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Nutritional and Metabolic Responses to Arginine Deficiency in Carnivores

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ABSTRACT The metabolic basis for the high dietary arginine requirement of the cat appears to be primarily the low activity of the enzyme pyrroline-5-carboxylate synthase (P5C synthase) in the intestinal mucosa. P5C synthase is required for de novo production of glutamyl-γ-semialdehyde, the immediate precursor for the synthesis of ornithine from glutamate. The next enzyme in ornithine synthesis, ornithine aminotransferase, in the cat intestinal mucosa shows low activity, which provides an additional barrier to ornithine and citrulline formation. It is suggested that the low activities of these enzymes corroborate other evidence that indicates that the cat evolved as a strict carnivore. The dog has a requirement for arginine intermediate between the cat and the rat, which is consistent with the dog having an omnivorous diet during its evolution. It is suggested that during periods of fasting, depletion of urea cycle intermediates in the cat results in some conservation of nitrogen while maintaining urea cycle enzymes at a relatively high level. However, after ingestion of animal protein (and arginine) the urea cycle of cats is capable of rapidly responding to the ammonia load, which rises from the deamination of amino acids. By this method of regulation the cat can respond rapidly to short-term fluctuations in protein intake.
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INDEXING KEY WORDS arginine • carnivore • urea cycle

The systematic classification of animals is based on taxonomic characteristics, which do not necessarily coincide with dietary and metabolic patterns. Although carnivores are usually associated with a diet of animal tissue, the order *Carnivora* contains mammals exhibiting a great diversity in dietary habits. The majority of the *Carnivora* are omnivores (e.g., ursids, procyonids and most canids); however, one small family (Ailuridae, greater and lesser panda) are strict herbivores, and only the *Felidea* are strict carnivores (1, 2).

If the present-day dietary habits of *Carnivora* reflect evolutionary history, their dietary patterns may have induced subtle changes in the metabolism of these mammals. Adaptations could take the form of either induction of pathways for the enhanced catabolism (or sequestration) of compounds in the diet or deletion of pathways required for the synthesis of nutrients universally present in the diet. The latter type of adaptation would save the animal the costs incurred in the synthesis of unnecessary enzymes but would increase the number of essential nutrients.

Many examples have been identified among the *Insecta*, of metabolic modifications of the first type. Insects have developed tolerances to allelochemicals, which appear to have coevolved in plants for protection against herbivory (3–5). There is also evidence that herbivorous mammals have developed strategies to deal with plant secondary compounds (6). The cat, in contrast to the omnivorous rat, exhibits metabolic modifications, predominantly of the second type. These modifications, which correlate

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with the strict carnivorous diet of the cat, include inabilities to convert carotene to vitamin A or linoleic to arachidonic acid and to synthesize adequate taurine under all dietary conditions. The inability of dietary tryptophan to provide adequate niacin for the cat could be interpreted as a combination of both types of adaptations (7, 8). A major metabolic adaptation of the first type is the reduced ability of the cat to conserve nitrogen, which results in a high protein requirement for maintenance.

A peculiarity of a strict carnivorous diet is that it always contains a high proportion of the energy as protein, a variable percentage as fat and a very low percentage as carbohydrate. Strict carnivores are not confronted with low protein (high carbohydrate) diets, which demand conservation of nitrogen. Rather their metabolism is oriented to the utilization of amino acids for gluconeogenesis and energy, and elimination of nitrogen. Consequently in the cat, the transaminases, e.g., alanine and aspartic aminotransferases, and the enzymes of the urea cycle do not adapt to the level of protein in the diet (9). The cat, therefore, contrasts with the dog, which is able to maintain nitrogen balance and to grow on diets much lower in nitrogen than the cat's (10–12; Smalley, K. A., Rogers, Q. R. and Morris, J. G., unpublished data). With respect to arginine, animal tissue proteins are well supplied with this amino acid, whereas plant tissues have a variable and frequently a low concentration of protein.

Arginine, an essential amino acid for carnivores

Until recently, arginine was regarded as a dispensable amino acid for adult carnivores. Rose and Rice (13) reported that "for the adult dog, arginine is not a dietary essential," as they were able to maintain body weight and nitrogen balance of mature dogs fed arginine-free diets.

