	22.6	<0.001	NS	<0.05
Phe	212[c] [11]	2336[a] [10]	278[c] [12]	1319[b] [11]	277.6	<0.001	NS	<0.08
Lys	1656 [11]	1804 [10]	1765 [12]	1779 [11]	163.1	NS	NS	NS

[1] AMPhe + Phe, basal diet supplemented with 0.5% DL-α-methylphenylalanine and 3.0% L-phenylalanine; LNAA, large neutral amino acid. Means within a row without a superscript letter in common are significantly different, $P < 0.05$.
[2] Dams in treatment groups 1, 3, and 4 were pair fed to those in group 2.
[3] Least-squares mean; *n* in brackets.

concentrations in maternal plasma. Rats fed the AMPhe + Phe diet had higher concentrations of phenylalanine and tyrosine in plasma than rats fed the basal diet. Concentrations of phenylalanine and tyrosine in maternal plasma did not differ significantly between rats fed the basal diet supplemented with LNAA1 and rats fed the basal diet, but were significantly lower in rats fed the AMPhe + Phe diet supplemented with LNAA1 than in rats fed the diet containing AMPhe + Phe alone.

The composition of LNAA2 (Table 1) was similar to the composition of LNAA1 except that it did not contain threonine and tyrosine, 2 of the amino acids in experiment 1 that were higher in concentration in fetal brain when rats were fed diets containing AMPhe + Phe than when fed the basal diet. LNAA3 contained the same amino acids as LNAA2, but the amount of inclusion in the diet was 50% higher. When responses to all 3 supplements were compared in a single experiment in which all diets also contained AMPhe + Phe, maternal weight gains and fetal body weights were not significantly different between the LNAA1, LNAA2, and LNAA3 treatment groups (**Table 7**). Fetal brain weights were also not significantly different between the LNAA1 and LNAA2 treatment groups, but were significantly higher in the LNAA3 group than in the other 2 groups. Additionally, food intake was significantly lower in the LNAA3 group than in the other 2 groups.

In experiment 3, the LNAA3 mixture was modified by including threonine and responses to LNAA3 and LNAA4 were compared. Fetal brain weight, fetal body weight, initial maternal body weight, and maternal weight gain were not significantly affected by treatment (**Table 8**). The mean (±SEM) fetal brain protein concentrations were 90.5 ± 3.4, 82.3 ± 3.1, 84.7 ± 3.7, and 82.5 ± 3.8 mg/g tissue, respectively, for the basal, AMPhe + Phe, AMPhe + Phe plus LNAA3, and AMPhe + Phe plus LNAA4 treatment groups; there were no significant differences between treatments. Food intake was significantly lower in rats fed the diet supplemented with LNAA4 than in rats fed the basal diet or

TABLE 6
Amino acid concentrations in maternal plasma (experiment 1)[1]

	Treatment[2]					Main effects and interactions: *P*		
	1: Basal (*n* = 10)	2: AMPhe + Phe (*n* = 10)	3: LNAA1 (*n* = 12)	4: AMPhe + Phe + LNAA1 (*n* = 11)	Pooled SEM	AMPhe + Phe	LNAA1	AMPhe + Phe × LNAA1
	μmol/L plasma				*μmol/L plasma*			
Thr	1334[3]	1377	1712	1811	177.6	NS	<0.05	NS
Ser	783	689	700	712	72.7	NS	NS	NS
Gly	426	404	441	502	37.2	NS	NS	NS
Val	440	544	471	580	27.0	<0.001	NS	NS
Met	121	147	123	134	10.5	NS	NS	NS
Ile	245	293	260	321	13.8	<0.001	NS	NS
Leu	340	411	364	441	20.4	<0.01	NS	NS
Tyr	160[c]	511[a]	166[c]	322[b]	35.1	<0.001	<0.05	<0.05
Phe	262[b]	3674[a]	113[b]	1107[b]	406.8	<0.001	<0.01	<0.01
Lys	3604	3709	2934	2953	234.8	NS	<0.01	NS

[1] AMPhe + Phe, basal diet supplemented with 0.5% DL-α-methylphenylalanine and 3.0% L-phenylalanine; LNAA, large neutral amino acid. Means within a row with different superscript letters are significantly different, $P < 0.05$.
[2] Dams in treatment groups 1, 3, and 4 were pair fed to those in group 2. For serine, *n* = 6, 9, 7, and 4 in groups 1–4, respectively.
[3] Least-squares mean.

