

# Urinary 3-methylhistidine and progressive winter undernutrition in white-tailed deer

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**Abstract:** Physiological indicators of muscle catabolism would aid assessment of winter nutritional restriction of ungulates, and urinary 3-methylhistidine has exhibited potential in this regard in several species. We examined the effect of chronic moderate and severe nutritional restriction during winter on urinary 3-methylhistidine:creatinine ratios in seven adult white-tailed deer (*Odocoileus virginianus*) and the relationship of these ratios to urinary urea nitrogen:creatinine ratios. Mean base line estimates of urinary 3-methylhistidine:creatinine ratio for the control and severely restricted deer (0.043 and 0.086  $\mu\text{mol}:\text{mg}$ , respectively) were similar ( $P = 0.280$ ) and remained unchanged in the control deer throughout the study. In contrast, mean 3-methylhistidine:creatinine ratios increased dramatically as nutritional restriction and cumulative mass loss progressed; the quadratic component of the data for the chronically restricted deer was significant ( $P < 0.001$ ). Likewise, there was a strong curvilinear relationship ( $R^2 = 0.82$ ) between cumulative mass loss (up to 29%) of the pooled deer and urinary 3-methylhistidine:creatinine ratios. Further, urinary urea nitrogen:creatinine ratios were strongly related to 3-methylhistidine:creatinine ratios ( $r^2 = 0.89$ ). Our study indicates that further investigation of 3-methylhistidine as an indicator of physical condition and muscle protein breakdown is warranted.

**Résumé :** Des indicateurs physiologiques du catabolisme musculaire faciliteraient l'évaluation des restrictions alimentaires des ongulés pendant l'hiver et, en ce sens, la 3-méthylhistidine urinaire a démontré des propriétés prometteuses chez plusieurs espèces. Nous avons étudié les effets de restrictions alimentaires chroniques modérées ou graves en hiver sur le rapport 3-méthylhistidine : créatinine urinaire de sept Cerfs de Virginie (*Odocoileus virginianus*) adultes, de même que la relation entre ce rapport et le rapport azote uréique : créatinine dans l'urine. En moyenne, les estimations de base du rapport 3-méthylhistidine : créatinine urinaire chez les témoins et les cerfs soumis à des restrictions de nourriture importantes (respectivement 0,043 et 0,086  $\mu\text{mol}:\text{mg}$ ) étaient semblables ( $P = 0,280$ ) et sont demeurées inchangées durant toute la durée de l'étude chez les cerfs témoins. En revanche, le rapport moyen 3-méthylhistidine : créatinine a augmenté considérablement à mesure que les restrictions alimentaires devenaient plus sévères et les pertes de masse plus importantes; la composante quadratique des données recueillies chez les cerfs soumis à une restriction chronique de nourriture était significative ( $P < 0,001$ ). De même, il y avait une relation curvilinéaire marquée ( $R^2 = 0,82$ ) entre la perte cumulative de masse (jusqu'à 29%) de tous les cerfs mis ensemble et le rapport 3-méthylhistidine : créatinine urinaire. De plus, le rapport azote uréique : créatinine urinaire était fortement relié au rapport 3-méthylhistidine : créatinine ( $r^2 = 0,89$ ). Notre étude démontre que l'utilité de la 3-méthylhistidine comme indicateur de la condition physique et du métabolisme des protéines musculaires vaut la peine d'être investiguée davantage.

[Traduit par la Rédaction]

## Introduction

Because winter poses the most direct challenge to the nutritional well-being, survival, and reproductive success of

northern ungulates, researchers and managers seek alternative, reliable means of assessing winter nutritional restriction and physical condition of these animals (Mautz 1978; Verme and Ullrey 1984; DelGiudice et al. 1992). Hanks (1981) sug-

Received September 24, 1997. Accepted June 29, 1998.

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gested that physiological assessments of condition would permit projection of population trends and resilience.

