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Quantitative excretion of 3-methylhistidine in urine of cats as a measure of in vivo skeletal muscle protein catabolism

Stanley L. Marks, Quinton R. Rogers, and James G. Morris

Department of Molecular Biosciences, School of Veterinary Medicine, University of California, Davis, CA USA

The purpose of this study was to evaluate the use of urinary 3-methylhistidine excretion as an index of in vivo skeletal muscle protein degradation in cats. The criterion for validation was the rapid and quantitative excretion of an intravenously administered dose of radiolabeled 3-methylhistidine (3-methyl-^{14}C). Four adult cats were maintained in individual metabolism cages and allowed free access to a purified diet (43.5% protein) and water for 4 weeks. The cats were then injected intravenously with 740 kBq 3-[^{14}C]methylhistidine dihydrochloride diluted in 1 mL of saline. Twenty-four-hour urine samples were collected for 7 days. Total radioactivity in each urine sample was determined by direct counting. Quench correction was determined by using an external standard. The mean (±SEM) cumulative urinary recovery of 3-[^{14}C]methylhistidine from the four cats was 94.9 ± 3.5% at 48 hr following radioisotope injection. The mean (±SEM) cumulative urinary recovery of radioactivity from the four cats was 103.9 ± 2.2% at 7 days following radioisotope administration. There was no detectable radioactivity found in expired CO_2 and negligible amounts (mean ± SEM: 0.6 ± 0.5%) in the feces. Chromatography of urinary amino acids and radioactive urine metabolites revealed no significant radioactivity in any other peak besides 3-methylhistidine. Acid hydrolysis of urine resulted in no increase in 3-methylhistidine content or urine, indicating that there is no significant acetylation of 3-methylhistidine in this species. On the basis of these results, 3-methylhistidine does not appear to be metabolized and should therefore be a valid index of in vivo skeletal muscle protein degradation in the cat. Urinary 3-methylhistidine excretion should be useful for studying how nutritional, hormonal, and other physiological or pathological factors cause losses or gains in skeletal muscle protein in this species. (J. Nutr. Biochem. 7:60–63, 1996.)

Keywords: 3-methylhistidine; muscle degradation; cats; feline

Introduction

Skeletal muscle constitutes the largest single tissue in the body of mature mammals.[1] Because muscle constitutes such a large proportion of the total body protein, changes in muscle protein metabolism often represent a major component of the total change in body protein metabolism. Despite this phenomenon, there has been a paucity of information on the contribution of protein turnover in skeletal muscle to overall body protein metabolism under a variety of pathophysiological conditions.[2,3] Studies of tissue protein breakdown in vivo are complicated by the extensive reutilization of amino acids.[4,5] The amino acid 3-methylhistidine (3-MH) has unique attributes associated with skeletal muscle metabolism. 3-MH is almost exclusively formed by the post-translational donation of a methionine methyl group to actin-bound histidine[6,7] and cannot be reutilized for protein synthesis after protein degradation. Because the major portion of total body 3-MH content is present in skeletal muscle[8] and is quantitatively excreted unchanged in the urine of rats and humans, the urinary excretion of 3-MH has been accepted as an index of in vivo skeletal muscle protein breakdown in these species.[7,9] 3-MH is also rapidly and

Address reprint requests to Dr. Quinton R. Rogers at the Department of Molecular Biosciences, School of Veterinary Medicine, University of California, Davis, CA 95616, USA.
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655 Avenue of the Americas, New York, NY 10010

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quantitatively excreted in the urine of cattle[10] and rabbits[11] but does not appear to be a valid index of skeletal muscle protein breakdown in sheep,[12] goats,[13] pigs,[14] or mice.[15]

There are several unique aspects of amino acid nutrition and metabolism in the feline that merit studies of skeletal muscle protein degradation. The protein requirement of cats is higher than that of other domestic mammalian herbivores and omnivores,[16,17] mainly because cats lack the ability to conserve nitrogen. In addition, cats are prone to a variety of nutritional, hormonal, and stress-related conditions that may affect protein turnover and overall body protein metabolism. Although 3-MH was reported to be present in feline urine by Tallan et al.,[18,19] there are no published studies to date reporting the quantitative fate of 3-MH in this species. The purpose of this study was to evaluate the use of urinary 3-MH excretion as an index of in vivo skeletal muscle protein degradation in cats. Validation of this measurement was dependent on the rapid and quantitative excretion of an intravenously administered dose of radiolabeled 3-MH.

