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ORIGINAL ARTICLE

Dietary supplementation with cholesterol and docosahexaenoic acid affects concentrations of amino acids in tissues of young pigs

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Abstract Cholesterol and docosahexaenoic acid (DHA) are important nutrients for neural development of infants. However, little is known about the effect of cholesterol or DHA on concentrations of amino acids (AA) in neonatal tissues. This study was conducted with the piglet (an established model for studying human infant nutrition) to test the hypothesis that dietary supplementation with the lipids may modulate AA availability in tissues. Sixteen newborn pigs were nursed by sows for 24 h and then assigned to one of four treatment groups, representing supplementation with 0.0% (control), 0.2% cholesterol, 0.2% DHA, or cholesterol plus DHA to the basal milk-formula. All piglets were euthanized at 49 days of age. In brain, cholesterol supplementation reduced ($P < 0.05$) concentrations of glutamate, serine, glutamine, threonine, β-alanine, alanine, methionine, isoleucine, leucine, and γ-aminobutyrate but increased ($P < 0.05$) concentrations of glycine and lysine, whereas DHA supplementation similarly affected ($P < 0.05$) concentrations of the same AA (except for isoleucine and lysine) and taurine. In addition, concentrations of most AA in liver, muscle and plasma were substantially altered by dietary supplementation of cholesterol and DHA in a tissue-dependent manner. Further, DHA reduced concentrations of carnosine in skeletal muscle, as well as ammonia in both plasma and brain. The results reveal that cholesterol and DHA can regulate AA metabolism and availability in various tissues of piglets. These novel findings have important implications for designing the next generation of infant formula to optimize neonatal growth and development.

Keywords Cholesterol · Docosahexaenoic acid · Amino acids · Pigs

Abbreviations

AA	Amino acids
BCAA	Branched-chain amino acids
Chol	Cholesterol
DHA	Docosahexaenoic acid
GABA	γ-Aminobutyrate

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Introduction

Dietary lipids can influence development of the central nervous system (Pond et al. 2003, 2008). Particularly, an increase in plasma concentrations of cholesterol, which is an essential constituent of all animal cells (especially of brain) and abundant in milk, is positively associated with enhancement of cerebrum weight gain and behavioral development (Boleman et al. 1998; Pond et al. 2008). Additionally, docosahexaenoic acid (DHA) is a component of complex lipids in membranes, nerve insulation, as well as a precursor for signaling molecules (including

prostaglandins). The availability of long-chain polyunsaturated fatty acids (including DHA) also modulates eicosanoid metabolism and myelination during growth spurt of the brain (Koletzko and Rodriguez-Palmero 1999; Martinez 1992). Interestingly, dietary DHA supply enhances visual and neurological development in preterm (Carlson et al. 1993) and term infants (Birch et al. 1998; Field et al. 2008; SanGiovanni et al. 2000), while promoting problem-solving and childhood intelligence (Willatts et al. 1998). The beneficial influences of dietary cholesterol and DHA on brain development are currently interpreted as modification of the membrane structure and functions of membrane-associated proteins (Fleith and Clandinin 2005). However, we are not aware of any study with an animal model to investigate potential influences of cholesterol or DHA on the availability of amino acids (AA) in the brain, some of which are important regulators of neurological function (El Idrissi 2008; Novelli and Tasker 2008; Tujioka et al. 2007).

Our recent studies showed that dietary supplementation with cholesterol and DHA up-regulates the arginine–nitric oxide pathway in various tissues of the neonatal pig (Li et al. 2008), an established animal model for studying infant nutrition (Ou et al. 2007; Tan et al. 2008). Increased production of physiological levels of nitric oxide [a neurotransmitter (Orlando et al. 2008; Suenaga et al. 2008)] may play an important role in neonatal development. Because nitric oxide synthesis is also modulated by AA other than arginine and citrulline (Wu and Meininger 2002), it is important to define changes in physiological levels of AA in the brain of lipid-supplemented piglets. Additionally, to determine whether the effects of DHA and cholesterol are specific to the neuronal tissue, we also analyzed AA in plasma, skeletal muscle and liver.

Materials and methods

Chemicals

HPLC-grade water and methanol were purchased from Fisher Scientific (Houston, TX). HPLC columns were products of Supelco Inc. (PA). Glutamate dehydrogenase, urease, and AA standards were procured from Sigma Chemicals (St. Louis, MO).