TABLE 7
Effect of different mixtures of large neutral amino acids (LNAAs) on selected fetal and maternal measures in rats receiving 0.5% DL-α-methylphenylalanine (AMPhe) and 3% L-phenylalanine (Phe) in their diets (experiment 2)[1]

	Treatment[2]			
	1: AMPhe + Phe + LNAA1 (n = 6)	2: AMPhe + Phe + LNAA2 (n = 7)	3: AMPhe + Phe + LNAA3 (n = 7)	Pooled SEM
Fetal				
Brain weight (g)	0.136[b,3]	0.138[b]	0.150[a]	0.00316
Body weight (g)	3.07[a]	3.07[a]	3.11[a]	0.0682
Maternal				
Food intake (g/d)	13.1[a]	13.0[a]	11.3[b]	0.405
Initial body weight (g)	314[a]	318[a]	308[a]	4.94
Body weight gain (g)	33.6[a]	39.1[a]	33.5[a]	3.89

[1] Means within a row with different superscript letters are significantly different, $P < 0.05$.
[2] Dams were fed ad libitum.
[3] Least-squares mean.

the AMPhe + Phe diet. Food intakes of rats fed diets supplemented with LNAA3 and LNAA4 were not significantly different.

Fetuses of rats fed the diet containing AMPhe + Phe alone had higher concentrations of glycine, tyrosine, phenylalanine, and lysine and lower concentrations of valine, isoleucine, and leucine in brain than fetuses of rats fed the basal diet (**Table 9**). Arginine, which was not measured in experiment 1, also was higher in the AMPhe + Phe group. Threonine and serine, which were measured in experiment 1, did not resolve sufficiently during chromatography to enable accurate measurement. Fetuses of rats fed the diet containing AMPhe + Phe plus LNAA3 had higher brain concentrations of valine, methionine, and isoleucine and lower concentrations of glycine, phenylalanine, lysine, and arginine than fetuses of rats fed the diet containing AMPhe + Phe alone. Fetuses of rats fed the diet containing AMPhe + Phe plus LNAA4 had higher concentrations of methionine and lower concentrations of glycine, phenylalanine, and lysine in brain than did fetuses of rats fed the diet containing AMPhe + Phe alone. Glycine concentrations were higher in fetal brains of rats fed LNAA4 than in those fed LNAA3. In general, concentrations of LNAAs in fetal brain, other than threonine, which was not measured, were not significantly different between the LNAA3 and LNAA4 treatments. Concentrations of valine and isoleucine in the fetal brains of rats fed AMPhe + Phe plus LNAA3 and concentrations of valine, isoleucine, and leucine in the fetal brains of rats fed AMPhe + Phe plus LNAA4 were lower than the concentrations of these LNAAs in fetal brains of rats fed the basal diet. The concentration of phenylalanine was higher in fetal brains of rats fed the diets containing AMPhe + Phe plus LNAA3 or LNAA4 than in rats fed the basal diet.

The addition of LNAAs to diets containing AMPhe + Phe tended to result in lower food intake (Tables 3, 7, and 8). Because reduced food intake might limit fetal growth or improvements in amino acid profiles, a final experiment was conducted to test the effects of LNAA4 supplementation of the diet under conditions in which rats fed the basal and the AMPhe + Phe diets were pair fed to rats fed the AMPhe + Phe plus LNAA4 diet. Food intakes, weight gains of dams, and fetal body and brain weights were lower in rats fed the AMPhe + Phe diet than in rats fed the basal diet (**Table 10**). Maternal body weight gains were higher in rats fed the AMPhe + Phe plus LNAA4 diet than in rats fed the basal diet; however, fetal body and brain weights were not significantly different between rats fed the AMPhe + Phe plus LNAA4 diet and those fed the basal diet.

