Energy expenditure of free-living reindeer estimated by the doubly labelled water method

Geir Gotaas[1], Eric Milne[2], Paul Haggarty[2] & Nicholas J. C. Tyler[1,3]

[1] Department of Arctic Biology and Institute of Medical Biology, University of Tromsø, N-9037 Tromsø, Norway (2g@mail.com).
[2] Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen AB21 9SB, United Kingdom.
[3] *Present address:* Department of Biology, University of Tromsø, N-9037 Tromsø, Norway.

Abstract: The doubly labelled water (DLW) method was used to measure total energy expenditure (TEE) in three male reindeer (*Rangifer tarandus tarandus*) aged 22 months in winter (February) while the animals were living unrestricted at natural mountain pasture in northern Norway (69°20'N). The concentrations of ^{2}H and ^{18}O were measured in water extracted from samples of faeces collecred from the animals 0.4 and 11.2 days after injection of the isotopes. Calculated rates of water flux and CO_2-production were adjusted to compensate for estimated losses of ^{2}H in faecal solids and in methane produced by microbial fermentation of forage in the rumen. The mean specific TEE in the three animals was 3.057 $W \cdot kg^{-1}$ (range 2.436 - 3.728 $W \cdot kg^{-1}$). This value is 64% higher than TEE measured by the DLW method in four captive, non-pregnant adult female reindeer in winter and probably mainly reflects higher levels of locomotor activity in the free-living animals. Previous estimates of TEE in free-living *Rangifer* in winter based on factorial models range from 3.038 $W \cdot kg^{-1}$ in female woodland caribou (*R. t. caribou*) to 1.813 $W \cdot kg^{-1}$ in female Svalbard reindeer (*R. t. platyrhynchus*). Thus, it seems that existing factorial models are unlikely to overestimate TEE in reindeer/caribou: they may, instead, be unduly conservative. While the present study serves as a general validation of the factorial approach, we suggest that the route to progress in the understanding of field energetics in wild ungulates is via application of the DLW method.

Key words: cervid, energetics, *Rangifer*.

Rangifer, 20 (4): 211–219

Introduction

The main advantage of the doubly labelled water (DLW) method for measuring CO_2-production (Lifson *et al.,* 1955; Lifson & McClintock, 1966) over alternative methods fot measuring energy expenditure, such as respirometry, is that it permits the study of subjects living unrestricted in their natural environment. All published estimates of TEE in free-living reindeer and caribou (*Rangifer tarandus*) are based on factorial models in which activity budgets have been combined with the known energetic costs of different types of behaviour (e.g. Boertje, 1985; Fancy, 1986; Tyler, 1987). An advantage of factorial models has been their low cost compared to the DLW method but with the reduction in the cost of isotopes and improvement in mass spectrometry, the difference in price between the two procedures has been greatly

reduced. One weakness of factorial models is that collection of data is labour intensive and time consuming. Furthermore, activities are usually assigned to just a few standard categories (e.g. lying down, standing, grazing, walking), while largely ignoring the variation in energy expenditure within each of these categories (e.g. the change in energy costs associated with running at different velocities or over different substrates (e.g. Thing, 1977; White & Yousef, 1978)). Lastly, factorial models are normally based on standard energy costs and mean activity budgets so that between animal variation in metabolism and levels of activity is inevitably overlooked (Parker *et al.*, 1990; Midwood *et al.*, 1994).

The DLW method has been used to measure rates of energy expenditure in more than 60 species of mammals (Nagy, 1994) but only six species of ruminants (Fancy *et al.*, 1986; Nagy *et al.*, 1990; Parker *et al.*, 1990; Midwood *et al.*, 1994; Nagy & Knight, 1994; Gotaas *et al.*, 1997; Haggarty *et al.*, 1998). Reluctance to apply the DLW-method in ruminants has been due in part to uncertainty regarding (i) the effect of the large volume of water in the gastrointestinal system relative to the total body water volume on the rate of equilibrarion of isotopically labelled water and (ii) the importance of 'non-water loss' of ^{2}H in microbially produced methane, in faecal solids and through sequestration in *de novo* synthesised tissue (e.g. Midwood *et al.*, 1993).

The primary aim of the present study was to estimate TEE in reindeer living unrestricted at natural mountain pasture in winter using the DLW method. Under such conditions, the need to catch animals for sampling presents considerable practical problems. To overcome these practical problems we have developed an approach which requires only the sampling of faeces (Gotaas & Tyler, 1995; Gotaas *et al.*, 1997). The concentration of injected markers (^{2}H and ^{18}O) can then be measured in water extracted from faeces samples. The secondary aim of the study was to compare estimates of TEE based on DLW measurements (adjusted for non-water losses of ^{2}H) in free-living animals with TEE determined in captive reindeer (also using DLW, Gotaas *et al.*, 1997) and TEE estimated in free-living caribou and Svalbard reindeer using factorial models (Boertje, 1985; Fancy, 1986; Tyler, 1987).

