Utilization of Nitrogen and Macro-Minerals in Response to Nutritional Status in Clinically Normal Adult Cats

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Abstract: Five male cats were used to examine utilization of nitrogen and macro-minerals (calcium, phosphorus and magnesium) in response to food restriction and subsequent repletion. For the first week, each cat was daily given 135 g of dry cat food (baseline period), followed by a restriction period for 1 week; during this period, daily food was individually restricted to 40% of the amount consumed by each cat during the baseline period. Food provision was then returned to the daily 135 g for the final week (recovery period). Fecal weight changed in association with changes in daily food intake, but urine volume changed less with the periods. Fecal and urinary excretion of nitrogen rapidly decreased during the restriction period, but the decreases were smaller than the decrease in nitrogen intake, leading to net nitrogen loss. On the other hand, the food restriction had relatively smaller effects on retention of macro-minerals, and calcium retention was not significantly affected by daily food provision, although the plasma concentration of magnesium was increased during the restriction period and tended to return during the recovery period. Nitrogen retention was increased by the removal of food restriction, but did not exceed the original level of nitrogen retention during the baseline period. These findings suggested that restriction of diet had a serious effect on nitrogen balance, and the impaired protein nutrition might not be easily recovered by subsequent nutritional repletion.

Key words: Cats, Nitrogen, Mineral, Utilization

Introduction

Protein metabolism of cats is known to be different from that of other mammals such as men and rats [10], e.g., the lower enzyme activities of the synthesis of metabolites is related to the urea cycle in the intestinal mucosa [9, 15], the lower efficiency of hepatic synthesis of taurine [4], and insignificant synthesis of niacin from tryptophan catabolism [8]. Furthermore, hepatic enzyme activities related to protein catabolism were more independent of dietary protein intake in adult cats [14]. These findings would be related to the eating habits of cats as strict carnivores [1] and be closely associated with a higher requirement of dietary protein in cats than in other mammals [7, 8].

Obese cats, unlike obese humans and dogs, cannot
endure food deprivation, and minimal food intake was often associated with an occurrence of hepatic lipidosis [2]. The etiology of this liver disease has been suggested to be the accumulation of lipid within hepatocytes resulting from limited synthesis of apolipoprotein B required for VLDL release from the liver, because obese cats showed remarkably more net nitrogen loss during the voluntary fast than obese humans and rats [1].

Taking together the characteristics of protein metabolism in cats with intolerance to food deprivation in obese cats, not only obese cats but also lean cats are expected to suffer significantly impaired protein nutrition caused by food restriction, although there is little quantitative information on protein utilization in response to nutritional restriction in cats. The purpose of this study was to examine changes in nitrogen balance during food restriction and the subsequent repletion in clinically normal lean cats. In addition, utilization and plasma concentrations of calcium, phosphorus and magnesium were also examined, because dietary protein intake has been reported to affect the requirements of minerals in clinically normal cats [4, 7].

**Materials and Methods**

*Animals:* Five male cats which appeared to be non-obese and clinically normal on the basis of physical examination findings were used in the experiment. All the cats were individually housed in a metabolism cage and cared for according to the principles outlined in the NIH Guide for the Care and Use of Laboratory Animals [13]. They were kept in a temperature-controlled room (24 ± 2°C) with artificial light provided from 06:00 to 18:00 daily.

*Diet and protocol:* For at least 2 mo. before the experiment, each cat was given daily 135 g of dry cat food (crude protein 34.6%, calcium 1.38%, phosphorus 1.02%, magnesium 0.15% of dry matter; all these constituents met or exceeded the dietary requirements for growing cats [12, 13]) at 17:00. This amount of food was almost equal to that consumed on an ad libitum basis (data not shown), and this food was highly palatable. The experiment for 3 weeks consisted of three phases of daily food provision. For the first week, the cats were given 135 g/d of the food (baseline period) after the pre-experimental period, followed by the food restriction period for the next week (restriction period).

During this period, daily food intake was individually restricted to 40% of the amount consumed by each cat during the baseline period. For the last week, they were given daily 135 g of the food again (recovery period). Water was available throughout the study. Feces and urine were separately collected every 24 hr at 17:00. To prevent possible loss of ammonia-nitrogen and crystallization of minerals, urine was collected in a glass bottle containing 5 ml of 20% (vol/vol) sulfuric acid. On the final day of each period and on the 4th day of the restricted period and recovery period, blood was collected by jugular vein puncture.

*Analyses:* Food and water intakes were daily calculated from the differences between the provision and the refusal. Collection and preparation of feces and urine and determination of nitrogen and minerals were described earlier [4, 5]. During the baseline period, feces and urine samples were pooled and analyzed, and, during the restriction and recovery periods, daily excretion was determined. Plasma protein was precipitated with 10% (wt/vol) trichloroacetic acid, and calcium, inorganic phosphorus and magnesium concentrations in the supernatant were analyzed as described earlier [4].

