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Comparative Biochemistry and Physiology Part A 126 (2000) 295–303

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Body composition in mink (*Mustela vison*) kits during 21–42 days postpartum using estimates of hydrogen isotope dilution and direct carcass analysis

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Received 13 January 2000; received in revised form 3 April 2000; accepted 14 April 2000

Abstract

We compared carcass analysis and hydrogen isotope dilution methods to measure total body water (TBW) and body composition in a small altricial carnivore, the mink. Dilution space (D) of mink at 21–42 days of age ($n = 20$), was determined after subcutaneous administration of tritiated water. The same animals were then used to determine TBW and body composition by carcass analysis and to derive predictive empirical relationships between TBW and total body fat, protein and energy. A separate validation set of 27 kits was used to test the accuracy of predicting body composition from TBW. D overestimated TBW by a consistent and predictable 4.1% ($R^2 = 0.999$, $P < 0.001$). Our estimates of fat, protein and energy content, using equations derived from TBW, were not significantly different than those obtained from direct carcass analysis ($P > 0.980$) in either the initial or validation set of mink. TBW was shown to decrease from 81 to 76% and total body protein to increase from 14 to 19% of LBM of the kits from 21 to 42 days of age. Although a rapidly changing hydration state was apparent in neonates, we conclude that when this is taken into account, accurate estimates of body composition can be obtained from hydrogen isotope dilution.

Keywords: *Mustela vison*; Mink; Carnivore; Isotope dilution; Carcass analysis; Fat; Protein; Energy; Body composition

1. Introduction

Studies of nutrition, growth and energetics in mammals often rely on the ability to accurately measure body composition of individuals. Direct measurements of body composition involve sacrificing the animal, grinding the carcass and taking a representative subsample for analysis of water, fat and protein content. The disadvantages of this method are that the animal must be killed and therefore cannot be studied longitudinally, it is expensive and not appropriate for studying protected species, and especially in studies involving large animals grinding the carcass can pose technical difficulties. Thus, the use of reliable indirect methods for determining body composition of live animals is important to many types of studies. Various alternative indirect methods for determining body composition have been used, but it is important to validate these across species.

One indirect method of determining body composition is the use of hydrogen isotope dilution

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1095-6433/00/$ - see front matter
PII: S1095-6433(00)00212-9

(tritiated water, HTO, or deuterium oxide, D_2O; Nagy, 1987; Costa, 1987; Oftedal and Iverson, 1987). This method assumes complete mixing of injected labeled water with the body water of an animal, allowing the calculation of total body water (TBW). Body composition of the animal is then calculated based upon the findings that water and protein content of lean body mass (LBM) tend to be relatively constant among mammals, especially of a given species and age (Spray and Widdowson, 1950; Reid et al., 1955, 1963; Reilly and Fedak, 1990; Gales et al., 1994). The advantages of using isotope dilution include that methods are relatively inexpensive and are also easy to perform on larger animals. But the primary advantage is that measurements are made on live animals such that individuals can be studied longitudinally. For instance, changes in body composition of individuals estimated from hydrogen isotope dilution have been used to assess energy and material fluxes and costs of specific events in the life cycle of several species (Bowen et al., 1992; Iverson et al., 1993; Oftedal et al., 1993; Coltman et al., 1998). However, potential disadvantages of these methods may arise from the number of assumptions that are made in calculations. Hence validation experiments on a range of species are important to understanding the reliability of isotope dilution techniques for estimating body composition. Such experiments require the measurement of body composition directly by carcass analysis and indirectly by isotope dilution in the same individuals.

A major assumption of isotope dilution techniques is that the isotope mixes with and labels body water only. While in most cases there has been a very close correspondence found between dilution space and TBW by desiccation (reviewed in Nagy and Costa, 1980; Speakman, 1997), it is now reasonably well-established that dilution space overestimates the body water of the animal (as determined by carcass analysis) due to the incorporation of hydrogen isotopes into exchangeable sites in organic constituents (Ussing, 1938). These overestimates have been reported to range from 0.5 to 5%, however, in some cases, errors in the carcass analysis itself may have compounded the estimated error. An average overestimate of $\sim 4.5\%$ has been found across studies of a number of vertebrate species (Speakman, 1997).