Protein concentrations in fetal brain did not differ significantly between rats fed the basal diet and those fed the AMPhe + Phe diet (**Table 11**). Protein concentrations were lower, however, in fetal brains of rats fed the AMPhe + Phe plus LNAA4 diet than in those fed the basal diet. There were no significant differences between treatments in protein synthesis as measured

TABLE 8
Comparison of the effectiveness of large neutral amino acid supplement 3 (LNAA3) and LNAA4 in alleviating the adverse effects of 0.5% DL-α-methylphenylalanine (AMPhe) and 3% L-phenylalanine (Phe) on selected fetal and maternal measures (experiment 3)[1]

	Treatment[2]				
	1: Basal (n = 9)	2: AMPhe + Phe (n = 9)	3: AMPhe + Phe + LNAA3 (n = 8)	4: AMPhe +Phe + LNAA4 (n = 8)	Pooled SEM
Fetal					
Brain weight (g)	0.153[a,3]	0.135[a]	0.146[a]	0.142[a]	0.00601
Body weight (g)	3.06[a]	2.98[a]	3.05[a]	3.06[a]	0.0748
Maternal					
Food intake (g/d)	13.5[a,b]	14.1[a]	12.1[b,c]	11.7[c]	0.484
Initial body weight (g)	309[a]	309[a]	309[a]	307[a]	1.43
Body weight gain (g)	36.9[a]	36.2[a]	33.4[a]	27.2[a]	3.87

[1] Means within a row without a superscript letter in common are significantly different, $P < 0.05$.
[2] Dams in treatment groups 1, 3, and 4 were pair fed to those in treatment group 2.
[3] Least-squares mean.

TABLE 9
Amino acid concentrations in fetal brain (experiment 3)[1]

	Treatment[2]				
	1: Basal (n = 9)	2: AMPhe + Phe (n = 8)	3: AMPhe + Phe + LNAA3 (n = 7)	4: AMPhe + Phe + LNAA4 (n = 8)	Pooled SEM
	μmol/L plasma				
Gly	1268[c,3]	3218[a]	1463[c]	1843[b]	101.0
Val	308[a]	170[c]	227[b]	199[b,c]	13.1
Met	97[a,b]	53[b]	135[a]	129[a]	19.6
Ile	147[a]	74[c]	106[b]	89[b,c]	8.71
Leu	256[a]	185[b]	226[a,b]	211[b]	14.3
Tyr	182[b]	280[a]	247[a,b]	258[a]	23.0
Phe	226[c]	3069[a]	1204[b]	1499[b]	224.6
Lys	936[b]	1422[a]	796[b]	979[b]	75.3
Arg	78[c]	132[a]	92[b,c]	108[a,b]	9.30

[1] AMPhe + Phe, basal diet supplemented with 0.5% DL-α-methylphenylalanine and 3.0% L-phenylalanine; LNAA, large neutral amino acid. Means within a row without a superscript letter in common are significantly different, $P < 0.05$.
[2] Dams in treatment groups 1, 3, and 4 were pair fed to those in group 2. For arginine, n = 8, 8, 7, 8 in groups 1–4, respectively.
[3] Least-squares mean.

in vitro by using a synthetic messenger RNA template and microsomal and pH 5 enzyme fractions from fetal brain.

DISCUSSION

The inclusion of 0.5% AMPhe and 3.0% L-phenylalanine in the diet from day 11 to day 20 of gestation resulted in lower fetal brain weights and, in 2 of 3 experiments, lower fetal body weights than were observed in rats fed the basal diet. This is consistent with the findings of Brass et al (21), who developed this model of maternal PKU, and others (14) who used PCPA to induce maternal PKU in rats.

