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Prolonged winter undernutrition and the interpretation of urinary allantoin:creatinine ratios in white-tailed deer

Glenn D. DelGiudice, Ken D. Kerr, L. David Mech, and Ulysses S. Seal

Abstract: The urinary allantoin:creatinine (A:C) ratio (expressed in micromoles of allantoin to micromoles of creatinine) has shown potential as an index of recent winter energy intake in preliminary controlled studies of elk (*Cervus elaphus*) involving mild condition deterioration (up to 11% loss of body mass). To ensure reliable nutritional assessments of free-ranging cervids by measuring A:C ratios of urine in snow, it is essential to extend this work. We assessed the effect of moderate and severe winter nutritional restriction on urinary A:C ratios of captive white-tailed deer (*Odocoileus virginianus*) that lost up to 32% body mass and related these ratios to metabolizable energy intake (MEI), body-mass loss, and other reported nutritional indicators. Deer in the control group were fed a low-protein, low-energy diet ad libitum, whereas deer in the treatment group were fed restricted amounts of the same diet. MEI was below the winter maintenance requirement for all deer, but was lower ($P = 0.029$) in treatment deer than in control deer. Percent body-mass loss differed between the two groups as the study progressed, and represented the full range of physiological tolerance (0–32% loss). Mean A:C ratios of control deer, which lost up to 17.4% body mass, showed a slight increasing ($P = 0.086$) trend, whereas initially similar A:C ratios of severely restricted deer increased ($P = 0.0002$) markedly by the eighth week (0.52 vs. 0.09 μmol:μmol). The urinary A:C ratio was not related ($P = 0.839$) to recent (2 days prior to urine sampling) MEI, but there was a marginally significant relation ($r^2 = 0.42$, $P = 0.110$) between the A:C ratio and cumulative percent mass loss. The urinary A:C ratio was directly related to urinary urea nitrogen:creatinine ($r^2 = 0.59$, $P < 0.0001$) and 3-methylhistidine:creatinine ($r^2 = 0.43$, $P < 0.0001$) ratios. This study confirms that elevated and increasing A:C ratios may be due either to increasing energy intake or to accelerated tissue catabolism and increased endogenous contributions to urinary allantoin excretion.

Résumé : Le rapport allantoïne : créatinine (A : C) de l'urine s'est montré un indice prometteur de l'absorption récente d'énergie en hiver au cours d'études préliminaires dans des conditions contrôlées chez des Wapitis (*Cervus elaphus*) qui ont subi une légère détérioration de leur condition physiologique (perte de masse allant jusqu'à 11 %). Pour que les rapports A : C de l'urine relevés dans la neige permettent d'estimer les conditions nutritionnelles des cervidés en liberté, il fallu étendre le champ de recherche. Nous avons mesuré les rapports A : C urinaires chez des Cerfs de Virginie (*Odocoileus virginianus*) en captivité qui ont perdu jusqu'à 32 % de leur masse et avons tenté de relier ces rapports à l'absorption d'énergie métabolisable (MEI), à la perte de masse et aux autres indicateurs nutritionnels disponibles. Les cerfs du groupe témoin ont été gardés à un régime faible en protéines et en énergie et se nourrissaient ad libitum, alors que les cerfs du groupe expérimental étaient soumis au même régime mais recevaient des portions rationnées. L'absorption d'énergie métabolisable a été inférieure au seuil de maintien en hiver chez tous les cerfs, mais a été encore plus basse ($P = 0{,}029$) chez les cerfs traités que chez les cerfs témoins. La perte de masse en pourcentage différait entre les groupes et la différence s'accentuait à mesure qu'avançait l'étude et représentait tout l'éventail de tolérance (perte de 0–32 %). Les rapports A : C moyens chez les cerfs témoins, qui ont perdu jusqu'à 17,4 % de leur masse, avaient légèrement tendance à augmenter ($P = 0{,}086$), alors que les rapports A : C des cerfs expérimentaux fortement privés de nourriture, semblables aux précédents au départ, avaient subi une augmentation très forte ($P = 0{,}0002$) à la 8^e semaine (0,52 vs. 0,09 μmol : μmol). Le rapport A : C urinaire n'était pas relié ($P = 0{,}839$) à l'absorption récente d'énergie métabolisable (2 jours avant le prélèvement d'urine), mais il y avait une relation presque significative ($r^2 = 0{,}42$, $P = 0{,}110$) entre A : C et le pourcentage cumulatif de perte de masse. Le rapport A : C de l'urine est

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directement proportionnel aux rapports azote uréique : créatinine (r^2 = 0,59, P < 0,0001) et méthyl-3-histidine : créatinine (r^2 = 0,43, P < 0,0001) de l'urine. Cette étude confirme que des rapports A : C élevés ou croissants peuvent être attribuables à une augmentation de l'absorption d'énergie ou au catabolisme accéléré des tissus et à l'augmentation des contributions endogènes à l'excrétion urinaire d'allantoïne.