Nutritional restriction of deer (*Odocoileus* spp.) and other mammals is associated with fat mobilization and degradation of endogenous protein, but there is far less physiological tolerance of the latter (Cahill 1970; Torbit et al. 1985; Heymsfield and Williams 1988; DelGiudice et al. 1990). Because skeletal muscle protein accounts for most of total body protein, it is of primary importance to whole-body protein metabolism (Daniel et al. 1977; Harris and Milne 1978). Thus, physiological indicators of muscle catabolism may aid assessment of winter nutritional restriction of deer.

The use of urinalysis to assess nutritional restriction and changes in body composition has been studied extensively (Tallen et al. 1955; Cowgill and Freeberg 1957; Young et al. 1972; Long et al. 1975; Harris and Milne 1978; Ballard and Tomas 1983; Hovell et al. 1987; Forbes 1988; Hoffer 1988), but only recently has this knowledge been applied to cervids. Studies of captive and free-ranging cervids have examined the effects of winter nutritional restriction, and in some cases refeeding, on urinary urea nitrogen, electrolytes, hydroxyproline, and allantoin (Eriksson and Valtonen 1974; Warren et al. 1982; DelGiudice et al. 1987, 1988, 1990, 1991a, 1991b, 1994a, 1994b, 1997; Parker et al. 1993; Garrott et al. 1996; Vagnoni et al. 1996). Urinary urea nitrogen has been studied most thoroughly and has exhibited potential for use in evaluating nutritional or dietary restriction and indicating changes in endogenous protein degradation and fat loss (Warren et al. 1982; DelGiudice et al. 1987, 1991a, 1994a, 1994b, 1997; Case 1996; Moen and DelGiudice 1997).

Urinary  $N^T$ -methylhistidine, or 3-methylhistidine, a methylated amino acid, was first identified in urine by Tallen et al. (1955). It occurs primarily ( $\geq 90\%$ ) in actin and myosin of skeletal muscle, and there is no exogenous source of this chemical for herbivores (Asatoor and Armstrong 1967; Johnson et al. 1967). Studies employing radio-labelled 3-methylhistidine have reported urinary 3-methylhistidine to be a reliable indicator of muscle protein degradation in humans, rats, cattle, rabbits, chicks, and frogs (Cowgill and Freeberg 1957; Young et al. 1972; Long et al. 1975; Harris and Milne 1978; Ballard and Tomas 1983) but not sheep or pigs (Harris and Milne 1980, 1981). In species where 3-methylhistidine has shown potential, it was not reutilized for protein synthesis after release during protein breakdown, only a small proportion was converted into an *N*-acetyl derivative, and most was excreted quickly and quantitatively in the urine (Young et al. 1972; Long et al. 1975; Harris and Milne 1978).

Although changes in urinary excretion of 3-methylhistidine and 3-methylhistidine:creatinine ratios have been associated with alterations in nutrition and body mass, the reliability of these characteristics for indexing muscle protein breakdown has been debated (Young et al. 1973; Haverberg et al. 1975; Nagabhushan and Narasinga Rao 1978; Young and Munro 1978; Ballard and Tomas 1983; Rennie and Millward 1983). At the center of this debate are questions concerning quantities of 3-methylhistidine in urine contributed by skeletal muscle versus nonskeletal muscle sources at various nutritional states, and this is related not just to respective pool sizes and relative contents of 3-methylhistidine, but to turn-

over rates of protein as well (Nishizawa et al. 1977; Ballard and Tomas 1983; Rennie and Millward 1983).

To our knowledge there are no published studies addressing the relationship between nutrition and urinary 3-methylhistidine of deer. Our objective in this initial effort was to assess the effect of chronic moderate and severe nutritional restriction during winter on urinary 3-methylhistidine:creatinine ratios of white-tailed deer (*Odocoileus virginianus*) and to relate these ratios to mass loss and urea nitrogen:creatinine ratios reported previously (DelGiudice et al. 1994a).