In both gestational models and in postnatal models of PKU in rats and mice, the administration of phenylalanine with AMPhe or PCPA has been reported to increase the concentrations of phenylalanine and tyrosine and to decrease the concentrations of certain LNAAs in fetal brain (10–14, 21, 31). In agreement with these reports, AMPhe + Phe consistently resulted in lower fetal brain concentrations of isoleucine and valine and in 1 of 2 experiments resulted in significantly lower concentrations of leucine than did the basal diet. The high glycine concentration in rats fed AMPhe + Phe agrees with the reports of Brass et al (21) and Su et al (31) and with the reports of Dienel (32) and Huether et al (33) involving postnatal rat models. The changes in leucine, isoleucine, valine, phenylalanine, tyrosine, threonine, glycine, serine, lysine, and arginine concentrations in fetal brain in this study are consistent with those observed previously in this laboratory (31).

Administrations of individual LNAAs or mixtures of LNAAs have been reported to increase brain protein synthesis in postnatal murine models of PKU (12, 34), and to improve fetal brain growth in a PCPA model of maternal PKU in rats (13, 14). This led various investigators to suggest that competition between phenylalanine and other LNAAs for uptake into the brain limits the availability of one or more LNAAs for protein synthesis and, consequently, for brain growth and development (10–15).

The results of the present investigation indicate that supplementation of the diet of gestating dams with a mixture of LNAAs ameliorates, but does not fully prevent, the adverse effects of AMPhe + Phe on fetal brain growth and tends to normalize the

TABLE 10
Influence of large neutral amino acid supplement 4 (LNAA4) on selected fetal and maternal measures in rats subjected to experimental phenylketonuria (experiment 4)[1]

	Treatment[2]			
	1: Basal (n = 9)	2: AMPhe + Phe (n = 10)	3: AMPhe + Phe +LNAA4 (n = 12)	Pooled SEM
Fetal				
Brain weight (g)	0.149[a,3]	0.126[b]	0.149[a]	0.00216
Body weight (g)	2.98[a]	2.68[b]	3.15[a]	0.0736
Maternal				
Food intake (g/d)	10.0[a]	9.4[b]	10.0[a]	1.57
Initial body weight (g)	285[a]	283[a]	287[a]	1.19
Body weight gain (g)	24.3[b]	17.8[c]	30.3[a]	1.60

[1] AMPhe + Phe, basal diet supplemented with 0.5% DL-α-methylphenylalanine and 3.0% L-phenylalanine. Means within a row with different superscript letters are significantly different, $P < 0.05$.
[2] Dams in treatment groups 1 and 2 were pair fed to those in treatment group 3.
[3] Least-squares mean.

TABLE 11
Effects of large neutral amino acids (LNAAs) on brain protein content and protein synthesis of fetal brain from rats subjected to experimental phenylketonuria (experiment 4)[1]

	Treatment			
	1: Basal	2: AMPhe + Phe	3: AMPhe + Phe + LNAA4	Pooled SEM
Brain protein (mg/g tissue)	74.7[a] [9][2]	72.6[a,b] [10]	69.4[b] [12]	1.13
Phenylalanine incorporated (pmol/incubation)[3]	892[a] [7]	1080[a] [9]	1202[a] [12]	106.0

[1] AMPhe + Phe, basal diet supplemented with 0.5% DL-α-methylphenylalanine and 3% L-phenylalanine. Means within a row without a superscript letter in common are significantly different, $P < 0.05$.
[2] Least-squares mean; *n* in brackets.
[3] *See* text for complete description of the in vitro assay of protein synthesis.

fetal brain amino acid profile. This response cannot be attributed solely to transport interactions at the blood-brain interface because there was a tendency for the concentrations of isoleucine, valine, and methionine to be lower in fetal plasma as well as fetal brain with AMPhe + Phe treatment. Furthermore, the addition of LNAAs to the diet containing AMPhe + Phe resulted in markedly lower phenylalanine concentrations not only in fetal brain but also in fetal blood and maternal blood.