In a study (14), to define the essential amino acids for the cat, individual amino acids were sequentially deleted from an otherwise complete diet to test their essentiality. With the exception of arginine, no acute untoward consequences occurred other than body weight loss and reduced feed intake. However, within an hour of consuming a single meal of a purified diet containing all the essential nutrients with the exception of arginine, near adult cats exhibited sialorrhea (frothing at the mouth), ataxia, emesis and tetanic spasms with emprosthotonus. In severe cases the clinical signs included cyanosis and death from respiratory failure (15, 16). Arginine deficiency in the cat is the most rapidly induced nutrient deficiency observed in any mammal.

When immature dogs were given a meal of an arginine-free diet they also developed sialorrhea, emesis and muscle tremors similar to those observed in the cat, but the condition did not progress to tetany (17, 18). Older dogs exhibited emesis when allowed to voluntarily consume an arginine-free diet, but force-feeding was necessary to induce sialorrhea and muscle tremors (19).

A consistent finding in both cats and dogs associated with the severe clinical signs following consumption of an arginine-free diet has been marked postprandial rise in circulating concentration of ammonia (table 1). Also in cats, and to a lesser extent in dogs, hyperglycemia accompanied the hyperammonemia (table 1). Hyperammonemia and some of the same clinical signs (seizures and coma) observed in cats have been reported in ferrets given arginine-devoid diets (20). Table 2 summarizes major clinical signs observed in these three species, following ingestion of an arginine-free diet. Ferrets appear more tolerant than cats or dogs to

TABLE 1

Plasma concentrations of ammonia and glucose following ingestion of meals with or without arginine[1]

Animal	Plasma concn			
	Ammonia		Glucose	
	+ Arg	− Arg	+ Arg	− Arg
	μg/ml		*mg/dl*	
Cat	1.4 ± 0.07	14.0 ± 0.9	88 ± 1.5	271 ± 45
Dog, A	2.9 ± 0.3	6.0 ± 0.9	177 ± 17	272 ± 11
B	3.1 ± 0.2	4.3 ± 0.2		
Ferret	2.4 ± 0.4	43.3 ± 3.3	NR	

[1]Values are means ± SEM. References for data: cat (16) eight cats/treatment; dog A (17) three puppies/value; dog B (19) four dogs/treatment; ferret (20) five ferrets/treatment, five experiments.

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TABLE 2

Clinical signs of arginine deficiency in carnivores and the rat[1]

Sign	Cat	Ferret	Dog	Rat
Depression of food intake	+	NR	+	+
Sialorrhea	+	NR	+	–
Emesis	+	+	+	–
Muscle tremors	+	+	+*	–
Coma	+	+	–	–

[1]Symbols: +, present; –, absent; NR, not reported; *when force-fed. References for data as for table 1, rats, ref. 23.

hyperammonemia. They do not show clinical signs at ammonia concentrations of 2000 μg/dl plasma and survive concentrations in excess of those associated with death in cats.

The "normal" serum or plasma concentrations of ammonia in cats, dogs and ferrets appear to be higher than those in humans: [cats 89 ± 3 to 174 ± 18 μg/dl (16); dogs 31 ± 1 to 120 ± 36 μg/dl (17, 19); ferrets 200–400 μg/dl (20); humans 17–80 μg/dl (20)]. Whether high circulating concentrations of ammonia are a consistent characteristic of carnivores needs further study.

Both dogs (17–19) and cats (21) exhibit orotic aciduria when the diet contains inadequate concentrations of arginine (table 3). The increased orotic acid production is presumably a result of ammonia being shunted into pyrimidine biosynthesis rather than to ureagenesis. It has been suggested by Tremblay et al. (22) that carbamoyl phosphate synthesized in the mitochondrion is not totally compartmentally isolated and can be used for pyrimidine biosynthesis in the cytosol. Below the arginine "requirement," orotic aciduria is inversely correlated with the arginine content of the diet, and appears to be the most sensitive index of arginine adequacy.

Orotic acid excretion may be elevated in growing cats and dogs given arginine-inadequate diets in the absence of other clinical signs of deficiency. Enhanced urinary excretion of citrate occurs in dogs following ingestion of an arginine-inadequate diet. While the excretion is much greater in the dog than the cat (table 3), neither approach the urinary output per unit body weight of the immature rat (23) given an arginine-deficient diet, 49 mg/kg body weight.

