Effects of fasting and refeeding on body composition of captive gray wolves (*Canis lupus*)

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Abstract: We examined the effects of fasting and refeeding on body composition in 9 captive adult gray wolves, *Canis lupus* (6 males, 3 females), during May–June 1995. Body composition was estimated by the technique of tritiated water dilution. Wolves were immobilized and weighed, base-line blood samples were taken, tritiated water was injected, and additional blood samples were taken before fasting, after 10 d of fasting, and again after 2 d of refeeding. Male wolves lost 8% ($P = 0.0001$) and females lost 7% body mass ($P = 0.01$) during the 10 d. Males lost 54% of this mass in water, 28% in fat, and 18% in protein/ash; females lost 58% in water, 20% in fat, and 22% in protein/ash. Upon refeeding, male wolves consumed an average of 6.8 kg (15.3% body mass) of deer meat per day and females consumed 6.4 kg (18.7% body mass). All wolves regained their initial mass. Males regained 24% of this mass in water, 70% in fat, and 6% in protein/ash; females regained 35% in water, 51% in fat, and 14% in protein/ash. This study provided evidence that after prolonged fasting, captive wolves could quickly and efficiently regain lost body mass after refeeding.

Résumé : Nous avons étudié les effets du jeûne et du retour à l'alimentation normale sur la composition corporelle de 9 Loups gris (*Canis lupus*) adultes gardés en captivité (6 mâles, 3 femelles) en mai–juin 1995. La composition corporelle a été estimée par la technique de dilution d'eau tritiée. Les loups ont été immobilisés et pesés, des échantillons sanguins de base ont été prélevés, de l'eau tritiée a été injectée et des échantillons additionnels de sang ont été prélevés avant le jeûne, après 10 jours de jeûne et de nouveau après 2 jours de retour à l'alimentation. Les mâles ont perdu 8% ($P = 0,0001$) et les femelles, 7% ($P = 0,01$) de leur masse corporelle au cours des 10 jours de jeûne. Les mâles ont perdu 54% de cette masse en eau, 28% en graisses et 18% en protéines/cendres; les femelles ont perdu 58% en eau, 20% en graisses et 22% en protéines/cendres. Quand ils ont pu se réalimenter, les loups mâles ont consommé en moyenne 6,8 kg (15,3% de leur masse corporelle) de viande de cerf par jour et les femelles, 6,4 kg (18,7% de leur masse corporelle). Tous les loups ont retrouvé leur masse initiale. Les mâles ont repris 24% de cette masse en eau, 70% en graisses, 6% en protéines/cendres et les femelles, 35% en eau, 51% en graisses et 14% en protéines/cendres. Il semble donc que, après un jeûne prolongé, les loups en captivité soient capables de regénérer la masse perdue rapidement et efficacement lorsqu'ils sont réalimentés.
[Traduit par la Rédaction]

Introduction

Wild gray wolves (*Canis lupus*) kill prey sporadically, often going days between feedings (Mech 1970). During the interprandial period, wolves must rely upon endogenous catabolism, resulting in loss of body mass. Generally, both fat and protein are catabolized in fasted mammals (Forbes 1987); however, the relative amounts relied upon by fasted wolves are unknown. When possible, wolves engorge, sometimes eating several kilograms in a single feeding (Mech 1970). How this feeding either reestablishes or alters subsequent body composition is unknown. DelGiudice et al. (1987*b*) fasted wolves for 10 days and monitored endogenous catabolism by measuring constituents in blood and urine. Fasting resulted in decreased concentrations of serum urea nitrogen, triglycerides, and thyroid hormones (triiodothyronine), but wolves continued to excrete urinary urea nitrogen, reflecting protein catabolism. After observing this phenomenon of feeding and engorging in wild wolves, and as an extension of the above previous study, we investigated the dynamics of body composition changes and the efficiency with which wolves regained lost mass after a limited feeding opportunity. Herein we describe a controlled experiment with captive wolves in which we mimicked this feeding pattern and employed the method of tritiated water dilution (Prentice et al. 1952) to describe alterations in body composition in vivo.