Perhaps the most important assumption of using isotope dilution to estimate body composition is the premise that TBW can be used to accurately predict LBM, total body fat (TBF), protein (TBP) and energy (TBE) content. Despite the large number of validation studies in mammals which have been conducted to understand the relationship between TBW determined using carcass analysis versus isotope dilution, relatively few (Rumpler et al., 1987; Reilly and Fedak, 1990; Farley and Robbins, 1994; Hilderbrand et al., 1998) have attempted to directly relate carcass proximate composition measures to the estimates of body fat and protein using TBW derived from isotope dilution. Given the significance of using TBW in studies of body composition and energetics in mammals, the accuracy of these assumptions is important to verify among species.

In the present study we compared carcass analysis and hydrogen isotope dilution methods to measure body composition in a young growing carnivore, the mink (*Mustela vison*). Our objectives were to: (1) assess changes in body composition of young growing mink kits; (2) determine the predictive relationship between isotope dilution and carcass desiccation in the determination of TBW; (3) determine the predictive relationship between TBW and components of body mass; and (4) assess the ability to accurately predict LBM, TBF, TBP and TBE composition using isotope dilution space and TBW.

2. Materials and methods

2.1. Animals

A total of 47 suckling mink kits of the standard black phenotype were used in this experiment as part of a larger study on mink development and physiology (Layton, 1998). The animals used in isotope studies ($n = 20$) consisted of four males and four females at 21 days old, two females and one male at 28 days old, and five females and four males at 42 days. A validation set of 27 additional animals aged 21 days (one male, two females), 28 days (eight males, seven females) and 42 days (five males, four females) was used to assess the average predictive value of equations derived from the first 20 animals. All animals were housed in the breeder shed at the Nova Scotia Agricultural College Fur Unit, Truro, NS. Litters were housed

together with their dam in wire-mesh cages equipped with a nest box. In addition to milk ingestion, the animals were offered a conventional mink diet based on fish and slaughterhouse offal and water was supplied by an automatic nipple drinker. The daily care and management of the animals, as well as euthanasia, was carried out in accordance with the guidelines of the Canadian Council on Animal Care (CCAC, 1993).

2.2. Hydrogen isotope dilution

Dilution space (D) was measured using HTO according to Oftedal and Iverson (1987). The animals were separated from their mother and weighed to the nearest 0.1 g. An initial blood sample was collected by clipping a toe nail and drawing blood directly into a heparinized capillary tube, which was immediately sealed at both ends with clay. Tritiated water in saline solution (5 μCi g^{-1}) was then administered subcutaneously in the loose skin of the neck. To accurately measure the quantity of isotope delivered, the syringe used was weighed to the nearest 0.1 mg before and after administration. Approximately 18.4 μCi kg^{-1} body mass was injected per animal.

Isotope equilibration was assumed to take ~ 30 min, based on a previous study of mink kits (Oftedal, 1981). Thus, two sequential blood samples were collected as described above at 40 and 60 min following administration to allow evaluation of whether equilibrium had been reached. During the equilibration period the animals were removed from any sources of food or water and were kept warm by placing them in an artificial nest containing a hot water bottle and cotton towels. As kits had no access to water, nor was it likely they urinated as this requires physical stimulation by mother, loss or gain of label was not likely to have occurred during the brief equilibration time.

Blood samples in the heparinized capillary tubes (~ 20–40 μl) were refrigerated until analysis 2–3 days later. At this time, the sealed tubes were centrifuged and then broken into segments such that one segment contained the plasma sample. The plasma and tube segment were weighed to the nearest 0.1 mg. The plasma was then blown into a scintillation vial containing 5 ml of scintillation fluid (Fisher Scintiverse II) and the tube segment was reweighed. The scintillation vial was sealed, shaken vigorously and tritium activity counted on a Beckman LS 3801 liquid scintillation counter. A blank sample (plasma from an unlabelled mink) was analyzed during each counting run. Internal standards were used for quench correction and count levels were converted to disintegrations per minute (DPM) per mg plasma, which contains 92.2% water in mammalian species (Altman, 1961). This procedure was based on methods previously developed (Oftedal, 1981) and used due to logistical constraints at the time which precluded distillation of plasma samples. Dilution space (D, g) was calculated from the amount (g) of HTO injected, the specific activity of the HTO solution and the specific activity of HTO in plasma corrected for background activity of the blank sample.

