Effect of arginine-free diet on plasma and tissue amino acids in young and adult ferrets

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A single meal of arginine-free diet caused severe hyperammonemia in young (2-month-old) ferrets, whereas adult (18-month-old) ferrets did not develop hyperammonemia after identical treatment. To explore the mechanism of hyperammonemia induced by arginine-free diet, we tested the effect of a single meal of arginine-free diet on the amino acid levels in plasma, liver, and kidney of young and adult ferrets. Plasma and tissues were obtained three hours after feeding the diets. Arginine-containing diet caused a rapid increase in plasma arginine and ornithine (compared to fasting levels) only in young ferrets, indicating that these amino acids were absorbed more rapidly in young ferrets than in adult ferrets. Young ferrets fed arginine-free diet had lower levels of plasma arginine than those fed arginine-containing diet, whereas in adult ferrets no differences were observed in plasma arginine levels between arginine-free and arginine-containing diet groups. Both young and adult ferrets had low levels of hepatic ornithine. However, only young ferrets became hyperammonemic after ingesting an arginine-free diet. A decreased synthesis or an increased catabolism of arginine in young ferrets may explain the rapid development of hyperammonemia resulting from ingestion of an arginine-free diet.

Keywords: arginine; amino acids; hyperammonemia; ferrets

Introduction

Arginine is a primary amino acid which plays a major role in protecting mammals against ammonia intoxication via urea synthesis. In addition, L-arginine plays several important roles in the regulation of metabolism.[1-3] Earlier studies of Rose[4] indicated that young rats need dietary arginine for optimum growth and positive nitrogen balance, whereas adult rats do not require dietary arginine. L-Arginine is often classified as an intermediate between the dispensable and indispensable amino acids because rats can store some nitrogen without dietary arginine and endogenous synthesis of arginine occurs in rats. Recent reports indicate that in unstressed animals and humans, arginine may be classified as a semidispensable amino acid, although it becomes indispensable in various clinically important states such as starvation, injury, or stress.[1,2] These reports indicate that adult mammals including humans may not, under certain conditions, meet all of their arginine requirement by endogenous synthesis.

Dietary arginine was also generally regarded as a dispensable amino acid for adult carnivores. However, recent experiments provide evidence that the response to the ingestion of an arginine-free diet (AFD) differs widely among various species and within different age-groups of the same species. For example, feeding an AFD to rats for two weeks caused growth retardation without signs of hyperammonemia,[5,6] whereas a single meal of an AFD to juvenile ferrets

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and near-adult cats produced severe hyperammonemia and encephalopathy.[7-12] Immature and mature dogs, when fed an AFD using a stomach tube, also show profuse salivation, vomiting, tremors, and hyperglycemia.[13-15] These studies suggest that a dietary source of arginine is essential for young cats, dogs, and ferrets, and probably for other carnivorous animals.

We have reported previously that single feeding of an AFD caused a severe hyperammonemia and encephalopathy in young ferrets whereas similar treatment in adult ferrets did not cause hyperammonemia or any other sickness.[10,11] The objective of this investigation was to compare the metabolic changes in young and adult ferrets following control or arginine-free meal in order to understand the susceptibility of young ferrets (and probably of other carnivores) to arginine deficiency. Previously, we have reported the effect of arginine-free diet on the plasma and urinary levels of glucose, urea, orotic acid, and ammonia,[10] and on the urea cycle enzymes in the liver and kidney of young and adult ferrets.[11] In the present paper, we have compared the effect of single feeding of AFD on the plasma and liver amino acid levels in young and adult ferrets.

Materials and methods

Animals

Two-month-old (young) or eighteen-month-old (adult) male, sable-coated ferrets, vaccinated for canine distemper, were purchased from Marshall Research Farm, North Rose, NY. They were housed in groups of two or three in cages with grid flooring, in an isolation room with controlled light and temperature (22–23°C, 12 hour light/dark cycle).