[Traduit par la Rédaction]

Introduction

Obvious management implications of the relationship of winter nutrition to the performance of northern ungulate populations have prompted broad and intensive study of the effects of nutritional restriction on physical condition and physiological indicators of nutritional status. Two primary objectives have been to identify characteristics and means of data collection that would facilitate sensitive, practical, and cost-effective assessments of nutritional restriction or condition.

Collection and chemical analysis of urine in snow (snow-urine) has potential as a practical, non-invasive means of assessing the nutritional status of northern ungulates (DelGiudice et al. 1988, 1989, 1991, 1994*a*, 1995, 1997; DelGiudice 1995; Garrott et al. 1996; Moen and DelGiudice 1997; Ditchkoff and Servello 1999; Pils et al. 1999). During the past 2 decades, chemicals in the urine of wild ungulates have been studied under controlled and field conditions to evaluate their potential for use in nutritional assessments. Most recently, urinary allantoin, expressed as the allantoin: creatinine (A:C) ratio, has been proposed as an indicator of recent (2–3 days prior to urination) metabolizable energy intake (MEI) by elk (*Cervus elaphus*) during winter (Vagnoni et al. 1996; Garrott et al. 1997). Urinary allantoin is an end-product of metabolism of purine bases that are derived from nucleic acid catabolism during tissue turnover, from digestion of nucleic acids in feed, and from ruminal microbial fermentation of nucleic acids, which is directly affected by the animal's nutritional plane (Antoniewicz and Pisulewski 1982; Chen et al. 1990*a*, 1990*b*; Puchala and Kulasek 1991).

Vagnoni et al. (1996) and Garrott et al. (1997) reported positive correlations of A:C ratios with recent energy intake of elk experiencing body-mass gains and maximum mean mass losses of 9–11%. However, because free-ranging ungulates lose up to 33% of their body mass during winter (Davenport 1939; Moen and Severinghaus 1981; Severinghaus 1981; DelGiudice et al. 1992), Vagnoni et al. (1996) emphasized the importance of carrying out additional studies of cervids that would improve our understanding of endogenous contributions to urinary allantoin excretion associated with severe nutritional restriction and body-condition deterioration. This is especially critical to reliable interpretations of A:C ratios from snow-urines of free-ranging cervids.

Urinary allantoin has been studied more thoroughly in domestic ruminants, whose 24-h excretion was also positively correlated with energy intake over short time periods when there was little change in physical condition (Chen et al. 1990*a*, 1990*b*, 1992; Verbic et al. 1990; Giesecke et al. 1993, 1994; Puchala et al. 1993). Cattle and sheep fed maintenance diets have shown pronounced species differences in endogenous allantoin excretion, and the effects of short-term fasting on allantoin excretion have been inconsistent (Walker and Faichney 1964*a*, 1964*b*; Rys et al. 1975; Antoniewicz and Pisulewski 1982; Fujihara et al. 1987; Chen et al. 1990*a*, 1990*b*; Puchala and Kulasek 1991). None of these studies involved prolonged nutritional restriction or broad ranges of body-mass loss.

In the light of previous findings, we hypothesized that when animals primarily experience varying degrees of nutritional restriction and prolonged undernutrition accompanied by greater body-mass losses than have been reported thus far, the relationship between MEI and urinary excretion of allantoin (or A:C ratio) may be less apparent. Consequently, our objective in this preliminary effort was to assess the effect of long-term moderate and severe winter nutritional restriction on urinary A:C ratios of captive white-tailed deer (*Odocoileus virginianus*) and relate these ratios to MEI, body-mass loss, and other indicators of nutritional status.

Materials and methods

Adult (≥1.5 years old) white-tailed deer (four pregnant females, three males) were maintained in outdoor pens (15.5 × 30.0 m) near Grand Rapids, Minnesota. The study period was 4 February – 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°C and –9.7, –6.8, 2.1, 12.7, and 24.1°C, respectively (DelGiudice et al. 1994*b*).

Two males and two females were assigned randomly to the treatment group and one male and two females to a control group. Deer were fed a high-protein (11.1% crude protein), high-energy (12498 kJ digestible energy/kg) pelleted diet ad libitum until 11 February (DelGiudice et al. 1990, 1994*a*). Base-line data were collected on 4 February from 08:00 to 12:00. We anesthetized deer by injecting 100–150 mg xylazine HCl and 200–650 mg ketamine HCl. Deer were weighed and blood-sampled, and urine was collected by catheterization (DelGiudice et al. 1994*b*).