2.3. Carcass analysis

After the above procedure was completed the animals ($n = 20$) were euthanised with an intra cardiac injection of Euthanyl® (0.44 ml kg^{-1}). The validation set of additional mink ($n = 27$) were also euthanised in the same manner. Digestive contents in the stomach and the intestinal tract were removed and each carcass frozen whole in an airtight container at -50°C until analysis. The carcass was then placed whole in a blender, thawed, and homogenized. The homogenate was weighed and freeze-dried to determine dry matter and water content (AOAC, 1984) and then ground in a coffee grinder. Subsamples of the finely ground powder were then analyzed for proximate composition. Crude protein was determined by the Dumas method (Ebeling, 1968) using a FP-228 nitrogen (N) determinator (Leco, MI, USA) and multiplying N by a factor of 6.25 (AOAC, 1984). Total lipid content was determined gravimetrically by petroleum ether extraction following acid hydrolysis using a 1047 hydrolysing unit and Soxtec system HT (Fisher Scientific).

2.4. Calculations and data analysis

Carcass analysis in the first 20 study animals provided all initial empirical data (g) for total body water (TBW_C), total body protein (TBP_C) and total body fat (TBF_C). Carcass lean body mass (LBM_C) was calculated as body mass − TBF_C. TBW_C was then regressed against D to

derive a predictive equation for calculating TBW_C from D (i.e. TBW_{est})(see Results). The relationships between TBW_C and LBM_C, and between TBP_C and LBM_C, in these 20 animals were then used with TBW_{est} to estimate LBM_{est}, TBP_{est} and TBF_{est} from water spaces in both these and the 27 validation animals. Total body gross energy (TBE, kJ) was calculated from body composition of kits assuming an energy density of 39.5 and 23.5 kJ g^{-1} of fat and protein, respectively (Schmidt-Nielsen, 1980). Data were analyzed using a combination of ANOVA with post hoc separation tests (Bonferroni/Dunn) and regression analyses using StatView 4.1 for the Macintosh. Data are presented as means $\pm$ S.E., unless otherwise stated.

3. Results

3.1. Total body water determination using isotope dilution followed by carcass analysis

The mink kits used in the main study ranged from 82 to 475 g, but averaged 116, 187 and 348 g at days 21, 28 and 42, respectively (Table 1). Although males tended to be larger than females at a given age, there were no significant differences in body composition or in relationships between components of body composition between sexes ($P > 0.7$, ANOVA). Hence, data for males and females were combined in all further analyses. Equilibration of HTO was confirmed in most individuals by 40 min., as evidenced by the isotope concentration in the serial blood samples not differing by more than 1%, and both values were averaged for equilibration concentration. In several cases, the 60 min. sample was slightly more concentrated than the 40 min. sample, thus in these cases we used only the latter value. Proportional dilution space (D) declined from 79.3 to 73.7% of body mass in mink kits from 21 to 42 days of age (Table 1). However, a strong linear relationship was evident between dilution space and body mass of mink kits across ages (Fig. 1a). Carcass TBW (TBW_c) declined from 76.5 to 70.5% across ages and was similarly related to body mass of kits (Fig. 1a), however the slope of the relationship between TBW_c and body mass was slightly more shallow than for that of D, reflecting an overestimation of body water by isotope dilution.

When TBW_c was regressed against D, a strong ($R^2 = 0.999$) predictive relationship was found ($TBW_c = 1.608 + 0.950\ D$, Fig. 1b). Throughout the lowest and highest ranges of TBW measured, D consistently overestimated TBW by an average of $4.1 \pm 0.85\%$. Assuming the carcass desiccation values to be the 'true' values for TBW, we used the equation in Fig. 1b to estimate TBW from D for each individual (Table 1). There was no significant difference between these TBW estimates and carcass TBW ($P > 0.9$, paired t-test).