## Materials and methods

### Experimental design and data collection

We maintained seven adult ( $\geq 1.5$  years) white-tailed deer (four pregnant females, three males) in individual outdoor pens (15.5  $\times$  30.0 m) near Grand Rapids, Minnesota. The study was conducted from 4 February to 5 May 1988. Monthly mean minimum and maximum temperatures from January to May were  $-23.3$ ,  $-23.8$ ,  $-9.9$ ,  $-2.8$ , and  $7.2^\circ\text{C}$  and  $-9.7$ ,  $-6.8$ ,  $2.1$ ,  $12.7$ , and  $24.1^\circ\text{C}$ , respectively.

Two males and two females were assigned randomly to the treatment group and one male and two females to the control group. Until 11 February, deer were fed ad libitum a high-protein (11.1% crude protein), high-energy (2990 kcal digestible energy/kg) pelleted diet (DelGiudice et al. 1990, 1994a). Base-line data were collected on 4 February between 08:00 and 12:00. Using a pole syringe we anesthetized deer by injecting 100–150 mg xylazine HCl and 200–650 mg ketamine HCl. Once induced, deer were weighed and blood and urine were collected (DelGiudice et al. 1994a).

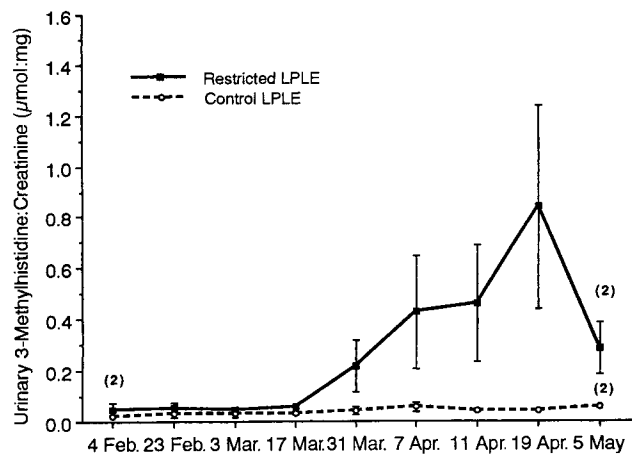
Treated deer (i.e., restricted diet) were provided with a low-protein (7.0% crude protein), low-energy (1900 kcal digestible energy/kg) pelleted diet at 0.2–1.0 kg per deer each day from 11 February to 5 May 1988, except for 15–19 April (see below). From 11 February to 15 April, each control deer received the same diet ad libitum as the treated deer. We believed ad libitum provision of this diet to be more realistic for a control group in a winter nutrition study, as opposed to an unnatural, high-quality diet (DelGiudice et al. 1994a). To simulate and examine the effect of acute severe nutritional restriction (such as might accompany a snowstorm) on control deer, we restricted all seven deer to 0.2 kg of feed per day from 15 to 19 April; ad libitum feeding was resumed for control deer from 19 April (after handling) to 5 May. Overall, mean daily feed intake was 0.53 kg (95% confidence interval 0.47–0.59 kg) and 0.28 kg (95% confidence interval 0.06–0.50 kg) for the control and restricted deer, respectively. Maximum cumulative mass loss was greater in restricted deer (17.0–32.2%) than in control deer (7.0–17.4%) (DelGiudice et al. 1994a). Deer were dependent on snow for water until late February, when we provided water ad libitum.

From 11 February to 5 May we anesthetized and handled all deer as described above at 1- to 2-week intervals. On average, 10 and 62 min elapsed between induction of anesthesia and blood and urine sampling, respectively (DelGiudice et al. 1994a).

### Laboratory and statistical analyses

Urinary creatinine was determined spectrophotometrically with an ABA-100 bichromatic autoanalyzer using modifications of the method of Jaffe (1886). Urinary 3-methylhistidine was analyzed using cationic-exchange resin and spectrophotometry (Vielma et al. 1981; Fitch et al. 1986). Urinary 3-methylhistidine is expressed as a ratio to creatinine (micromoles:milligrams) to correct for differences in hydration and as an estimate of fractional catabolic rate (Haverberg et al. 1975; Coles 1980; Elia et al. 1981; Ballard and Tomas 1983).