Diets

Water and stock diet (Cat Chow, Ralston Purina Co., St. Louis, MO) were provided ad libitum. A synthetic diet containing free amino acids, vitamins, corn starch, sucrose, and salt mixture was prepared as prescribed previously,[8] except that corn oil was used instead of turkey fat. An AFD was prepared by substituting alanine for an isonitrogenous amount of arginine. The slight increase of alanine required was compensated for by decreasing the carbohydrate content.

Experimental design

Ferrets were fasted overnight and fed ad libitum, at 9:00 a.m. either the arginine-containing diet (ACD) or AFD. At 12:00 noon, ferrets were anesthetized by keeping in a glass chamber containing ether for about 1 minute, and 3 mL of blood was collected by cardiac puncture into chilled heparinized tubes (Vacutainer) and centrifuged at 8000*g* for 15 minutes in a refrigerated centrifuge. A portion of the plasma was stored at −20°C. Between 12:00 noon and 12:30 p.m., animals were sacrificed. Livers and kidneys were removed, frozen in liquid nitrogen, and stored at −20°C.

Amino acid analysis

Plasma was deproteinized with 3% (w/v) sulfosalicylic acid for amino acid determination. Portions of liver were pulverized in liquid nitrogen and a 10% (w/v) homogenate was prepared in 3% sulfosalicylic acid. Amino acids in plasma and tissues were determined using high performance liquid chromatography (Waters Associates) with a gradient programmer and an integrator. Chromatographic separations were performed with a reverse phase C18 column as described previously.[16]

Ammonia assay

Liver, kidney, and brain samples were homogenized in chilled perchloric acid (1 M). Homogenates were centrifuged at 10,000*g* for 15 minutes at 0–2°C, and the supernatants were neutralized to pH 7.00 with 1 N sodium hydroxide. Ammonia in the supernatants and plasma was assayed by glutamate dehydrogenase reaction.[17] Ammonia-free water was used as a blank and also for preparing all reagents.

Statistical analysis

Student's *t* test was used to calculate the statistical difference. P values <0.05 were considered statistically significant.

Results

Young ferrets fed an arginine-free meal became hyperactive two hours after ingesting the diet. Within 30 minutes of the onset of hyperactivity, they became prostrate and subsequently developed coma. The typical symptoms for hyperammonemia such as irritability and seizures were observed in several ferrets. Adult ferrets did not develop hyperammonemia and encephalopathy after a single meal of AFD. There was no significant difference in the amount of food intake between control and arginine-deficient groups. Young or adult ferrets in both groups ate about 15–20 g of diet (0.7 to 0.9 g of nitrogen). When expressed per kilogram body weight, the food intake in young ferrets was slightly higher than in the adult ferrets.[10] Adult ferrets did not develop hyperammonemia or encephalopathy even after eating an arginine-free diet for 7 days.

Plasma and liver ammonia levels in young or adult ferrets fasted for 16 hours were not significantly different from those fed ACD (*Tables 1* and *2*). Previously, we have reported that young ferrets fed a single meal of AFD exhibited elevated levels of ammonia in plasma, liver, kidney, and brain as compared to the levels in ferrets fed ACD.[11] In contrast, the ammonia concentration in plasma, kidney and brain of similarly treated adult ferrets was not elevated (*Table 3*).

Plasma levels of serine, glutamine, alanine, and γ-ABA were significantly higher in adult ferrets fasted for 16 hours than similarly treated young ferrets (*Table 4*). There were no significant differences in plasma and the liver levels of other amino acids of young and adult

Table 1 Amino acids in plasma of young and adult ferrets fed arginine-containing or arginine-free meal