Animals and diets

Contemporary neonatal female pigs from the Texas Tech University swine herd (Lubbock, TX) were allowed to nurse their dams for 24 h to receive colostrum, then transported to the Animal and Food Science Building and housed in individual cages in a temperature-controlled room designed for neonatal pig research. They were assigned randomly within litter to one of four nutritionally adequate sow-milk replacer formulas containing: (1) 0.0% cholesterol and DHA, (2) 0.20% cholesterol and zero DHA, (3) zero cholesterol and 0.2% DHA, or (4) 0.2% cholesterol and 0.2% DHA (Li et al. 2008). The doses of cholesterol and DHA were based on the previous studies (Boleman et al. 1998; Pond et al. 2008). The experiment was performed in four blocks of four pigs each (total of 16 pigs). All diets were fed ad libitum to each individually housed pig throughout the 49 day experiment. Diets were fed in liquid form (4 parts water to 1 part dry formula) four times daily at 0,800, 1,200, 1,700, and 2,200) starting at 60 mL/feeding during day 1 and gradually increasing to 100 mL/feeding by day 3. Pigs were initially trained to eat the diet as a gruel (50% water and 50% dry formula on day 4; only the dry formula by day 5). On day 49, at 2 h after feeding, blood samples (3 mL) were obtained from the jugular vein of conscious pigs, immediately followed by euthanasia with an overdose of pentobarbital and collection of cerebrum, liver and skeletal muscle. Blood was centrifuged at 3,000*g* and 4°C for 10 min to obtain plasma. All plasma and tissue samples were frozen rapidly in liquid nitrogen and stored at −80°C for AA analysis. This research was approved by the Texas Tech University Animal Use and Care Committee.

Analyses of AA, ammonia and urea

AA were determined using HPLC methods involving pre-column derivatization with *o*-phthaldialdehyde (Wu et al. 1997; Wu and Meininger 2008). Briefly, 100 mg of tissue was homogenized in 2 ml of 1.5 M $HClO_4$ and then neutralized with 1 ml of 2 M K_2CO_3. Samples were centrifuged at 2,000×*g* for 10 min, and the supernatant fluids were used for AA analysis. The HPLC system consisted of: (1) a model 600E Powerline multisolvent delivery system with 100-μL heads, a model 712 WISP Autosampler, and a Millennium-32 workstation; (2) a model 474 scanning fluorescence detector (excitation 340 nm, emission 450 nm, gain 100); and (3) a Supelco C_{18} reverse-phase guard column (4.6 mm × 5 cm, 20–40 μm) coupled to a Supelco C_{18} reverse-phase column (4.6 mm × 15 cm, 3 μm). AA in samples were quantified on the basis of known amounts of standards. Ammonia (the sum of ammonia plus ammonium) was determined using glutamate dehydrogenase (Wu 1997). Urea was analyzed using urease and glutamate dehydrogenase (Wu 1995).

Statistical analysis

Values are expressed as mean ± SEM. This study involved a 2 × 2 factorial design. Therefore, data were subjected to two-way factorial ANOVA (SAS, Cary, NC), which

provided probability values (P) for treatment means (cholesterol and DHA) and their interactions. $P < 0.05$ was taken to indicate statistical significance.

Results

Amino acid concentrations in plasma

Glycine was the most abundant free AA in pig plasma, followed by glutamine, proline, and alanine (Table 1). Dietary supplementation with cholesterol, DHA, or both affected ($P < 0.05$) concentrations of various AA in plasma of piglets (Table 1). Specifically, cholesterol supplementation increased ($P < 0.05$) concentrations of asparagine, serine, taurine, tyrosine, methionine, valine, isoleucine, leucine and lysine but decreased ($P < 0.05$) concentrations of threonine and alanine in plasma. In addition, DHA supplementation enhanced ($P < 0.05$) concentrations of asparagine, serine, glutamine, histidine, threonine, arginine, β-alanine, tyrosine, tryptophan, methionine, valine, isoleucine, leucine, ornithine and lysine in plasma. GABA was undetectable in piglet plasma (<1 μmol/L). Circulating levels of other AA were not altered in response to cholesterol or DHA treatment. There were cholesterol × DHA interaction effects on plasma concentrations of most AA.