Treated deer were fed a low-protein (7.0% crude protein), low-energy (7942 kJ digestible energy/kg) pelleted diet at a restricted level of 0.2–1.0 kg per deer each day from 11 February to 5 May, except for 15–19 April (see below). Each control deer was fed the same diet ad libitum from 11 February to 15 April (DelGiudice et al. 1994*b*). We restricted all seven deer to 0.2 kg of feed per day from 15 to 19 April to simulate the acute severe nutritional restriction that might accompany a snowstorm (DelGiudice et al. 1994*b*, 1998). We resumed ad libitum feeding for control deer from 19 April after handling to 5 May. Mean daily feed intake during the study 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, and maximum cumulative mass loss was 17.0–32.2% in restricted deer versus 7.0–17.4% in control deer (DelGiudice et al. 1994*b*). MEI was calculated by multiplying digestible energy intake by 0.85 (Hobbs et al. 1982). We anesthetized and handled all deer from 11 February to 5 May as described above. Additional details are presented in DelGiudice et al. (1994*b*).

Chemical and statistical analyses

Urinary creatinine concentrations were determined spectrophotometrically with a ABA-100 bichromatic autoanalyzer using modifications of the method of Jaffe (1886). Urinary allantoin concentrations were assayed to the nearest microgram per millilitre using modifications of the colorimetric method of Young and Conway (1942). Allantoin concentrations are reported in micromoles of allantoin to micromoles of creatinine (A:C ratio) to correct for differences in hydration among individuals.

We analyzed the temporal patterns of recent (i.e., 2 days prior to handling) mean daily MEI (kJ/kg$^{0.75}$ body mass) and urinary A:C ratio in deer of the two 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). Fixed effects of the model were diet, time, time^2, diet × time, and diet × time^2. Deer were random "subject" effects in the model. We did not examine potential sex effects; however, data from the males were within the bounds of variability of those of the females (DelGiudice et al. 1994*b*, 1998). Heterogeneous autoregressive and compound-symmetry covariance structures were selected to account for within-deer correlations for MEIs and A:C ratios, respectively (Wolfinger 1993). Mixed-effects repeated-measures polynomial regression models were used to evaluate the dependence of the A:C ratio on recent MEI, percent body-mass loss, and urinary urea nitrogen:creatinine (UN:C) and 3-methylhistidine:creatinine (3-MeH:C) ratios in deer. In these cases, we used compound-symmetry covariance structures and fit the following polynomials:

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

where t is the time-dependent predictor (recent MEI, percent body-mass loss, urinary UN:C ratio, or urinary 3-MeH:C ratio). Null hypotheses (H_0) tested included equality of initial or base-line (i.e., β_0) MEI and A:C ratios of the control and restricted groups, as well as whether the temporal patterns of A:C ratios of control and restricted deer had slopes that departed linearly or curvilinearly from 0 (i.e., $\beta_1 = 0$ and $\beta_2 = 0$, respectively). We selected the highest order polynomial that was statistically significant ($\alpha = 0.05$; Neter et al. 1990).

Results

Initially, recent MEIs did not differ between the treated and control deer (H_0: equal intercepts, $F_{[1,5]} = 3.96$, $P = 0.103$). Subsequently, however, MEI increased in the control deer ($F_{[2,40]} = 3.86$, $P = 0.029$), except during 15–19 April (Fig. 1).

Temporal patterns of urinary A:C ratios differed significantly between restricted and control deer ($F_{[2,46]} = 9.45$, $P = 0.001$) (Fig. 2), even though initially there was no significant difference (H_0: equal intercepts, $F_{[1,5]} = 1.40$, $P = 0.289$) (Fig. 2). Urinary A:C ratios of control deer increased slightly throughout the study (H_0: $\beta_1 = 0$, $T_{46} = 1.59$, $P = 0.119$; H_0: $\beta_2 = 0$, $T_{46} = 1.76$, $P = 0.086$), whereas those of restricted deer exhibited a pronounced increase by 7 April (H_0: $\beta_1 = 0$, $T_{46} = 2.59$, $P = 0.013$; H_0: $\beta_2 = 0$, $T_{46} = 3.98$, $P = 0.001$). There was no significant relation between recent MEI and urinary A:C ratio (H_0: $\beta_1 = 0$, $T_{43} = 0.21$, $r^2 = 0.01$, $P = 0.839$) of the seven deer (Fig. 3), but there was a marginally significant curvilinear relation between A:C ratios and progressive percent mass loss (H_0: $\beta_3 = 0$, $T_{41} = 1.63$, $r^2 = 0.42$, $P = 0.110$) (Fig. 4). Short-term severe nutritional restriction (15–19 April) had no apparent or consistent effect on the A:C ratios of the already restricted deer or the control deer (Table 1). The urinary A:C ratio was significantly related to the urinary UN:C (H_0: $\beta_1 = 0$, $T_{49} = 9.50$, $r^2 = 0.59$, $P < 0.001$) and 3-MeH:C ratios (H_0: $\beta_1 = 0$, $T_{49} = 5.65$, $r^2 = 0.43$, $P < 0.001$).

