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Protein Metabolism: Adaptation

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Abstract

Scientific research has shown that as carnivores, cats have obligatory requirements for nutrients that are not essential for many other mammals. A higher maintenance requirement for protein is one example. This has been attributed to the inability to downregulate nitrogen catabolism secondary to the consumption of an almost-exclusive vertebrate prey diet. Studies support that the feline can adapt protein oxidation to dietary intake provided their protein requirement is met. However, their inability to downregulate nitrogen catabolism may not fully explain their high-protein requirement. One recently proposed model suggests that the cat has a high-protein requirement because of its high-endogenous glucose demand that is met by obligatory amino acid-based gluconeogenesis. It is postulated that the high-protein requirement is the result of amino acids entering gluconeogenesis to supply the glucose needs of the brain and other tissues requiring glucose.

Glossary of Abbreviations

AP: Adequate Protein
HP: High Protein
LP: Low Protein
ME: Metabolizable Energy
MP: Medium Protein

The domestic cat (*Felis catus*) is the only member of the family *Felidea* in which nutritional requirements have been studied extensively.[1] Scientific research has shown that as carnivores, cats have obligatory requirements for nutrients that are not essential for many other mammals. Their nutritional and metabolic idiosyncrasies are believed to be the result of evolutionary adaptations to a diet consisting mainly of animal tissue.[1] As a result, there are some nutrients, such as arginine, taurine, niacin, and vitamins A and D, that are nonessential in many mammals that are essential in felines.[1] The essentiality of these nutrients is often the result of synthesis rates *in vivo* that are insufficient to meet their needs.

Carnivores also have a higher maintenance requirement for dietary protein compared to noncarnivores.[2] The significant difference in protein requirements between carnivores and omnivores is demonstrated by the findings that the rat can maintain body weight, nitrogen balance and carcass nitrogen when fed diets containing 3.5 to 4.5% metabolizable energy (ME) as protein,[3,4] whereas the minimum protein requirement for maintenance in the cat is 16% ME.[2,5]

Dietary protein is required to provide essential amino acids and the nitrogen needed to synthesize dispensable amino acids and other nitrogen-containing compounds. Therefore, is the cat's high-protein requirement due to a high requirement for one or more essential amino acids or dispensable nitrogen?[1] Research supports that the essential amino acid requirements for the growing kitten generally are similar to other growing mammals.[1,6] Cats are able to control the activity of enzymes in the first irreversible step of essential amino acid degradation to some extent, explaining why they do not have a high requirement for essential amino acids.[1,7]

This leaves the possibility that the protein requirement is driven by the need for dispensable nitrogen. Early findings to support a high requirement for dispensable nitrogen came from an *in vitro* study reporting no difference in the activity of some hepatic aminotransferases and urea cycle enzymes in cats consuming high- (54% ME) and low-protein (14% ME) diets.[8] The result is a continuously high rate of nitrogen loss compared to other species that adapt to variations in protein calories by regulating protein oxidation and turnover.[9] Similar metabolic inflexibility has been reported in other carnivores, such as vultures, barn owls, alligators, and trout.[10-14] Conversely, studies in mink have reported that rates of amino acid decarboxylation and protein oxidation are regulated based on protein intake, even when protein is supplied near or below the requirements.[15-17] These findings support some metabolic flexibility in carnivores.[18] Studies conducted in omnivorous and herbivorous species fed high- and low-protein diets under similar experimental conditions have reported significant changes in enzyme activities.[5,19-25]

Based on the Rogers, et al. (1977) study, the high-protein requirement in cats seems to reflect a high obligatory rate of protein oxidation caused by an inability to downregulate the enzymes of urea cycle synthesis in order to conserve nitrogen when consuming a low-protein diet.[8] Reports that cats have higher endogenous nitrogen excretion on a protein-free diet, compared to omnivores,[26] and during times of food deprivation[27] provide further support for this hypothesis. It is important to point out a few limitations of this classic *in vitro* study. The enzyme

assays used in this project were optimized for rats and not cats,[28,29] and enzyme activity was reported as maximal activity, which may or may not reflect physiological conditions. The cats in this study were fed adequate protein concentrations, thus the findings did not test metabolic flexibility at or below requirements.[28,29]

Rogers and Morris (2002) suggest that the basis for the difference between cats and other noncarnivorous animals can be better understood in the context of the mechanisms available to the animal to conserve nitrogen when necessary or to oxidize the surplus when provided.[30] The mechanisms are based on four levels of control of urea cycle enzymes:

1) substrate regulation based on the amount of nitrogen entering the cycle from ammonia or aspartate;
2) allosteric regulation of carbamoylphosphate synthase 1 by N-acetyl glutamate (NAG);
3) control of the urea cycle by increasing and decreasing ornithine; and
4) up- and downregulation of the enzymes involved in urea synthesis.[30]

These researchers argue that while the first three levels of regulation are present in the cat, it is the fourth level, the inability to downregulate the nitrogen catabolic enzymes, that results in obligate nitrogen loss and a high-nitrogen requirement.[30] Therefore, the concentration of urea cycle intermediates may be more important in the control of ureagenesis.[30,31]

Future work based on that study provide additional details but also raise additional questions. An *in vivo* study by Lester, et al. (1999) reported no change in protein oxidation in a group of cats fed diets with varying amounts of energy coming from protein.[32] One potential limitation of the study was that protein oxidation was calculated rather than measured by indirect calorimetry. The end result may have represented nitrogen flux rather than oxidation.[28] Other *in vitro* and *in vivo* studies reported that cats have some ability to adapt to increases in dietary protein intake in terms of ureagenesis and gluconeogenesis.[5,28,31,33] However, none of the studies evaluated diets supplying protein at a concentration less than the cat's minimum requirement.

In one of these *in vivo* studies, using indirect calorimetry, Russell, et al. (2002) investigated substrate oxidation when cats were fed moderate- (35% ME) or high-protein (52% ME) diets.[28] Both treatment groups exceeded the cat's minimum requirement of protein (16% ME) for maintenance.[28] They found that protein oxidation increased when cats were fed the high-protein diet. The authors concluded that cats are more capable of adjusting protein metabolism than previously thought based on the enzyme data.[28] However, only diets exceeding the minimum requirement for protein were evaluated, and adaptation to these protein concentrations could easily be explained by allosteric and substrate/intermediate level regulation of the urea cycle and/or change in liver size.[5]

A second study evaluated protein oxidation in cats fed diets with protein concentrations below, at and above their requirement to test their ability to adapt substrate oxidation to dietary macronutrient concentration.[5] Semi-purified diets containing protein at 7.5% (low protein, LP), 14.2% (adequate protein, AP), 27.1% (medium protein, MP), and 49.6% (high protein, HP) of calories were fed in a modified cross-over design.[5] Using indirect respiration calorimetry and nitrogen balance to measure substrate oxidation, they reported that the ratio of protein oxidation: protein intake was higher when cats consumed the LP diet compared to the other three diets.[5] Provided the diet contained adequate protein to meet the cat's minimum requirement, protein oxidation closely matched protein intake, a finding consistent with that reported by Russell, et al. (2002).[5,28]

To explore the possibility that protein oxidation exceeded intake due to poor energy intake when the cats were consuming the LP diet, the investigators ran a follow-up study. They fed the MP diet (27.1% protein calories) in the same amounts as the cats voluntarily reduced their intake while being fed the LP diet. They determined that protein oxidation for this energy-restricted subgroup was similar to cats fed the MP diet to meet their energy needs. This finding suggests that energy balance may be independent of the cat's inability to adapt protein oxidation to low concentrations of dietary protein.[5] Overall, the findings supported their hypothesis that cats would adapt protein oxidation to dietary intake provided their protein requirement was met; however, if dietary intake was below their protein requirement, cats would be unable to decrease protein oxidation enough to maintain nitrogen balance.[5]

Overall, the limited metabolic flexibility in cats to adapt to low-protein diets may be the result of evolutionary adaptations to a diet consisting primarily of protein.[1] The ability to upregulate the urea cycle aids in protecting against ammonia toxicity after a high-protein meal and permits the utilization of the carbon skeletons from amino acids for gluconeogenesis.[5] This high rate of protein oxidation only becomes a detriment when consuming a diet in which the protein content is below the cat's minimum requirement. In this situation, the cat exceeds its ability to adapt and faces a negative nitrogen balance, whereas most omnivores would continue to thrive.[5]

However, the findings from these studies may have another meaning when considered in the context of Waterloo's explorations of the dual regulation of the urea cycle by substrate supply (reactive regulation) and urea cycle enzyme adaptations (adaptive regulation).[9] Herein, the argument is that adaptive changes to urea cycle enzymes are not necessary given the rapid and automatic regulation of carbamoylphosphate synthase 1.[9] Applied to the cat, it may be that the feline reacts rather than adapts to dietary protein although with the same net result.[34]