Amino acid concentrations in brain

Glutamate was the most abundant free AA in the brain of pigs, followed by glutamine, taurine, aspartate, γ-aminobutyrate (GABA), and threonine (Table 2). Dietary supplementation with cholesterol increased ($P < 0.05$) concentrations of serine, glycine, lysine and γ-aminobutyrate (GABA), but decreased ($P < 0.05$) concentrations of glutamate, glutamine, threonine, beta-alanine, methionine, isoleucine, and leucine in the brain. Dietary DHA enhanced ($P < 0.05$) concentrations of glycine, taurine, alanine and GABA, reducing concentrations of glutamate, glutamine, threonine, methionine, and leucine in this tissue. There was a trend ($P < 0.06$) that dietary DHA reduced proline concentration in the brain. Significant interactions between cholesterol and DHA were observed for glutamate,

Table 1 Plasma concentrations (nmol/ml) of amino acids and ammonia in piglets fed diets supplemented with cholesterol and DHA

	Control	Cholesterol	DHA	Chol + DHA	P value (Chol)	P value (DHA)	P value (chol × DHA)
ALA	408 ± 11	400 ± 7	435 ± 22	345 ± 15	0.006	0.365	0.016
ASN	109 ± 9	134 ± 10	218 ± 14	113 ± 9	0.003	0.001	<0.001
ASP	13 ± 0.9	14 ± 1.1	14 ± 0.8	12 ± 0.9	0.689	0.358	0.159
β-ALA	12 ± 0.9	13 ± 0.6	19 ± 1.8	16 ± 1.5	0.286	0.001	0.11
CYS	141 ± 10	146 ± 10	158 ± 14	152 ± 9	0.955	0.302	0.596
GLN	513 ± 8	516 ± 24	656 ± 68	544 ± 19	0.174	0.043	0.153
GLU	105 ± 8	113 ± 9	123 ± 7	115 ± 13	1.00	0.316	0.404
GLY	996 ± 53	951 ± 27	1105 ± 94	1074 ± 107	0.632	0.16	0.928
HIS	57 ± 4	100 ± 5	128 ± 9	71 ± 7	0.314	0.007	<0.001
ILE	120 ± 6	156 ± 11	216 ± 14	138 ± 5	0.049	0.002	<0.001
LEU	131 ± 5	198 ± 13	283 ± 12	153 ± 11	0.014	<0.001	<0.001
LYS	172 ± 8	217 ± 23	422 ± 19	270 ± 30	0.028	<0.001	0.001
MET	74 ± 3	81 ± 4	144 ± 7	96 ± 8	0.005	<0.001	0.001
ORN	83 ± 4	108 ± 6	163 ± 11	121 ± 9	0.329	<0.001	0.001
PHE	56 ± 4	110 ± 7	116 ± 7	70 ± 6	0.529	0.11	<0.001
PRO	440 ± 29	468 ± 32	536 ± 50	480 ± 37	0.693	0.183	0.301
SER	131 ± 7	143 ± 9	304 ± 14	213 ± 14	0.005	<0.001	0.001
TAU	90 ± 6	139 ± 9	123 ± 11	140 ± 6	0.002	0.059	0.073
THR	399 ± 14	384 ± 25	634 ± 45	502 ± 33	0.037	<0.001	0.087
TRP	48 ± 3	82 ± 3	120 ± 6	70 ± 6	0.113	<0.001	<0.001
TYR	133 ± 12	165 ± 14	275 ± 19	171 ± 8	0.02	<0.001	<0.001
VAL	280 ± 11	377 ± 9	439 ± 22	282 ± 8	0.049	0.039	<0.001
Ammonia	53 ± 4	51 ± 3	38 ± 2	40 ± 3	0.616	<0.001	0.407

Values are mean ± SEM, $n = 4$ per treatment group

Chol cholesterol, *CYS* cysteine + ½ cystine, *Ammonia* NH_3 + ammonium

Table 2 Concentrations (nmol/mg tissue) of amino acids and ammonia in the brain of piglets fed diets supplemented with cholesterol and DHA