Discussion

By providing ad libitum and restricted amounts of the pelleted winter diet to our control and treatment deer, we succeeded in ensuring that the two groups had different mean daily MEIs (kJ/kg$^{0.75}$ body mass) during most of the study. Consequently, this resulted in a difference in mean cumulative mass loss between the two groups as the study progressed (DelGiudice et al. 1994*b*), and the cumulative mass losses (0–32%) of the seven deer represented the full range of the species' physiological tolerance (DeCalesta et al. 1975, 1977; Moen and Severinghaus 1981; Severinghaus 1981; Torbit et al. 1985; DelGiudice et al. 1992).

At mean MEIs (65–442 kJ/kg$^{0.75}$ per day) that remained below winter maintenance for captive deer (561 kJ/kg$^{0.75}$ per day; Ullrey et al. 1970; Robbins 1983) throughout the study, the absence of a relation between recent daily MEIs (2 days prior to urine sampling) and urinary A:C ratios was in contrast to the findings of studies of captive elk experiencing positive and negative energy balance (Vagnoni et al. 1996; Garrott et al. 1997). Additional evidence for the absence of such a relation in our deer was obtained when towards the end of the study, MEI was reduced further (65.3–86.6%) in all subjects and effects on A:C ratios were inconsistent (Table 1). Most noteworthy was that high A:C ratios in treatment deer that had already lost the most body mass (22.5–29.0%) either remained high (Nos. 79 and 92) or increased markedly (No. 65; Table 1).

The winter maintenance requirement of captive elk, 552 kJ MEI/kg$^{0.75}$ per day, is similar to that of captive white-tailed deer (Robbins 1983; Jiang and Hudson 1992). The primary nutritional difference between this deer study and the two elk studies was that in the latter the mean MEIs ranged from submaintenance to well above maintenance level (e.g., 200–1200 kJ/kg$^{0.75}$; Garrott et al. 1997). Most of the mean A:C ratios (33) related to recent mean MEI were in the maintenance range or above (Garrott et al. 1997). Thirteen of the mean MEIs related to mean A:C ratios in the elk studies were below maintenance level, and only 5 of those were below 400 kJ/kg$^{0.75}$ per day (Fig. 2 in Garrott et al. 1997). Further, changes in body mass from the two elk studies ranged from a maximum mean loss of 11% to a gain of 9%, whereas in this study, mean body-mass losses ranged from 4.2 to 24.2% and individual mass losses from 0 to 32.2%.

The curvilinear relation between percent mass loss and urinary A:C ratios for the treatment and control deer indicates the importance of considering more than the effect of recent MEI when interpreting A:C ratios. This was unexpected, based on findings from captive elk and domestic ruminants experiencing smaller body-mass losses (Chen et al. 1990*a*; Verbic et al. 1990; Balcells et al. 1991; Vagnoni et al. 1996; Garrott et al. 1997). The increasing trend of A:C ratios in the nutritionally restricted deer began at about 24% body-mass loss (Fig. 4), and values were comparable to those from captive elk with mean MEIs at and above winter maintenance and gaining body mass (Garrott et al. 1997). A:C ratios of severely restricted deer comparable to and higher than those of control deer were occurring when body-mass losses were as moderate as 8%.

Our data from deer in negative energy balance and losing body mass indicate that factors other than MEI affect A:C

Fig. 1. Recent (2 days prior to urine sampling) mean metabolizable energy intake (MEI) of captive adult white-tailed deer (*Odocoileus virginianus*) fed either restricted (treatment) or ad libitum (control) amounts of a low-protein, low-energy (LPLE) commercial diet from 11 February to 5 May 1988 at Grand Rapids, Minnesota. Prior to the study, all deer were maintained on a high-protein, high-energy commercial diet, but consumption was not monitored prior to initiation of the study. Sample sizes were four and three deer in the treatment and control groups, respectively.