**Fig. 1.** Mean ( $\pm$  SE) urinary 3-methylhistidine:creatinine ratios for control and treated white-tailed deer fed either ad libitum or severely restricted amounts of a low-protein, low-energy (LPLE) commercial diet at Grand Rapids, Minnesota, from 4 February to 5 May 1988. Sample sizes were 3 and 4 for the control and treated groups, except where indicated in parentheses.



We evaluated the temporal pattern of 3-methylhistidine:creatinine in deer from treatment and control groups by fitting a mixed-effects repeated-measures analysis of covariance (ANCOVA) model to the log-transformed data (Ware 1985; SAS PROC MIXED, SAS Institute Inc. 1996). The model contained fixed effects for diet, time, time<sup>2</sup>, diet  $\times$  time, and diet  $\times$  time<sup>2</sup>; deer were modelled as random "subject" effects. Using the techniques of Wolfinger (1993), a first-order antedependence covariance structure was selected to account for the within-deer correlations. A mixed-effects repeated-measures polynomial regression model was used to evaluate dependence of 3-methylhistidine:creatinine on percent mass loss in deer. In this case, a compound symmetry covariance structure was employed. We fit polynomials of the form

$$Y = \beta_0 + \beta_1 t + \beta_2 t^2 + \beta_3 t^3$$

where  $t$  is the time-dependent predictor, percent mass loss. We selected the highest order polynomial that was statistically significant ( $\alpha = 0.05$ ; Neter et al. 1990).

## Results

Temporal patterns of urinary 3-methylhistidine in the treatment group differed significantly from those of the controls ( $F_{[2,46]} = 10.90$ ,  $P < 0.0001$ ). Initially, 3-methylhistidine:creatinine ratios in controls versus treatment deer were not significantly different ( $H_0$ : equal intercepts,  $F_{[1,5]} = 1.46$ ,  $P = 0.280$ ) (Fig. 1). However, whereas values in the controls remained nearly constant through time ( $H_0$ :  $\beta_1 = 0$ ,  $T_{46} = 0.86$ ,  $P = 0.392$ ;  $H_0$ :  $\beta_2 = 0$ ,  $T_{46} = 1.50$ ,  $P = 0.140$ ) (Fig. 1), 3-methylhistidine:creatinine values in treated deer exhibited a marked increase by 31 March ( $H_0$ :  $\beta_1 = 0$ ,  $T_{46} = 2.42$ ,  $P = 0.018$ ;  $H_0$ :  $\beta_2 = 0$ ,  $T_{46} = 4.42$ ,  $P < 0.0001$ ) (Fig. 1).

Similarly, a significant curvilinear relationship was observed between 3-methylhistidine:creatinine and progressive percent mass loss in the seven deer ( $H_0$ :  $\beta_0 = 0$ ,  $T_6 = -0.06$ ,  $P = 0.951$ ;  $H_0$ :  $\beta_1 = 0$ ,  $T_{41} = 1.42$ ,  $P = 0.162$ ;  $H_0$ :  $\beta_2 = 0$ ,  $T_{41} = -2.53$ ,  $P = 0.015$ ;  $H_0$ :  $\beta_3 = 0$ ,  $T_{41} = 4.29$ ,  $P = 0.0001$ ;  $R^2 = 0.82$ ) (Fig. 2). The single observation with 32% mass loss (triangle in Fig. 2) was excluded from the regression

analysis. This deer was near death and was the only animal to experience  $>29\%$  mass loss during the study. This deer's final 3-methylhistidine:creatinine ratio did not adhere to the general trend of values and may reflect a breakdown of "normal" physiological processes as it approached death. Until more data are available for such extreme mass loss, we think it prudent to limit statistical inference to the range of mass loss values  $\leq 29\%$ .

The 3-methylhistidine:creatinine ratios were strongly related ( $r^2 = 0.89$ ) to urea nitrogen:creatinine ratios (Fig. 3). Short-term (4 days) acute severe nutritional restriction had a minimal effect on urinary 3-methylhistidine:creatinine values in control deer, but had a dramatic effect on the ratios of the treated deer (Table 1).