	Control	Cholesterol	DHA	Chol +DHA	*P* value (Chol)	*P* value (DHA)	*P* value (chol × DHA)
ALA	0.97 ± 0.04	0.94 ± 0.03	1.06 ± 0.04	0.68 ± 0.06	<0.001	0.065	0.001
ASN	0.13 ± 0.01	0.15 ± 0.01	0.16 ± 0.01	0.11 ± 0.01	0.15	0.255	<0.001
ASP	2.01 ± 0.19	2.15 ± 0.11	2.13 ± 0.23	1.51 ± 0.08	0.162	0.14	0.038
β-ALA	0.06 ± 0.001	0.05 ± 0.001	0.06 ± 0.001	0.03 ± 0.001	0.002	0.016	0.05
CYS	0.20 ± 0.02	0.20 ± 0.01	0.18 ± 0.01	0.17 ± 0.01	0.68	0.116	0.68
GABA	1.53 ± 0.11	1.88 ± 0.13	1.90 ± 0.12	1.01 ± 0.08	0.034	0.041	<0.001
GLN	7.49 ± 0.38	5.95 ± 0.22	6.12 ± 0.30	4.35 ± 0.21	<0.001	<0.001	0.693
GLU	8.46 ± 0.15	7.57 ± 0.35	8.71 ± 0.41	5.72 ± 0.31	<0.001	0.027	0.006
GLY	1.08 ± 0.07	1.53 ± 0.09	1.14 ± 0.06	2.24 ± 0.14	<0.001	0.001	0.005
HIS	0.10 ± 0.01	0.08 ± 0.001	0.08 ± 0.001	0.08 ± 0.001	0.102	0.102	0.23
ILE	0.08 ± 0.001	0.08 ± 0.01	0.09 ± 0.01	0.06 ± 0.01	0.018	0.311	0.081
LEU	0.14 ± 0.01	0.13 ± 0.01	0.12 ± 0.01	0.10 ± 0.01	0.04	0.023	0.303
LYS	0.15 ± 0.01	0.18 ± 0.01	0.17 ± 0.01	0.18 ± 0.01	0.022	0.154	0.352
MET	0.06 ± 0.001	0.05 ± 0.001	0.05 ± 0.001	0.03 ± 0.001	<0.001	0.013	0.134
ORN	0.02 ± 0.001	0.02 ± 0.001	0.03 ± 0.001	0.02 ± 0.001	0.337	0.337	0.337
PHE	0.06 ± 0.01	0.07 ± 0.01	0.06 ± 0.01	0.05 ± 0.01	0.512	0.14	0.065
PRO	0.97 ± 0.04	1.06 ± 0.08	0.92 ± 0.04	0.89 ± 0.05	0.639	0.059	0.283
SER	0.85 ± 0.05	0.92 ± 0.05	0.99 ± 0.06	0.57 ± 0.02	0.004	0.055	<0.001
TAU	2.10 ± 0.11	2.24 ± 0.11	2.14 ± 0.05	1.66 ± 0.08	0.089	0.013	0.006
THR	1.46 ± 0.08	1.20 ± 0.05	1.39 ± 0.10	0.94 ± 0.04	<0.001	0.039	0.202
TRP	0.02 ± 0.001	0.02 ± 0.001	0.02 ± 0.001	0.02 ± 0.001	1.00	1.00	1.00
TYR	0.14 ± 0.01	0.13 ± 0.01	0.14 ± 0.01	0.13 ± 0.01	0.313	0.569	0.909
VAL	0.17 ± 0.01	0.16 ± 0.01	0.16 ± 0.01	0.13 ± 0.01	0.12	0.082	0.335
Ammonia	0.26 ± 0.02	0.24 ± 0.02	0.16 ± 0.02	0.18 ± 0.02	0.259	<0.001	0.384

Values are mean ± SEM, $n = 4$ per treatment group

Chol cholesterol, *CYS* cysteine + ½ cystine, *GABA* γ-aminobutyrate, *Ammonia* NH_3 + ammonium

asparagine, serine, histidine, alanine, tryptophan, tyrosine and GABA in the brain ($P < 0.05$).

