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Technical Note: The Use of a Compartmental Model to Estimate the De Novo Production Rate of N^{τ}-Methylhistidine in Cattle[1,2]

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ABSTRACT: Urinary N^{τ}-methylhistidine (NMH) excretion has been used as an index of muscle protein breakdown in cattle. An alternative means to estimate muscle proteolysis in cattle is to estimate the de novo production of NMH from plasma kinetics isotopically. Three crossbred steers (average 229 kg) were given a 5.0-mg bolus intravenous injection of [methyl-$^2H_3$-N^{τ}-methylhistidine (d_3-NMH), after which 16 serial blood samples and three consecutive 24-h urine samples were taken. The enrichment of NMH in plasma was determined by gas chromatography-mass spectrometry, and compartmental analysis of the kinetic data was performed using the SAAM modeling program. The NMH production rates per day (NMHPR, micromoles per day) were 732, 782, and 725, and the fractional breakdown rates (FBR, percentage per day) were 1.61, 1.72, and 1.58 as determined by urinary excretion of NMH, by a three-pool catenary model (plasma kinetics, Model A), and by a more descriptive, three-pool model with two response curves (both plasma and urine kinetics, Model B), respectively. Model A and B estimates of NMHPR and FBR were similar ($P > .25$) to those of estimates obtained from urinary NMH excretion. Kinetic modeling also allows calculation of compartment mass and flux of NMH between compartments and indicates that when NMH exists the muscle pool it is rapidly excreted via the urine. In conclusion, kinetic modeling offers an alternative approach to estimating the NMH production rate.

Key Words: Methylhistidine, Isotopes, Kinetics, Cattle, Compartmental Models

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Introduction

The turnover of myofibrillar proteins is often calculated from urinary excretion of N^{τ}-methylhistidine (**NMH**) and has been validated for use in cattle studies (Harris and Milne, 1981; McCarthy et al., 1983). Upon degradation of actin and fast-twitch myosin, NMH is released and, not reutilized or modified to any significant extent, it is rapidly excreted in the urine (Young et al., 1972).

To date, no attempt at integrating tracer and tracee data into a comprehensive whole-animal kinetic model of NMH metabolism in cattle or any other species has been reported. The development of the SAAM/CONSAM computer modeling program (Berman and Weiss, 1978) has made possible the detailed analysis of tracer observations at multiple sites in the body and has facilitated the interpretation of findings in the context of a physiologically identifiable kinetic model.

An alternative method of quantifying NMH production in vivo is to measure isotopically the de novo production, rather than excretion. The first objective of this research was to develop compartmental models based on the kinetics of a stable isotope of NMH ([methyl-$^2H_3$-N^{τ}-methylhistidine, **d_3-NMH**) in plasma and urine. Second, the model-calculated values for the NMH production rate and fractional turnover rate of myofibrillar protein for steers were compared with the values calculated from the measurement of urinary NMH.

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Materials and Methods

Animals and Experimental Protocol. Three crossbred steers (average BW 229 kg) were obtained from Department of Animal Science ruminant nutrition farm at Iowa State University. The steers had ad libitum access to a finishing diet and water throughout the duration of the study. Seven days before the start of the experiment, the steers were transferred to metabolism cages designed for the collection of urine. On the morning of study, one catheter was inserted into the jugular vein to facilitate both tracer injection and withdrawal of blood. Approximately 1 h later, d_3-NMH (MSD Isotopes, Montreal, Canada) with a bolus dose of approximately 30 μmol dissolved in 10 mL of sterile saline was injected into the catheter and was flushed with an additional 10 mL of saline. Blood (10 mL) was collected and transferred to EDTA-coated tubes 1, 2, 5, 10, 15, 30, 45, 90, 150, 210, 270, 330, 720, 1,440, 2,880, and 4,320 min postinjection. Blood was directly placed in an ice bath until it could be centrifuged, after which plasma was collected and stored at –70° C. During the first 3 d of the tracer experiment, consecutive 24-h total urine outputs were collected. Aliquots of each urine collection were kept frozen at –20° C. Additional baseline blood and urine samples were collected before the injection to subtract background enrichment from the experimental samples.