Amino Acid μmol/L	2-month-old ferrets		18-month-old ferrets	
	ACD	AFD	ACD	AFD
Aspartic acid	3 ± 0.4	68 ± 11[a]	2 ± 0.3[b]	2 ± 0.3[b]
Glutamic acid	46 ± 5	158 ± 23[a]	52 ± 4	49 ± 1[b]
αAminoadipate	2 ± 0.2	6 ± 0.5[a]	1 ± 0.1[b]	1 ± 0.1[b]
Aspargine	103 ± 8	77 ± 8	33 ± 0.9[b]	45 ± 5
Serine	136 ± 6	170 ± 9[a]	78 ± 2.3[a,b]	95 ± 10[b]
Glutamine	92 ± 5	194 ± 13[a]	216 ± 13[a]	214 ± 14
Histidine	63 ± 4	58 ± 3	32 ± 1[b]	41 ± 4
Glycine	250 ± 19	234 ± 9	136 ± 7[b]	179 ± 5[a]
Threonine	276 ± 7	235 ± 6[a]	84 ± 6[b]	112 ± 15[b]
Citrulline	22 ± 2	20 ± 2	7 ± 0.5[b]	9 ± 0.7[b]
Arginine	102 ± 7	24 ± 3[a]	62 ± 3[b]	66 ± 5[b]
Alanine	286 ± 18	437 ± 43[a]	119 ± 3[b]	122 ± 7[b]
Taurine	22 ± 4	101 ± 11[a]	26 ± 3	41 ± 8
γABA	2 ± 0.2	4 ± 0.7[a]	1 ± 0.2[b]	2 ± 0.2[a]
Tyrosine	62 ± 2	174 ± 13[a]	31 ± 2[b]	36 ± 2[b]
Methionine	72 ± 5	69 ± 2	23 ± 2[b]	35 ± 5[b]
Valine	231 ± 10	234 ± 12	100 ± 8[b]	106 ± 8[b]
Phenylalanine	86 ± 2	95 ± 3	31 ± 0.5[b]	40 ± 4[b]
Isoleucine	94 ± 5	111 ± 50	30 ± 2[b]	43 ± 5[b]
Leucine	106 ± 5	127 ± 5[a]	52 ± 6[b]	54 ± 4[b]
Ornithine	16 ± 2	4 ± 0.5[a]	3 ± 0.2[b]	4 ± 0.3
Lysine	106 ± 12	100 ± 8	57 ± 6[b]	75 ± 8
Ammonia	59.1 ± 4.5	2196 ± 102[a]	47.4 ± 6.2	44 ± 3.9

Values are means ± SEM (n = 5).
[a] Statistically significant difference ($P<0.05$) when compared with ACD in the same age group.
[b] Statistically significant difference as compared with 2-month-old ferrets in the same diet group.

Table 2 Amino acids in livers of young and adult ferrets fed arginine-containing or arginine-free meal

Amino acid μmol/g	2-month-old ferrets		18-month-old ferrets	
	ACD	AFD	ACD	AFD
Aspartic acid	0.43 ± 0.06	1.8 ± 0.05[a]	0.4 ± 0.04	0.45 ± 0.04[b]
Glutamic acid	1.5 ± 0.09	1.8 ± 0.06[a]	0.97 ± 0.12[b]	0.93 ± 0.03[b]
Aspargine	0.15 ± 0.02	0.12 ± 0.02	0.13 ± 0.01	0.10 ± 0.01[a]
Serine	0.26 ± 0.04	1.0 ± 0.15[a]	0.16 ± 0.01	0.16 ± 0.02[b]
Glutamine	0.08 ± 0.01	0.11 ± 0.01[a]	0.10 ± 0.01	0.05 ± 0.01[a,b]
Histidine	0.24 ± 0.03	0.46 ± 0.05[a]	0.20 ± 0.01	0.2 ± 0.01[b]
Glycine	0.29 ± 0.03	0.57 ± 0.06[a]	0.34 ± 0.02	0.4 ± 0.03
Threonine	0.48 ± 0.08	1.22 ± 0.17[a]	0.11 ± 0.01	0.13 ± 0.02[b]
Citrulline	0.02 ± 0.001	0.02 ± 0.004	0.05 ± 0.01[b]	0.05 ± 0.01[b]
Arginine	0.16 ± 0.01	0.29 ± 0.04[a]	0.11 ± 0.01	0.24 ± 0.06
Alanine	0.44 ± 0.08	1.92 ± 0.15[a]	0.34 ± 0.04	0.40 ± 0.03[b]
Taurine	3.22 ± 0.3	2.75 ± 0.14	2.86 ± 0.09	3.19 ± 0.17
BAIB	0.13 ± 0.02	1.14 ± 0.14[a]	0.11 ± 0.02	0.16 ± 0.01[b]
Tyrosine	0.03 ± 0.003	0.16 ± 0.02[a]	0.02 ± 0.003	0.03 ± 0.001[b]
Methionine	0.03 ± 0.005	0.13 ± 0.02[a]	0.03 ± 0.003	0.04 ± 0.004[b]
Valine	0.21 ± 0.03	0.32 ± 0.05	0.09 ± 0.01[b]	0.13 ± 0.01[b]
Phenylalanine	0.07 ± 0.01	0.08 ± 0.02	0.04 ± 0.01	0.04 ± 0.002[b]
Isoleucine	0.07 ± 0.01	0.18 ± 0.03[a]	0.04 ± 0.01	0.04 ± 0.01[b]
Leucine	0.09 ± 0.01	0.23 ± 0.04[a]	0.07 ± 0.01	0.09 ± 0.01[b]
Ornithine	0.18 ± 0.04	0.09 ± 0.02	0.04 ± 0.01[b]	0.05 ± 0.01
Lysine	0.05 ± 0.01	0.84 ± 0.17[a]	0.05 ± 0.01	0.07 ± 0.01[b]
N-Acetylglutamate	14 ± 2.5	56 ± 2.2[a]	23 ± 2.2	17 ± 2.7[b]
Ammonia	1.0 ± 0.1	6.9 ± 0.4[a]	1.5 ± 0.05[b]	2.2 ± 0.3[a,b]