Table 1
Body composition of suckling mink kits determined by carcass analysis and estimated by hydrogen isotope dilution[a]

	Age (days)			ANOVA (P)
	21 ($n = 8$)	28 ($n = 3$)	42 ($n = 9$)	
Body mass (g)	116.1 ± 7.84^a	187.1 ± 7.01^b	348.1 ± 23.19^c	<0.001
Carcass analysis				
%TBW	76.5 ± 0.57^a	73.9 ± 0.65^b	70.5 ± 0.71^c	<0.001
%TBP	13.5 ± 0.21^a	14.4 ± 0.45^a	17.5 ± 0.32^b	<0.001
%TBF	5.8 ± 0.69	7.7 ± 0.64	6.9 ± 0.63	0.270
TBW/LBM (%)	81.3 ± 0.29^a	80.0 ± 0.48^a	75.8 ± 0.49^b	<0.001
TBP/LBM (%)	14.4 ± 0.15^a	15.6 ± 0.49^a	18.8 ± 0.36^b	<0.001
Isotope dilution[b]				
%D space	79.3 ± 1.01^a	$76.8 \pm 0.62^{a,b}$	73.7 ± 1.07^b	0.004
$\%TBW_{est}$	76.1 ± 0.97^a	$73.7 \pm 0.60^{a,b}$	70.7 ± 1.03^b	0.004
$\%TBP_{est}$	13.6 ± 0.23^a	13.9 ± 0.16^a	17.6 ± 0.23^b	<0.001
$\%TBF_{est}$	6.1 ± 1.21	8.3 ± 0.79	6.5 ± 1.28	0.647

[a] Means in a row with different superscripts were significantly different.

[b] TBW_{est} calculated from D and equation in Fig. 1b and converted to a percentage of body mass. TBP_{est} calculated from equations in Fig. 2a, b and converted to a percentage of body mass. TBF_{est} calculated as $100 - \%LBM_{est}$ from equation in Fig. 2a.

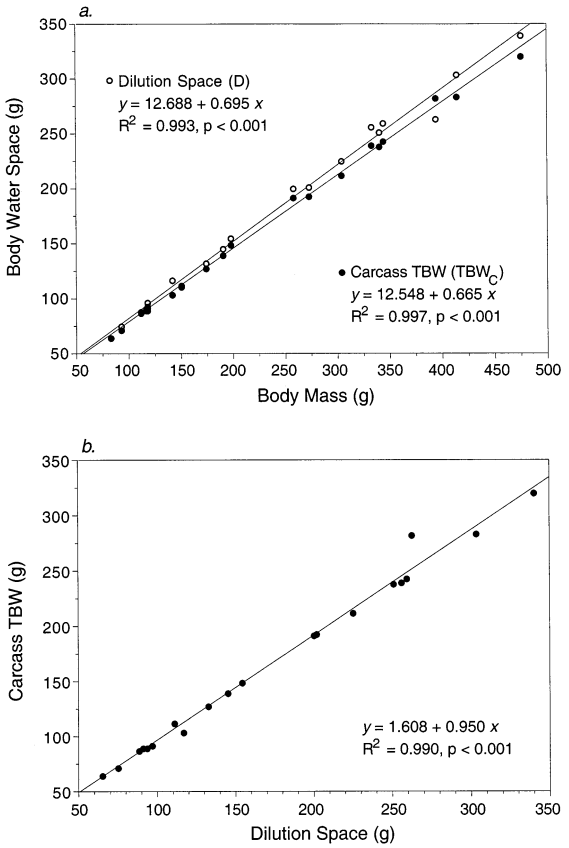

Fig. 1. (a) Relationship between body mass and dilution space and mass and carcass body water in mink kits from 21 to 42 days of age ($n = 20$). Standard errors (S.E.) of the intercepts for D and TBW_c were 3.653 and 2.049, respectively. S.E. of the slopes for D and TBW_c were 0.014 and 0.008, respectively. Residual S.E. of predicted y on x for D and TBW_c were 7.416 and 4.160, respectively; (b) Relationship between dilution space and carcass TBW in mink kits from 21 to 42 days of age ($n = 20$). S.E. of the intercept and slope were 4.252 and 0.022, respectively. Residual S.E. of predicted y on x was 8.142.