Analysis of Plasma N^{τ}-Methylhistidine. One milliliter of plasma and 50 μL of 1-methylhistidine (**1MH**) internal standard (3.5 m*M*) were added to a plastic sample tube (12 mL). Then, 3 mL of 1.5 *N* perchloric acid was added, and the tube was vortexed and centrifuged at 2,300 × *g* for 15 min at 5° C. The supernatant was poured onto a prepared cation exchange column (Dowex-50W in the hydrogen form, Sigma Chemical, St. Louis, MO). The NMH and internal standard (1MH) were eluted from the column with 25% NH_4OH into a 20-mL scintillation vial. The eluant was heated to 65° C in a block and dried under a stream of nitrogen gas. To form derivatives, 100 μL of acetonitrile (Regis Chemical, Morton Grove, IL) and 100 μL of MTBSTFA (N-methyl-N-(t-butyldimethylsilyl) trifluroeacetamide, Regis Chemical) were added to the dried sample and allowed to incubate overnight at room temperature.

The sample containing derivatives was transferred into an injection vial (Sun Brokers, Wilmington, NC), capped, and analyzed by gas chromatography-mass spectroscopy (Hewlett-Packard gas chromatogram/mass selective detector, Model 5890/5970B, Avondale, PA). The column used was a 25 m × .22 mm i.d. × .11 μm film thickness, cross-linked methyl silicone gum phase capillary column (HP-1, Hewlett-Packard). The major ion fragments for NMH and d_3-NMH were monitored by using selective ion monitoring. The NMH was monitored at 238 atomic mass units (amu) and its stable isotope, d_3-NMH, at 241 amu, whereas 1MH was detected at 340 amu. The NMH in plasma was quantified from a linear peak height standard curve. When d_3-NMH was used as tracer, the enrichment, d_3-NMH/NMH, was quantified in plasma by first subtracting the natural background enrichment, d_3-NMH/NMH, from detected d_3-NMH.

Analysis of Urine N^{τ}-Methylhistidine. Unlike plasma, urine contains substantial amounts of 1-methylhistidine, which makes 1MH an impractical internal standard. Therefore, dilabeled [1,1]-$^{18}O_2$-1-methylhistidine (**$^{18}O_2$-1MH**; 40 μL of a 2,955 μ*M* stock solution) was used as the internal standard to quantify NMH in urine. A urine sample was prepared by transferring 1 mL to a microfuge tube and removing particulate matter by spinning for 3 min. From this tube, 100 μL of urine was pipetted into a plastic sample tube (12 mL) with 40 μL of internal standard. In addition, 1 mL of double-deionized H_2O was added to the tube, and the contents were acidified with 5 μL of 3 *N* HCl. The urine sample was then vortexed and poured over a cation-exchange column and subsequently eluted with 25% ammonium hydroxide. The eluent was collected in a scintillation vial and then dried with a stream of nitrogen gas on a heating block at 65° C. After it was dry, the urea in the sample was hydrolyzed with 1 mL of urease solution (3.5 mg/100 mL; jack bean urease Type IX, Sigma) by incubation on a heating block for 2 h at 37° C. The sample was again dried on the heating block and derivatives were formed by adding 100 μL of acetonitrile and 100 μL of MTBSTFA; the sample was incubated overnight at room temperature. The sample containing derivatives was transferred into an injection vial and injected in a Hewlett-Packard gas chromatograph/mass selective detector. The retention time for NMH was the same in urine as in plasma and exhibited the same mass spectra. The $^{18}O_2$-1MH had a retention time similar to that of natural 1MH, but major ions from the mass spectra were 4 amu heavier. The NMH and d_3-NMH were quantified by peak height as explained for plasma analysis, except that $^{18}O_2$-1MH replaced 1MH in the calculations.