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Methods

This study was conducted in east-central Minnesota (45°16'N, 92°55'W) during May–June 1995. Ten captive adult (3–5 years old) wolves (6 males, 4 females) were used in this study and cared for in accordance with the principles and guidelines of the Canadian Council on Animal Care. All wolves were born in captivity of wild-born parents. Wolves were housed in outdoor pens with attached kennels (2 × 4 m) for isolation of individuals. Wolves were fed vehicle-killed white-tailed deer (*Odocoileus virginianus*) and were supplied water ad libitum. All wolves were vaccinated annually for

rabies, canine distemper, canine parvovirus, infectious canine hepatitis, and leptospirosis. All were regularly treated for ecto- and endo-parasites and maintained on heartworm (*Dirofilaria immitis*) prophylaxis (Kreeger et al. 1990).

Forty-eight hours prior to this study, all food and fecal material were removed from the pens, but water was provided. The purpose of the 24-h fast prior to base-line measurements was to approximate an ingesta-free mass in all wolves. On day 1 of the study, wolves (FED group) were immobilized in the morning with 10 mg/kg ketamine and 1 mg/kg promazine administered intramuscularly (Kreeger 1992). Wolves were weighed and a base-line 10-mL blood sample was obtained from the right cephalic vein.

Immediately after the initial sample was taken, a known amount of physiological saline solution (1.17–1.23 g) containing 250 μCi (1 Ci = 37 GBq) tritiated water (THO) per gram was injected into the left cephalic vein. The THO was kept frozen until just before administration to prevent evaporation. To avoid any possible artifact from the injection site, subsequent 10-mL blood samples were taken from the right cephalic vein 30, 60, 90, and 120 min after injection of the THO to insure isotope equilibration (Coleman et al. 1972). Blood samples were allowed to clot, then centrifuged, and sera were stored until radioactive counts were made. Upon recovery from anesthesia, wolves were returned to their respective pens and fasted for 10 d. Deposition of feces was monitored daily to estimate clearance of the alimentary system. Water intake was monitored daily by measuring the amount lost from a known volume, although individual wolf consumption and accidental spillage could not be precisely determined.

On day 10 of the study, fasted wolves (FASTED group) were immobilized as before and weighed, and blood sampling and THO injection as described for day 1 were repeated. All wolves recovered from the anesthesia during the afternoon and were separated for feeding. Each wolf was provided with skinned and boned deer meat equal to approximately 25% of its mass. The feeding of each animal was monitored; the animal was considered sated when it ceased feeding and began to look for a feed-cache site. At this time all remaining feed was removed and weighed.

On day 11, the wolves were fed in the same manner as on day 10 and the amount of feed consumed was recorded for each wolf. On day 12, no food was given and all water was removed. On day 13, after being fasted for 48 h to allow clearing of the alimentary system (ingesta-free), wolves (REFED group) were again immobilized and weighed and blood sampling and THO injection as described for day 1 were repeated.

Activity of THO was counted in 0.2-mL aliquots of serum in scintillation vials with a liquid scintillation analyzer (Packard Tricarb 1900CA) autocorrected by an internal quench curve. Counting efficiency was determined with a set of sealed tritium quenched standards and an automatic external curve. Net background counts were zero per minute. Instrument counting efficiency was 40%. Activity counts were corrected for percent carryover from previous THO injections before total body water (TBW) was calculated. TBW was calculated on the basis of the dilution principle, $V_2 = C_1V_1/C_2$, where V_2 is body fluid volume, C_1V_1 is the quantity of isotope injected (total counts), and C_2 is the concentration of the isotope in serum at equilibrium (Forbes 1994).

The dilution of THO is an overestimate of TBW because the tritium atom exchanges with stable hydrogen in body tissues (Kodama 1971). This overestimate is thought to usually average 3–4% (Torbit et al. 1985*a*). Accordingly, all TBW estimates in this study were reduced by 3.5% prior to calculation of other values.