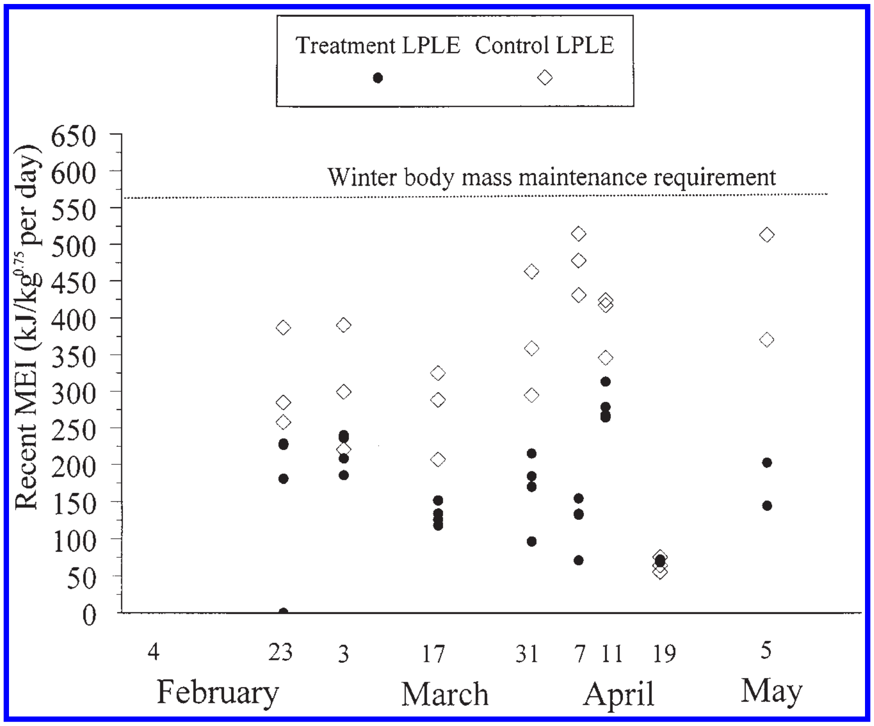


Fig. 2. Urinary allantoin:creatinine ratios of captive adult white-tailed deer maintained on a high-protein, high-energy commercial diet through 10 February 1988 and fed either restricted (treatment) or ad libitum (control) amounts of a low-protein, low-energy (LPLE) commercial diet from 11 February to 5 May 1988 at Grand Rapids, Minnesota. Sample sizes were four and three deer in the treatment and control groups, respectively.

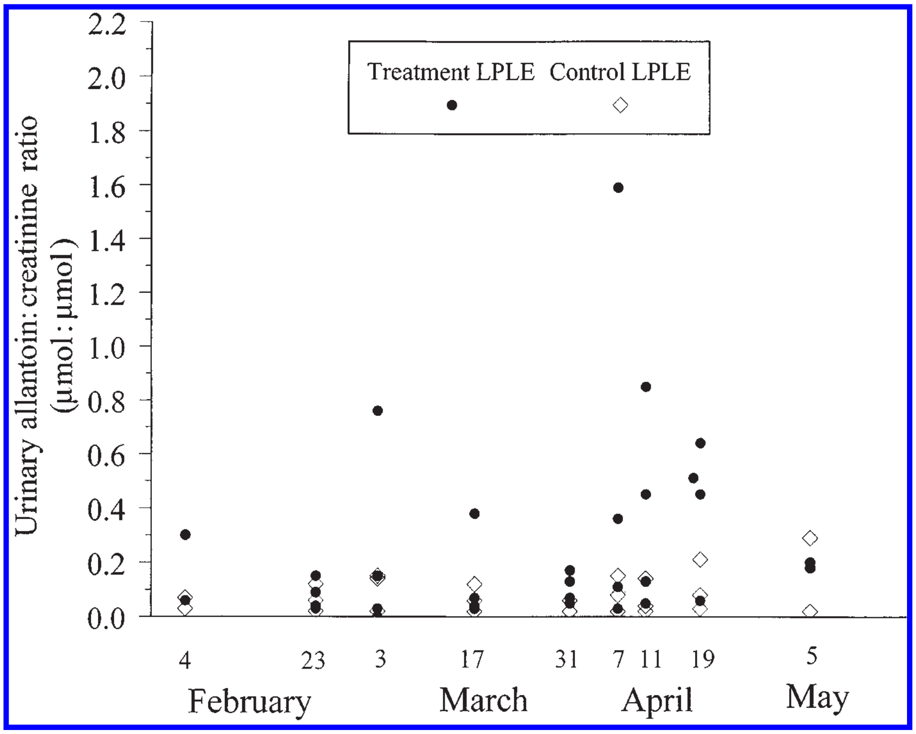
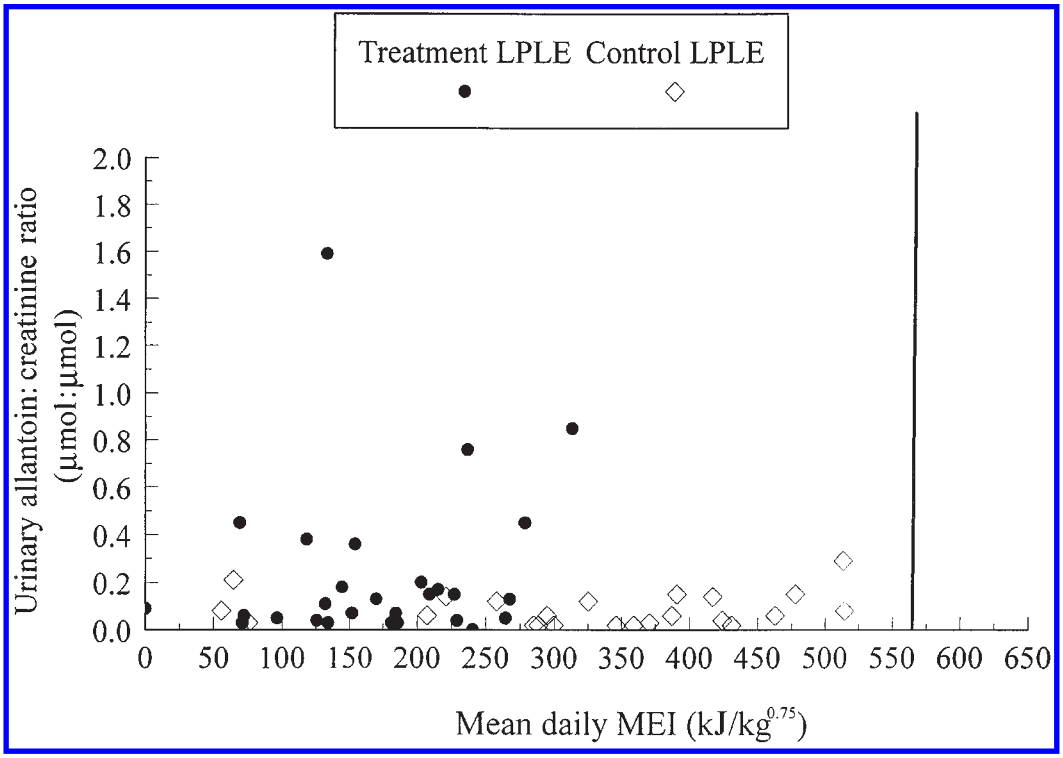