Methods

Experimental procedures

Experiments were conducted from 7th February to 11th March 1996 on five young (22 months) male reindeer (*R. t. tarandus*) A, B, C, D, E, mean body mass (BM) 61.1 (s = 3.6, range 54.5 - 65.0) kg, in a herd of approximately 350 free-ranging, semi-domesticated reindeer in Finnmark, Norway (69°20'N). The herd was gathered and the experimental animals were caught by hand and weighed to 0.1 kg on an electronic balance (Alpha 2001C, Farmer Tronic, Denmark). Four of the animals (B, C, D and E) were injected with sufficient sterile, pyrogen-free $^{2}H_{2}{}^{18}O$ (160 ml, approximately 10% enrichment of both isotopes) to enrich the body water ^{2}H and ^{18}O by approximately 300 ppm (part per million) excess. The fifth animal (A) was injected with 160 ml sterile physiological saline. All doses were injected through the body wall on the left hand side of the animal directly into the dorsal sac of the rumen at a point approximately 5 cm caudal to the 14th rib and approximately 5 cm ventral to the apex of the transverse process of the 2nd lumbar vertebrum (Engebretsen, 1975). The exact amount of DLW injected was determined gravimetrically by weighing the dose syringes with their needles to 0.001 g before and after injection.

One sample of faeces (approximately 25 g wet weight) was collected from each animal prior to injection of DLW for determination of the background levels of ^{2}H and ^{18}O in the body water. Samples were taken from the rectum and put into screw-cap plastic vials which were immediately sealed and then stored at -20 °C until analysed.

Following injection of the isotopes the animals were tethered and provided with food (lichens *Cladonia* spp.) and snow *ad lib.* until new samples of faeces were collected (as above) at approximately 11 h post-injection at which time the isotopes were assumed to have reached equilibrium in the body water pool (Gotaas & Tyler, 1995). Thereafter all five animals were released back into the herd in which they then moved freely, grazing on natural mountain pasture for the remainder of the experiment. We were subsequently unable to locate one of the experimental animals (reindeer D) but the remaining four animals were recaught, weighed and samples of faeces were collected at 11, 22 and 32 days post-injection in the manner described above.

Water was extracted from two or three portions of each sample of faeces (each portion approximately 2 g wet weight) by vacuum sublimation (Midwood, 1990). The sublimated water was combined by sample and stored in 5 ml cryo-tubes (Greiner Labortechnik, Germany) at -20 °C until analysed.

Ambient temperatures during the field trial were recorded at the Norwegian Meteorological Institute weather-station at Cuovddatmohkki, approximately 12 km from where the herd was grazing.

Analyses

The concentration of ^{2}H and ^{18}O was measured in sublimated faeces water, in diluted solutions of the dose and in the diluting water. ^{18}O was determined after equilibration of $H_2{}^{18}O$ with CO_2 (Midwood *et al.*, 1992) on a SIRA 12 isotope ratio mass spectrometer (VG Isogas, Middlewich, UK). $^{2}H_2O$ was converted to hydrogen gas by zinc reduction (Wong *et al.*, 1987), with the modification that 500 mg zinc was used for the reduction, and the ^{2}H content was determined using a SIRA 10 isotope ratio mass spectrometer (VG Isogas). All analyses of ^{2}H and ^{18}O were performed in at least three replicates and the mean ppm values were used in subsequent calculations (within sample CV <0.450% for ^{2}H and <0.066% for ^{18}O).

Calculations

Body water pool size, rate constants for disappearance of ^{2}H and ^{18}O (k_D and k_O), water flux (J_{H2O}) and rate of CO_2-production (r_{CO2}) were calculated using the two-point method. The difference in rates of disappearance of ^{2}H and ^{18}O was assessed from analysis of samples collected at equilibrium and on day 11 of each trial. Parameters were calculated according to Coward (1990), Schoeller & Coward (1990) and Haggarty *et al.* (1994), while errors were estimated according to Cole *et al.* (1990) and Haggarty *et al.* (1994). To investigate the effect of an increase in the study duration on the two-point calculations, r_{CO2} and TEE were also calculated using the samples collected on day 22 and 32, respectively, as the last-point sample.

The parameters J_{H2O} and r_{CO2} were calculated as follows:

$$J_{H2O} = \frac{k_D \cdot N_D}{(f_1 \cdot X) + (1-X)} \qquad \text{(equation 1)}$$

and

$$r_{CO2} = \frac{k_O \cdot N_O - [(f_2 \cdot X \cdot J_{H2O}) + (1-X) \cdot J_{H2O}]}{2 \cdot f_3} \qquad \text{(equation 2)}$$

The fractionation factors f_1 ($^{2}H_2O$ [vapour] : $^{2}H_2O$ [liquid]), f_2 ($H_2{}^{18}O$ [vapour] : $H_2{}^{18}O$ [liquid]) and f_3 ($C^{18}O_2$ [gas] : $H_2{}^{18}O$ [liquid]) were taken as 0.94, 0.99 and 1.04, respectively (Schoeller & Coward, 1990). X denotes the proportion of the total water loss assumed to undergo fractionation and was calculated using an iterative approach (Haggarty *et al.*, 1998). Initially, X was set at 0.4, and all parameters were calculated for each animal based on this value. A new value for X was then obtained using the following equation:

$$X = \frac{TEE \cdot 1000 \cdot 0.45}{2.418 \cdot J_{H2O}} \qquad \text{(equation 3)}$$

where TEE is given in $MJ \cdot d^{-1}$, J_{H2O} is given in $g \cdot d^{-1}$, 0.45 is the proportion of heat lost evaporatively in reindeer (Folkow & Mercer, 1986) and 2.418 $kJ \cdot g^{-1}$ is the latent heat of evaporization of water (Blaxter, 1989). The iterative calculation of X and TEE was repeated until no further change in eithet parameter was observed, at which point the mean value of X for the three reindeer was 0.55.