Values are means ± SEM (n = 5).
[a] Statistically significant difference ($P<0.05$) when compared with ACD in the same age group.
[b] Statistically significant difference as compared with 2-month-old ferrets in the same diet group.

Table 3 Amino acids in kidneys of young and adult ferrets fed arginine-containing or arginine-free meal

Amino acid μmol/g	2-month-old ferrets		18-month-old ferrets	
	ACD	AFD	ACD	AFD
Aspartic acid	1.1 ± 0.10	1.33 ± 0.16	0.63 ± 0.13[b]	0.48 ± 0.09[b]
Glutamic acid	2.8 ± 0.04	2.6 ± 0.17	2.47 ± 0.15	2.17 ± 0.10
Aspargine	0.13 ± 0.01	0.10 ± 0.01	0.07 ± 0.02	0.05 ± 0.007
Serine	0.70 ± 0.03	0.76 ± 0.09	0.49 ± 0.12	0.31 ± 0.05[b]
Glutamine	0.45 ± 0.03	0.75 ± 0.07	0.35 ± 0.07	0.16 ± 0.01[a,b]
Histidine	0.2 ± 0.02	0.25 ± 0.07	0.16 ± 0.05	0.10 ± 0.01
Glycine	1.08 ± 0.05	1.23 ± 0.17	1.02 ± 0.32	0.55 ± 0.09[b]
Threonine	0.55 ± 0.10	0.46 ± 0.06	0.17 ± 0.05[b]	0.20 ± 0.04[b]
Citrulline	0.05 ± 0.003	0.06 ± 0.005	0.06 ± 0.01	0.04 ± 0.005
Arginine	0.02 ± 0.003	0.02 ± 0.004	0.01 ± 0.004	0.01 ± 0.002
Alanine	1.27 ± 0.13	2.6 ± 0.42	1.16 ± 0.25	0.85 ± 0.18[b]
Taurine	4.2 ± 0.14	4.1 ± 0.27	2.37 ± 0.41[b]	1.97 ± 0.21[b]
BAIBA	0.04 ± 0.004	0.04 ± 0.004	0.02 ± 0.006	0.01 ± 0.001[b]
Tyrosine	0.12 ± 0.01	0.21 ± 0.04	0.08 ± 0.02	0.06 ± 0.009[b]
Valine	0.18 ± 0.04	0.24 ± 0.03	0.15 ± 0.05	0.1 ± 0.02[b]
Phenylalanine	0.09 ± 0.01	0.1 ± 0.01	0.01 ± 0.001[b]	0.04 ± 0.008[a,b]
Isoleucine	0.44 ± 0.08	0.08 ± 0.02	0.02 ± 0.003[b]	0.04 ± 0.007[a,b]
Leucine	0.13 ± 0.01	0.13 ± 0.02	0.02 ± 0.003[b]	0.06 ± 0.013[a,b]
Ornithine	0.32 ± 0.04	0.07 ± 0.01	0.04 ± 0.009[b]	0.03 ± 0.002[b]
Lysine	0.41 ± 0.05	0.16 ± 0.01[a]	0.22 ± 0.09[b]	0.08 ± 0.01[a]
Ammonia	2.8 ± 0.3	12.6 ± 1.0[a]	3.7 ± 0.21	3.7 ± 0.1[b]