Urea cycle intermediates as substitutes for arginine

Since arginine acts anaplerotically to stimulate ureagenesis in the Krebs-Henseleit cycle, the other intermediates of the cycle should substitute for arginine to enhance the synthesis of urea and removal of ammonia. In the cat, the addition of ornithine (at about twice the minimal requirements of arginine for growth) to an arginine-free diet

TABLE 3

Urinary output of orotate and citrate in cats and dogs per unit body weight following ingestion of diets containing various levels of arginine

Animal	Arg %	Urinary orotate	Arg %	Urinary citrate
	% in diet	*mg/(kg body wt · d)*	*% in diet*	*mg/(kg body wt · d)*
Cat	1.66	0.2	1.0	0.19
	0.4	7.2	0.6	0.35
Puppy	1.1	0.3	1.1	1.1
	0.56	0.7	0.56	1.1
	0.0	21–69	0.0	10
Mature dog	1.1	0.0	1.1	1.2
	0.56	0.0	0.56	ND
	0.0	1.8–4.6	0.0	2.1

[1]Arg % is the percentage of arginine as free-base in the diet. References for data: cat (21) four cats/treatment, except 1.66% level, n = 10; puppies (17, 18) three puppies/treatment and two (at 0.2) or four (0.0) observations; mature dog (19) three or four dogs/treatment. ND, no data.

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prevented hyperammonemia and associated clinical signs (15, 16). However, even high concentrations of ornithine (about 10 times the minimal requirements of arginine for growth) did not permit maintenance of body weight by kittens (24). Apparently sufficient ornithine, or other urea cycle intermediates, entered the liver to stimulate ureagenesis, but arginine, as an intermediate of the urea cycle, was not released from the liver (due to the high activity of the enzyme arginase) to enter the circulation for tissue growth.

When kittens were given diets containing either arginine (at 1.66% free base) or an equimolar concentration of citrulline, they grew at comparable rates (24). However, this concentration of arginine is approximately twice the minimal requirement to maximize growth. The equimolar substitution of citrulline for arginine at the minimal arginine requirement for growth resulted in inferior growth rates, orotic aciduria, and occasional instances of emesis (Johannsen, C., Morris, J. G. and Rogers, Q. R., unpublished results).

The substitutions of arginine by ornithine and citrulline in the diets of young puppies (7–10 wk of age) and older puppies (17–20 wk of age) has been examined at concentrations approximating the minimal arginine requirements for maximal weight gain (18). In these studies the arginine requirement for maximal body weight gain was 0.4% of the diet, whereas the requirement to minimize urinary orotic acid was 0.53% of the diet. Ornithine added to an arginine-free diet somewhat reduced emetic episodes and promoted either a greater rate of body weight gain (young puppies) or a reduced rate of body weight loss (older puppies). In one experiment with young puppies, the addition of citrulline to an arginine-free diet resulted in weight gains similar to those obtained with an arginine-containing diet. However, in the other experiment with older puppies, the diet containing citrulline promoted lower rates of body weight gain than the arginine diet (18).

The foregoing experiments demonstrate that ornithine, when present in adequate concentrations, protects cats and dogs against the hyperammonemia that follows the consumption of an arginine-free meal. Although ornithine was an ineffective precursor for arginine for maintenance of body weight of the cat, it may be capable of being partially utilized for growth by dogs. In both the cat and the dog, citrulline is not as efficiently utilized as arginine; however, if present in high enough concentrations, it can totally substitute for arginine. The ability of low dietary concentrations of ornithine to reduce emesis in dogs and of high dietary concentrations to totally suppress it in cats suggests that inadequate urea cycle intermediates are present in the liver for the removal of the ammonia load produced following catabolism of arginine-devoid diets containing amino acids. Stewart and associates (25) measured the concentrations of ornithine, citrulline and arginine in the livers of cats given arginine-devoid diets. They confirmed that cats showing hyperammonemia had low concentrations of ornithine in the liver. They also demonstrated that the ornithine concentrations in the livers from cats were lower than those in the livers from rats fed an arginine-devoid meal.