The calculated values for J_{H2O} and r_{CO2} were adjusted to compensate for non-water losses of ^{2}H in faecal solids and microbially produced methane (CH_4) in the following manner. Total faecal dry matter production was estimated assuming an intake of lichen (*Cladonia* spp.) of 15 g dry matter per kg BM per day (Gotaas *et al.*, unpubl.) and a lichen digestibility of 86.2% (Sletten & Hove, 1990). Furthermore, we assumed an apparent water flux due to loss of ^{2}H in exchangeable positions in reindeer faeces corresponding to 0.152 g $H_2O \cdot g^{-1}$ faeces dry matter (Gotaas *et al.*, 2000). The loss of ^{2}H incorporated into CH_4 was estimated following the procedure of Midwood *et al.* (1989), assuming a ratio r_{CO2} to r_{CH4} of 20 in reindeer in winter (Gotaas *et al.*, unpubl.). We also assumed that the apparent water flux due to this loss of ^{2}H in CH_4 corresponded to 1.05 g $H_2O \cdot l^{-1}$ CH_4 (Midwood *et al.*, 1989). The sequestration of ^{2}H through incorporation into *de novo* synthesised fat was assumed to be negligible because the estimated rate of fat synthesis in young reindeer in the experimental herd during winter was <10 $g \cdot d^{-1}$ (Christiansen *et al.*, 1997). The total apparent water flux due to non-water losses of ^{2}H was used to adjust the rate of CO_2-production assuming that each gram overestimation of H_2O was equivalent to an underestimation of r_{CO2} of 0.598 l (Haggarty *et al.*, 1994).

r_{O2} was calculated from r_{CO2} using a respiratoty quotient (RQ) value of 0.91 (estimated from the chemical composition of lichen, Sletten & Hove, 1990; Soppela *et al.*, 1992)) according to Black *et al.* (1986).

Finally, TEE ($kJ \cdot d^{-1}$) was calculated according to the equation of Brouwer (1965) as follows:

$$TEE = 16.18 \cdot r_{O2} + 5.02 \cdot r_{CO2} - 2.17 \cdot r_{CH4} - 5.99 \cdot UNL \quad \text{(equation 4)}$$

where r_{O2}, r_{CO2} and r_{CH4} are given in $l \cdot d^{-1}$, while UNL is Urinary Nitrogen Loss given in $g \cdot d^{-1}$ (estimated by iteration as: UNL = TEE·Digestible energy in diet·Digestibility of diet·Nitrogen in diet, where TEE is in $kJ \cdot d^{-1}$, digestible energy is in $g \cdot kJ^{-1}$ and nitrogen content is in $g \cdot g^{-1}$ dry matter).

Statistical analysis

Changes in the body mass of each animal and in the concentrations of ^{2}H and ^{18}O in the body water of the control animal during the course of the trial were examined by linear regression (least squares) analysis. The significance of regression coefficients was determined using *t*-tests.

Values of TEE calculated from study durations of 22 and 32 days were compared with values calculated from a study duration of 11 days using Mann-Whitney *U*-tests.

Two-tailed tests were used throughout. H_0 was rejected at $P \geq 0.05$ in all tests.

The experiments described in this article have been conducted in accordance with current regulations for experimental research involving live animals in Norway.

Results

Background concentration of isotopes

The concentration of ^{2}H and ^{18}O (Table 1) did not change significantly in the control animal during the 32 day period covered by sample collection ($P > 0.05$).

Body mass

The body mass of the reindeer (Table 2) did not change significantly during the 32 day period covered by sample collection ($P > 0.05$).

Ambient temperature

The mean of the standard daily middle temperature during the period from 7th to 19th February was -17.6 °C; from 7th February to 1st March it was -14.0 °C and from 7th February to 11th March the mean was -11.6 °C (Table 3).

Dilution spaces

The isotope dilution space volumes differed only slightly between individuals and ranged from 68.8% to 70.7% of body mass for N_D and from 67.3% to 69.8% of body mass for N_O. The value for N_O was higher, while the value for N_D was lower in reindeer E compared to reindeer B and C. Consequently, the dilution space ratio (N_D:N_O) was lower in this animal (0.985) compared to the other two (N_D:N_O = 1.051 and 1.040, respectively) (Table 4).