Concentration ratios of amino acids in fetal brain relative to fetal plasma and in fetal plasma relative to maternal plasma, calculated from treatment means in Tables 4, 5, and 6, are shown in **Table 12**. The inclusion of AMPhe + Phe to the basal diet tended to result in lower fetal brain–to–fetal plasma and fetal plasma–to–maternal plasma concentration ratios of isoleucine, valine, leucine, and methionine than observed with the basal diet alone. The ratios averaged 16% lower for fetal brain to fetal plasma and 41% lower for fetal plasma to maternal plasma (**Table 13**). In a previous study (31), the concentration ratios of isoleucine, valine, leucine, and methionine in response to AMPhe + Phe averaged 15% lower for fetal brain to fetal plasma and 46% lower for fetal plasma to maternal plasma than with a basal diet. The addition of LNAAs to the diet containing AMPhe + Phe tended to prevent changes in the fetal brain–to–fetal plasma and fetal plasma–to–maternal plasma concentration ratios of these amino acids (Table 12 and Table 13), although only leucine and methionine fetal brain–to–fetal plasma concentration ratios appeared to be restored fully to the ratios extant in animals fed the basal diet. It is tempting to speculate that the primary effects of the mixture of LNAAs are twofold: *1*) to reduce the phenylalanine concentration in maternal blood, thereby limiting the interference of phenylalanine with the transport of other amino acids to the fetus, and *2*) to reduce, secondarily, the concentration of phenylalanine in fetal blood, thereby limiting the interference of phenylalanine with the transport of other amino acids into fetal brain.

The LNAA1 mixture was based on the profile of amino acids that Binek-Singer and Johnson (12) administered by injection into mice in a preweaning model of PKU. The amount of the mixture included in the diet in the present study was an educated guess based on an investigator's experience with amino acid mixtures in diets nutritionally adequate in total protein but marginally adequate or excessive in a single amino acid (35). It was obvious in experiment 1 that LNAA1 improved, but did not restore, fetal brain amino acid profiles compared with those of rats consuming the basal diet. Tyrosine clearly was not needed in the mixture because the addition of AMPhe + Phe to the basal diet resulted in higher tyrosine concentrations in fetal brain and maternal and fetal plasma. Brain threonine concentrations also were higher. Because threonine is a precursor of glycine (36, 37), the concentration of which was markedly higher in brains of fetuses from rats fed the AMPhe + Phe diet than in those fed the basal diet, it seemed desirable to omit threonine from the mixture of LNAAs. The mixture lacking threonine and tyrosine (ie, LNAA2) did not improve fetal brain growth in rats receiving AMPhe + Phe (Table 7), but increas-

TABLE 12
Concentration ratios of selected large neutral amino acids (LNAAs) in fetal brain (FB) relative to fetal plasma (FP) and in FP relative to maternal plasma (MP) (experiment 1)[1]

	Treatment							
	1: Basal		2: AMPhe + Phe		3: LNAA1		4: AMPhe + Phe + LNAA1	
	FB:FP	FP:MP	FB:FP	FP:MP	FB:FP	FP:MP	FB:FP	FP:MP
Thr	1.61	0.40	2.64	0.34	1.62	0.42	2.17	0.27
Ser	2.59	0.33	3.66	0.41	2.38	0.37	2.94	0.33
Gly	3.49	0.75	6.74	1.05	3.40	0.74	4.73	0.78
Val	0.59	0.91	0.47	0.57	0.58	0.88	0.51	0.63
Met	0.75	1.26	0.64	0.56	0.82	1.39	0.75	0.88
Ile	0.62	0.70	0.46	0.42	0.58	0.68	0.54	0.54
Leu	0.70	0.81	0.65	0.61	0.71	0.80	0.70	0.65
Tyr	0.79	1.36	0.90	0.73	0.78	1.68	0.80	1.06
Phe	0.71	0.81	0.96	0.64	0.80	2.46	0.79	1.19
Lys	0.49	0.46	0.74	0.88	0.49	0.60	0.52	1.30

[1] AMPhe + Phe, basal diet supplemented with 0.5% DL-α-methylphenylalanine and 3% L-phenylalanine. All values are derived from the means in Tables 4, 5, and 6.