Fig. 3. Urinary allantoin:creatinine ratios versus recent (2 days prior to urine sampling) mean daily metabolizable energy intake (MEI) of captive adult white-tailed deer fed restricted (treatment) or ad libitum (control) amounts of a low-protein, low energy (LPLE) commercial diet from 11 February to 5 May 1988 at Grand Rapids, Minnesota. Sample sizes were four and three deer in the treatment and control groups, respectively. The thick vertical line demarcates the winter maintenance requirement of captive white-tailed deer (561 kJ/kg$^{0.75}$ body mass per day; Ullrey et al. 1970).



ratios when deer undergo acute severe energy restriction or long-term nutritional restriction and body-mass loss. The elevation of the urinary A:C ratio with severe restriction could be attributable to either an increase in 24-h excretion of urinary allantoin, a reduction in 24-h excretion of creatinine, or both. There are insufficient published data to determine the relative importance of these two mechanisms to the changes in A:C ratio; however, by integrating chemistry data from collections of snow-urines of free-ranging elk and simulation modeling of their physiology, DelGiudice et al. (2001) presented evidence that supports the importance of diminishing urinary creatinine output to the elevation of UN:C ratios with prolonged nutritional restriction. Further, short-term severe energy restriction can reduce urinary creatinine output as well (Van Niekerk 1962).

The literature on domestic ruminants and additional data from our deer provide some useful insights. Endogenous urinary allantoin excretion differs among species. Using intragastric infusion to preclude the influence of diet on urinary allantoin production, Chen et al. (1990*a*) reported that endogenous urinary allantoin excretion was 421 µmol/kg$^{0.75}$ per day in cattle and 92 µmol/kg$^{0.75}$ per day in sheep. We estimated A:C ratios associated with these endogenous allantoin outputs to be about 0.7 and 0.1–0.2 µmol:µmol, respectively. Using 24-h urinary allantoin and creatinine output from cattle and sheep at maintenance (Fujihara et al. 1987; Chen et al. 1990*a*; Puchala et al. 1993; Giesecke et al. 1994), we calculated mean urinary A:C ratios of 3.8 and 0.6 µmol:µmol, respectively. Consequently, we determined that in cattle and sheep at maintenance, allantoin derived from tissue turnover (i.e., endogenous) contributes roughly 17–19% of the allantoin to the A:C ratio. This is similar to findings of Fujihara et al. (1991) for domestic sheep and goats.

Although our deer were experiencing various degrees of negative energy balance throughout the study, their A:C ratios were more comparable to those of sheep than those of cattle. As in sheep, this may be attributable to low levels of xanthine oxidase activity in the blood of deer (Chen et al. 1990*a*). Similarly, the predicted endogenous A:C ratio of captive elk (0.1–0.2 µmol:µmol by extrapolation; Fig. 2 in Garrott et al. 1997) and A:C ratios of elk at maintenance (approximately 0.3–0.4 µmol:µmol) were much closer to those of sheep. Using data from Maloiy et al. (1970), we determined that A:C ratios of red deer were even lower than those of sheep fed similar diets. These findings suggest that the 24-h urinary excretion of allantoin by wild ungulates is relatively low, but the endogenous contribution to maintenance A:C ratios may also be about 17–19%.