Marker disappearance

Based on previously reported rates of marker disappearance (e.g. Gotaas *et al*, 1997), we allowed the experimental trials to run for 30 days to comply with the recommendation that DLW-studies should have a duration of 2-3 biological half-lives ($t_{1/2}$) of isotope markers. In fact, the rate constants ranged from 0.098 to 0.126 for k_D and from 0.128 to 0.161 for k_O (Table 4), corresponding to average values of $t_{1/2}$ of 6.03 days for ^{2}H and 4.68 days for ^{18}O, respectively. All calculations were therefore performed using data obtained from samples collected at equilibrium and 11 days after injection of DLW (i.e. 1.8

Table 1. Concentration of ^{2}H and ^{18}O (parts per million; ppm) in the control reindeer (22 months old male reindeer) throughout the winter trial.

Reindeer A	7 February	19 February	1 March	11 March	Mean (*s*)
^{2}H (ppm)	138.5	138.4	139.2	138.4	138.6 (0.4)
^{18}O (ppm)	1974.0	1974.3	1974.6	1973.6	1974.1 (0.4)

Table 2. Live body mass (kg) of four free-living, sub-adult (22 months old) male reindeer throughout the winter trial.

Reindeer	7 February	19 February	1 March	11 March	Mean (s)
A	63.0	63.5	63.0	61.5	62.8 (0.9)
B	62.0	63.5	63.5	62.0	62.8 (0.9)
C	missing value	64.5	65.0	62.5	64.0 (1.3)
E	56.0	55.5	56.5	54.5	55.6 (0.9)

Table 3. Means standard daily middle temperature (T_{middle}), mean minimal temperature (T_{min}) and the maximal temperature (T_{max}) in the study area throughout the winter trial.

	T_{middle} (ſ); range; *n*	T_{min} (ſ); range; *n*	*Tmax* (ſ); range; *n*
7 - 19 February	-17.8 °C (5.0); -8.5 to -29.8; 13	-25.4 °C (6.4); -9.9 to -35.2; 13	-12.6 °C (4.7); -7.0 to -25.0; 13
7 February - 1 March	-14.0 °C (7.0); -1.7 to -29.8; 24	-21.2 °C (9.2); -4.6 to -35.2; 24	-9.2 °C (5.9); -0.6 to -25.0; 24
7 February - 11 March	-11.6 °C (7.4); +0.5 to -29.8; 34	-18.0 °C (9.6); -3.2 to -35.2; 34	-6.9 °C (6.3); +4.0 to -25.0; 34

half lives for ^{2}H and 2.4 half lives for ^{18}O).

CO_2-production and total energy expenditure

The mean r_{CO2}, adjusted to compensate for loss of ^{2}H in methane and in faecal solids during the experimental period, was 707 l·d^{-1} (range 584 - 794; Table 4), equivalent to a mean specific TEE of 3.057 W·kg^{-1} (range 2.436 - 3.728; Table 4). The highest individual value for specific TEE was 1.53 times the lowest individual value.

Effect of expanding the experimental period on estimated TEE

The effect of expanding the experimental period was assessed by repeating all calculations using the samples collected on day 22 and day 32, respectively, as the last-point value rather than the samples collected on day 11. The mean specific TEE based on a study duration of 22 days was 3.414 W·kg^{-1} (range 2.115 - 5.379; Table 5) while the mean value based on a study duration of 32 days was 2.290 W·kg^{-1} (range 1.508 - 2.537; Table 5). Neither value differed significantly from the mean value of 3.057 W·kg^{-1} (Table 5) calculated based on a study duration of 11 days ($P>0.05$).

Discussion

The present study is the fitst to report estimates of energy expenditure in a free-living wild ungulate based on direct measurement of physiological parameters recorded over a significant period of time. We have demonstrated three key points. First, we have shown that the doubly labelled water method can be successfully applied under field conditions even in the middle of winter at very low ambient temperatures. Second, we have detected unexpectedly large individual variation in TEE in animals of the same sex and age living under identical conditions. Third, we have demonstrated that existing factorial models are unlikely to overestimate TEE in reindeer/caribou: they may, instead, be unduly conservative.

The mean TEE of 3.057 W·kg^{-1} recorded in the three male reindeer in the present study is remarkably similar to Fancy's (1986) estimate of TEE in free-living adult female woodland caribou (*R. t. caribou*) in March-April (3.038 W·kg^{-1}, Table 6) which was based on a factorial model. Both this value and ours in the present study, however, are substantially greater than factorial model estimates of TEE of free-living adult female Alaska caribou (*R. t. granti*) in January-February (2.222 W·kg^{-1}, Table 6) and free-living adult female Svalbard reindeer (*R. t. platyrhynchus*) in winter (1.813 W·kg^{-1}, Table 6). The latter two estimates are quite similar to the mean TEE of four captive adult female reindeer (*R. t. tarandus*) kept in semi-outdoor paddocks in February, measured using DLW (1.866 W·kg^{-1}, Table 6). It is, of course, impossible to investigate the reasons underlying the differences between these various estimates of TEE, as they are likely to differ from case to case. The present study serves, rather, as a general validation of the factorial approach. We suggest, further, that the route to progress in the understanding of field energetics of wild ungulates is via application of the DLW method.

