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# Urinary metabolites as an index of body condition in wintering white-tailed deer *Odocoileus virginianus*

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We tested whether snow-urine ratios to creatinine of urea nitrogen (U:C), potassium (K:C), allantoin (A:C) and 3-methylhistidine (M:C) could be used to determine when to initiate an emergency feeding program in white-tailed deer *Odocoileus virginianus*. Food distribution to 11 experimental adult deer was gradually reduced over 64 days to simulate the conditions occurring for wild deer during winter. At the end of the partial fasting period, experimental deer had lost 19% of body mass on average. The animals were then fed *ad libitum* during a 13-day recovery period. A control group of four deer was fed *ad libitum* during the entire study. Control deer lost 6% of body mass during the experiment. Results for U:C and K:C ratios suggest that they were unreliable as indicators of physical condition of white-tailed deer during winter, at least within the physiological range and sample size considered in this study. A:C ratios showed fluctuations that were congruent with current knowledge of fasting physiology. A:C ratios of experimental deer relative to control deer, however, increased significantly only after 64 days of partial fasting, when animals had lost 19% of body mass. At that time it may already be too late to launch an effective feeding program. K:C and A:C ratios also increased during the recovery period, illustrating the potential difficulty of determining whether such an increase results from starvation or from resumed food intake. Concentrations of 3-methylhistidine in the snow remained too low to be detected, due to dilution. We conclude that, under the limits of this study, none of the creatinine ratios represents an accurate index of body condition to determine when to initiate an emergency feeding program.

*Key words: allantoin, creatinine, deer, fasting, Odocoileus virginianus, physical condition, potassium, urea nitrogen, urine, winter*

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It has been suggested that the nutritional status of ruminants can be assessed from levels of different metabolic end products found in urine (e.g. allantoin, urea nitrogen, sodium, potassium, cortisol and 3-methylhistidine) because appearance of metabolites in the urine is correlated with energy intake of the animal (e.g. elk: Vagnoni et al. 1996, Garrott et al. 1997, White et al. 1997, Pils et al. 1999; white-tailed deer: DelGiudice & Seal 1988, DelGiudice et al. 2000). Urine samples can easily be obtained during winter in the presence of snow, even from a free-ranging population. This non-invasive method of collecting snow-urine to monitor wild animals has recently stimulated interest among wildlife managers. There are some concerns, however, about its limitations as an indicator of physical condition (for review see Saltz & White 1991, Case 1994, DelGiudice 1995, Saltz et al. 1995, White et al. 1995a, White et al. 1995b, Moen & DelGiudice 1997, Pils et al. 1999, Del Giudice et al. 2000).

Deep snow over an extended period of time can result in starvation of more than 40% of the white-tailed deer *Odocoileus virginianus* population at the northern limit of its range (Potvin et al. 1981). Postmortem analysis showed that mortality would occur following 30-37% loss of body mass in adult deer (Davenport 1939, de Calesta et al. 1975, Moen & Severinghaus 1981, Severinghaus 1981). An emergency feeding program began in 1997 in southeastern Québec to reduce starvation losses during severe winters (Rioux et al. 1998, Etcheverry et al. 2001, Ouellet et al. 2001), but when to initiate supplemental feeding is not precisely understood. At present, the emergency feeding program is launched on the basis of cumulative snow-sinking depth, which is positively correlated with winter mortality rate (Potvin & Breton 1992).

The physiology of fasting is an active area of research and has been well described in the literature (e.g. Cahill 1976, Cherel & Le Maho 1985, Torbit et al. 1985, Belkhou et al. 1990, Saltz et al. 1995): glycogen reserves of animals become exhausted within a few hours of fasting (phase-I of fasting physiology) and to survive a long period of reduced food intake, mammals and birds rely mostly on fat to fuel metabolism (phase-II) rather than protein of muscular origin. When lipid reserves have been depleted, lean tissue is metabolized (phase-III). Such fasting physiology is reflected in the rate of body mass loss over time, which is high and declines in phase-I, low during phase-II and increases to become high again in phase-III.