Values are means ± SEM (n = 5).
[a] Statistically significant difference ($P<0.05$) when compared with ACD in the same age group.
[b] Statistically significant difference as compared with 2-month-old ferrets in the same diet group.

Table 4 Fasting amino acids levels in plasma and livers of young and adult ferrets

Amino acid	Plasma		Liver	
	2-month-old	18-month-old	2-month-old	18-month-old
	μmol/L[a]		μmol/g[a]	
Aspartic acid	2.1 ± 0.3	2.4 ± 0.4	0.5 ± 0.04	0.6 ± 0.05
Glutamic acid	53 ± 2.5	60 ± 8.1	1.5 ± 0.08	1.2 ± 0.07
Aspargine	45 ± 2.9	42 ± 4.0	0.11 ± 0.01	0.11 ± 0.01
Serine	77 ± 4.5	111 ± 11	0.09 ± 0.001	0.15 ± 0.01
Glutamine	149 ± 6.1	298 ± 35	1.0 ± 0.08	0.13 ± 0.01[b]
Histidine	34 ± 1.2	41 ± 2.7	0.22 ± 0.01	0.20 ± 0.01
Glycine	143 ± 7.4	191 ± 16	0.42 ± 0.02	0.33 ± 0.03
Threonine	80 ± 5.7	90 ± 6.7	0.17 ± 0.01	0.13 ± 0.01
Citrulline	11 ± 0.4	11 ± 0.5	0.016 ± 0.001	0.04 ± 0.004
Arginine	72 ± 4.5	69 ± 8.1	0.14 ± 0.01	0.09 ± 0.01
Alanine	119 ± 5.7	162 ± 16	0.29 ± 0.02	0.45 ± 0.03
Taurine	18 ± 2.5	25 ± 1.3	2.45 ± 0.04	2.89 ± 0.04
γABA	0.2 ± 0.04	2.4 ± 0.1	—	—
BAIBA	1.7 ± 0.20	5.3 ± 0.5[b]	0.04 ± 0.001	0.12 ± 0.01
Tyrosine	32 ± 2.0	41 ± 3.1[b]	0.029 ± 0.001	0.032 ± 0.002
Methionine	22 ± 1.2	24 ± 2.2	0.014 ± 0.001	0.023 ± 0.001
Valine	126 ± 8.6	113 ± 9.8	0.11 ± 0.005	0.12 ± 0.01
Phenylalanine	44 ± 2.5	40 ± 2.7	0.04 ± 0.001	0.041 ± 0.002
Isoleucine	54 ± 2.5	37 ± 4.5	0.047 ± 0.001	0.043 ± 0.003
Leucine	77 ± 3.7	61 ± 5.8	0.075 ± 0.006	0.086 ± 0.006
Ornithine	5.1 ± 0.20	4.4 ± 0.4	0.057 ± 0.008	0.042 ± 0.004
Lysine	54 ± 4.5	81 ± 10	0.064 ± 0.001	0.059 ± 0.005

[a] Values are means ± SEM (n = 5).
[b] Statistically significant difference as compared with 2-month-old ferrets.

ferrets that were fasted for 16 hours (*Table 4*). A single meal of ACD to young ferrets caused an increase in plasma arginine, ornithine, histidine, citrulline, asparagine, threonine, glycine, alanine, and valine indicating rapid absorption of these amino acids. However, except for valine, these amino acids were not altered in similarly treated adult ferrets (*Tables 1* and *4*). Compared to fasted ferrets, both young and adult ferrets fed ACD had decreased levels of glutamine.