CO_2-production and total energy expenditure

The mean TEE in the free-living reindeer reported here was 64% higher than the mean TEE of four captive reindeer estimated using DLW (Gotaas *et al*, 1997). The present study used young male animals while Gotaas *et al.* (1997) used non-pregnant adult females; however, neither the difference in the age or the sex of the animals is likely to explain so large a difference in TEE. Silver *et al.* (1969) for instance, found no significant difference in fasting metabolic rate between male and

Table 4. Body mass (BM; kg), study duration (days), dilution spaces (N) and rate constants (k) for ^2H and ^{18}O, water flux (J_{H2O}), rate of CO_2-production (r_{CO2}) and O_2-consumption (r_{O2}) and total energy expenditure (TEE; $MJ \cdot d^{-1}$ and $W \cdot kg^{-1}$) in three free-living, male reindeer (22 months old) in winter. r_{CO2} has been adjusted to compensate for loss of ^2H_2O in faecal dry solids and in microbially produced methane (CH_4).

Reindeer		B	C	E	Mean (s)
BM	kg	62.8	64.5	55.8	61.0 (4.6)
Study duration	d	11.15	11.16	11.12	11.14 (0.02)
N (mol) ± SE	N_D	2463 ± 12	2519 ± 12	2128 ± 10	2370 (212)
	N_O	2344 ± 10	2423 ± 11	2160 ± 10	2309 (135)
	$N_D:N_O$	1.051	1.040	0.985	1.025 (0.035)
k (d^{-1}) ± SE	k_D	0.098 ± 0.001	0.120 ± 0.002	0.126 ± 0.002	0.115 (0.015)
	k_O	0.128 ± 0.0006	0.155 ± 0.0004	0.161 ± 0.0005	0.148 (0.017)
J_{H2O} ±SE	$mol \cdot d^{-1}$	250 ± 3	312 ± 4	278 ± 5	280 (31)
	$g \cdot d^{-1}$	4504	5621	5010	5045 (559)
r_{CO2}	$l \cdot d^{-1}$	584	741	794	707 (109)
r_{O2}	$l \cdot d^{-1}$	641	814	872	776 (120)
TEE	$MJ \cdot d^{-1}$	13.21	16.76	17.96	15.98 (2.47)
	$W \cdot kg^{-1}$	2.436	3.008	3.728	3.057 (0.647)

SE is the standard error of the calculated rate constants/fluxes.

Table 5. Estimates of total energy expenditure, TEE ($W \cdot kg^{-1}$), in three free-living, sub-adult (22 months old) male reindeer (B, C, E) in winter calculated using alternative end-point samples (11, 22 or 32 days). Values with the same superscript are not significantly different (Mann-Whitney *U*-test, $P \geq 0.05$).

Study duration	TEE ($W \cdot kg^{-1}$)				
(days)	B	C	E	Mean (s)	CV
11	2.436	3.008	3.728	3.057 (0.647)^{a,b}	21.2
22	2.115	2.748	5.379	3.414 (1.731)^{a}	50.7
32	1.508	2.826	2.537	2.290 (0.693)^{b}	30.3

female white-tailed deer (*Odocoileus virginianus*). Similarly, factors such as age, rate of growth and level of feeding account for only 6% of the total variance in basal metabolic rate (BMR) in sheep (Graham *et al.*, 1974). The ambient temperature in the study area was periodically below the lower critical temperature of reindeer in winter of -30 °C (Nilssen *et al.*, 1984). Consequently, it is likely that the elevated TEE in our free-living reindeer was due at least in part to thermoregulatory thermogenesis. Keeping in mind the high insulative value of reindeer fur (Folkow & Mercer, 1986), however, even the lowest temperature recorded (-35.2 °C) is unlikely to have represented a substantial thermal load for the animals. The most likely explanation is that the high specific TEE in free-living reindeer compared to captive individuals, is caused mainly by a higher level of locomotor activity.

The present study revealed large individual variation in TEE between animals of the same age and sex living under identical conditions, with the highest value (3.728 $W \cdot kg^{-1}$) being 53% higher than the lowest value (2.436 $W \cdot kg^{-1}$). Individual variation in TEE of similar magnitude has been recorded in previous DLW studies with captive female reindeer (range 1.662 - 2.055 $W \cdot kg^{-1}$ (n = 4) in winter and 2.504 - 4.623 $W \cdot kg^{-1}$ (n = 4) in summer (Gotaas *et al.*, 1997)) and in captive male sheep (range 1.800 - 2.848 $W \cdot kg^{-1}$ (n = 4) (Midwood, 1990)). It is not possible to ascertain whether the variation in TEE in either of these studies was a consequence of differences in minimal metabolic rate or differences in the level of locomotor activity of the animals. The fact that individual variation in BMR in humans has been reported as being only 6-7% (Garby & Lammert, 1994), however, suggests that differences in the level of locomotor activity is a more likely explanation of large individual differences in TEE such as those observed in the present study. Resting metabolic rate (RMR) has often been taken as being representative of the 'basal' state due to the inherent difficulties in defining a basal metabolic rate in ruminants. McEwan (1970) reported values of RMR ranging from 1.61 to 2.04 $W \cdot kg^{-1}$ in two female barren ground caribou (*R. t. groenlandicus*), a difference of 27%. This is con-

Table 6. Estimates of the mean specific total energy expenditure (TEE, W·kg^{-1}) of reindeer/caribou (*Rangifer tarandus*) in winter assessed using either the doubly labelled water method (DLW) or factorial models (FACT) based on respirometry measurements.