TABLE 13
Percentage decreases in the distribution ratios of selected large neutral amino acids (LNAAs) in fetal brain (FB) relative to fetal plasma (FP) and in FP relative to maternal plasma (MP) (experiment 1)[1]

	Percentage decrease due to AMPhe + Phe		Percentage decrease due to AMPhe + Phe + LNAA1	
	FB:FP	FP:MP	FB:FP	FP:MP
	%		%	
Val	20	37	14	31
Met	15	56	0	30
Ile	26	40	13	23
Leu	7	25	0	20
Val + Met + Ile + Leu	16	41	6	27
Val + Ile + Leu	17	51	8	25
Val + Ile	23	38	13	27

[1] AMPhe + Phe, basal diet supplemented with 0.5% DL-α-methylphenylalanine and 3% L-phenylalanine. Calculated from distribution ratios in Table 12.

ing the dietary supplementation by 50% (ie, LNAA3) resulted in significantly higher brain weights. This higher level of LNAA supplementation, however, resulted in lower food intake.

Diets for rats and other simple-stomached animals are easily imbalanced with regard to threonine when other amino acids are provided in excess (38–40). Because of the possibility that the supplemental amino acids (LNAA3) increased the need for threonine, this amino acid was reintroduced into the mixture (LNAA4). In an experiment to compare LNAA3 and LNAA4 (Table 8), the negative control (AMPhe + Phe treatment) did not result in low fetal brain weights ($P > 0.05$) and, therefore, the intended comparison was not possible. The tendency of LNAA4 to depress food intake may have partly offset its otherwise positive effects. Therefore, a final experiment was carried out to test the effect of LNAA4 under conditions in which all treatments were pair fed to the AMPhe + Phe plus LNAA4 group rather than to the AMPhe + Phe group as in the first 3 experiments. With use of this pair-feeding regimen, the adverse effect of AMPhe + Phe on fetal brain weight was prevented by supplementation of the diet with LNAA4; however, protein concentrations of fetal brain were ≈7% lower than those of the untreated controls.

Weight gains and the efficiencies of food utilization for weight gain of dams were higher in rats fed the AMPhe + Phe plus LNAA4 diet than in those fed the basal diet. The reason for this is not clear. All rats had limited food intake as a result of pair-feeding. Perhaps the intake of one or more amino acids was particularly limiting for the growth of dams fed the basal diet and this limitation was overcome by the amino acids contained in LNAA4. Alternatively, or in addition, the increase in weight gain may have reflected a change in body composition.

Brain protein synthesis as assessed in a cell-free system with a synthetic template did not differ significantly between treatments. This suggests that neither ribosomal activity nor the activity of the pH 5 enzyme fraction was reduced by AMPhe + Phe treatment or increased by LNAA. This contrasts with the results of Binek-Singer and Johnson (12) in a preweaning mouse model in which they observed alterations in the polyribosomal profile indicative of reduced initiation of protein synthesis and a decreased rate of peptide elongation in brain tissue of mice injected with AMPhe and phenylalanine—effects that were prevented by the injection of a mixture of LNAAs.

The present studies indicate that the adverse effect of a dietary supplement of 0.5% AMPhe plus 3.0% L-phenylalanine from day 11 to day 20 of gestation on fetal growth and fetal brain weight in a rat model of maternal PKU was ameliorated but not completely prevented by supplementation of the diet of the dam with a mixture of several LNAAs. Significant alterations in the concentrations of branched-chain amino acids (leucine, isoleucine, and valine) and other amino acids persisted in fetal brain when the mixture of LNAAs was included in the diet of phenylketonuric dams.