Young ferrets fed AFD had lower levels of plasma arginine than those fed ACD, whereas in adult ferrets there were no differences in plasma arginine levels between the control and arginine-free group. Aspartic acid levels in young ferrets fed AFD were significantly elevated over the controls, although the levels varied widely in individual animals. The aspartate levels were not elevated in the samples taken 90 minutes after feeding the diet (data not shown). AFD did not cause a statistically significant change in plasma lysine levels of young and adult ferrets. Plasma glutamine levels in adult ferrets fed control diet were significantly higher than the similarly treated young ferrets and were comparable to those of young ferrets fed AFD. AFD caused an increase in plasma glutamine levels only in young ferrets (*Table 1*).

The plasma alanine level of young ferrets fed ACD was significantly higher than that in similarly treated adult ferrets. An AFD treatment caused further increase in plasma alanine in young ferrets, whereas there was no significant difference in the plasma alanine level between adult ferrets fed ACD and AFD. Young ferrets fed AFD exhibited significantly lower levels of plasma ornithine than those fed ACD in the same age-group. Plasma ornithine levels in adult ferrets fed ACD were similar to those fed AFD. However, the plasma ornithine value in young ferrets fed AFD was comparable to similarly treated adult ferrets.

Young ferrets fed ACD had increased hepatic levels of serine, glycine, and ornithine as compared to those in fasted ferrets (*Tables 1* and *4*). However, in adult ferrets, these amino acids were not altered following ACD treatment. Compared to young ferrets fed ACD, AFD-treated young ferrets had significantly elevated hepatic levels of several amino acids (except for ornithine, citrulline, valine, phenylalanine, and taurine) whereas, in adult ferrets, these amino acids were not significantly different from those in ACD-treated animals.

The hepatic levels of ornithine and glutamic acid in young ferrets fed ACD were significantly higher than similarly treated adult ferrets (*Table 2*). Young ferrets fed AFD had significantly higher levels of almost all amino acids, (except for glycine, taurine, and ornithine) than those in similarly treated adult ferrets. Feeding with AFD resulted in marked elevation of hepatic lysine in young ferrets compared to their adult counterpart (*Table 2*).

N-acetylglutamate in the liver of young ferrets fed AFD was significantly elevated as compared to those fed control diet. Such increase was not seen in adult ferrets fed AFD (*Table 2*).

The levels of ornithine, asparagine, lysine, leucine, isoleucine, and valine in kidneys of young ferrets fasted for 16 hours were significantly higher than that in similarly treated adult ferrets (*Table 5*). The levels of lysine, isoleucine, and ornithine were significantly lower in young ferrets fed AFD than those fed ACD. In adult ferrets, AFD caused a decrease in lysine levels only (*Table 3*).

Table 5 Fasting amino acids levels in kidneys of young and adult ferrets

Amino acid μmol/g	2-month-old ferrets	18-month-old ferrets
Aspartic acid	0.91 ± 0.06	0.88 ± 0.12
Glutamic acid	2.19 ± 0.30	2.83 ± 0.07
Aspargine	0.31 ± 0.04	0.085 ± 0.003[a]
Serine	0.82 ± 0.06	0.70 ± 0.005
Glutamine	0.36 ± 0.02	0.69 ± 0.16
Histidine	0.22 ± 0.01	0.20 ± 0.02
Glycine	1.45 ± 0.13	1.03 ± 0.006
Threonine	0.44 ± 0.04	0.30 ± 0.04
Citrulline	0.034 ± 0.011	0.051 ± 0.003
Arginine	0.007 ± 0.002	0.02 ± 0.003
Alanine	1.50 ± 0.1	1.56 ± 0.11
Taurine	1.24 ± 0.06	3.07 ± 0.35[b]
BAIBA	0.5 ± 0.08	0.033 ± 0.003[b]
Tyrosine	0.22 ± 0.04	0.12 ± 0.02[b]
Valine	0.54 ± 0.05	0.18 ± 0.005[b]
Phenylalanine	0.28 ± 0.02	0.089 ± 0.005[b]
Isoleucine	0.72 ± 0.05	0.035 ± 0.003[b]
Leucine	0.23 ± 0.04	0.097 ± 0.002[b]
Ornithine	0.5 ± 0.06	0.067 ± 0.007[b]
Lysine	0.72 ± 0.03	0.20 ± 0.01[b]