3.2. Body composition by carcass analysis

In the above 20 mink kits, relative TBP_C increased from 13.5–14.4% at 21–28 days to 17.5% at 42 days (Table 1). In contrast, TBF_C was quite variable and did not differ significantly among ages, averaging $\sim 6.6\%$. LBM_C also did not change across ages studied, averaging $93.4 \pm 0.42\%$. However, the composition of LBM_C changed significantly. The water content of LBM_C (TBW_C/LBM_C) decreased from 81.3% at 21 days to 75.8% at 42 days, while the protein content of LBM_C (TBP_C/LBM_C) increased at these times from 14.4 to 18.8% (Table 1).

In order to estimate body composition from isotope dilution data, equations were derived for predicting LBM and TBP from TBW using the carcass analyses of the 20 mink kits (Fig. 2a, b). Consistent with changes over time in the composition of LBM_C, both the relationship between LBM_C and TBW_C and that between TBP_C and LBM_C were significantly better fit by curvilinear equations with lowest residuals. Using these relationships, TBP and TBF were estimated for these same mink kits and are shown for illustrative purposes in Table 1. TBE_C calculated from TBF_C and TBP_C of kits increased from 649.1 ± 71.67 kJ at 21 days to 2418.4 ± 236.32 kJ at 42 days. The relationship of TBE_C (y) to TBW_C (x) was also best fit by a second order curvilinear equation

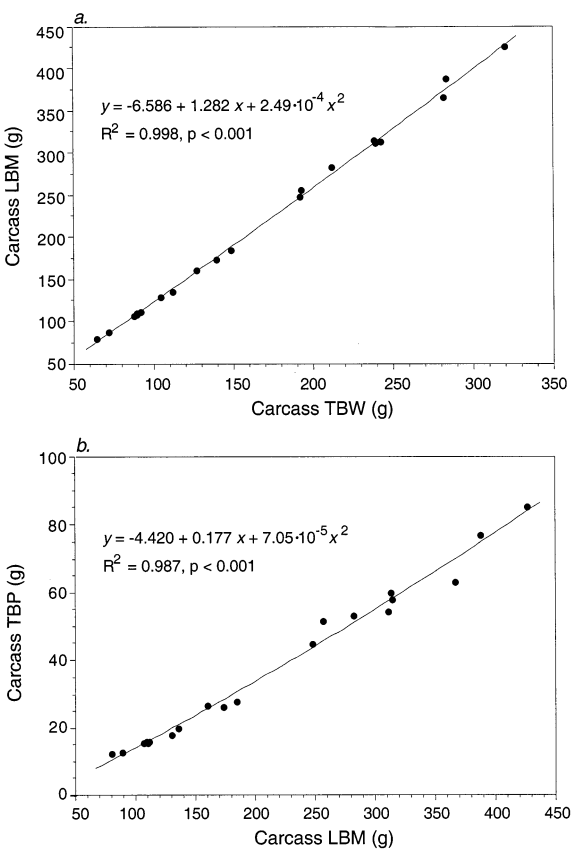

Fig. 2. (a) Relationship between carcass TBW and carcass lean body mass for mink from 21 to 42 days of age ($n = 20$). S.E. of the intercept and slope terms were 6.440, 0.082 (x) and 2.205×10^{-4} (x^2), respectively. Residual S.E. of predicted y on x was 4.916; (b) Relationship between carcass lean body mass and carcass protein for mink from 21 and 42 days of age ($n = 20$). S.E. of the intercept and slope terms were 3.310, 0.033 (x) and 6.726×10^{-5} (x^2), respectively. Residual S.E. of predicted y on x was 2.790.

Table 2
Body composition of a validation set of mink kits determined by carcass analysis and estimated from TBW space[a,b]