The estimate of TBW includes both tissue water and water found in the gut. Gut water must be subtracted from TBW before body composition can be estimated (Forbes 1987; Torbit et al. 1985*a*). Gut water could not be measured directly because no wolves were euthanized. However, gut water in domestic dogs (*C. l. familiaris*) that were fasted for 24 h was 3% of TBW (Cizek 1954), therefore we used 3% as the portion of TBW attributable to gut water in the FED and REFED groups. Accordingly, ingesta-free water content (corrected TBW) was calculated by subtracting 3% of TBW from TBW. Since gut water falls rapidly in fasting dogs (Cizek 1954), we presumed that percent gut water in wolves fasted for 10 d (FASTED group) would be minimal.

Since the wolves were fasted for 24–48 h prior to testing, ingesta-free body mass was presumed to be equal to the mass of the wolf. Lean body mass (LBM) was calculated by dividing the corrected TBW by 0.73, a value representing the proportion of water in the fat-free wet mass of most mature animals (Sheng and Huggins 1979; Forbes 1994). Fat was calculated by subtracting LBM (i.e., fat-free wet mass) from body mass (BM). Percent body water was determined by dividing the TBW volume (L) by BM (kg) corrected for gut water.

BM and percent fat among groups and between the sexes were compared using one- and two-way analysis of variance (ANOVA). Differences were considered significant at $P \leq 0.05$. Values are reported as means ± standard error (SE).

Results

One female wolf was removed from data analyses of body composition because of sampling error. Relative to base-line values, male wolves lost 8% ($P = 0.0001$) and females lost 7% mass ($P = 0.01$) during the 10-d fast (Table 1). Males lost 54% of this mass in water, 28% in fat, and 18% in protein/ash; females lost 58% in water, 20% in fat, and 22% in protein/ash. After refeeding, both males and females regained their initial mass (Table 1). Males regained 24% of this mass in water, 70% in fat, and 6% in protein/ash; females regained 35% in water, 51% in fat, and 14% in protein/ash. Males differed from females in BM ($P = 0.0004$), TBW ($P = 0.0002$), LBM ($P = 0.0002$), and fat ($P = 0.04$) in all groups.

Male wolves consumed an average of 6.9 kg (15.5% BM) deer meat on the first day of refeeding and 6.6 kg (15.0% BM) on the second day; females consumed 6.5 kg (19.1% BM) on the first day and 6.3 kg (18.3% BM) on the second day. There was no difference between males and females in either the absolute amount consumed ($P \geq 0.47$) or the amount consumed relative to BM ($P \geq 0.07$). Defecations ceased in <48 h after feeding, indicating that the alimentary system had cleared prior to retesting.

Discussion

To our knowledge, this is the first report of the use of THO to determine body composition in wolves. Percent TBW of wolves (68.5 ± 0.9%) compared favorably with calculations for normal domestic dogs (68.3%; Sheng and Huggins 1979), which supports the methods used in this study. However, we caution that these data probably represent relative as opposed to absolute values. Although the technique of body composition estimation by dilution of THO is well accepted, it does not represent a high degree of precision (Forbes 1987). This is due, in part, to errors in the administration and retrieval of THO, such as incomplete intravenous injection or loss of THO in urine, feces, and vomitus and through respiration. Moreover, the techniques for studying body water composition in vivo work well in normal individuals, but become less precise as nutritional status becomes

Table 1. Changes in body composition of 9 adult wolves (6 males, 3 females; FED group) fasted for 10 d (FASTED group), refed in excess of their requirements for 2 days, and then fasted for 2 more days before retesting (REFED group).

	BM (kg)	Percent BM[a]	TBW (L)	Percent TBW[b]	LBM (kg)	Fat (kg)	Percent fat[c]
FED group							
Males	49.3±3.7	100.0	31.1±1.7	69.4±2.0	42.6±2.4	5.7±1.6	11.1±2.5
Females	37.2±3.9	100.0	22.5±2.9	70.9±3.9	30.8±4.0	3.0±1.5	9.1±5.0
FASTED group							
Males	45.3±3.5	91.9	29.0±1.8	67.9±1.7	39.7±2.4	4.6±1.3	9.8±2.3
Females	34.6±3.6	93.0	20.9±2.4	69.2±3.6	28.6±3.3	2.5±1.3	8.1±4.7
REFED group							
Males	49.5±4.0	100.3	30.0±2.0	66.3±1.7	41.0±2.8	7.5±1.5	15.0±2.2
Females	37.1±4.1	99.6	21.8±2.7	69.4±1.1	29.9±3.7	3.8±0.7	11.1±1.4

Note: Values are reported as means with standard errors. BM, body mass; TBW, total body water; LBM, lean body mass. See the text for calculations.