Values are means ± SEM (n = 5).
[a] Statistically significant difference as compared with 2-month-old ferrets.

Discussion

Our observation that young and adult ferrets fed ACD excreted higher amounts of orotic acid than other animals suggests that ferrets have limited or impaired ability to detoxify ammonia into urea via the urea cycle.[10] These studies indicate that similar to young ferrets, adult ferrets also require dietary arginine, but the need is not as acute as in young ferrets. Adult ferrets did not develop hyperammonemia even after eating an arginine-free diet for seven days.[10] The increase in orotic acid excretion in the young and adult ferrets on control diet could be due to low levels of ornithine in the liver (*Table 2*).

Several possibilities have been suggested to explain the metabolic basis of the rapid development of hyperammonemia in cats following a single meal of an AFD.[12] These include decreased or nonfunctional enzymes, enzyme inhibition, impaired activation of enzymes, decreased substrate concentration, or an impaired transport of ornithine across the mitochrondrial membrane. The possibility of decreased or nonfunctional enzymes appears unlikely because the activities of urea cycle enzymes in the liver and kidney of young ferrets fed ACD or AFD were comparable to those

found in adult ferrets or rats.[11] The fact that ferrets recover (and their ammonia returns to normal levels) within 6 to 7 hours after eating AFD indicate that they have a normal functioning urea cycle.

The observation that hyperammonemia and encephalopathy were prevented in young ferrets by supplying dietary arginine[9] indicates that arginine is the key metabolite required for the detoxification of ammonia. We have previously reported[9] that ip injection of ornithine shortened the period of encephalopathy and increased the rate of ammonia detoxification. This indicates that in young ferrets, the rate of ammonia production is greater than the rate of detoxification in the absence of arginine or ornithine. Stewart et al.[12] suggested that low levels of hepatic ornithine are probably responsible for making cats susceptible to hyperammonemia following an AFD. However, this explanation appears unlikely because, similar to cats, both young and adult ferrets exhibited low levels (as compared to that in rat) of hepatic ornithine. However, only young ferrets became hyperammonemic after ingesting an AFD.

Young ferrets fed AFD had very high levels of hepatic lysine as compared to ferrets fed ACD. Such increase was not seen in the adult ferrets fed AFD. Lysine has been shown to antagonize arginine in rat,[18-19] dog,[20] and in several other species. Such high levels of lysine were also found in adult cats fed AFD.[12] Dietary lysine also induces fatty liver which is completely prevented by supplying arginine.[21] We have shown that young ferrets fed AFD exhibited elevated levels of free fatty acids and total lipids as compared to those fed control diet.[22]

Young ferrets fed AFD had a significantly higher level of arginine in their liver as compared to that in ferrets fed ACD. However, the increased level of arginine was not sufficient to overcome hyperammonemia because the levels of several other amino acids were also increased in the ferrets fed AFD. Such a dramatic increase in the levels of several amino acids was not observed in either fasted young and adult ferrets or in adult ferrets fed AFD. The accumulation of several amino acids in liver is not surprising because liver is the primary site of the metabolic fate of several amino acids. Since ferrets recovered within 6 to 7 hours after eating the diet, it appears that they have a normal functioning of the urea cycle. The increase in orotic acid in ferrets fed AFD indicate that they also have the ability to detoxify ammonia by diverting it towards pyrimidine biosynthesis.