Methods and materials

Animals

Four adult specific pathogen-free cats (2 males and 2 females), originating from the Nutrition and Pet Care Center, University of California at Davis, were used as experimental animals. The body weight of the cats at the time the pulse dose of 3-[^{14}C]methylhistidine dihydrochloride was given ranged from 2,867 to 4,070 g (mean ± SEM: 3,383 ± 251 g). The cats were maintained according to the Guide for the Care and Use of Laboratory Animals (NRC 1985) and the Animal Welfare Act. The protocol was approved by the Committee for Care and Use of Laboratory Animals, University of California, Davis.

Diet

The cats were adapted to the purified diet as shown in *Table 1* for 4 weeks prior to commencement of the study. The cats had free access to the same purified diet and to water throughout the study. The dietary concentration of protein, vitamins, and minerals met or exceeded the National Research Council (1986) requirements.

Design

Four cats were individually maintained in metabolism cages (0.66 × 0.66 × 0.76 m) that were contained in open circuit metabolic chambers that permitted the collection of total feces, urine, and expired CO_2. The metabolic chamber (0.79 × 0.79 × 0.94 m) provided a constant temperature of 21°C, light (12 hr light: 12 hr dark), and an air flow of ~2 L/min. The intake air passed through a 2 L container of soda lime to remove CO_2. The air outlet was connected in series to two 1 L flasks fitted with aerators containing 500 mL of ethanolamine-ethylene glycol monomethyl ether (1:2) (Fisher Scientific, Pittsburgh, PA USA) for the collection of CO_2. The serial flask design allowed the collection of total CO_2 from the metabolic chamber. Seven hundred and forty kBq of radiolabeled 3-MH dihydrochloride (3-methyl-^{14}C) (97% pure), (Amersham, Arlington Heights, IL USA) diluted in 1 mL saline was administered intravenously to the cats. Total CO_2 was collected every 12 h for 7 days, total urine was collected for 7 days, and feces were collected for 21 days.

Table 1 Composition of the purified diet

Ingredients (manufacturer)	Amount (g/kg)
Casein (New Zealand Milk Products, Santa Rosa, CA USA)	217.5
Soybean protein (Archer Daniels Midland Co., Decatur, IL USA)	217.5
Starch (Melojel, food-grade cornstarch, National Starch and Chemical Corp., Bridgewater, NJ USA)	190.5
Granulated sugar (Westco Products, Sacramento, CA USA)	200.0
Animal tallow (Florin Tallow, Dixon, CA USA)	100.0
Mineral mix[22]	50.0
Vitamin mix[22]	10.0
Choline chloride (Dupont, Highland, IL USA)	3.0
Taurine (Taisho Pharmaceutical Co., Torrance, CA USA)	1.5
Carageenan (FMC Corp., Philadelphia, PA USA)	10.0

Methods and materials

Total CO_2 was trapped in a known volume of ethanolamine-ethylene glycol monomethyl ether (1:2) which was changed every 12 hr. The method of Jeffay and Alvarez[20] was followed. Carbon-14 enrichment in expired CO_2 was determined by counting (Packard 2000CA Liquid Scintillation Analyzer, Downers Grove, IL USA) 1 mL of the ethanolamine-ethylene glycol monomethyl ether in 14 mL of Scintisol (Isolab, Akron, OH USA). Urine was collected in a container maintained in an ice bath. The container was changed and the volume of urine was measured at the end of every 24 hr period. One milliliter of urine was mixed with 9 mL of Scintisol for the determination of radioactivity. An aliquot of urine from each day was chromatographed on a Beckman 7300 automatic amino acid analyzer-flow liquid-scintillation system (Fullerton, CA USA) for the determination of amino acids and identification of the radioactive urine metabolites. The amount of N-acetyl-3-MH was determined by measurement of the 3-MH concentration before and after hydrolysis with 6 mol/L of HCl heated for 24 hr at 100°C. Urine creatinine was determined by utilizing a modification of the Jaffe method with picric acid and sodium hydroxide.[21] Feces from each cat were collected for 21 days of the study, weighed, placed in plastic containers along with 50 mL of water, and frozen. Feces were thawed overnight and then homogenized (Polytron homogenizer, Brinkman Instruments, Westbury, NY USA). Homogenate (100 to 150 mg) was placed in a Teflon-capped glass counting vial and decolorized by successive additions of 0.2 mL of perchloric acid (11.7 mol/L) and 0.4 mL of hydrogen peroxide (8.8 mol/L) followed by incubation at 70°C for 3 hr. The resulting digest was then dissolved in 10 mL of Scintisol for the determination of radioactivity. Values in the text are means ± SEM.