Kinetic Modeling. The response curves of d_3-NMH in plasma and urine were evaluated using the Simulation, Analysis, and Modeling/Conversational SAAM (SAAM/CONSAM) computer program (Boston et al., 1981). The tracer data were interpreted with linear compartmental models, which were described by a linear first-order differential equation for each compartment in the

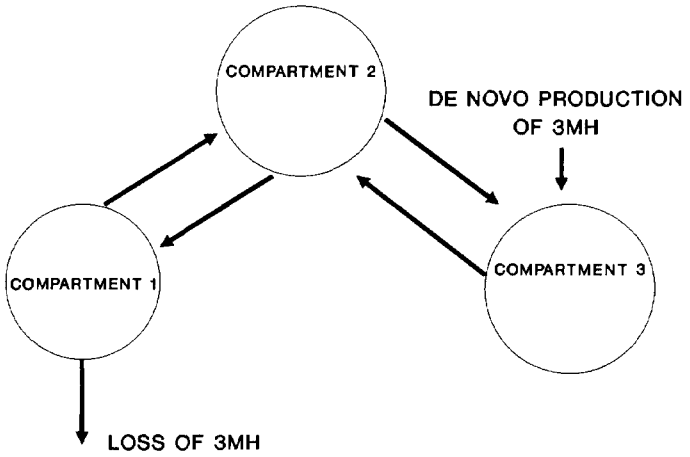

Figure 1. A graph illustration of a three-compartment kinetic model used for Models A and B. Model A was configured with plasma kinetics entered into Compartment 1 and the model had an exit out of the system from Compartment 1. Model B, a more descriptive, three-compartment model, was configured with two response curves, the plasma kinetic entered into Compartment 1 and urine kinetics as an exit via Compartment 1 into a urine sink. 3MH = N^{τ}-methylhistidine.

model (see Berman and Weiss, 1978). The first compartmental model constructed was a catenary three-compartment model (Model A; Figure 1), configured by entering the molar enrichment of plasma d_3-NMH:natural NMH into Compartment 1 over time. These data were used in conjunction with prior knowledge of NMH metabolism in cattle (Harris and Milne, 1981) in constructing the model. Harris and Milne (1981) have demonstrated in cattle the presence of body pools of free NMH in blood and muscle. There is also a dipeptide pool of NMH (balenine; Harris and Milne, 1987) in muscle.

Based on the recovery of radiolabeled NMH (Harris and Milne, 1981; McCarthy et al., 1983), after NMH is released from the polypeptides, actin and myosin, it is rapidly excreted in the urine of cattle. Therefore, if Model A is truly an accurate representation of NMH metabolism, then the exist out of the system from Compartment 1 of Model A should be a urinary exit. The exit out of the system from Compartment 1 of Model A was then described by the response curve of urine d_3-NMH. The urinary data were expressed as a fraction of the dose. This more descriptive model (Model B, illustrated in Figure 1) included two response curves (compared to only one for Model A): the urinary excretion of NMH as an exit from Compartment 1 and the plasma response curve entered in Compartment 1.

In constructing these models we attempted to minimize residual sum of squares; a three-compartment model produced a lower sum of squares than did a two-compartment model.

In addition, the tracer models of Figure 1 define steady-state pool sizes and fluxes, which, along with a de novo production, were calculated for each model. These calculations can be found in the SAAM manual (Berman and Weiss, 1978). The de novo production rate could be used to calculate a fractional degradation (breakdown) rate (**FBR**) for the myofibrillar proteins. The FBR was calculated by the following equation: FBR = NMH production rate divided by the total protein-bound NMH pool. To calculate the total protein-bound NMH pool, it was assumed that the percentage of muscle on a live weight basis was equal to 33% and the concentration of protein-bound NMH was equal to .6 μmol/g of muscle.

Statistical Analysis. The means for the NMH compartmental models and urinary production and turnover rates were compared by paired *t*-test (Steel and Torrie, 1980).