Statistical significance was determined by analysis of variance with the general linear model procedures of SAS [16]. As for daily intake of food and water, urinary and fecal excretion and nitrogen and mineral balance, factors considered were the period, day within the period and the animal. When the period effect was significant (P < 0.05), differences between periods were determined by analysis of variance that considered the period and animal as factors. In addition, comparisons of data for the baseline period and each sampling day were also conducted by analysis of variance that considered the period and animal as the factors.

*Results*

Because feces and urine were individually pooled during the baseline period for 7 days, the figures, except for the plasma concentration of minerals, present one baseline data and seven data for each following period. Fig. 1 shows changes in daily food and water intake, urine volume and fecal weight with daily food provision. Fecal weight in association with food intake was rapidly decreased by the food restriction (Fig. 1A and B). Both daily food intake and fecal weight returned to the level
Fig. 1. Changes in daily food and water intakes, urine volume, and fecal excretion in response to daily food provision. (A) shows daily food and water intakes, and (B) shows urine volume and fecal weight. The table shows the comparison of periods. I, II, and III are baseline period, restriction period, and recovery period, respectively. NS: P > 0.10. *: Significantly different from the data during the baseline period (P < 0.05).

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<tr>
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Fig. 2. Changes in nitrogen balance in response to daily food provision. (A) shows nitrogen intake, fecal and urinary excretion of nitrogen, and (B) shows apparent absorption and retention of nitrogen. The table shows the comparison of periods. I, II, and III are the baseline period, restriction period, and recovery period, respectively. NS: P > 0.10. *: Significantly different from the data during the baseline period (P < 0.05).

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during the baseline period after the removal of food restriction, although it tended to be rather higher than that during the baseline period. Daily water intake was also decreased by the food restriction (Fig. 1A). The intake was also rapidly increased by the subsequent removal of food restriction, and for the first few days during the recovery period, it tended to be higher than that during the baseline period. In contrast, changes in urine volume in response to daily food provision were relatively small, although they tended to be slightly decreased for the last few days of the restriction period (Fig. 1B).

Effects of the food restriction and subsequent repletion on nitrogen balance are shown in Fig. 2. The food restriction caused a 60% decrease in nitrogen intake, but only a 39% decrease was seen in fecal nitrogen excretion (Fig. 2A). The decrease in urinary nitrogen excretion caused by the food restriction was also smaller (32%), although it had significantly decreased after 3 days of the restriction period (Fig. 2A). As a consequence of the smaller decreases in nitrogen excretion into feces and urine, nitrogen retention was negative throughout the restriction period (Fig. 2B).

Nitrogen retention rapidly increased with the increase in daily food provision, because of the increase in nitrogen intake over the excretion. Nevertheless, the retention during the recovery period never exceeded
that during the baseline period. Although nitrogen intake for the first few days during the recovery period tended to be higher than that during the baseline period, fecal nitrogen excretion was also higher, resulting in an amount similar to that of nitrogen retention.

Figure 3 shows the balance of calcium, phosphorus and magnesium in response to changes in daily food provision. Urine was minor as an excretion route of calcium (<9% of intake) throughout the study (Fig. 3A), and urinary excretion of phosphorus and magnesium was also minor during the baseline period (<14% for phosphorus and <12% for magnesium) (Fig. 3B, C).

The food restriction and subsequent repletion caused a rapid decrease and increase in fecal calcium excretion, respectively (Fig. 3A). The extent of changes in fecal calcium excretion was comparable with that in calcium intake, except on days 4 and 6 of the recovery period. As a result, daily food provision hardly affected calcium retention (Fig. 3D). In contrast to calcium balance, phosphorus retention was significantly decreased by the food restriction, and the subsequent removal of the restriction tended to restore phosphorus retention to the level during the baseline period (Fig. 3E). Magnesium retention was hardly affected by the food restriction because of a rapid decrease in the fecal excretion, which was similar to the changes in calcium retention during the restriction period (Fig. 3F), but, in contrast to calcium retention, magnesium retention during the recovery period was significantly higher than that during the restriction period.

Changes in plasma concentrations of these minerals in response to the change in daily food intake are shown in Fig. 4. Plasma concentrations of calcium, inorganic phosphorus and magnesium were differently affected by daily food provision. Plasma calcium concentrations hardly changed throughout the study (Fig. 4A), in accordance with the changes in calcium retention. Plasma concentrations of inorganic phosphorus were also constant during the restriction period, but the removal of food restriction increased the plasma concentrations each day for at least 7 days (Fig. 4B). Conversely, plasma magnesium concentrations were higher during the restriction period than those during the baseline period (Fig. 4C). The concentrations during the recovery period tended to return to those during the baseline period, although the concentrations were still higher within 7 days.