Amino acid concentrations in liver

Taurine was the most abundant free AA in the liver of pigs, followed by glycine, proline, alanine, glutamate, glutamine, and aspartate (Table 3). Compared with other tested tissues, changes in hepatic concentrations of individual or total AA were modest in cholesterol- and DHA-supplemented pigs. However, dietary supplementation with cholesterol reduced the concentrations of glutamine, threonine, beta-alanine, alanine, methionine, isoleucine, but increased concentrations of lysine in the liver (Table 3). In contrast, dietary supplementation with DHA increased ($P < 0.05$) concentrations of asparagine, but decreased ($P < 0.05$) concentrations of glycine, glutamine, beta-alanine and taurine in the liver. Significant interactions between cholesterol and DHA were observed for hepatic aspartate, glutamate, serine, histidine, glycine, taurine, alanine and ornithine concentrations ($P < 0.05$).

Amino acid and carnosine concentrations in skeletal muscle

Concentrations of carnosine were higher than those of any AA in pig skeletal muscle (Table 4). In this tissue, taurine and glutamine were the most abundant β- and α-AA, respectively. Dietary supplementation with cholesterol increased ($P < 0.05$) concentrations of glutamate and leucine, but decreased concentrations of aspartate, histidine, glycine, and ornithine in skeletal muscle (Table 4). Of particular interest, dietary supplementation with DHA enhanced ($P < 0.05$) intramuscular concentrations of the following AA: aspartate, glutamate, asparagine, serine, glutamine, histidine, taurine, alanine, tyrosine, tryptophan, methionine, isoleucine, phenylalanine, leucine and lysine. In contrast, the DHA treatment decreased ($P < 0.05$) concentrations of beta-alanine, ornithine and carnosine in muscle (Table 4). Significant interactions between cholesterol and DHA were observed for all nutritionally nonessential AA, except for alanine, proline and cysteine ($P < 0.05$).

Table 3 Concentrations (nmol/mg tissue) of amino acids and ammonia in the liver of piglets fed diets supplemented with cholesterol and DHA

	Control	Cholesterol	DHA	Chol + DHA	*P* value (Chol)	*P* value (DHA)	*P* value (chol × DHA)
ALA	3.91 ± 0.35	4.64 ± 0.35	4.17 ± 0.53	2.88 ± 0.19	0.492	0.083	0.026
ASN	0.80 ± 0.06	0.85 ± 0.05	1.05 ± 0.10	1.03 ± 0.05	0.882	0.011	0.673
ASP	1.49 ± 0.15	0.97 ± 0.04	1.26 ± 0.09	1.23 ± 0.09	0.017	0.848	0.03
β-ALA	0.40 ± 0.02	0.39 ± 0.01	0.26 ± 0.02	0.20 ± 0.02	0.072	<0.001	0.218
CYS	0.20 ± 0.01	0.20 ± 0.01	0.20 ± 0.01	0.20 ± 0.01	0.672	0.672	0.734
GLN	3.37 ± 0.26	2.64 ± 0.14	2.98 ± 0.17	2.50 ± 0.20	0.011	0.218	0.543
GLU	3.72 ± .020	3.67 ± 0.15	3.11 ± 0.20	4.16 ± 0.22	0.032	0.766	0.021
GLY	6.46 ± 0.11	7.49 ± 0.53	6.62 ± 0.43	5.20 ± 0.16	0.623	0.018	0.008
HIS	0.75 ± 0.03	0.77 ± 0.02	0.85 ± 0.05	0.60 ± 0.03	0.006	0.318	0.002
ILE	0.28 ± 0.02	0.25 ± 0.02	0.29 ± 0.02	0.30 ± 0.01	0.491	0.111	0.131
LEU	0.57 ± 0.03	0.50 ± 0.03	0.54 ± 0.04	0.51 ± 0.03	0.159	0.772	0.527
LYS	0.48 ± 0.02	0.42 ± 0.02	0.48 ± 0.05	0.44 ± 0.02	0.117	0.717	0.795
MET	0.08 ± 0.001	0.08 ± 0.001	0.08 ± 0.001	0.10 ± 0.01	0.148	0.275	0.06
ORN	0.41 ± 0.02	0.60 ± 0.03	0.50 ± 0.06	0.46 ± 0.07	0.171	0.639	0.049
PHE	0.19 ± 0.01	0.19 ± 0.01	0.18 ± 0.01	0.19 ± 0.01	0.833	1.00	0.531
PRO	4.83 ± 0.27	4.92 ± 0.12	4.95 ± 0.28	4.86 ± 0.25	0.991	0.904	0.721
SER	1.06 ± 0.07	1.40 ± 0.10	1.41 ± 0.09	1.00 ± 0.09	0.687	0.783	0.002
TAU	9.90 ± 0.88	10.36 ± 0.43	9.56 ± 0.37	6.81 ± 0.42	0.061	0.005	0.014
THR	0.90 ± 0.09	0.79 ± 0.04	0.80 ± 0.04	0.88 ± 0.03	0.728	0.867	0.116
TRP	0.07 ± 0.001	0.07 ± 0.001	0.07 ± 0.001	0.07 ± 0.001	0.918	0.478	0.611
TYR	0.23 ± 0.01	0.21 ± 0.01	0.25 ± 0.02	0.24 ± 0.01	0.312	0.105	0.863
VAL	0.61 ± 0.04	0.51 ± 0.02	0.54 ± 0.03	0.53 ± 0.02	0.091	0.394	0.198
Ammonia	0.35 ± 0.03	0.33 ± 0.02	0.23 ± 0.02	0.22 ± 0.02	0.264	<0.001	0.338