Results

The SAAM modeling program was used to describe the kinetics of d_3-NMH in three steers. After the bolus injection of tracer, the enrichment of d_3-NMH:NMH decreased very rapidly in plasma during the first 6 h, but the rate of decline was much lower during the last 72 h (Figure 2). In addition, d_3-NMH also accumulated in urine more rapidly during the first 24 h than during the next 48 h (Figure 2). Two variants of a three-compartment model were used, one to describe the response curve in plasma (Model A, Figure 1) and one to describe response curves of both plasma and urine simultaneously (Model B, Figure 1). Both of these compartmental models were adequate in describing NMH kinetics in steers and produced similar steady-state compartment mass and transfer rates between compartments (Figure 3). The plasma curves, shown in Figure 2 for Model A, produced a good agreement between the observed and the calculated values by the model. The same curves were also produced by Model B (curves not shown). The de novo production rate of NMH calculated in steady state by Models A and B and the FBR of myofibrillar protein were compared with values calculated from the quantification of urinary NMH (Table 1). Estimated values by these models were not different from estimates calculated from the quantification of urinary NMH excretion ($P > .25$). There were also no differences between Models A and B ($P > .25$).

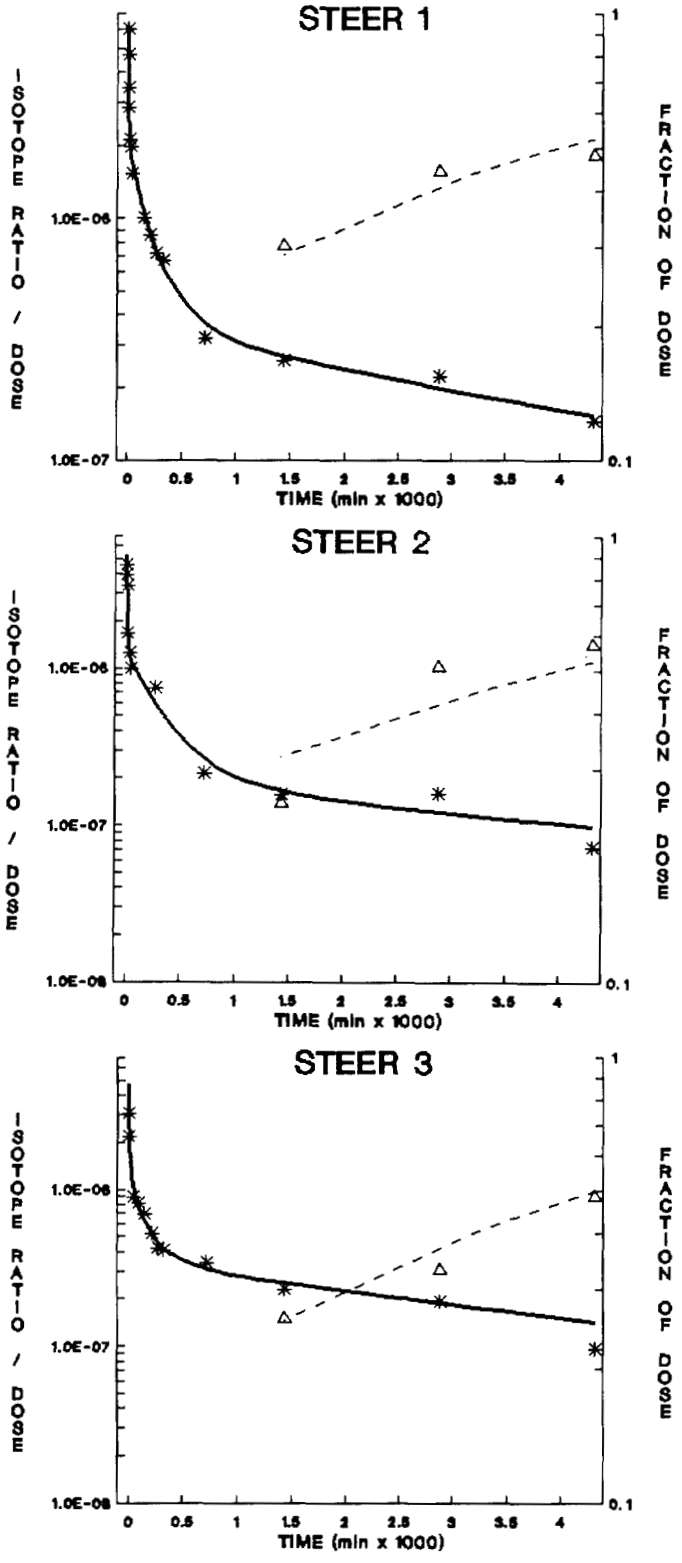

Figure 2. The decay curves of the [methyl-$^2H_3$]-N^{τ}-methylhistidine:N^{τ}-methylhistidine isotopic ratio per dose in plasma for each steer. The asterisks represent the observed data points and the solid line represents data produced by SAAM using Model A. The fraction of [methyl-$^2H_3$]-3-methylhistidine that accumulated in urine after a bolus injection of tracer is presented on the Y2 axis for each steer. The triangles represent the observed data points, and the dotted line represents data produced by SAAM using Model B.

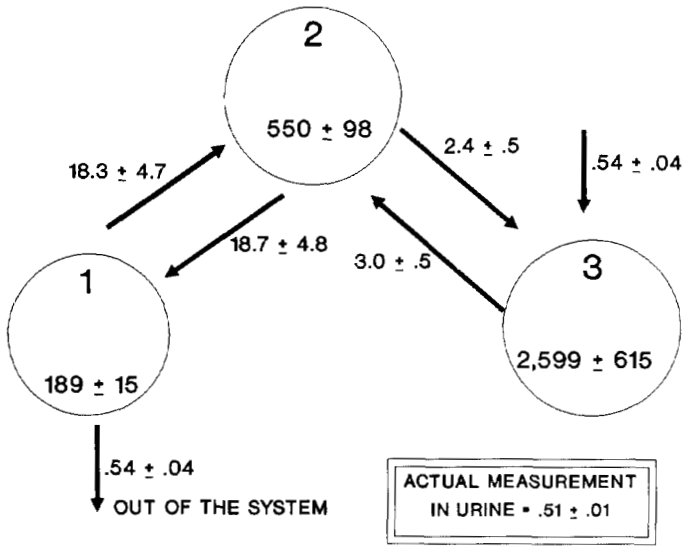