Results

Analysis of the urine samples for total radioactivity from the four cats indicated quantitative recovery within 48 hr of the 3-[^{14}C]methylhistidine injection. The cumulative recoveries of urinary 3-[^{14}C]methylhistidine are expressed as a percent

of the total injected dose and plotted against time for the two male and two female cats (*Figure 1*). Cumulative urinary recovery of 3-[^{14}C]methylhistidine from the four cats was 94.9 ± 3.5% at 48 hr following radioisotope injection. Cumulative urinary recovery of radioactivity from the four cats was 103.9 ± 2.2% at 7 days following radioisotope administration. There was no detectable radioactivity found in expired CO_2 and negligible amounts (0.6 ± 0.5%) in the feces. Urinary amino acids and radioactive urine metabolites were determined chromatographically on an automatic amino acid analyzer-flow liquid-scintillation system. No substantial radioactivity was found in any peak other than that of 3-MH. Acid hydrolysis of urine resulted in no increase in 3-MH content or urine, indicating that there is no acetylation of 3-MH in this species.

Discussion

The rapid and quantitative recovery of radioactivity in feline urine suggest that the urinary excretion of 3-MH is a suitable index of skeletal muscle protein breakdown in cats. The urinary recoveries of radioactivity ranged from 88 to 104% of the administered dose at 48 hr following administration. Three of the four cats had excreted 100% of the administered dose by day 4, and one cat (male) had excreted 97.8% of the administered dose at this time. These values are consistent with the rapid rate of excretion found in rats[7] and humans.[9] The results in *Figure 1* demonstrate that recoveries among cats varied appreciably during the first 24 to 48 hr following radioisotope administration. The most likely reason for the delayed excretion of 3-MH in one female cat was incomplete voiding of urine at 24 hr following radioisotope administration. The average frequency of urination for cats has been reported to range from 2.4 to 3.0 per day.[23] It is thus plausible that this cat voided partially immediately prior to injection of the radioisotope and voided much of the administered radioisotope present in the urine on day 1 during the second day following administration.

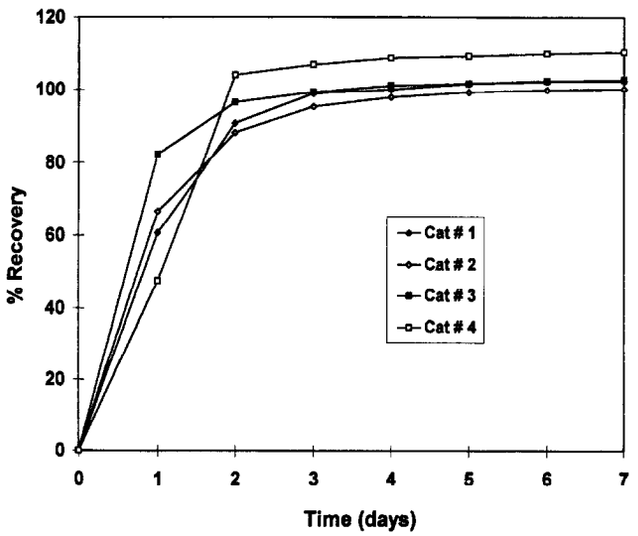

Figure 1 Cumulative recoveries of 3-[^{14}C]methylhistidine (percent of dose) in urine from two female cats (■; □) and two male cats (◆; ◇).

3-MH did not undergo metabolic degradation in the cats. This conclusion is based on the cumulative recovery of 3-MH in the urine, the lack of detectable radioactivity found in expired CO_2, and the negligible quantity found in the feces. The radiolabel detected above background in feces from cat #3 (2.0% of the administered dose) was attributed to urine contamination. The lack of acetylation of 3-MH in the cat contrasts with the large proportion acetylated by mature rats[7] and eliminates the need to hydrolyze the urine of feline subjects prior to determination of total 3-MH excretion. Determination of urinary amino acids and radioactive urine metabolites on an automatic amino acid analyzer-flow liquid-scintillation system showed the presence of only one radioactive peak. The time taken to elute this peak corresponded with that of a 3-MH standard. The inability to oxidize 3-MH and its rapid and quantitative excretion as the original compound suggests that 3-MH excretion provides a valid index of in vivo skeletal muscle protein degradation in the cat. Although the gastrointestinal tract and skin contribute to 3-MH excretion in man and rats, the contribution from these extraintestinal tissues appears to be small.[24,25] Preliminary studies of urinary 3-MH output and creatinine concentrations have been performed on two adult cats maintained on purified diets (43.5% protein) (*Table 1*). The mean urinary creatinine and 3-MH output was 78.4 ± 3.8 and 5.8 ± 0.4 mg/24 hr, respectively, with a 3-MH:creatinine ratio of 0.07. Urinary 3-MH excretion should be useful for studying how nutritional, hormonal, and other physiological or pathological factors affect skeletal muscle protein degradation in this species.

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