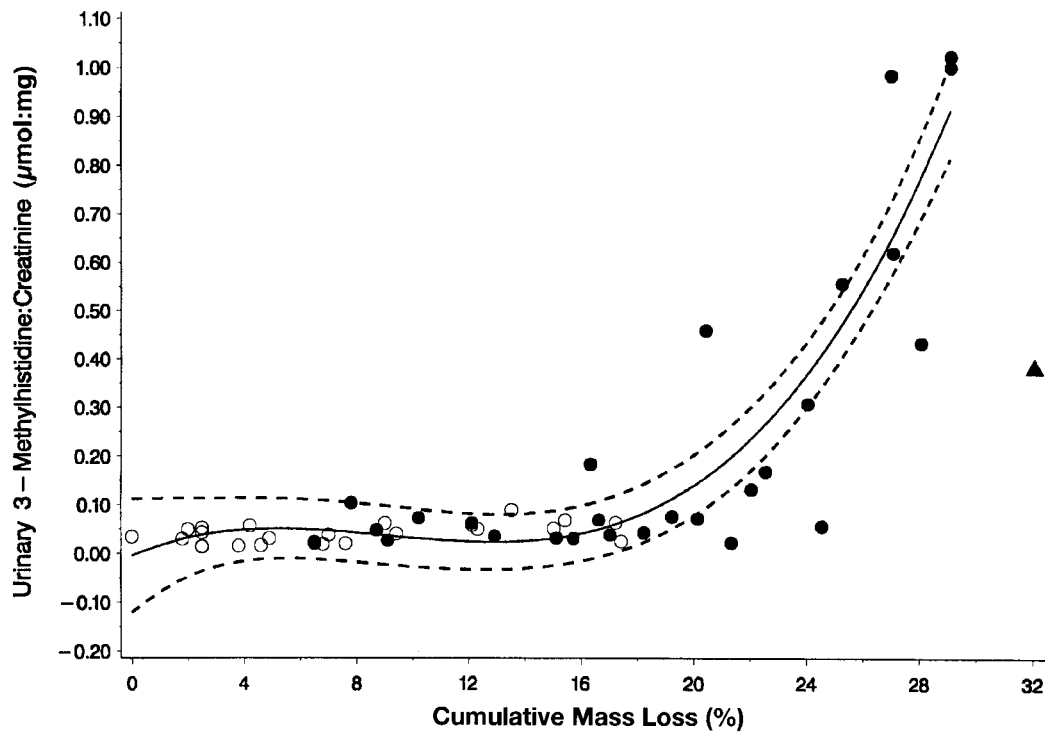
## Discussion

The mean mass-specific digestible energy intake of the treated deer averaged less than half that of the control deer during most of the study and resulted in significantly greater cumulative mass loss (DelGiudice et al. 1994a). Assuming that urinary 3-methylhistidine is a reliable indicator of muscle protein catabolism, the similar and stable mean urinary 3-methylhistidine:creatinine values ( $\mu\text{mol:mg}$ ) for control and severely restricted deer through 17 March indicated that the fractional rates of catabolism of myofibrillar protein were comparable in the two groups. Divergence of mean urinary 3-methylhistidine:creatinine ratios between the two deer groups by 31 March, associated with mass losses of 12.9–24.0% (mean = 19.4%) and 2.5–15.4% (mean = 7.2%) (DelGiudice et al. 1994a), suggested that the mean fractional rate of catabolism of muscle in the restricted deer increased 17-fold over base-line values by 18–19 April. Ballard and Tomas (1983) suggested that the daily percentage of muscle protein degraded could be estimated using single urine specimens. Applying their equation to our data, an instantaneous estimate of the mean daily percentage of muscle protein degraded by severely restricted deer increased from 0.25 to 4.20%, whereas in controls it increased from 0.12 to 0.21% between 4 February and 19 April. By 19 April, three of the four restricted deer had lost at least 28% of their base-line body mass.