Study type	State of animals	Subspecies	Sex	Reproductive status	BM (kg)	Age	Time of year	TEE (mean (s); *n* (W·kg^{-1})	Source
DLW	Free-living	*R.t. tarandus*	m		61.0	22 months	Feb	3.057 (0.647); 3	Present study
	Captive	*R.t. tarandus*	f	non-pregnant	81.3	adult	Feb	1.866 (0.166); 4	Gotaas *et al.*, 1997
FACT	Free-living	*R.t. granti*	f	unknown	107.0	adult	Jan - Feb	2.222	Boertje, 1985
	Free-living	*R.t. caribou*	f	unknown	100.0	adult	Mar - Apr	3.038	Fancy, 1986
	Free-living	*R.t. platyrhynchus*	f	non-pregnant	51.8	adult	1st March	1.813	Tyler, 1987

siderably higher than the variation in BMR reported for humans but is still small compared to the individual differences in TEE recorded in free-living reindeer in the present study. We therefore conclude that the large variation in the specific TEE of our reindeer probably reflects individual differences in the level of locomotor activity.

Marker disappearance

The mean half-lives of ^{2}H and ^{18}O in the present study (6.03 days and 4.68 days, respectively) are surprisingly low and indicate a rapid turnover of both isotopes. By comparison, the reported $t_{1/2}$-values for isotopes of H and O in captive reindeer and caribou in winter range from 8.4 to 14.0 days for ^{2}H and ^{3}H, respectively, and from 6.1 to 8.9 days for ^{18}O (e.g. Larsen & Blix, 1985; Fancy *et al.*, 1986; Gotaas & Tyler, 1995; Gotaas *et al.*, 1997). The short half-life values and, consequently, high J_{H2O} observed in rhe free-living reindeer in the present study, was most probably due to the combined effect of (i) a highet respiratory evaporative water loss associated with high TEE (Folkow & Mercer, 1986) and (ii) ingestion of snow while feeding from craters in the snow. Similar differences in body water parameters between captive and free-living individuals of the same species have been found in black-tailed deer (*O. h. sitkensis*); Parker *et al.* (1993) found that the water flux in summer was more than twice as high in free-living compared to captive animals, a finding they attributed to the increased evaporative water loss and greater moisture content of the diet.

Dilution spaces

The theoretical range of dilution space ratios (N_D:N_O) in reindeer has been estimated at 1.038 - 1.062 (Gotaas *et al.*, 1997). The dilution space ratio of two of the animals in the present study fitted comfortably inside this tange while in a third animal the ratio was substantially lower (0.985). This animal appeared neither unusually lean, nor did any of the calculated parameters (rate constants, r_{CO2}, TEE, etc.) differ between this and the two other DLW-dosed reindeer. At present, therefore, we have no explanation for the low dilution space ratio in this reindeer.

Effect of expanding the experimental period on calculated TEE

The mean TEE did not change significantly aftet increasing the duration of the study from 11 to 22 and 32 days, respectively. Schoeller & Taylor (1987), who investigated the precision of the DLW method as a function of increasing study duration in humans, concluded that 'The mean values for each metabolic period were not statistically different from those of the first 7 d and the precision at 14 d was quite similar to those ptedicted from the model in which we assumed that we were limited by analytical error of the isotopic analyses. The precision for longer periods, however, became progressively worse than the predicted precision.' We found that the standard deviation of our estimates of TEE tended to increase when the duration of the study was expanded from 11 to 22 and 32 days, respectively, probably because at this stage the concentrations of both isotopes were close to the background values.

Conclusions and perspectives

The tesults presented here demonstrate the usefulness of the DLW method fot estimating TEE in a free-living ruminant such as reindeer in winter. The large differ-

ences in TEE between free-living and captive reindeer, most likely due to differences in the level of locomotor activity, indicate that prediction of the TEE of free-living reindeer from studies of captive animals is not necessarily valid.

The data presented here raise several questions related to the energetics of free-living reindeer. For instance, what is the basis and extent of the individual differences in TEE between animals of the same sex and age living under identical conditions? What is the basis of the large difference between free-living and captive reindeer in winter? Is the difference likely to be equally large in summer? We have suggested that differences in levels of locomotor activity may be a majot determinant of individual variation in energy expenditure. The development of new, high capacity activity data loggers (van Oort *et al.,* 1999a; b) presents an excellent opportunity for simultaneous recording of activity and estimation of TEE using the DLW method. The combination of these two techniques would represent a novel approach for the study of individual variation in TEE and would provide a better description of the energetics of animals living in their natural habitat.