Potassium in urine is conserved via reabsorption in the kidneys and fasting ruminants display a low potassium excretion rate (Gans & Mercer 1984, Schmidt & Gutleb 1997). Starving ruminants, however, show high values, reflecting cell destruction and potassium release from tissues in moribund animals (DelGiudice 1995). Urea nitrogen in urine also reflects a complex physiology because it is affected by protein intake, energy intake, tissue catabolism, diuresis and urea recycling (Warren et al. 1982, Case 1994, White et al. 1995b, Moen & DelGiudice 1997). Saltz & White (1991) and DelGiudice et al. (1994) first reported a curvilinear increase of U:C with increasing body mass loss. Allantoin is the end product of purine degradation in ruminants and thus should be more accurate than urea nitrogen in describing the physical status of white-tailed deer (Garrott et al. 1996). 3-methylhistidine occurs in actin and myosin of skeletal muscles. There are no exogenous sources of this metabolite for herbivores, and so it could be used as an index of protein breakdown and physical condition of deer (DelGiudice et al. 1998).

We tested whether certain urinary metabolites (i.e. ratios of allantoin, urea nitrogen, potassium and 3-methylhistidine to creatinine) found in snow-urine samples could be used to determine body condition of free-ranging individuals. Body condition in this study refers to percent of starting body mass, i.e. prior to a food restriction period (Cabanac 2003). Furthermore, we wished to determine whether we could use those metabolites to signal when to trigger an emergency feeding program for deer.

## Material and methods

From December 1997 to March 1998, 12 experimental and four control adult female white-tailed deer were kept in individual pens (4 × 5 × 2.5 m) on a deer farm in St-Valérien, Québec, Canada. All animals were pregnant. Prior to the beginning of the experiment, deer were fed once a day with a mixture of grass, alfalfa hay and oat grain for a three-week habituation period to food and habitat. Then they were fed a pelleted feed (winter feed: a mixture of sawdust, ground yellow corn, barley grain, oat grain, ground soy bean, beet pulp, cane molasses, limestone and dicalcic phosphate, with lignosulfate as a binding agent) that included 35% maple *Acer* sp. sawdust, with fibers 4-5 mm in length (Ouellet et al. 2001). The protein content (9.7%) and fiber content of the pellet feed resembled that of natural forage usually consumed by wild deer in winter, i.e. twigs. Throughout the experiment, ca 0.15 kg/day of first-cut hay was also supplemented to reflect the minimal amount of food with long fibers available to wild deer. From 7 January to 11 March (64 days), we gradually reduced the

amount of food provided daily to the experimental deer to induce a 20% reduction in body mass (referred to as partial fasting). Such proportional mass loss is expected in deer that are nutritionally stressed at the end of winter (DelGiudice et al. 1992, Mrosovsky 1990, Worden & Perkins 1995). In the first week of partial fasting, we provided 60% of the food consumed during the preceding week. Thereafter, we gradually decreased the amount of food provided on an individual basis, to reach the target of 20% body mass reduction. For unknown reasons, one individual did not lose substantial weight during the partial fasting period, and this deer was excluded from the results. From 12 to 25 March (i.e. days 65-77 since the beginning of the experiment) deer were fed *ad libitum*, with a diet composed of 75% winter feed (see above) and 25% wood (aspen sawdust with a high soluble cell content (59.6%) of neutral digestible fiber), husk (alternate pellet feed with soy husk as the fiber source) and/or hay (a second-cut grass hay dominated by orchard grass). We termed this 13-day period the recovery period for experimental deer. The control group of four individuals was fed the winter feed diet *ad libitum* plus the first-cut hay supplement throughout the experiment. The effect on body mass of different diets offered to deer during a recovery period has been previously discussed (Ouellet et al. 2001). That work concluded that the use of pelleted feeds should be favoured to achieve rapid recovery of undernourished white-tailed deer in winter (Etcheverry et al. 2001, Ouellet et al. 2001). Animals were cared for in accordance with the principles and guidelines of the Canadian Council on Animal Care.