Young et al. (1973) reported that changes in 3-methylhistidine:creatinine ratios reflected changes in 24-h urinary excretion of 3-methylhistidine in fasted humans, and Haverberg et al. (1975) showed that 40 and 45% increases in urinary excretion of 3-methylhistidine per unit body mass and 3-methylhistidine:creatinine ratios, respectively, accompanied a mean 35% decline in body mass of rats during 14 days of protein and energy restriction. Similarly, 3-methylhistidine:creatinine ratios of undernourished patients were more than double those of normal children (Nagabhushan and Narasinga Rao 1978). In contrast, in protein-restricted rats, 24-h urinary 3-methylhistidine excretion ( $\mu\text{mol/day}$ ), urinary 3-methylhistidine output per unit body mass, and 3-methylhistidine:creatinine ratios decreased 81, 71, and 68%, respectively (Haverberg et al. 1975).

Our analysis of data pooled from the control and treatment deer (Fig. 2) demonstrated that mass loss explained a substantial portion of the variability of 3-methylhistidine:creatinine ratios ( $R^2 = 0.82$ ). However, the results were

**Fig. 2.** Predicted values (solid line) of urinary 3-methylhistidine:creatinine  $\pm$  90% confidence limits (broken lines) based on observed cumulative mass loss in seven adult white-tailed deer that consumed varying amounts of a low-protein, low-energy commercial diet at Grand Rapids, Minnesota, from 4 February to 5 May 1988.  $\circ$ , observed values for controls;  $\bullet$ , observed values of nutritionally restricted deer;  $\blacktriangle$ , an outlier value (see Results).



**Table 1.** Effect of short-term (15–19 April) extreme nutritional deprivation on cumulative mass loss and urinary 3-methylhistidine:creatinine (3-MeH:C) and urea nitrogen:creatinine (UN:C) ratios of slightly and highly undernourished white-tailed deer, Grand Rapids, Minnesota, sampled on 11 and 19 April 1988.

Deer No.	Sex	Before severe deprivation			After severe deprivation		
		Cumulative mass loss (%)	Urinary 3-MeH:C ( $\mu$ mol:mg)	Urinary UN:C (mg:mg)	Cumulative mass loss (%)	Urinary 3-MeH:C ( $\mu$ mol:mg)	Urinary UN:C (mg:mg)
<b>LPLE restricted<sup>a</sup></b>							
79	M	29	1.02	28.1	>29.0	1.88	53.1
92	F	27.1	0.62	20.1	29	1	51.9
47	F	15	0.03	2.1	17	0.04	6.4
65	M	22.5	0.17	6.1	28.1	0.43	22.2
Mean		23.4	0.46	14.1	>25.8	0.84	33.4
SE		3.1	0.23	6.1		0.4	11.5
<b>LPLE control</b>							
55	F	4.7	0.03	2.7	7	0.04	8.1
6	F	2	0.05	3.2	17.4	0.03	3.8
66	M	15.1	0.05	3.8	17.2	0.06	6.9
Mean		7.3	0.04	3.2	13.9	0.04	6.3
SE		4	0.01	0.3	3.4	0.01	1.3

**Note:** Cumulative mass loss (since 4 February) and urinary UN:C ratio data are from DelGiudice et al. (1994a). Deer 79 and 92 died on 18 and 19 April, respectively; the mass of deer 79 was not obtained at death.

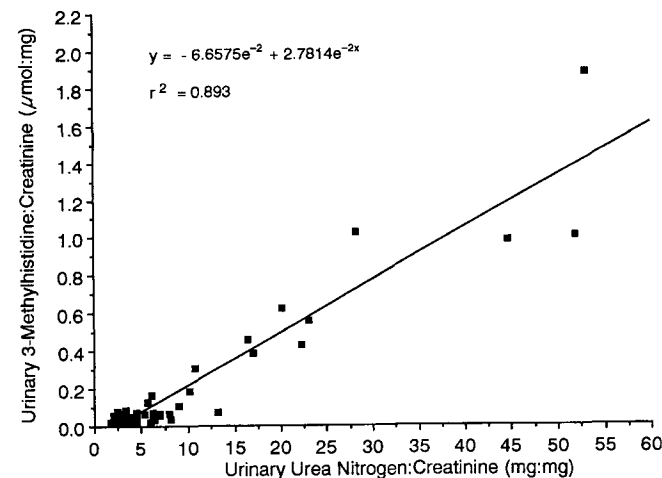
<sup>a</sup>Until this period of extreme nutritional restriction (15–19 April), the slightly undernourished group was fed a low-protein, low-energy (LPLE) diet (see the text) ad libitum, and the highly undernourished group was fed restricted amounts of the LPLE feed.