Arginine synthesis in carnivores

Children and adults (26, 27), postpubertal and pregnant swine (28), and rats (29, 30) do not appear to require a dietary source of arginine for maintenance. However, consumption of an arginine-free meal by adult cats can be a life-threatening event, whereas in adult dogs a similar meal may result only in emesis. These nutritional responses indicate that there are fundamental differences between these species in the synthesis or metabolism of arginine.

While the synthesis of arginine is an integral component of the urea cycle, the high concentration of arginase in the liver prevents the release of arginine into the general circulation. Arginine synthesis from citrulline occurs in vivo in the kidney (31), which has a lower concentration of arginase than liver. The source of this citrulline was originally thought to be liver (32), but the recent elegant studies by Windmueller and associates (33–36) have shown that for rats, citrulline is primarily produced in the small intestine. The mucosa of the small intestine metabolizes glutamate to produce end products that include ornithine, citrulline, alanine, proline and ammonia. In the rat intestine, glutamine is a preferred substrate to glucose; some 20–30% of the plasma glutamine is extracted on each passage of blood

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through the intestine. As the concentration of glutamine is generally the highest of all the free amino acids in plasma, production of citrulline by the intestine of the rat is continuous even in the absence of incoming glutamine or glutamic acid. However, the venous-arterial concentration difference of glutamine across the intestine is less for the cat than the dog (36).

The first enzyme involved in the catabolism of glutamine in the intestine is a phosphate-dependent glutaminase. This enzyme occurs in the intestinal mucosa of all animals surveyed (37), but the activity in the jejunum of the cat is less than that of the dog or rat. Whether this difference is a significant factor in regard to citrulline synthesis in the cat remains to be investigated. Glutaminase from the intestine of the rat has an apparent K_m for glutamine only one-tenth of that of the liver enzyme. This difference in K_m makes the intestine a very important site for the metabolism of glutamine at all times, in the absorptive and in the postabsorptive state (36). Intestinal glutaminase unlike kidney glutaminase is not induced by acidosis. While there is no evidence to suggest that glutaminase activity of the intestine is regulated, the production of citrulline would be influenced by concentration of circulating glutamine, which is highest in the postprandial period. Consequently in animals such as the rat, production of citrulline would be enhanced in the postprandial period when the circulating levels of ammonia (and glutamine) are highest. Thus the urea cycle would be provided with intermediates even though the diet was devoid of arginine.

By the mid 1970's, researchers had noted the presence in the rat intestine of many of the enzymes for the synthesis of citrulline and proline. These included carbamoylphosphate synthase (38, 39), P5C reductase (40), ornithine carbamoyltransferase (38–40), and ornithine aminotransferase (OAT) (40–42). Resolution of the initial critical step in the pathway of ornithine synthesis in intestinal tissue (the reduction of glutamate to glutamyl γ-semialdehyde) was first described by Jones and co-workers (43–46). They showed that the enzyme P5C synthase responsible for the reduction of glutamate required ATP, Mg^{2+} and NADPH. No activity of P5C synthase in cat intestinal mucosa was found by Costello et al. (47) using a chemical method. However, Rogers and Phang (48) using a more sensitive coupled isotopic method showed the activity per gram of intestinal mucosa of the cat to be only 18% that of the rat. However, on a per unit body weight basis, the total activity of the cat was only 5% that of the rat (table 4). These findings provide a metabolic basis for the apparent inability of the cat to synthesize adequate ornithine and hence citrulline to prevent hyperammonemia. A further limitation to ornithine and citrulline synthesis in the intestine of the cat is the low activity of the enzyme OAT. The intestine of the cat has only about one-sixth the activity of that of the rat, table 5 (Costello, M. J., Morris, J. G. and Rogers, Q. R., unpublished data). The low activities of OAT and P5C synthase would virtually preclude any de novo synthesis of ornithine in the intestine. Also, the low activity of P5C synthase in the mucosa of the cat would indicate that de novo synthesis of proline by this tissue would be limited. However, proline is a dispensable amino acid for the cat (14) and proline apparently does not spare arginine and protect against hyperammonemia (Buffington, C. A., Rogers, Q. R. and Morris, J. G., unpublished data). Apparently adequate proline is released from the liver and or kidney in the normal catabolism of ornithine to provide the body needs.