[a]Percent change in mass of the FASTED and REFED groups compared with the base-line mass of the FED group.

[b]Percent TBW equals the volume of TBW (L = kg) divided by BM (less gut water).

[c]Percent fat equals the mass of fat divided by BM (less gut water).

more abnormal. The FED group of wolves had 10.4 ± 2.2% body fat (males and females), which would be considered lean, and this percentage decreased after fasting. Any error in assuming the water content of LBM (73% in this study) greatly magnifies any error in fat estimation (Forbes 1987). We are unaware of any reports on the water content of LBM in fasted canids. Such data do exist for undernourished white-tailed deer (Torbit et al. 1985*a*), but when these percentages were applied to data in this study, they resulted in little numerical change. Additionally, the application of formulae for ruminants to monogastric animals may be questionable. Such an error in the water content of LBM may be demonstrated by the numerically higher percentage of fat in both males and females after refeeding. If these data are accurate, we are at a loss to explain the physiological mechanisms that would account for increased fat deposition after a short period of refeeding.

Unlike previous studies with white-tailed and mule (*O. hemionus*) deer (Robbins et al. 1974; Torbit et al. 1985*a*; DelGiudice et al. 1990), we were unable to calculate the protein and ash content of wolves because we did not euthanize them for a direct calculation, and we were unable to locate relevant data or predictive equations for dogs from the literature. Thus, the dynamics of change in body composition in this study reflect changes in three components: body water, fat, and protein/ash. Ash normally represents <5% BM in deer (Robbins et al. 1974; Torbit et al. 1985*a*; DelGiudice et al. 1990). The simple separation of the body into fat and lean compartments, as in this study, has been considered useful for a variety of investigations (Sheng and Huggins 1979).

It appeared that wolves simultaneously catabolized protein and fat during fasting, and they had not reached the point where fat reserves were depleted and protein catabolism was accelerated (Torbit et al. 1985*b*; DelGiudice et al. 1987*a*, 1990). Wolves in the wild have been observed to go without feeding for at least 17 days (Mech 1970); a longer period of fasting may have reflected accelerated protein catabolism.

Upon refeeding, wolves consumed 15–19% of their BM before satiation. The females consumed more than the males on both days of refeeding, although the differences were not significant. Also of interest was that after refeeding, the percentage of fat was numerically, though not significantly, higher than at the beginning of the study. These data suggest that regained mass was distributed differently upon refeeding in this study. We caution, however, that the changes in body composition noted in this study may differ somewhat from changes that may be experienced by wild wolves. Net energy balance influences the composition of gains and losses in mammals (Forbes 1987). The activity budgets probably differed markedly between captive and wild wolves in that the wolves used in this study led fairly sedentary lives compared with free-ranging wolves. Also, we offered only skinned and boned, highly digestible meat upon refeeding, whereas in the wild, wolves would eat meat, hide, hair, and viscera (Mech 1970). Since it takes more energy to deposit a gram of fat (12 kcal) than a gram of protein (8.66 kcal), the composition of food consumed after fasting may have an appreciable effect on subsequent body composition (Spady et al. 1976). Studies in humans have shown that the composition of the diet fed to undernourished subjects had a marked effect on the distribution of fat and LBM (Forbes 1987), and this phenomenon may apply to wolves feeding following fasting. The energy expended during fasting and the composition of the food eaten during refeeding would thus probably differ between captive and wild wolves.

This study provided evidence that after prolonged fasting, wolves could quickly and efficiently regain lost mass after consuming an amount of feed that potentially would be available after they had killed an adult ungulate in the wild. Furthermore, these data suggest that the composition of the regained mass could be different, for a short period at least, from body composition prior to the initiation of fasting.

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