The catabolism of endogenous protein accelerates with severe or prolonged dietary energy restriction of deer (Torbit et al. 1985; Moen and DelGiudice 1997), which presumably would increase endogenous allantoin excretion. Urinary UN:C ratios of our study deer were directly related (r^2 = 0.52, P = 0.04) to their cumulative body-mass losses (DelGiudice et al. 1994*b*); urea is the end-product of both dietary and endogenous protein metabolism. Further, the urinary 3-MeH:C ratio of these deer was even more strongly related (r^2 = 0.82, P = 0.0001) to their body-mass loss (DelGiudice

Fig. 4. Relationship of urinary allantoin:creatinine ratios to cumulative body-mass loss in captive adult white-tailed deer that consumed varying amounts of a low-protein, low-energy (LPLE) commercial diet from 11 February to 5 May 1988 at Grand Rapids, Minnesota. Sample sizes were four and three deer in the treatment and control groups, respectively.

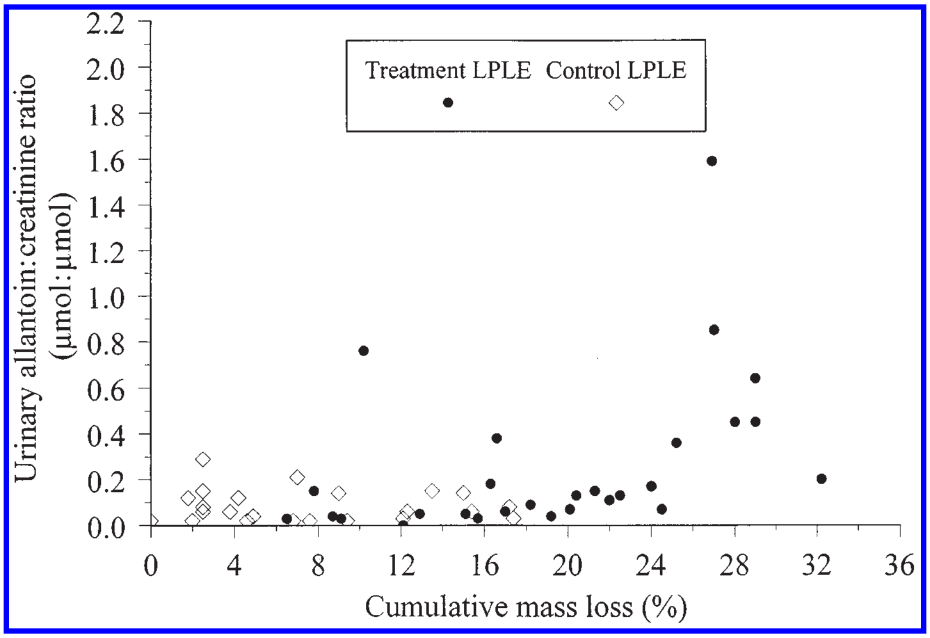


Table 1. Effect of short-term (15–19 April) severe nutritional restriction on recent (2 days prior to handling) daily mass-specific metabolizable energy intake (MEI) and urinary allantoin:creatinine (A:C) ratios of slightly and highly food-restricted white-tailed deer (*Odocoileus virginianus*) sampled on 11 and 19 April 1988 in Grand Rapids, Minnesota.

		Before severe restriction			After severe restriction		
Deer No.	Sex	Cumulative mass loss (%)	MEI ($\text{kJ/kg}^{0.75}$)	Urinary A:C ratio (µmol:µmol)	Cumulative mass loss (%)	MEI ($\text{kJ/kg}^{0.75}$)	Urinary A:C ratio (µmol:µmol)
LPLE restricted diet[a]							
79	M	29	279	0.45	>29.0		0.51
92	F	27.1	313	0.85	29		0.64
47	F	15	264	0.05	17	72	0.06
65	M	22.5	268	0.13	28.1	69	0.45
Mean		23.4	281.0	0.37	>25.8	70.4	0.42
SE		3.1	11.2	0.18		1.5	0.13
LPLE control diet							
55	F	4.7	424	0.04	7	64	0.21
06	F	2	346	0.02	17.4	76	0.03
66	M	15.1	417	0.14	17.2	56	0.08
Mean		7.3	395.8	0.07	13.9	65.2	0.11
SE		4	25.0	0.04	3.4	5.7	0.05

Note: Cumulative mass loss (since 4 February) data are from DelGiudice et al. (1994*b*). Deer 79 and 92 died on 18 and 19 April, respectively. Recent mean daily MEI could not be calculated for these deer, but it was limited to 65–70 $\text{kJ/kg}^{0.75}$ body mass or less.