	Age (days)			Overall ($n = 27$)
	21 ($n = 3$)	28 ($n = 15$)	42 ($n = 9$)	
Body mass (g)	124.5 ± 9.86	169.6 ± 9.66	389.7 ± 21.83	237.9 ± 22.97
% TBW_c	75.1 ± 0.61	74.9 ± 0.48	69.8 ± 0.45	73.2 ± 0.56
% LBM_c	93.0 ± 0.48	93.3 ± 0.42^a	92.2 ± 0.31	92.9 ± 0.27
% LBM_{est}	92.6 ± 0.64	94.2 ± 0.36^b	92.4 ± 0.62	93.5 ± 0.33
% TBP_c	13.2 ± 0.22	14.0 ± 0.15^a	17.6 ± 0.33	15.1 ± 0.37
% TBP_{est}	13.6 ± 0.27	14.8 ± 0.20^b	17.5 ± 0.21	15.6 ± 0.30
% TBF_c	7.0 ± 0.48	6.7 ± 0.42^a	7.8 ± 0.31	7.1 ± 0.27
% TBF_{est}	7.3 ± 0.64	5.8 ± 0.36^b	7.6 ± 0.62	6.6 ± 0.33

[a] Subscript c, carcass analysis; subscript est, estimated from TBW.
[b] LBM, TBP and TBF estimates were calculated from TBW_c and the equations derived from the separate set of mink in Fig. 2a,b and converted to a percentage of body mass. TBF_{est} calculated as $100 - \%LBM_{est}$. Estimated overall LBM, TBP and TBF did not differ from carcass analysis of overall LBM, TBP and TBF, respectively ($P > 0.05$, paired t-test). When divided into age groups, estimated values differed from carcass analysis for all components at 28 days ($P < 0.05$, i.e. means with different superscripts), but did not differ from one another at 21 or 42 days ($P > 0.40$, paired t-test).

($y = 64.65 + 4.99x + 0.02x^2$, $R^2 = 0.965$, $P < 0.001$).

3.3. Validation of body composition estimates from water space

In the separate validation set of 27 mink kits, body composition was determined by carcass analysis. These kits had a similar range in body mass (98–482 g) and relative proportions of TBW, TBP and TBF were also similar to that of the main study mink (Table 2). Again, there were no significant differences in body composition between sexes ($P > 0.7$, ANOVA), hence data from males and females were combined. The water space of these mink were then used to test the validity of the equations derived (Fig. 2) for estimating body composition. Across all 27 validation mink, estimates of LBM, TBP and TBF were highly correlated with carcass analysis of the same components (Fig. 3a–c). In all cases the intercept was not significantly different than 0 ($P > 0.2$) and the slopes were not significantly different than 1 ($P > 0.2$). However, LBM_{est} was most accurately predicted, while TBF_{est} was quite variable; TBP_{est} was somewhat intermediate (Fig. 3a–c). The average percent error in estimates was 0.6% for LBM, 3.3% for TBP and -6.6% for TBF. Across all 27 mink, estimates of LBM, TBP and TBF were not significantly different than carcass analyses ($P > 0.05$, paired t-test, Table 2). However, when split into age groups, estimated versus carcass analysis did not differ at 21 and 42 days, but did differ significantly at 28 days (Table 2). When 28 day old mink were removed, errors for all estimated components were $< 0.9\%$. TBE estimated from TBW (1593 ± 194.5 kJ) was also highly correlated to that calculated from carcass analysis (1591 ± 184.5 kJ) ($R^2 = 0.961$, $P < 0.001$). TBE_{est} did not differ from TBE_C (average % difference $0.4 \pm 2.06\%$, $P > 0.9$, paired t-test).

4. Discussion

Hydrogen isotope dilution has become a widely used and well-validated means with which to measure the total body water (TBW) of live animals (reviewed in Speakman, 1997). However, the use of the TBW measured, to then estimate the fat, protein and energy content of the body of live animals has not been as well studied. Early investigations (Pace and Rathbun, 1945) used a constant factor across all mammals, applied to TBW values, to estimate components of body mass. However, a number of more recent studies have demonstrated that empirical relationships derived by regression of TBW against components of body mass for each given species is a far more accurate and reliable method (Reid et al., 1963; Rumpler et al., 1987; Reilly and Fedak, 1990; Farley and Robbins, 1994; Hilderbrand et al.,

1998). Since interspecific extrapolation is likely to reduce the accuracy of predictions (Reilly and Fedak, 1990), it is important that a broad range of species be studied. Additionally, although it may not in practice be possible to derive empirical relationships for every species, measurements from closely-related species are likely to provide the next best alternative for reliable calculations. Relatively few such studies have been conducted in carnivores and especially in growing juveniles. Our results confirm not only the accuracy of measuring TBW by isotope dilution, but provide new data on the empirical relationship between TBW and total body fat (TBF), protein (TBP) and energy (TBE) in a young carnivore, the mink.