The general model of protein turnover in mammals proposed by Waterloo (1999) states that the lower limit of amino acid catabolism is dictated by the rate of whole-body protein turnover

and obligatory nitrogen loss.[9,35] One study evaluating urea kinetics in the cat reported protein turnover to be one-half to one-third that in other mammals.[31,35] The results did not explain the cat's need to catabolize amino acids at the high rates reported in numerous other studies.[31,35] The authors concluded that the high-protein requirement of the cat remains unexplained but is probably not due solely to its inability to downregulate hepatic protein catabolism in response to variations in dietary protein intake.[31,34]

More recently, a model was proposed by Eisert[35] to explain this paradox. In summary, the model says, "… cats do not have a high-protein requirement per se, but rather a secondarily high elevated protein requirement in response to a high endogenous glucose demand."[35] The hypotheses that serve as the foundation for the model include:

1) The cat has a relatively large brain for its size and hence a secondarily high metabolic demand for glucose. The cat has developed specific metabolic strategies that do not include hyperketonemia to meet this demand while consuming a low-carbohydrate diet.
2) Amino acids enter gluconeogenesis at a rate to meet the endogenous glucose demand independent of dietary carbohydrate intake (obligatory gluconeogenesis).
3) Obligatory amino acid-based gluconeogenesis results in endogenous nitrogen losses that exceed the amount predicted for a carnivore the size of the cat and therefore increase the minimum protein requirement in cats above that of other noncarnivorous species.[35]

A review of the natural feline diet based on published databases concluded that a prey-based diet supplies insufficient carbohydrates to meet the high, ongoing endogenous glucose demand of the cat.[35] This endogenous glucose demand stems from the cat's relatively large brain for a mammal of its size.[35,36] Using published data on brain mass and whole blood-glucose utilization, a predictive allometric model was developed to compare the glucose demand of the cat brain with other mammals.[35-37] The result was that with the exception of primates, the relative brain-glucose demand of the cat expressed on a metabolic body weight basis was the greatest in all the mammals evaluated.[35] The brain-glucose demand reported by this model represented 30% of the measured gluconeogenesis in the cat following an overnight fast.[35,38] It is proposed that the discrepancy between the amount of carbohydrate derived from the cat's natural diet and the cat's high-glucose needs is met through endogenous gluconeogenesis.[35]

Eisert (2011) continues to explore whether the glucose requirement of the cat brain is a sufficient explanation for the cat's elevated protein requirement. In order to evaluate this, the theoretical nitrogen costs of brain-glucose demand calculated from the brain-glucose demand of the cat estimated in the predictive allometric model is compared to the endogenous urinary nitrogen losses reported in the cat.[26,35] Endogenous urinary nitrogen losses were used as a proxy for total amino acid oxidation. Using this theoretical approach, there was close agreement in cats between nitrogen loss predicted from brain-glucose demand and published endogenous urinary nitrogen losses.[26,35] The author concludes that this finding supports the hypothesis that the cat's high-endogenous nitrogen losses are the consequence of augmentation of its minimal nitrogen losses by obligatory gluconeogenesis.[35]

Two potential criticisms to the model are raised and addressed by the author.[35] The first is that obligate gluconeogenesis reduces metabolic flexibility and leaves the cat incapable of adapting to a low-protein diet. This point is refuted by offering the hypothesis that the risks of a transient negative nitrogen balance in a feline consuming a high-protein diet are relatively small compared to the compromise in brain function or other organ systems due to low-glucose concentrations. The second criticism is why maintain obligatory gluconeogenesis in domestic cats provided with high-carbohydrate, low-protein diets? Eisert (2011) argues that the modern cat is likely consuming a diet that provides at least 30% of the calories from protein.[35] Therefore, failure to adapt to a higher carbohydrate diet by reducing gluconeogenesis from protein is unlikely to carry a risk of protein deficiency.[35] This is further underscored by evolutionary pressure to maintain the current metabolic status quo.[35]

In conclusion, the cat's inability to downregulate hepatic catabolic capacity at low-protein intakes may only partially explain this carnivore's high-nitrogen requirement. An emerging argument suggests that cats have evolved a high capacity for gluconeogenesis from amino acids to solve the dilemma of how to survive on a high-protein, prey-based diet as a small mammal with a large brain.[35] While arguably the overarching model proposed by Eisert (2011) requires more direct scientific support, the hypotheses and ideas are intriguing and provide a platform for future studies. Certainly this model emphasizes the interrelatedness and interdependence of protein, fat and carbohydrate in the feline diet. Future studies are needed to better understand how dietary composition impacts nutrient utilization and requirements.

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