Urine samples were collected in the snow on days 1, 8, 21, 29, 64, 69 and 77, on the same days that deer were weighed (± 0.5 kg). All individual pens were carefully cleaned and covered with fresh snow the day before each sampling session. Urinary analyses for creatinine, urea nitrogen and potassium concentrations were performed on a Vitros analyzer (Johnson and Johnson Clinical Diagnostics Inc, Rochester, New York). Allantoin concentrations were analyzed by high-performance liquid chromatography (HPLC), using C18 Waters Column, Spectra 100 UV-Vis detector (Spectra-Physics) and SP4100 Computing integrator (Spectra-Physics), according to the technique described by Chen et al. (1993). Concentrations of 3-methylhistidine were determined by HPLC using LC 18 AccQ. tag amino acid Column 3.9 mm X 150 mm (4 mm), fluorescence detector Waters 420 and HPLC pump Waters 600. We used AccQ-Fluor Reagent Kit® (No. 052880) and AccQ. tag Eluent A Concentrate ® (Wat052890). The 3-methylhistidine and amino acid standards were purchased from Sigma. Urea nitrogen, potassium and allantoin concentrations were expressed as ratios to creatinine (U:C, K:C and A:C, respectively) in mg/mg (DelGuidice 1995).

We conducted an analysis of variance (ANOVA) for repeated measures using the MIXED Procedure (SAS 1990), with Least Squares Means Statement (LSM) as *post hoc* comparisons to test the effect of treatment on body mass and on the above urinary metabolite ratios over time. U:C, K:C and A:C ratios were log-transformed to ensure normality. To avoid lengthy presentations of statistical results, only the lowest or highest probabilities are given for a certain series of comparisons. The relationships between body mass loss and urinary metabolites were tested with an analysis of covariance (ANCOVA) using the MIXED Procedure (SAS 1990) with individual as a random effect. Statistics were conducted using individual values but results presented in Figures 1-3 are expressed as means with the standard deviation (mean ± SD) whereas Figure 4 shows individual values.

## Results

### Body mass

All animals consumed their entire daily food ration during the restriction period. During the partial fasting period, mean body mass of experimental deer gradually diminished from 56.8 to 48.8 kg. The range in individual body mass loss was 15-26% of original body mass, for a mean 18.8 ± 2.7% body mass reduction in 64 days ($F_{6,73} = 18.98$, $P < 0.0001$; Fig. 1).

During the first day of the recovery period, daily food intake of experimental deer remained low, but gradually increased over the next 4-5 days to reach

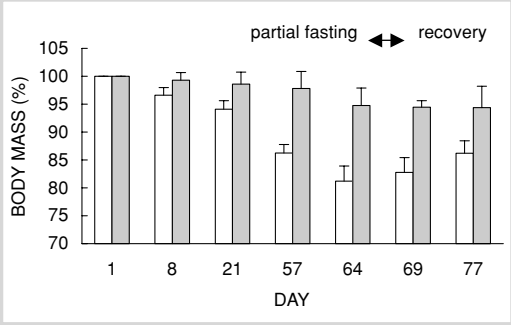


Figure 1. Mean body mass (± SD) of 11 experimental (□) and four control (■) white-tailed deer during partial fasting (days 1-64) and recovery (days 65-77), expressed as percentage of body mass on day 1.

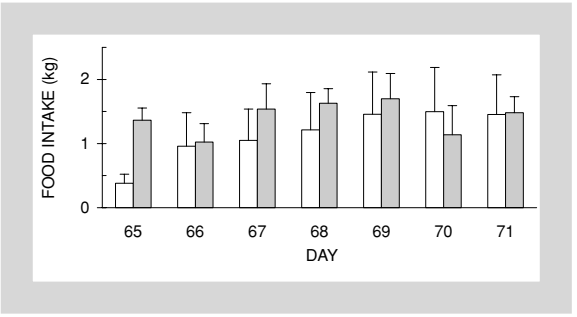


Figure 2. Mean daily food consumption (± SD) of 11 experimental (□) and four control (■) white-tailed deer during the first week of recovery (days 65-71).

similar levels as those of control deer (Fig. 2). On the 5th and 13th days of recovery (days 69 and 77), mean body mass of the experimental deer increased to 47.1 and 48.8 kg (17 and 14% reduction in body mass, respectively), resulting in a gain of 5% in body mass (relative to day 1) over 13 days.

Even though the four control deer had access to *ad libitum* food, they showed a slow but continuous loss in body mass from day 1 to day 77. Mean body mass loss was 5.2% (± 3.1%) on day 64 and 5.6% (± 3.8%) on day 77 (a loss of approximately 3 kg; see Fig. 1). Body mass of the experimental and control groups differed statistically after the first eight days of the experiment ($P < 0.01$).