Acknowledgements

This study was supported by the Norwegian Reindeer Husbandry Research Council, the University of Tromsø and the Scottish Office Agriculture, Environment and Fisheries Department. G. Gotaas is grateful for a Joseph-Bech-Europa-Studienreisestipendium awarded by the F.V.S. Foundation, Hamburg, Germany. We thank reindeer herder Mattis A. Sara for assistance in the field.

References

Black, A. E., Prentice, A. M. & Coward, W. A. 1986. Use of food quotients to predict respiratory quotients for the doubly-labelled water method of measuring energy expenditure. – *Human Nutrition: Clinical Nutrition* 40: 381–391.

Blaxter, K. 1989. *Energy metabolism in animals and man* Cambridge: Cambridge University Press.

Boertje, R. D. 1985. An energy model for adult female caribou of the Denali herd, Alaska. – *Journal of Range Management* 38: 468–473.

Brouwer, E. 1965. Report of Sub-commirtee on Constants and Factors. – *In:* K. L. Blaxter (ed.). *Energy Metabolism,* pp. 441–443. London: Academic Press.

Christiansen, H. R., Tyler, N. J. C. & Johansen, O. 1997. Bruker simler i Finnmark kroppsreserver om vinteren? (Do female reindeer in Finnmark deplete their body reserves in winter?) – *Reindriftsnytt* 31 (3): 15-19 (In Norwegian).

Cole, T. J., Franklin, M. & Coward, W. A. 1990. Estimates of error. – *In:* A. M. Prentice (ed.). *The doubly-labelled water method for measuring energy expenditure – Technical recommendations for use in humans,* pp. 69-89. Vienna: International Atomic Energy Agency.

Coward, W. A. 1990. Calculation of pool sizes and flux rates. – *In:* A. M. Prentice (ed.). *The doubly-labelled water method for measuring energy expenditure - Technical recommendations for use in humans,* pp. 48-68. Vienna: International Atomic Energy Agency.

Engebretsen, R. H. 1975. Topography of internal organs of reindeer (*Rangifer tarandus*). – *Acta Veterinaria Scandinavica* Suppl. 57: 1–18.

Fancy, S. G. 1986. *Daily energy budgets of caribou: a simulation approach.* Ph.D. thesis, University of Alaska (Fairbanks).

Fancy, S. G., Blanchard, J. M., Holleman, D. F., Kokjer, K. J. & White, R. G. 1986. Validation of doubly labeled water method using a ruminant. – *American Journal of Physiology* 251: R143-R149.

Folkow, L. P. & Mercer, J. B. 1986. Partition of heat loss in resting and exercising winter- and summer-insulated reindeer. – *American Journal of Physiology* 251: R32-R40.

Garby, L. & Lammert, O. 1994. Between-subjects variation in energy expenditure: estimation of the effect of variation in organ size. – *European Journal of Clinical Nutrition* 48: 376–378.

Gotaas, G. & Tyler, N. J. C. 1995. Faeces is a reliable source of body water for measuring tritium in reindeer in summer and in winter. – *Rangifer* 15: 63–70.

Gotaas, G., Milne, E., Haggarty, P. & Tyler, N. J. C. 1997. Use of feces to estimate isotopic abundance in doubly labeled water studies in reindeer in summer and winter. – *American Journal of Physiology* 273: R1451-R1456.

Gotaas, G., Milne, E., Haggarty, P. & Tyler, N. J. C. 2000. Loss of deuterium in faecal solids and by sequestration in reindeer: effect on doubly labelled water studies. – *Rangifer* 20 (1): 31–41.

Graham, N. McC., Searle, T. W. & Griffiths, D. A. 1974. Basal metabolic rate in lambs and young sheep. – *Australian Journal of Agricultural Research* 25: 957–971.

Haggarty, P., Franklin, M. F., Fuller, M. F., McGaw, B. A., Milne, E., Duncan, G., Christie, S. L. & Smith, J. S. 1994. Validation of the doubly labeled water method in growing pigs. – *American Journal of Physiology* 267: R1574-R1588.

Haggarty, P., Robinson, J. J., Ashton, J., Milne, E., Adam, C. L., Kyle, C. E., Christie, S. L. & Midwood, A. J. 1998. Estimation of energy expenditure in free-living red deer (*Cervus elaphus*) with the doubly labelled water method. – *British Journal of Nutrition* 80: 263–272.

Larsen, T. S. & Blix, A. S. 1985. Seasonal changes in total body water, body composition and water turnover in reindeer. – *Rangifer* 5 (1): 2–9.

Lifson, N. & McClintock, R. 1966. Theory of use of the turnover rates of body water for measuring energy and material balance. – *Journal of Theoretical Biology* 12: 46–74.

Lifson, N., Gordon, G. B. & McClintock, R. 1955. Measurement of total carbon dioxide production by means of $D_2{}^{18}O$. – *Journal of Applied Physiology* 7: 704–710.

McEwan, E. H. 1970. Energy metabolism of barren ground caribou (*Rangifer tarandus*). – *Canadian Journal of Zoology* 48: 391–392.