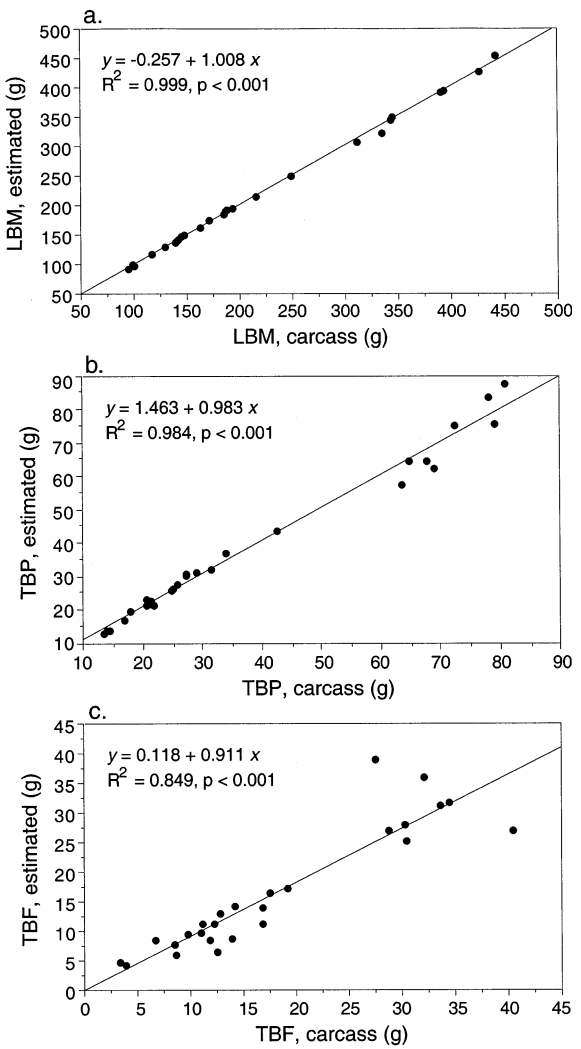

Fig. 3. Relationships between body composition determined by carcass analysis and estimated from TBW space in a validation set of mink kits ($n = 27$): relationships between: (a) carcass LBM and estimated LBM; (b) carcass TBP and estimated TBP; and (c) carcass TBF and estimated TBF. See Table 2 legend for calculations of LBM_{est}, TBP_{est} and TBF_{est}. S.E. of the intercepts were 1.809, 1.104 and 1.571 for a, b and c, respectively. S.E. of the slopes were 0.007, 0.025 and 0.077 for a, b and c, respectively. Residual S.E. of predicted y on x was 4.113, 2.986 and 4.095 for a, b and c, respectively.

When estimating body water by hydrogen isotope dilution techniques, the isotope administered is assumed to mix only with body water. However, the isotope is also known to exchange with organic molecules containing hydrogen or amino groups resulting in loss of isotope from, and overestimation of, TBW by 1–5% (Oftedal and Iverson, 1987; Speakman, 1997). Results from our study concur with those found for black bears *Ursus americanus*, and several species of pinnipeds, as well as other mammalian taxa (Nagy and Costa, 1980; Farley and Robbins, 1994; Speakman, 1997; Bowen and Iverson, 1998; Hilderbrand et al., 1998). Our overestimate (4.1%) was slightly higher than that found for black bears (3.7%) and pinnipeds (3.3%), as well as adult mink (1–3%; Wamberg, 1996), but similar to the average overestimation found for most mammals studied (4.3%; Speakman, 1997).

The use of isotope dilution in small animals, such as in the present study, is associated with some technical difficulties. This is especially true in the early growth phase, when the mink kits weighed as little as 80 g. Due to the small size of the kits only small quantities of blood could be collected and only one analysis per sample was performed. We presumed that the problems associated with small animal size would be likely to cause larger errors in measuring body water than would direct carcass analysis. This was especially the case since the carcass was able to be processed whole without prior preparation, thus avoiding any loss of fluids and minimizing evaporation. Nevertheless, our dilution spaces measured from isotope dilution were very tightly and consistently correlated with carcass analyses (Fig. 1a, b).