REFERENCES

1. Mabry CC, Denniston JC, Nelson TL, Son CD. Maternal phenylketonuria. A cause of mental retardation in children without the metabolic defect. N Engl J Med 1963;269:1404–8.
2. Lenke RR, Levy HL. Maternal phenylketonuria and hyperphenylalaninemia. An international survey of the outcome of untreated and treated pregnancies. N Engl J Med 1980;303:1202–8.
3. Rouse B, Lockhart L, Matalon R, et al. Maternal phenylketonuria pregnancy outcome: a preliminary report of facial dysmorphology and major malformations. J Inherit Metab Dis 1990;13:289–91.
4. Lenke RR, Levy HL. Maternal phenylketonuria—results of dietary therapy. Am J Obstet Gynecol 1982;142:548–53.
5. Koch R, Hanley W, Levy H, et al. A preliminary report of the collaborative study of maternal phenylketonuria in the United States and Canada. J Inherit Metab Dis 1990;13:641–50.
6. Smith I, Glossop J, Beasley M. Fetal damage due to maternal phenylketonuria: effects of dietary treatment and maternal phenylalanine concentrations around the time of conception. J Inherit Metab Dis 1990;13:651–7.
7. Drogari E, Beasley M, Smith I, Lloyd JK. Timing of strict diet in relation to fetal damage in maternal phenylketonuria. Lancet 1987;2:927–30.
8. Pueschel SM, Hum C, Andrews M. Nutritional management of the female with phenylketonuria during pregnancy. Am J Clin Nutr 1977;30:1153–61.
9. Kecskemethy HH, Lobbregt D, Levy HL. The use of gelatin capsules for ingestion of formula in dietary treatment of maternal phenylketonuria. J Inherit Metab Dis 1993;16:111–8.
10. Andersen AE, Avins L. Lowering brain phenylalanine levels by giving other large neutral amino acids. Arch Neurol 1976;33:684–6.
11. Berry HK, Bofinger MK, Hunt MM, Phillips PJ, Guilfoile MB. Reduction of cerebrospinal fluid phenylalanine after oral administration of valine, isoleucine, and leucine. Pediatr Res 1982;16:751–5.
12. Binek-Singer P, Johnson TC. The effects of chronic hyperphenylalaninaemia on mouse brain protein synthesis can be prevented by other amino acids. Biochem J 1982;206:407–14.
13. Berry HK, Butcher RE, Brunner RL, Bray NW, Hunt MM, Wharton CH. New approaches to treatment of phenylketonuria. In: Mittler P, ed. Research to practice in mental retardation. Biomedical aspects.