**Discussion**

The decrease in daily food provision to 40% of *ad libitum* food intake especially reduced nitrogen retention. The food restriction decreased fecal and urinary excretion of nitrogen, but the decreases were relatively smaller than the decrease in nitrogen intake. As a result, nitrogen retention exhibited a net loss during the food restriction period. The decrease in nitrogen retention was 53.3% of the decrease in nitrogen intake, which was higher than the proportions for calcium (8.8%) and phosphorus (21.4%). This suggests that restriction of diet is more likely to have a detrimental effect on protein nutrition than on mineral balance.

The removal of food restriction rapidly increased nitrogen retention, but during the recovery period nitrogen retention was never greater than that during the baseline period. Taken together with easily decreased nitrogen retention caused by the food restriction, these findings suggest that adult cats cannot fully recover nitrogen loss induced by a transient restriction of food, even when they were subsequently allowed to consume their food. Alternatively, it might take a longer time to recover the nitrogen loss completely. Although previous reports suggested that food restriction caused an occurrence of hepatic lipodisosis in obese cats [1, 2], the current study suggests that a deterioration of the dietary protein-energy status has a potentially worse impact on protein nutrition in non-obese cats also. Furthermore, our suggestion implies that feeding of a highly acceptable diet would be favorable not only from the psychological aspect of the cat’s owner but also from the nutritional aspect of the cat itself, because food intake is often and easily reduced by an unpalatable diet [10].

Compared with nitrogen retention, mineral retention was not so strikingly worsened by the food restriction. In fact the reduction of daily food provision did not

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**Fig. 3.** Changes in balance of calcium (A and D), phosphorus (B and E), and magnesium (C and F) in response to daily food provision. (A), (B) and (C) are intake and fecal and urinary excretion of these minerals, and (D), (E) and (F) show the retention. The table shows the comparison among periods. I, II, and III mean baseline period, restriction period, and recovery period, respectively. NS: P>0.10. *: Significantly different from the data during the baseline period (P<0.05).
Plasma concentrations

(A) Calcium

(B) Inorganic phosphorus

(C) Magnesium

lead to a further decrease in calcium retention, although calcium retention during the baseline period exhibited a slightly negative value. Since about 99% of body calcium exists in bone, this finding suggests that harmful effects on bone homeostasis caused by dietary protein-energy restriction are not so great in adult cats. Alternatively, the intake of minerals might meet the mineral requirements even during the period of dietary protein-energy restriction. Our recent study on infant calves showed that both nitrogen retention and calcium retention were decreased by nutritional restriction, and retention of these two elements is closely related, suggesting a large dependence of nitrogen retention in response to the nutritional restriction on nitrogen balance in bone [5]. The discrepant results for adult cats and growing calves may be due to the difference in physiological stages, i.e., maintenance and growth. The inactive bone turnover in adult animals may result in tolerance of dietary protein-energy restriction. Alternatively, higher activity of protein catabolism in cats as strict carnivores is also likely to cause different changes in nitrogen retention and calcium retention.

Interestingly, plasma concentrations of calcium, inorganic phosphorus and magnesium were differently changed in response to daily food provision. The finding that the plasma calcium concentration was constant throughout the study would be due to the well-known strict regulation of extracellular calcium concentration [3]. On the other hand, the plasma concentration of inorganic phosphorus was not affected by the food restriction, but increased during the recovery period, although the reason is not known. The plasma magnesium concentration was increased by the food restriction, but tended to return to the original concentration when daily food provision increased to the amount during the baseline period. This increase in the plasma magnesium concentration in response to dietary protein-energy restriction may be a specific feature of adult cats. Our preliminary study showed that the plasma magnesium concentration was constant during fasting for 7 days in adult sheep (Funaba et al., unpublished data). In addition, the mineral metabolism of cats was slightly different from that of humans and rats, when high-protein diet was fed [4]. In view of the higher concentration of magnesium within the cell than extracellular fluid [3], the higher plasma concentration may reflect the release of magnesium from the cell, although the physi-
ological significance of the changes in plasma concentrations in cats should be clarified in future studies.

In summary, the current study suggested that there is impaired nitrogen balance in response to dietary protein-energy restriction, without an effect on bone nitrogen turnover. In addition, because nitrogen retention might be difficult to completely recover by the subsequent removal of food restriction, we claim that dietary protein-energy restricted, which will be easily elicited by feeding an unpalatable diet [1], should be avoided in adult cats.

Acknowledgments

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References