There was a four-fold increase in the hepatic levels of N-acetylglutamate in ferrets fed AFD. In these ferrets, the increased levels of hepatic N-acetylglutamate correlated well with increased levels of arginine (*Tables 4* and *5*). L-arginine is an allosteric activator of N-acetylglutamate synthetase.[23] N-acetylglutamate is an essential cofactor for the carbamyl phosphate synthetase I (CPS I), which is the first step for urea synthesis in liver. Earlier studies of Stewart and Walser[24] indicate that the levels of N-acetylglutamate were increased in rats injected with amino acid mixture. The increase was unaffected by the inclusion or deletion of arginine in the amino acid mixture. Therefore, they concluded that a moderate load of amino acid mixtures activates ureagenesis by increasing N-acetylglutamate, and that this autoregulatory mechanism becomes saturated at large doses of amino acid which results in hyperammonemia. Ferrets fed control diet did not have elevated levels of N-acetylglutamate in the liver, overruling this hypothesis (*Table 4*). Arginine deficiency or ammonia appears responsible for the observed increase in hepatic levels of N-acetylglutamate.

The branched chain amino acids and the aromatic amino acids compete for transport across the blood brain barrier.[25] The ratio of branched chain amino acids to aromatic amino acids in plasma has been reported to be decreased in condition of fulminant hepatic failure.[26,27] The ratio of valine + leucine + isoleucine/phenylalanine + tryptophan was significantly lower in young ferrets fed AFD as compared to that in ferrets fed ACD (1.7 vs. 2.9). However, in adult ferrets, there was no significant difference in the ratio between ferrets fed AFD and ACD (2.7 vs. 2.9, *Table 1*).

Although arginine is synthesized in large amount by the liver, the high activity of hepatic arginase normally prevents release of any arginine into the blood.[28] Among extrahepatic tissues, the kidney has the greatest capacity to convert citrulline into arginine, and the level of activity varies among species. The citrulline required for this process is synthesized in the small intestine, released in the blood, and taken up by the kidney. It has been shown that the daily production of arginine by the kidney and the daily requirement of additional dietary arginine to achieve high growth rates in young rats are the same.[29] The synthesis of arginine or an uptake of citrulline by kidney appears to be normal in young ferrets because arginine and citrulline concentration in kidneys of young ferrets was similar to that in adult ferrets.

The observation is that fasting levels of ammonia and amino acids in the plasma and liver of young and adult ferrets were comparable. During fasting, body proteins are catabolized causing release of arginine to supply other intermediates of the urea cycle that are needed for the synthesis of urea from ammonia. Since the tissue proteins of animal origin contain sufficient arginine, it is unlikely that a strict carnivore will have a total deficiency of dietary arginine. Only under conditions such as stress or challenge with free amino acids the arginine requirement may become absolute. The disorder of arginine deficiency also occurs naturally in the animal that resembles the ferret. Minks, another member of the same Mustela genus, fed a purified diet containing adequate arginine developed a severe fatal disease only during the period of active fur growth.[30] Production of fur, an arginine-rich cellular product, appears to have caused the arginine deficiency, because the fatal disorder was completely prevented by arginine supplementation.

Although several papers have been published in re-

cent years describing the effect of AFD in cats, dogs, ferrets, and other carnivores, the comparison of such effects becomes difficult because of the differences in the age of animals used in these studies.[7-14] The comparison becomes more difficult due to the use of general terms such as near adult, young adult, immature, and growing, to describe the age of animals. Additional studies on the effect of AFD in various age-groups of ferrets, cats, and dogs may help to elucidate the susceptibility of carnivores to a lack of dietary arginine. It is also important to note that these amino acid values are from the samples obtained 3 hours after feeding the specified diets. The three-hour time period was selected in the present study because plasma ammonia levels and the severity of sickness were observed at that time. An analysis of plasma and tissue amino acids at various time intervals after feeding may help to further elucidate the mechanism of hyperammonemia induced by AFD in young ferrets.

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