Vol 3. Baltimore: University Park Press, 1977:229–39.
14. Brunner RL, Vorhees CV, McLean MS, Butcher RE, Berry HK. Beneficial effect of isoleucine on fetal brain development in induced phenylketonuria. Brain Res 1978;154:191–5.
15. McSwigan JD, Vorhees CV, Brunner RL, Butcher RE, Berry HK. Amelioration of maze deficits from induced hyperphenylalaninemia in adult rats using valine, isoleucine, and leucine. Behav Neural Biol 1981;33:378–84.
16. Vorhees CV, Acuff-Smith KD, Weisenburger WP, Minck DR, Berry HK. Branched chain amino acids improve radial-arm maze acquisition and water maze forced-choice learning in rat offspring exposed in utero to hyperphenylalaninemia. Neurotoxicol Teratol 1992;14:35–41.
17. Kelly CJ, Johnson TC. Effects of *p*-chlorophenylalanine and α-methylphenylalanine on amino acid uptake and protein synthesis in mouse neuroblastoma cells. Biochem J 1978;174:931–8.
18. Delvalle JA, Dienel G, Greengard O. Comparison of α-methylphenylalanine and *p*-chlorophenylalanine as inducers of chronic hyperphenylalaninaemia in developing rats. Biochem J 1978;170:449–59.
19. Lane JD, Schöne B, Langenbeck U, Neuhoff V. Characterization of experimental phenylketonuria. Augmentation of hyperphenylalaninemia with α-methylphenylalanine and *p*-chlorophenylalanine. Biochim Biophys Acta 1980;627:144–56.
20. Koe BK, Weissman A. *p*-Chlorophenylalanine: a specific depletor of brain serotonin. J Pharmacol Exp Ther 1966;154:499–516.
21. Brass CA, Isaacs CE, McChesney R, Greengard O. The effects of hyperphenylalaninemia on fetal development: a new animal model of maternal phenylketonuria. Pediatr Res 1982;16:388–94.
22. Hanulak AT, Hull EM. Behavioral deficits in a rat model of maternal PKU. Psychobiology 1987;15:75–8.
23. Sadava D, Sutcliffe D. The effects of maternal hyperphenylalaninemia on learning in mature rats. Life Sci 1988;43:1119–23.
24. Strupp BJ, Bunsey M, Levitsky DA, Hamberger K. Deficient cumulative learning: an animal model of retarded cognitive development. Neurotoxicol Teratol 1994;16:71–9.
25. Ad Hoc Committee on Standards for Nutritional Studies. Report of the American Institute of Nutrition Ad Hoc Committee on Standards for Nutritional Studies. J Nutr 1977;107:1340–8.
26. Ad Hoc Committee on Standards for Nutritional Studies. Second report of the American Institute of Nutrition Ad Hoc Committee on Standards for Nutritional Studies. J Nutr 1980;110:1726.
27. Umbreit WW. Reactions of α-methyl amino acids. In: McElroy WD, Glass HB, eds. A symposium on amino acid metabolism. Baltimore: The Johns Hopkins Press, 1955:48–54.
28. Lerner MP, Johnson TC. Regulation of protein synthesis in developing mouse brain tissue. Alteration in ribosomal activity. J Biol Chem 1970;245:1388–93.
29. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. J Biol Chem 1951;193:265–75.
30. SAS Institute, Inc. SAS user's guide: statistics, version 5 ed. Cary, NC: SAS Institute, Inc, 1985.
31. Su C-L, Austic RE, Strupp BJ, Levitsky DA. Improved model of maternal phenylketonuria in rats by use of lower dietary concentrations of α-methylphenylalanine and L-phenylalanine in a semipurified diet. Nutr Res 1998;18:989–1002.
32. Dienel GA. Chronic hyperphenylalaninemia produces cerebral hyperglycinemia in immature rats. J Neurochem 1981;36:34–43.
33. Huether G, Kaus R, Neuhoff V. Amino acid depletion in the blood and brain tissue of hyperphenylalaninemic rats is abolished by the administration of additional lysine: a contribution to the understanding of the metabolic defects in phenylketonuria. Biochem Med 1985;33:334–41.
34. Hughes JV, Johnson TC. Experimentally-induced and natural recovery from the effects of phenylalanine on brain protein synthesis. Biochim Biophys Acta 1978;517:473–85.
35. Davis AJ, Austic RE. Dietary protein and amino acid levels alter threonine dehydrogenase activity in hepatic mitochondria of *Gallus domesticus*. J Nutr 1997;127:738–44.
36. Bird MI, Nunn PB, Lord LAJ. Formation of glycine and aminoacetone from L-threonine by rat liver mitochondria. Biochim Biophys Acta 1984;802:229–36.
37. Ballèvre O, Cadenhead A, Calder AG, et al. Quantitative partition of threonine oxidation in pigs: effect of dietary threonine. Am J Physiol 1990;259:E483–91.
38. Leung PM-B, Rogers QR, Harper AE. Effect of amino acid imbalance on plasma and tissue free amino acids in the rat. J Nutr 1968;96:303–18.
39. Tews JK, Kim Y-WL, Harper AE. Induction of threonine imbalance by dispensable amino acids: relation to competition for amino acid transport into brain. J Nutr 1979;109:304–15.
40. Davis AJ, Austic RE. Dietary threonine imbalance alters threonine dehydrogenase activity in isolated hepatic mitochondria of chicks and