based on a small sample of deer ( $n = 7$ ) and likely under-represent the degree of heterogeneity of this relationship in natural populations. Nonetheless, the relationship in our sample is well defined and is consistent with the urinary

urea nitrogen:creatinine ratio – cumulative mass loss curve (DelGiudice et al. 1994a).

The marked effect of the short-term (4 days) severe nutritional restriction on the already restricted deer was mani-

**Fig. 3.** Relationship of urinary 3-methylhistidine:creatinine ratios with urinary urea nitrogen:creatinine ratios for seven adult white-tailed deer that consumed varying amounts of a low-protein, low-energy commercial diet at Grand Rapids, Minnesota, from 4 February to 5 May 1988 (urea nitrogen:creatinine values are from DelGiudice et al. 1994a).



festated in their deteriorated physical condition relative to the controls. Mean urea nitrogen:creatinine ratios doubled in both groups following deprivation (DelGiudice et al. 1994a), but whereas 3-methylhistidine:creatinine ratios almost doubled in the treated deer, they increased only 22–24% in two control deer and decreased in the third. These data suggest that 3-methylhistidine:creatinine ratios may be more indicative of long-term nutritional stress than urea nitrogen:creatinine ratios. Case (1996) reported a greater tendency for urinary urea nitrogen:creatinine ratios than for 3-methylhistidine:creatinine ratios to increase with decreasing kidney fat indices in free-ranging caribou (*Rangifer tarandus*). He suggested that 3-methylhistidine:creatinine may be more reflective of long-term undernutrition and poor condition.

Our study provides preliminary results indicating a potentially useful relationship among winter nutritional restriction, mass loss, and urinary 3-methylhistidine:creatinine ratios in white-tailed deer; however, our sample ( $n = 7$ ) was small. To better understand what the urinary 3-methylhistidine:creatinine ratios represent relative to protein degradation, detailed isotopic studies are needed to quantify the kinetics of 3-methylhistidine and the relative contributions from skeletal and nonskeletal muscle protein pools. Approximately 90% of the total body pool of 3-methylhistidine occurs in skeletal muscle, primarily in actin and myosin, which comprise 65% of muscle protein and 30–35% of total body protein in humans (Asatoor and Armstrong 1967; Johnson et al. 1967; Young and Munro 1978). Although skeletal muscle is the largest pool of 3-methylhistidine in the body, protein turnover rate in this pool (and subsequent release of 3-methylhistidine) should be compared with that in the smaller nonskeletal muscle tissues (e.g., gastrointestinal tract, skin) to determine their relative contributions to the urinary 3-methylhistidine excreted by deer. This question should be studied experimentally, randomizing deer to varying feeding regimes (e.g., dietary energy and protein deficiencies, fasting, optimal diets). Research should be carried out on the ef-

fect of nutritional restriction and condition deterioration on urinary creatinine excretion and on the actin and myosin content of 3-methylhistidine in the various body pools of deer (Young et al. 1973; Haverberg et al. 1975; Nishizawa et al. 1977; Young and Munro 1978).

## Acknowledgements

We gratefully acknowledge financial support from the Special Projects Foundation of the Minneapolis Big Game Club, National Rifle Association, Minnesota Department of Natural Resources, U.S. Fish and Wildlife Service, North Central Forest Experiment Station, Minnesota Zoological Society, and James Ford Bell Foundation. We thank M.D. Lewis and M.K. Twite for technical laboratory support and W.J. Paul and many student interns for assistance with animal care and handling. E.C. Hellgren and an anonymous reviewer provided useful comments on an earlier draft.

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