Carcass analysis itself may also be associated with errors. For instance, body water estimates can be affected by evaporation, fluid losses and incomplete drying, resulting in an underestimation of TBW. Errors in carcass analysis may potentially be more problematic in large animals due to fluid loss during the preparation (i.e. in sections) of the carcass for whole body grinding. However, as stated above especially in small animals, which can be processed whole in a single

container, our carcass analysis of body water is likely to be accurate as we ensured that complete drying occurred. Analyses of protein and fat content from carcass analyses also may be associated with errors. However, analysis of protein using total nitrogen and gravimetric fat analyses by extraction as used in this study are considered reliable methods (AOAC, 1984).

Our finding from carcass analyses that the relationship between TBW or TBP and LBM changed with days of age in mink kits likely reflects the relatively more hydrated states of newborn mammals (Spray and Widdowson, 1950; Adolph and Heggeness, 1971; Tauson, 1994). TBW decreased from 81 to 76% of LBM from 21 to 42 days in mink kits, which is consistent with that found in kittens from birth to 80 days (82–76%; Spray and Widdowson, 1950). TBP increased from 14 to 19% of LBM in mink kits, again similar to that found in kittens at 15–20% of LBM (Spray and Widdowson, 1950). On average, the LBM of mink kits in our study was comprised of 78.6% water and 16.5% protein, which is similar to the LBM of kittens at 40 days of age that is comprised of 78% water and 17% protein (Spray and Widdowson, 1950). However, if we had used a single value throughout all ages for the composition of LBM in mink kits, our estimates of TBF and TBP from isotope dilution would have been less precise. Thus, when working with altricial growing neonates, it is clear that a rapidly changing hydration state from birth should be taken into account in estimating water and protein contents of LBM. Spray and Widdowson (1950) found these changes to be the most rapid in the first 40 days of life in rats, rabbits, pigs and kittens, while changes become relatively minor by 80–120 days. When taking into account these changes in LBM composition with age (Fig. 2a, b), we were able to use TBW, either estimated from isotope dilution or by carcass analysis, to accurately estimate LBM, TBP, TBF and TBE. Our estimates of LBM, TBP, TBF and TBE were not significantly different than those obtained from direct carcass analysis across all mink (Tables 1 and 2; Fig. 3) which was also found for black bears and grey seals (*Halichoerus grypus*)(Reilly and Fedak, 1990; Farley and Robbins, 1994; Hilderbrand et al., 1998). Although the estimated composition values differed from carcass values in the 28-day validation mink, this may have been due in part to the fact that the empirical relationships used for these calculations at this age were derived based on only three animals in contrast to eight or nine individuals at 21 and 42 days of age. In our study, the relationship of fat content determined from carcass analysis or by estimation from derived equations was more variable than that for protein, which may in part have reflected the difficulties that were associated with procedures involved in accurately extracting carcass body fat. Small errors in the estimation of LBM from body water may also lead to relatively larger errors in estimated body fat due to the relationship TBF = body mass − LBM (Henen, 1991).

In conclusion, the relationship of TBW to isotope dilution space in young growing mink kits is consistent with that previously found for other mammals (Speakman, 1997). While changes postpartum in relationships between TBW and other components of body mass occur, when these factors are taken into account, accurate estimates of body composition are able to be obtained. Although changes in hydration state appear to be similar among other altricial mammalian neonates, it will be important to compare the empirical relationships of other components of body composition derived herein to that of other altricial neonates in order to understand the extent to which these relationships can be used interspecifically.

Acknowledgements

We would like to thank Jill Hendsbee for help with animal handling and analysis of samples. Funding for Kirsti I. Rouvinen-Watt was provided by Agri-Focus 2000 Technology Development Program and Agriculture and Agri-Food Canada; post graduate scholarships to Heather N. Layton were provided by Natural Sciences and Engineering Research Council (NSERC), the Nova Scotia Department of Agriculture and Marketing, the Nova Scotia Fur Institute, the Canada Mink Breeders Association; and an NSERC operating grant provided funding for Sara J. Iverson.

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