### Urea nitrogen:creatinine

The time*treatment interaction was statistically significant for U:C ratios ($F_{6,13} = 10.57$, $P < 0.0002$). Experimental and control deer were not statistically different ($P > 0.36$) on any one sampling day (mean U:C ratio of experimental deer for the partial fasting period was 8.6 ± 2.6; Fig. 3). However, the experimental group showed higher values on day 77 than for all previous days ($P < 0.04$) except day 64 ($P = 0.77$).

### Potassium:creatinine

The time*treatment interaction was also statistically significant for K:C ratios ($F_{6,71} = 5.41$, $P = 0.0001$). Experimental deer showed higher values than control deer on day 64, the last day of the partial fasting period (day 1-57: $P > 0.24$; day 64: $P = 0.0001$), and during recovery ($P < 0.0001$; see Fig. 3). For the experimental group, K:C ratios were much higher during the recovery period than the partial fasting period ($P < 0.0007$), however, within each period K:C ratios did not differ statistically over time (partial fasting: $P > 0.20$; recovery: $P = 0.061$). Accordingly, the difference recorded between

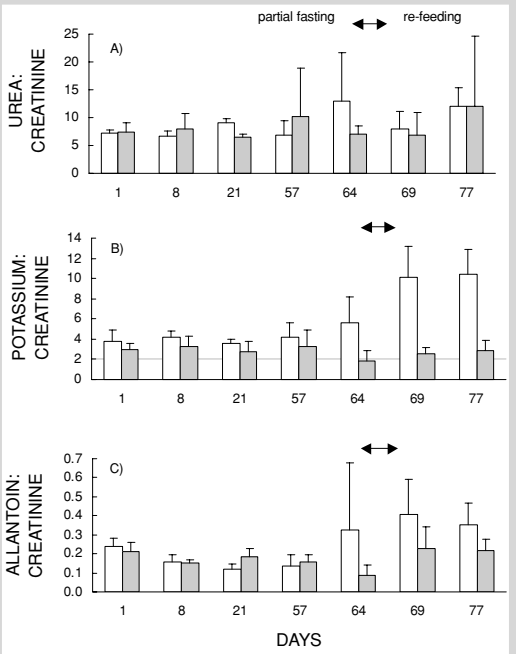


Figure 3. Mean (± SD) urea (A), potassium (B) and allantoin (C) to creatinine ratios (mg:mg) in urine of 11 experimental (□) and four control (■) white-tailed deer during partial fasting (days 1-64) and recovery (days 65-77).

the two treatment groups on day 64 partly resulted from a reduction in the K:C ratio for control deer compared to previous days ($P < 0.042$; mean plateau value for K:C of experimental deer for the partial fasting period was 4.3 ± 0.77).

### Allantoin:creatinine

The time*treatment interaction was statistically significant for A:C ratios ($F_{6,70} = 2.64$, $P = 0.023$). A:C ratios differed between experimental and control deer on day 64 ($P < 0.029$), the last day of the partial fasting period (see Fig. 3). This was due to an increase in A:C for experimental deer combined with a decrease for control deer. Indeed, A:C ratios for the experimental group decreased significantly from day 1 (0.236 ± 0.046) to day 8 (0.155 ± 0.040; $P < 0.033$), remained low through day 57 (0.137 ± 0.061; $P > 0.15$), then increased on day 64 (0.324 ± 0.355) compared to day 21 ($P = 0.027$) and day 57 ($P = 0.06$). Furthermore, the A:C ratio for the control group on day 64 was lower than on all other sampling days (days 1, 21, 69 and 77: $P < 0.011$; days 8 and 57: $P = 0.057$). For experimental deer, A:C ratios measured during the recovery period were higher than on all previous days ($P < 0.017$), except day 1 ($P = 0.079$).

### 3-Methylhistidine:creatinine

Concentrations of 3-methylhistidine were too low to be detected. Even on day 64 of experimentation (at a time when M:C ratios should have been highest), as many as eight out of 10 sampled deer showed no measurable results. The highest concentration recorded was 5.5 picomol/L from one individual that had lost 23% of body mass at that time (day 64).