Values are mean ± SEM, $n = 4$ per treatment group

Chol cholesterol, *CYS* cysteine + ½ cystine, *Ammonia* NH_3 + ammonium

Ammonia and urea concentrations in plasma and tissues

Dietary supplementation with DHA reduced ($P < 0.05$) ammonia concentrations in plasma (Table 1), brain (Table 2), and liver (Table 3), but not in skeletal muscle (Table 4), of pigs (Tables 1–4). In contrast, cholesterol supplementation did not affect ammonia concentrations in either plasma or the tissues (Tables 1–4). Urea concentrations in plasma, brain, and liver, or skeletal muscle did not differ among all groups of pigs (data not shown).

Discussion

AA are not only building blocks for tissue proteins but are also potent antioxidants, regulators of hormone secretion, and cell signaling molecules (Hu et al. 2008; Jobgen et al. 2006; Larson et al. 2007; Wang et al. 2007). Furthermore, AA are precursors for the synthesis of non-protein substances with biological importance, including nitric oxide, polyamines, neurotransmitters, amino sugars, purine and pyrimidine nucleotides, creatine, carnitine, porphyrins, melatonin, melanin, phospholipids, and sphingolipids (Fang et al. 2002; Hu et al. 2007; Li et al. 2007; Montanez et al. 2008). Polyamines (polycationic molecules) regulate gene expression, signal transduction, ion channel function, DNA and protein synthesis, as well as cell proliferation, differentiation, and function (Flynn et al. 2002; Rider et al. 2007; Sugita et al. 2007). Additionally, alterations in concentrations of glutamate, glutamine, aspartate, and GABA may play a role in the function and apoptosis of cells in the central nervous system and other organs (Mates et al. 2002; Rhoads et al. 1997). Likewise, a profound increase in concentrations of proline due to a deficiency of proline oxidase may be linked to the pathogenesis of schizophrenia, a neurological disease (Willis et al. 2008). Also, marked elevation of phenylalanine is a cause of impaired neurological development in children with phenylketonuria (PKU) caused by a deficiency of phenylalanine hydroxylase or its essential cofactor tetrahydrobiopterin (Thony et al. 2000). Although dietary cholesterol and DHA are known to affect cognitive development (Fleith and Clandinin 2005; Pond 2003) and immunity (Field et al. 2001, 2008), little is known about the effect of the lipids on AA metabolism in neonatal tissues of any species. This is the first report that dietary

Table 4 Concentrations (nmol/mg tissue) of amino acids, carnosine, and ammonia in gastrocnemius muscle of piglets fed diets supplemented with cholesterol and DHA