The low activities of P5C synthase and OAT substantially explain the metabolic basis for the cat's extreme intolerance of an arginine-free diet. Withholding food for a short period, e.g., overnight, depletes the urea cycle intermediates in the liver and in

TABLE 4

Comparative pyrroline-5-carboxylate (P5C) synthase activities of cat and rat intestines[1]

Source	P5C synthase activity of		
	Intestinal mucosa	Total intestine	Total/body wt
	nmol/(min · g mucosa)	*nmol/min*	*nmol/(min · kg body wt)*
Rat	22.9	154	308
Cat	4.2	63	16
Cat (as % of rat)	18.3%	41%	5.1%

[1]From Rogers and Phang (48).

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the circulation. When the cat is given an arginine-free meal, extensive deamination of amino acids occurs preparatory to their utilization for gluconeogenesis and for oxidation for energy. The urea cycle is unable to dispose of the ammonia at a rate commensurate with production, and hyperammonemia ensues. The dog appears to be more tolerant than the cat of hyperammonemia, and one would predict that the enzyme patterns in the dog are intermediate between the rat's and the cat's.

The dietary requirements for arginine of those carnivores studied to date (table 6) correlate with the above predicted enzyme patterns: the cat requiring the highest concentration, the dog, an intermediate level and the rat, the lowest. The low activities of P5C synthase and OAT in the intestinal mucosa of the cat are consistent with its having an evolutionary history as a strict carnivore. Reliance of the cat on the diet for precursors of urea cycle intermediates, rather than on de novo synthesis, results in their depletion in the postabsorptive period. The depleted urea cycle intermediates reduce the capacity of the liver to dispose of ammonia and decrease catabolism of dispensable amino acids. However, when a meal of animal protein is ingested, arginine becomes available to the liver, which rapidly increases its capacity for ureagenesis and disposal of the ammonia load that may arise from deamination of amino acids. This response is rapid, probably more rapid than could be achieved from synthesis of new regulatory enzymes, which have a time course of up to 30 h (51). Thus what at first may appear to be a defect in metabolism of the cat permits some conservation of nitrogen between meals and also enables the cat to rapidly respond to an ammonia load arising from catabolism of a high protein meal.

Arginine is well supplied in tissue proteins of animal origin, so the strict carnivore would never be confronted with a dietary lack of arginine following a meal. It is only when the cat is given a diet that contains nitrogenous sources that do not include arginine (or urea cycle intermediates) that its metabolic pattern is ineffective. Periods of food deprivation, which require catabolism of body protein, release arginine to supply intermediates of the urea cycle for ureagenesis.

TABLE 5

Comparison of the activity of ornithine aminotransferase (OAT) in tissues from rats and cats[1]

Tissue	OAT activity in Rat	Cat
	μmol/(min · g fresh tissue)	
Liver	1.6 ± 0.36	0.6 ± 0.14
Kidney cortex	1.3 ± 0.24	1.4 ± 0.23
Kidney medulla	2.0 ± 0.32	1.0 ± 0.21
Intestinal mucosa	1.9 ± 0.07	0.3 ± 0.07
Intestinal serosa	1.2 ± 0.20	0.2 ± 0.12
Skeletal muscle	0.3 ± 0.19	ND

[1]Costello, M. J., Morris, J. G. & Rogers, Q. R., unpublished data. ND, not determined. Values are means ± SEM; for rats, n = 3, for cats, n = 4.

TABLE 6

Dietary arginine requirements for maximal body weight gain and minimal orotate excretion of carnivores compared to the rat (% of diet)[1]

Species	Arginine requirement: Growth	Ref.	Minimal orotate excretion	Ref.
	% of diet		*% of diet*	
Dog				
(puppy)	0.4	(18)	0.56	(17)
(adult)			0.28	(19)
Cat	0.8	(21)	1.05	(21)
Mink	>0.8<1.2	(49)	NR	—
Ferret	0.4[2]	(20)	NR	—
Rat	>0.26<0.56	(50)	>0.56<0.84	(50)

[1]Diet as free base assuming 10% moisture in air-dried diets. NR, not reported. [2]To minimize ammonia concentration in blood.

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