Midwood, A. J. 1990. *Application of the doubly labelled water technique for measuring CO_2 production to sheep.* Ph.D. thesis, University of Aberdeen.

Midwood, A. J., Haggarty, P., McGaw, B. A. & Robinson, J. J. 1989. Methane production in ruminants: its effect on the doubly labeled water method. – *American Journal of Physiology* 257: R1488-R1495.

Midwood, A. J., Haggarty, P., Milne, E. & McGaw, B. A. 1992. Factors affecting the analysis of ^{18}O-enriched aqueous samples when using CO_2 equilibration in Vacutainers. – *Applied Radiation and Isotopes* 43: 1341–1347.

Midwood, A. J., Haggarty, P. & McGaw, B. A. 1993. The doubly labeled water method: errors due to deuterium exchange and sequestration in ruminants. – *American Journal of Physiology* 264: R561-R567.

Midwood, A. J., Haggarty, P., McGaw, B. A., Mollison, G. S., Milne, E. & Duncan, G. J. 1994. Validation in sheep of the doubly labeled water method for estimating CO_2 production. – *American Journal of Physiology* 266: R169-R179.

Nagy, K. A. 1994. Field bioenergetics of mammals: What determines field metabolic rates? – *Australian Journal of Zoology* 42: 43–53.

Nagy, K. A. & Knight, M. H. 1994. Energy, water, and food use by springbok antelope (*Antidorcas marsupialis*) in the Kalahari Desert. – *Journal of Mammalogy* 75: 860–872.

Nagy, K. A., Sanson, G. D. & Jacobsen, N. K. 1990. Comparative field energetics of two macropod marsupials and a ruminant. – *Australian Wildlife Research* 17: 591–599.

Nilssen, K. J., Sundsfjord, J. A. & Blix, A. S. 1984. Regulation of metabolic rate in Svalbard and Norwegian reindeer. – *American Journal of Physiology* 247: R837-R841.

Parker, K. L., White, R. G., Gillingham, M. P. & Holleman, D. F. 1990. Comparison of energy metabolism in relation to daily activity and milk consumption by caribou and muskox neonates. – *Canadian Journal of Zoology* 68: 106–114.

Parker, K. L., Gillingham, M. P., Hanley, T. A. & Robbins, C. T. 1993. Seasonal patterns in body mass, body composition, and water transfer rates of free-ranging and captive black-tailed deer (*Odocoileus hemionus sitkensis*) in Alaska. – *Canadian Journal of Zoology* 71: 1397–1404.

Schoeller, D. A. & Coward, W. A. 1990. Isotope fractionation corrections. – *In:* A. M. Prentice (ed.). *The doubly-labelled water method for measuring energy expenditure - Technical recommendations for use in humans,* pp. 90–113. Vienna: International Atomic Energy Agency.

Schoeller, D. A. & Taylor, P. B. 1987. Precision of the doubly labelled water method using the two-point calculation. – *Human Nutrition: Clinical Nutrition* 41: 215–223.

Silver, H., Colovos, N. F., Holter, J. B. & Hayes, H. H. 1969. Fasting metabolism of white-tailed deer. – *Journal of Wildlife Management* 33: 490–498.

Sletten, H. & Hove, K. 1990. Digestive studies with a feed developed for realimentation of starving reindeer. – *Rangifer* 10: 31–37.

Soppela, P., Nieminen, M. & Saarela, S. 1992. Water intake and its thermal energy cost in reindeer fed lichen or various protein rations during winrer. – *Acta Physiologica Scandinavica* 145: 65–73.

Thing, H. 1977. Behavior, mechanics and energetics associated with winter cratering by caribou in northwestern Alaska. – *Biological papers of the University of Alaska* 18: 1–43.

Tyler, N. J. C. 1987. Body composition and energy balance of pregnant and non-pregnant Svalbard reindeer during winter. – *Zoological Society of London Symposia.* 57: 203–229.

van Oort, B. E. H., Stokkan, K.-A. & Tyler, N. J. C. 1999a. Long term patterns of activity in relation ro photoperiod in free-ranging reindeer and sheep. – *Rangifer* Report No. 4: 87.

van Oort, B. E. H., Vik-Strønen, A., Barnes, B. M., Tobler, I., Stokkan, K.-A. & Tyler, N. J. C. 1999b. Activity loggers for reindeer. – *In:* Z. Zomborszky (ed.). *Advances in Deer Biology.* (Proceedings of the 4th Internarional Deer Biology Congress, Kaposvár, Hungary, 1998), p. 54. Pannon University of Agriculture, Kaposvár. ISBN: 963-9096-53-9.

White, R. G. & Yousef, M. K. 1978. Energy expenditure of reindeer walking on roads and on rundra. – *Canadian Journal of Zoology* 56: 215–223.

Wong, W. W., Lee, L. S. & Klein, P. D. 1987. Deuterium and oxygen-18 measurements on microliter samples of urine, plasma, saliva, and human milk. – *American Journal of Clinical Nutrition* 45: 905–913.

Manuscript received 21 October, 1999
accepted 30 March, 2000