### Urinary metabolites compared to body mass loss

A rise in metabolite ratios seemed to take place for the experimental group above a cumulative mass loss of ca 22% (Fig. 4). However, during the food restriction period, there was no significant relationship between A:C and U:C ratios and body mass loss of experimental deer ($F_{1,50} = 3.07$, $P = 0.09$, and $F_{1,51} = 0.55$, $P = 0.46$, respectively). However, K:C ratios increased with respect to body mass loss ($F_{1,50} = 12.39$, $P = 0.001$). The relationship between the various metabolites and body mass loss did not differ among individuals ($F_{10,50}$ = 0.38 to 0.56, $P > 0.05$).

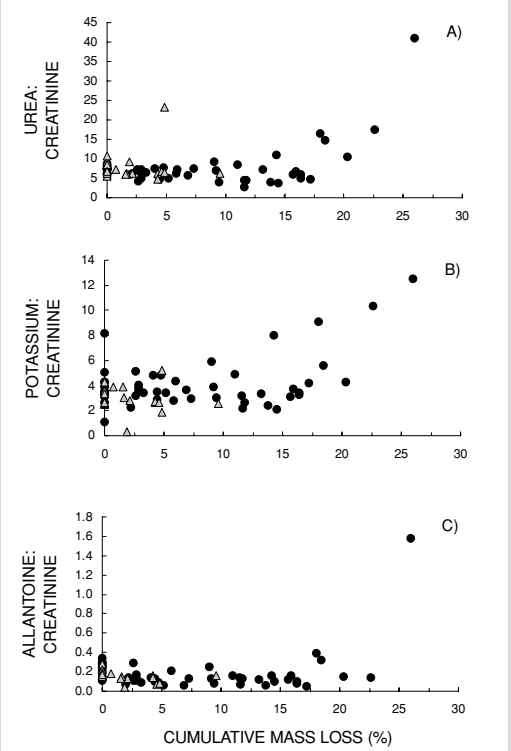


Figure 4. Urea (A), potassium (B) and allantoin (C) to creatinine ratios (mg:mg) in urine of 11 experimental white-tailed deer during partial fasting (●) and four control white-tailed deer (▲) compared to cumulative mass loss.

## Discussion

Reductions in body mass of experimental deer recorded in our study were similar to those of wild deer during winter (DelGiudice et al. 1992, Worden & Perkins 1995). In addition, the decline in body mass of the control deer was in agreement with results from other studies on ungulates fed *ad libitum* in winter (Holter et al. 1977, Severinghaus 1981, Mrosovsky 1990, Saltz & White 1991, DelGiudice et al. 1992, Case 1994, Fuller et al. 2001). This suggests that our protocol and conclusions could also apply to wild deer.

The barely detectable concentrations of 3-methylhistidine were likely due to the diluting effect of the snow, since urine collection was carried out using snow-urine sampling, and suggests that the protocol was inappropriate to detect 3-methylhistidine at such low concentrations. DelGiudice et al. (1998) reported a curvilinear relationship between M:C and body mass loss when they sampled urine directly from the urinary bladder of individual deer. Since we were seeking a non-invasive approach to monitor physical condition, we can not recommend the use of M:C ratios found in snow-urine as a tool to monitor physiology of wild animals.

We found that K:C and U:C ratios in urine were unreliable indices of the physical condition of adult white-tailed deer during the food restriction period. This was because the experimental and control groups could not be distinguished using either ratio over time during the restriction period. Saltz & White (1991) and DelGiudice et al. (1994) reported a curvilinear increase of U:C with increasing body mass loss, starting at body mass loss of ca 25-30%. Our discrepancy may be explained by the fact that our animals only lost 19% of body mass on average. Changes in potassium excretion rate have been reported comparing fasting (low rate) to starving (high rate) ruminants (Gans & Mercer 1984, DelGiudice 1995, Schmidt & Gutleb 1997), but again, our animals were not moribund.

Changes in allantoin excretion rate over the partial fasting period showed, however, some interesting variations that are congruent with current knowledge of fasting physiology. Phase-I would be marked by a decrease in excretion rate, as seen on the second sampling (day 8); phase-II, where ketone bodies and lipid reserves are utilized to fuel metabolism, would correspond to the constant low excretion rate that lasted for at least 50 days; and the critical phase-III, when lipid reserves become depleted and animals must burn endogenous protein reserves through gluconeogenesis, would be marked by an increase in A:C ratios, as seen on day 64. Mean body mass loss was 19% on day 64, which was a marked in-

crease compared to only 14% seven days before (see Figs. 1 and 4).