[a] 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.

et al. 1998), and 3-methylhistidine is derived solely from the catabolism of muscle tissue. Like the A:C ratios, the increasing trend of UN:C and 3-MeH:C ratios began at about 20–24% body-mass loss, which may indicate that fat reserves had reached a critical low level and the catabolism of endogenous protein was accelerating. Chen (1989) noted that 24-h urinary allantoin excretion in steers increased by 33% during the first 3 days of fasting; however, the results of studies of the effects of short-term fasting in sheep have been inconsistent (Rys et al. 1975; Fujihara et al. 1991). Five- to 8-day fasts in sheep have resulted in 80–87% reductions in 24-h urinary allantoin excretion (Fujihara et al. 1991).

As with domestic ruminants, 24-h urinary creatinine excretion is relatively stable in captive deer at or above a maintenance level of nutrition (Antoniewicz and Pisulewski 1982; Fujihara et al. 1987; Puchala et al. 1993; DelGiudice et al. 1995, 1997), and creatinine coefficients (millimoles excreted per kilogram of body mass in 24 h) are reasonably similar among mammalian species (Greenblatt et al. 1976; Wallin 1979; Cool 1992; DelGiudice et al. 1995). While we found little in the literature concerning the effects of nutritional restriction on the 24-h excretion of creatinine in deer or other wild ungulates, reductions greater than 33% have been noted with short-term dietary undernutrition of various domestic ruminant and monogastric species (Blaxter and Wood 1951; Van Niekerk 1962; Chetal et al. 1975; Long et al. 1981; Hovell et al. 1983, 1987; Fujihara et al. 1991). Whether ruminants adapt physiologically with more prolonged undernutrition, and how that might affect creatinine excretion, are not known, but we observed four- to five-fold increases in mean A:C ratios of the treated deer, with even larger increases for individual subjects. Assuming that excretion of allantoin remained stable, creatinine output would have to decrease by 50% just to double the A:C ratio. Consequently, it is very likely that on a mass-specific basis, increases in endogenous urinary allantoin and diminished creatinine excretion were both contributing to the elevated A:C ratios of nutritionally restricted deer.

The logical progression of research dictated that a study such as ours extend earlier efforts by determining whether moderate to severe winter nutritional restriction and body-mass losses similar to those expected in northern free-ranging ungulates affected urinary A:C ratios in a way not predicted by the boundaries and results of previous efforts. For relative and quantitative nutritional assessments, establishing reference values of urinary chemistries relative to known levels of energy intake and body condition is essential to accurate interpretation of their physiological meaning and significance. This has been widely acknowledged in the medical and veterinary sciences and clinics (Davidson and Henry 1969; Coles 1980; Benjamin 1981; Shils and Young 1988). Reference data, whether for their *relative* value or *actual* value, are at least as important for the interpretation of nutritional assessments of free-ranging wild ungulates and other animals (Seal et al. 1981; Harder and Kirkpatrick 1994; DelGiudice 1995; Vagnoni et al. 1996).

Our data suggest that higher or increasing energy intake is not the only cause of higher or increasing A:C ratios. Pils et al. (1999) reported A:C values from snow-urines of free-ranging elk calves that began increasing by mid-January to early February, earlier than for the cows sampled during each of five winters. By late March to early April of four winters, mean ratios increased to higher than 0.50 μmol:μmol. This was up to two times higher than those of the adult cows. With no previous reports addressing the effects of prolonged nutritional restriction and the full range of body-mass losses on A:C ratios in cervids, Pils et al. (1999) interpreted the calves' ratios as being directly related to MEI. According to Garrott et al. (1997), this could indicate an MEI (950 kJ/kg$^{0.75}$) far greater than the mass-specific winter maintenance requirement of elk, suggesting that calves should be gaining mass. Based on our study and an increased understanding of undernutrition and urinary A:C ratios, a more plausible explanation is that the early increasing trend of A:C ratios of the calves was indicative of greater nutritional restriction than in the cows. This is strongly supported by urinary UN:C ratios in snow-urines collected from known elk cows and calves and trends in calves per 100 cows in the same study area during two of the five winters (White et al. 1995).

Our study indicates that caution must be exercised when interpreting urinary A:C ratios of free-ranging ungulates. More rigorous quantification of the physiological relations influencing the urinary excretion of allantoin is required. Additional research should include simultaneous examination of the effects of short-term and prolonged nutritional restriction and recovery on body composition, and 24-h urinary output of allantoin and creatinine. Study focused on 24-h urinary excretion of endogenous allantoin, applying methods used with domestic ruminants (e.g., intragastric infusion), would be useful for improving our understanding of the influence of tissue catabolism on A:C ratios at various planes of nutrition and physical condition. Further, field studies sequentially estimating digestible energy intake of free-ranging ungulates while monitoring them using snow-urine A:C ratios as winter progresses may enhance the value of this characteristic as a nutritional index.

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