Note

Incomplete Urinary Excretion of 3-Methylhistidine in Young Female English Saanen Goats

Naoyuki NISHIZAWA, Frank M. TOMAS, Colin S. CHANDLER, Hiroyuki WATANABE, and Shin-ichi HAREYAMA

Department of Bioscience and Technology, Faculty of Agriculture, Iwate University, Morioka, Iwate 020, Japan
*CSIRO, Division of Human Nutrition, Kintore Avenue, Adelaide, South Australia 5000, Australia

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Skeletal muscle is the largest tissue mass in animals. Thus muscle protein turnover is an important factor in the over-all regulation of whole-body protein metabolism. In addition to its primary role in skeletal muscle, muscle protein also acts as a protein reserve which may contribute significantly to pregnancy and lactation. Production of milk protein would depend on body protein reserves whenever secretion of milk protein exceeds dietary protein intake, such as at the stage of early lactation after parturition. However, the role of muscle in these circumstances is poorly characterized.

N'-Methylhistidine (3-methylhistidine, 3MH) in muscle proteins, actin and myosin, is formed by the post-translational methylation of specific histidine residues. After degradation of these proteins it is released and rapidly and quantitatively excreted in urine and not reused for protein synthesis. Therefore, urinary excretion of 3MH has been widely used for measurement of overall muscle protein breakdown. Direct estimation of muscle protein breakdown in several farm animal species has also been done by measuring the rate of urinary excretion of 3MH. Goats are an experimental model for the dairy cow as well as other cattle. We reported data which suggest that urinary 3MH may be a useful index of muscle protein degradation in male Japanese Saanen goats. However, no one has examined whether urinary 3MH excretion in female Saanen goats is a reliable index of muscle protein degradation. This paper describes its attempted validation in this animal.

Three female English Saanen goats, aged 3 months and weighing 8.0—13.5 kg, were fed with a cereal-based diet consisting of Capra goat meal, Milling Industries Ltd, Dry Creek, SA, Australia) for 2 weeks. T-[14CH3]J3MH (532.8 MBq/mmol; Amersham International Pbc Buckinghamshire, England) was passed through a GLC50 W x 8 resin column to remove non-specific radioactivity. Animals were each injected with 740 kBq labeled 3MH into a jugular vein. Urine was then collected under 2 M HCl and thymol using metabolic cages fitted with urine-feces separators. Collection periods were over days 1, 2, 3—4, and 5—6. These urine samples were stored at -20°C until analyzed. The animals were given this diet ad libitum during the experimental period. To measure the total radioactivity in urine, 0.5 ml of the sample was mixed with 5 ml Pico-Floro 40 (Packard Instruments Inc., Downers Grove, IL, U.S.A.) and the radioactivity was measured with a liquid scintillation counter (Tri-Carb-1500, Packard Instruments Inc., Downers Grove, IL, U.S.A.). To examine metabolites of 3MH in urine the samples were desalted as described in a previous paper and then put on to a column (40 x 250 mm) of Aminex A-9 fritted to a LKB 4103 amino acid analyzer. The resin column was eluted with 0.1 M sodium citrate buffer (pH 4.65, 0.25 M/min) at 33°C and the eluate collected in a fraction collector. Each fraction was mixed with Pico-Floro 40 and the radioactivity measured. To further examine the chemical characteristics of the metabolites, a portion of the desalted sample was hydrolyzed in 2 M HCl at 104°C for 1 hr and put on the Aminex column after drying and reconsititution. N-acetyl-3MH was prepared by reacting 3MH in glacial acetic acid with acetic anhydride at room temperature for 18 hr. N-acetyl-3MH was detected in eluate fractions by the method reported previously after hydrolyzing the samples in 2 M HCl at 104°C for 2 hr.

Cumulative recovery of radioactivity in urine from two animals after an intravenous dose of radioactive 3MH was incomplete (Fig. 1). The data presented are from only two animals because one animal spilled the urine. The mean cumulative recovery of radioactivity was 18.5, 30.5, and 45.0% after days 1, 2, and 4, respectively. Total recovery for the experimental period of 6 days was only 55.4%. This result differs from those obtained from male Japanese Saanen goats and cattle, which showed nearly 100% recovery of a dose of radioactive 3MH, but agrees with those of Brown et al. who reported incomplete recovery of radioactivity from goats. The total recovery of radioactivity from urine in the male Japanese Saanen goats was 35.6, 55.4, and 94.5% at 1, 2, and 6 days after administration of labelled 3MH and reached nearly 100% after 7 days. Although a complete isotope balance and muscle analysis were not undertaken in this study, the incomplete recovery of radioactivity in urine suggests that 3MH may be incorporated into muscle dipeptide balene (3-15alanine-3MH) as occurs in sheep and pigs, or that the isotope is eliminated into exhaled CO2 as a result of catabolism of 3MH.

Chromatographic separation of metabolites in untreated urine from day 1 showed 5 peaks of radioactivity. An average of 76% of the radioactivity put on was eluted in the same position as authentic 3MH (fraction No. 52, Fig. 2). The metabolite at the position of fraction No. 8 was tentatively identified as N-acetyl-3MH because it co-eluted with authentic N-acetyl-3MH and radioactivity was transferred to the 3MH peak after acid hydrolysis. The peaks at fraction Nos. 12 and 15 were stable to mild acid hydrolysis and we also have evidence (not shown) that these metabolites are stable to 6 M HCl at 105°C for 24 hr. The metabolite at fraction No. 15 appears likely to be 1-methylimidazole-4-acetic acid as reported by Murray et al. but our attempts to characterize it were unsuccessful. A chromogen for imidazole acetic acid did not develop but the amounts in the eluate were probably below the detection limits.

The radioactivity eluted with the three metabolites contained in fractions 8—15 was only a relative small proportion (8 to 10%) of the total counts put on. The peak at fraction No. 38 contained 15.5% of the radioactivity put on to the column and appears to be an impurity injected with [14C]3MH. The most likely identity is 3-methylhistidine since authentic 1-methylhistidine elutes in the same position. Chromatography of the injectate showed this peak to account for only 2.8% of injected counts and the amount recovered on day 1 is consistent with total excretion of the injected dose. No counts were eluted in this position from urine collected on subsequent days (not shown). The presence of this impurity does not, however, affect the conclusion drawn from this study.

In conclusion, our observations show that only a small proportion of 3MH is excreted as metabolites in young, female English goats. However,
the poor recovery of injected radioactivity appears to indicate that the urinary excretion of 3MH from these goats does not provide a reliable measure of muscle protein degradation. This result implies also that each animal must be examined rigorously for the recovery of 3MH from urine and its metabolism. The question arises as to why male, Japanese Saanen goats which are a strain related to the animals used in this study do not metabolize or retain 3MH in the same way. This may be due to a sex or age effect as indicated by Brown et al.\textsuperscript{13} and also reported in mice\textsuperscript{18} and cockerels.\textsuperscript{19} These questions require further detailed study for their resolution.

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