Anecdotally, we noted that one female in our study had reached a physiological limit because she underwent spontaneous abortion on day 64, after losing 26% body mass. Interestingly, the A:C ratio of that individual was 1.58 at the time, which was 10 times higher than on day 1. Eventually, this female lost 29% of its body mass and recovered with difficulties thereafter. Similar increases in A:C (DelGiudice et al. 2000) and M:C (as collected from the urinary bladder; DelGiudice et al. 1998) have been shown in white-tailed deer, starting at ca 25% of body mass loss. These observations suggest that phase-III of starvation commences at a loss of ca 20-25% body mass in white-tailed deer.

Potassium, urea nitrogen and allantoin levels in urine reflect a complex physiology because they are affected by protein intake, energy intake, tissue catabolism, diuresis and urea recycling (Warren et al. 1982, Case 1994, White et al. 1995b, Moen & DelGiudice 1997). Therefore, it is possible that we failed to record substantial changes because our experimental deer were only partially fasting as they normally do in their natural environment in winter. In addition, ruminants digest a certain amount of microbial flora originating from the rumen or lower digestive tract, even when energy intake is insufficient to meet maintenance requirements (de Calesta et al. 1977, Holter & Hayes 1977, Church 1984, DelGiudice et al. 1987, Moen & DelGiudice 1997). Such microbial digestion certainly delays the physiological response to food restriction and further reduces the utility of A:C, U:C as well as K:C, as indicators of physical condition in free-ranging animals (de Calesta et al. 1977, Saltz et al. 1995, White et al. 1995b, Moen & DelGiudice 1997).

On the other hand, it is also possible that significant increases in metabolite ratios require starving animals and again our intention was not to kill the deer. Mammals survive fat utilization until depletion, however, they can not survive more than 30-50% depletion of protein reserves (Cahill 1976). In deer, several studies have reported that mortality occurs at losses of about 30-37% body mass (Davenport 1939, de Calesta et al. 1975, Moen & Severinghaus 1981, Severinghaus 1981). A:C ratios were elevated only after 64 days, when deer had lost on average 19% of body mass. Given that phase-III of fasting commences at ca 20-25% of body mass loss and that lethal body mass loss occurs at 30-37% (Davenport 1939, de Calesta et al. 1975, Severinghaus 1981), then it is likely that when metabolite ratios become significantly high, deer have only a few days before the effect of prolonged undernutrition becomes irreversible. This would be even more inaccurate for U:C ratios which would rise only after a 25-30% decrease in body mass (Saltz & White 1991, DelGiudice et al. 1994) and for K:C ratios which would rise only when the deer are moribund (Gans & Mercer 1984, DelGiudice 1995, Schmidt & Gutleb 1997). We also showed that malnourished captive deer take several days to increase food intake in the presence of *ad libitum* supplemental feed in winter. In addition, Etcheverry et al. (2001) suggested that wild deer spend additional time before finding food supplements and consuming a large proportion of the food provided. These problems, combined with the time required to analyze urine samples, tend to shorten the time window during which an effective emergency feeding program could be launched.

Our study also pointed out that K:C and A:C ratios increased during the recovery period relative to the partial fasting period, which underlines the fact that these ratios may be an index of food intake as documented in the literature on ungulates (DelGiudice et al. 1987, DelGiudice et al. 1992, White et al. 1997). Accordingly, it would be difficult to distinguish whether an increase in ratios resulted from starvation or increased food intake. This measure becomes even more complicated when the public offers food supplements to wild animals (Dumont et al. 2000).

## Conclusion

We were seeking a non-invasive approach to monitor physiological condition of white-tailed deer in winter. Even though experimental deer lost an average 19% of body mass due to partial fasting, our results showed that none of the snow-urine end products (urea nitrogen, potassium, allantoin and 3-methylhistidine to creatinine ratios) were satisfactory in describing the physiological status of the animals. The metabolites did not accurately measure body condition and thus could not be used to determine when to initiate an emergency feeding program in winter.

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