Figure 3. The steady-state compartment size (μmoles, mean ± SE) and transfer rate (μmoles/minute, mean ± SE) of N^{τ}-methylhistidine between compartments for the plasma model (Model A). Actual measurement in the urine represents quantitative collection of urine and measurement of N^{τ}-methylhistidine excretion rate.

Discussion

The developed model made use of linear, first-order differential equations; therefore, it is based on the assumption of steady-state kinetics in the animals during the experiment. No significant differences were found in individual steer plasma levels or in urinary production of NMH during the experiment, so the steers were assumed to be in steady state. There were no animal byproducts in the diets, so it was assumed that the only source of natural NMH was from the degradation of actin and myosin.

We have compared the in vivo kinetics of NMH in steers by two different models with that of the quantification of urinary excretion. The catenary three-compartment model (Model A) only required the sampling of plasma, whereas the plasma and urine model (Model B) required the sampling of plasma and urine. The models gave similar, if not identical, results and confirmed that the exit from Compartment 1 was via the urinary route. The models demonstrated that they could estimate the production rate of NMH and FBR of myofibrillar protein as effectively as the quantification of urinary excretion. Although the SE for urinary excretion was lower than for either Model A or B, only a limited sample size was used and it may be premature to conclude whether one model is more variable than another. Also, the compartmental models measured total de novo production rate rather than urinary excretion rate. In addition,

Table 1. N^{τ}-Methylhistidine (NMH) production and fractional breakdown rates of myofibrillar protein calculated from kinetic models and urinary collection

Model	Steer 1	Steer 2	Steer 3	Mean[a]	SE
Urinary					
NMH production rate[a], μmol/d	724	757	715	732	13
Fractional breakdown rate[b], %/d	1.61	1.53	1.53	1.61	.04
Kinetic Model A (plasma)[b]					
NMH production rate, μmol/d	666	885	796	782	64
Fractional breakdown rate, %/d	1.56	1.89	1.70	1.72	.10
Kinetic model B (plasma and urine)[c]					
NMH production rate, μmol/d	548	876	751	725	96
Fractional breakdown rate, %/d	1.28	1.87	1.61	1.58	.17