	Control	Cholesterol	DHA	Chol + DHA	*P* value (Chol)	*P* value (DHA)	*P* value (chol × DHA)
ALA	1.32 ± 0.09	1.55 ± 0.11	1.92 ± 0.18	1.82 ± 0.20	0.688	0.015	0.306
ASN	0.11 ± 0.01	0.13 ± 0.01	0.23 ± 0.02	0.16 ± 0.01	0.123	<0.001	0.009
ASP	0.11 ± 0.01	0.11 ± 0.01	0.19 ± 0.01	0.13 ± 0.01	0.003	<0.001	0.014
β-ALA	0.73 ± 0.07	0.50 ± 0.06	0.33 ± 0.02	0.39 ± 0.04	0.142	0.001	0.016
CYS	0.07 ± 0.001	0.08 ± 0.001	0.11 ± 0.01	0.09 ± 0.01	0.657	0.001	0.094
GLN	4.33 ± 0.47	8.82 ± 0.22	10.9 ± 0.44	8.24 ± 0.97	0.152	<0.001	<0.001
GLU	1.41 ± 0.12	1.55 ± 0.14	2.36 ± 0.11	1.69 ± 0.07	0.034	<0.001	0.004
GLY	2.40 ± 0.12	3.34 ± 0.31	2.13 ± 0.17	2.82 ± 0.20	0.002	0.083	0.571
HIS	0.28 ± 0.02	0.44 ± 0.04	0.38 ± 0.01	0.51 ± 0.04	0.001	0.01	0.673
ILE	0.06 ± 0.01	0.06 ± 0.01	0.12 ± 0.02	0.09 ± 0.01	0.251	0.001	0.251
LEU	0.09 ± 0.01	0.11 ± 0.01	0.22 ± 0.01	0.14 ± 0.01	0.008	<0.001	<0.001
LYS	0.20 ± 0.03	0.29 ± 0.01	0.35 ± 0.04	0.26 ± 0.02	1.00	0.027	0.004
MET	0.05 ± 0.001	0.06 ± 0.001	0.08 ± 0.01	0.06 ± 0.001	0.211	0.013	0.035
ORN	0.17 ± 0.02	0.12 ± 0.01	0.09 ± 0.001	0.09 ± 0.001	0.027	<0.001	0.017
PHE	0.05 ± 0.001	0.07 ± 0.01	0.09 ± 0.01	0.07 ± 0.01	0.862	0.021	0.006
PRO	2.00 ± 0.11	2.04 ± 0.09	1.90 ± 0.07	1.95 ± 0.10	0.627	0.307	0.968
SER	0.20 ± 0.01	0.30 ± 0.02	0.53 ± 0.04	0.29 ± 0.05	0.062	<0.001	<0.001
TAU	8.55 ± 0.37	10.1 ± 0.29	12.0 ± 0.40	9.44 ± 0.28	0.196	0.002	<0.000
THR	0.63 ± 0.07	0.86 ± 0.05	0.79 ± 0.02	0.71 ± 0.03	0.146	0.916	0.005
TRP	0.03 ± 0.001	0.03 ± 0.001	0.04 ± 0.001	0.04 ± 0.001	0.584	0.044	0.584
TYR	0.14 ± 0.01	0.18 ± 0.01	0.20 ± 0.01	0.18 ± 0.01	0.57	0.026	0.053
VAL	0.20 ± 0.01	0.20 ± 0.01	0.23 ± 0.02	0.21 ± 0.01	0.612	0.19	0.402
Carnosine	10.85 ± 0.70	9.35 ± 0.35	5.26 ± 0.16	6.37 ± 0.17	0.631	<0.001	0.007
Ammonia	0.24 ± 0.02	0.23 ± 0.02	0.22 ± 0.03	0.23 ± 0.03	0.578	0.338	0.814

Values are mean ± SEM, $n = 4$ per treatment group

Chol cholesterol, *CYS* cysteine + ½ cystine, *Ammonia* NH_3 + ammonium

cholesterol and DHA modulates AA concentrations in plasma, brain, muscle and liver of young pigs in a tissue-dependent manner.