[a]No statistical differences between model NMH production and fractional breakdown rates ($P > .25$).
[b]A compartmental model based on [methyl-$2H_3$]-N^{τ}-methylhistidine (d_3-NMH) plasma kinetics (see Figure 1).
[c]A compartmental model based on d_3-NMH plasma and urine kinetics (see Figure 1).

when the data are expressed as percentage of body weight the NMH production rates were not as variable. The mean FBR value calculated for Model A was 1.72 %/d (Table 1). The FBR values of 1.22%/d at a growth stage of 217 kg (Nishizawa et al., 1979), 1.41 %/d at a growth stage of 236 kg (Harris and Milne, 1981), and 2.07 %/d at a growth stage of 400 kg (Gopinath and Kitts, 1984) have been reported for cattle.

The advantages of this compartmental model are that 1) it does not require quantitative urine collection, 2) it measures the total production rate and is not dependent on the determination of free or conjugated forms, 3) it gives information about pool size and transfer rates, and 4) it does not require restraint of the animals for long periods.

The structural configurations of these models are not unique, and alternative arrangements may also be compatible with the data. It was possible with a simple plasma model (A) to construct a two-compartment model. The present construct represents a framework and methodological approach describing the steady-state NMH kinetics in the whole animal and constitutes a working theory for testing by further experimentation with designs that alter muscle protein breakdown.

Implications

The decay of [methyl-$^2H_3$]-N^{τ}-methylhistidine can be compartmentally modeled and seems to be reflective of N^{τ}-methylhistidine production in vivo. Kinetic compartmental modeling offers an alternative approach to determining the N^{τ}-methylhistidine production rate, and the preliminary data presented here suggest that it is as effective as quantifying urinary N^{τ}-methylhistidine.

Literature Cited

Berman, M., and M. F. Weiss. 1978. SAAM MANUAL. U.S. Department of Health, Education and Welfare Publ. No.(NIH) 78-180. U.S. Government Printing Office, Washington, DC.

Boston, R. C., P. C. Grief, and M. Berman. 1981. Conversational SAAM–an inter-reactive program for kinetic analysis of biological systems. Comp. Prog. Biomed. 13:111.

Gopinath, R., and W. D. Kitts. 1984. Growth, N^{τ}-methylhistidine excretion and muscle protein degradation in growing beef steers. J. Anim. Sci. 59:1262.

Harris, C. I., and G. Milne. 1981. The urinary excretion of N^{τ}-methyl histidine by cattle: validation as an index of muscle protein breakdown. Br. J. Nutr. 45:411.

Harris, C. I., and G. Milne. 1987. The identification of the N^{τ}-methyl histidine-containing dipeptide, balenine, in muscle extracts from various mammals and the chicken. Comp. Biochem. Physiol. 86B(2):273.

McCarthy, F. D., W. G. Bergen, and D. R. Hawkins. 1983. Muscle protein turnover in cattle of differing genetic backgrounds as measured by urinary N^{τ}-methylhistidine excretion. J. Nutr. 113:2455.

Nishizawa, N., Y. Yoyoda, T. Noguchi, S. Hareyama, H. Itabashi, and R. Funabiki. 1979. N^{τ}-methylhistidine content of organs and tissues of cattle and an attempt to estimate fractional catabolic and synthetic rates of myofibrillar proteins of skeletal muscle during growth by measuring urinary output of N^{τ}-methylhistidine. Br. J. Nutr. 42:247.

Steel, R.G.D., and J. H. Torrie. 1980. Principles and Procedures of Statistics: A Biometrical Approach (2nd Ed.). McGraw-Hill Book Co., New York.

Young, V. R., S. D. Alex, B. S. Baliga, H. N. Munro, and W. Muecke. 1972. Metabolism of administered 3-methylhistidine: Lack of muscle transfer ribonucleic acid charging and quantitative excretion as 3-methylhistidine and its N-acetyl derivative. J. Biol. Chem. 217:3592.