A novel and important observation from this work is that dietary supplementation with DHA markedly increased plasma concentrations of all branched-chain amino acids (BCAA) and glutamine in pigs (Table 1). In this study, all the pigs were in positive nitrogen balance as indicated by their normal weight gain and food intake did not differ among the treatment groups. Thus, because there is no de novo synthesis of BCAA in pigs, an increase in circulating levels of BCAA likely results from a reduction in their catabolism. A major site may be the small intestine, which degrades 40–50% of dietary BCAA in young pigs (Wang et al. 2008a, b; Wu 1998). It is now known that both epithelial absorptive cells and luminal microorganisms are responsible for the extensive degradation of BCAA in the pig small intestine (Chen et al. 2007). Our finding raised an important question of whether DHA may regulate intestinal BCAA metabolism. In DHA-supplemented pigs, increased entry of BCAA from the lumen of the gut into the portal vein will result in elevation of their concentrations in plasma (Kong et al. 2008). This, in turn, will increase the availability of circulating BCAA for uptake by extrahepatic tissues. One of these tissues is skeletal muscle, which represents approximately 40% of body weight and has relatively high activities of both BCAA transaminase and glutamine synthetase (Chang and Goldberg 1978). A major nitrogenous product of BCAA degradation in muscle is glutamine, which is released into the circulation. This may explain why supplementing DHA to the diet for young pigs increased plasma levels of glutamine (Table 1), a conditionally essential AA that is known to improve intestinal function and growth performance in the neonates (Wang et al. 2008a, b).

Ornithine is an intermediate of the urea cycle in the liver and small intestine of post-weaning pigs, where it reacts with carbamoylphosphate (derived from ammonia and bicarbonate) to yield citrulline (Wu and Morris 1998). Thus, although ornithine is not a substrate for protein synthesis, it plays an important role in the detoxification of ammonia, a metabolite of AA degradation that is extremely

toxic to the brain of mammals, particularly neonates (Wu et al. 2004). Another novel and important finding from this work is that dietary DHA supplementation increased ornithine concentrations in plasma (Table 1), while reducing concentrations of ammonia in both plasma and brain (Table 2). Ornithine was absent from the sow-milk replacer diet but can be synthesized from glutamine, glutamate, arginine and proline in pigs (Wu and Morris 1998). It is possible that DHA regulates ornithine synthesis in the small intestine by up-regulating expression of key genes such as pyrroline-5-carboxylate synthase and proline oxidase (Wu 1997). In support of this view, concentrations of arginine were also elevated in DHA-supplemented young pigs (Li et al. 2008). Future studies are necessary to elucidate the underlying mechanisms. Nonetheless, an increase in the availability of ornithine facilitates the removal of ammonia from the circulation (Wu and Morris 1998), therefore reducing the concentration of ammonia in the brain. This would indirectly contribute to health of the central nervous system.

Glutamate, aspartate, and GABA (a product of glutamate decarboxylation) are excitatory neurotransmitters (Ben-Ari et al. 2007). Interestingly, dietary supplementation with cholesterol plus DHA reduced concentrations of glutamate, aspartate and GABA in the brain (Table 2) despite no changes in plasma (Table 1). It is noteworthy that decreases in concentrations of these neurotransmitters in the brain are functionally associated with beneficial changes in the behavior of piglets supplemented with cholesterol or DHA (Pond et al. 2008). Available evidence shows that glutamine is actively hydrolyzed by the mitochondrial phosphate-dependent glutaminase in the brain to generate glutamate (Watford 1994). In addition, glutamate can be synthesized from BCAA by both cytosolic and mitochondrial BCAA transaminase in the brain. Glutamate–oxaloacetate transaminase activity is widely spread in animal tissues to synthesize aspartate from glutamate (Rhoads et al. 1992). Thus, we surmise that dietary supplementation with cholesterol or DHA may reduce the uptake of either acidic AA and/or their precursors (glutamine and BCAA), as well as the activity of glutamate decarboxylase for GABA synthesis by the brain. These effects of cholesterol and DHA are likely tissue-specific, because there were no decreases in concentrations of glutamate and aspartate in piglet skeletal muscle (Table 4). In the brain, a decrease in either glutamate availability or glutamate decarboxylase can result in reduced generation of GABA.

In conclusion, the results of the present study indicate that dietary supplementation with DHA and cholesterol markedly influence AA profiles in the plasma, brain, liver and skeletal muscle of young pigs. Importantly, the effects of cholesterol and DHA are tissue-specific, particularly with profound decreases in concentrations of excitatory AA and GABA in the brain but great increases in plasma ornithine as well as intramuscular BCAA and glutamine. In addition, DHA decreases concentrations of ammonia (a toxic substance) in both plasma and the brain. These novel findings suggest that cholesterol and DHA may play an important role in regulating AA metabolism, therefore impacting neurological growth and development of neonates.

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