TECHNICAL COMMENT

Estimation of Total Body Water in Pinnipeds Using Hydrogen-Isotope Dilution

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Hydrogen-isotope dilution methods have been used widely to study the body composition, milk intake, solid-food intake, and energy metabolism of pinnipeds (Costa 1987; Oftedal and Iverson 1987) and other vertebrates (Nagy 1987). Indeed, much of the current understanding of water balance and of the reproductive and foraging energetics of large mammals comes from studies of this group of species. An important assumption in the use of isotope dilution for these purposes is that the tracer mixes completely, and only, with total body water (TBW) of an animal that has been given a known quantity of deuterium oxide (D_2O) or tritiated water (HTO). It has been known for some time that a small fraction of hydrogen isotope is lost to rapidly exchangeable hydrogen atoms in organic constituents of the body (see, e.g., Ussing 1935). Hevesy and Jacobsen (1940) estimated that 0.5%–2% of body mass is accounted for by rapidly exchanging hydrogen atoms of isotopes in organic compounds. For this reason, hydrogen-isotope dilution space will usually overestimate TBW, and it may be necessary to apply an appropriate correction factor to dilution space. Although relationships between TBW and hydrogen-isotope dilution spaces have been studied in other mammalian groups (see, e.g., Nagy and Costa 1980), these relationships in pinnipeds are not well understood.

The only true validation of isotope-dilution space estimates of TBW depends on measurement by isotope dilution followed by carcass desiccation of the same individual. This has been done in a number of taxa, and the generally close correspondence between estimated dilution space and TBW measured by desiccation indicates that isotope dilution is a valid technique (Pinson 1952; Nagy and Costa 1980). However, recent practice varies considerably with respect to the estimation of TBW in pinnipeds from hydrogen-dilution space. In many studies, dilution space and TBW have been assumed to be equal; in others, it has been assumed that dilution space overestimates TBW by 4.0%; and in still others, authors do not state how TBW was estimated from dilution space. More recently, authors have used equations developed for single species (Reilly and Fedak 1990; Arnould et al. 1996). The purpose of this article is to review the relationship between TBW and hydrogen-isotope dilution space in pinnipeds and to develop a single predictive equation to estimate TBW in species for which data are lacking. Adopting such an approach will provide a consistent basis for comparative studies not only within pinnipeds but in relation to other mammalian taxa.

In the Otariidae (i.e., fur seals and sea lions), only one study has compared hydrogen-isotope dilution space with TBW derived from carcass analysis of the same individuals. Arnould et al. (1996) examined the accuracy of both HTO and D_2O as a means of estimating TBW in four pups and five adult female Antarctic fur seals (*Arctocephalus gazella*). HTO dilution space significantly overestimated TBW of pup and adult females, by an average of 1.9% ± 1.00% ($n = 9$). D_2O dilution space also overestimated TBW of adult females by an average of 1.7% ± 1.74% ($n = 5$), but this difference was not significantly different than zero. Using these data, Arnould et al. (1996) derived predictive regression equations to estimate TBW from either HTO or D_2O dilution space. However, in the case of HTO, the slope of the regression did not differ significantly from 1.0, and the intercept did not differ from zero. It is therefore not clear that the use of this relationship would improve the estimation of TBW compared to the use of estimates of HTO dilution space. However, given the small sample size, failure to reject the null hypothesis that the slope equals 1.0 may have simply reflected the low statistical power of the test.

In contrast to the study in otariids, hydrogen-isotope dilution space has been compared with carcass desiccation in four species of the Phocidae (i.e., hair seals). Reilly and Fedak (1990) administered both D_2O and HTO to four grey seals (*Halichoerus grypus;* two pups and two adults) that were subsequently desiccated. Isotope dilution overestimated TBW in all four animals. The mean overestimation was 2.8% for D_2O and 4.0% for HTO, although there was no significant difference between isotopes (paired *t*-test, $t = 0.87$, df = 3, $P = 0.45$). Lydersen et al. (1992) found that HTO dilution space overesti-

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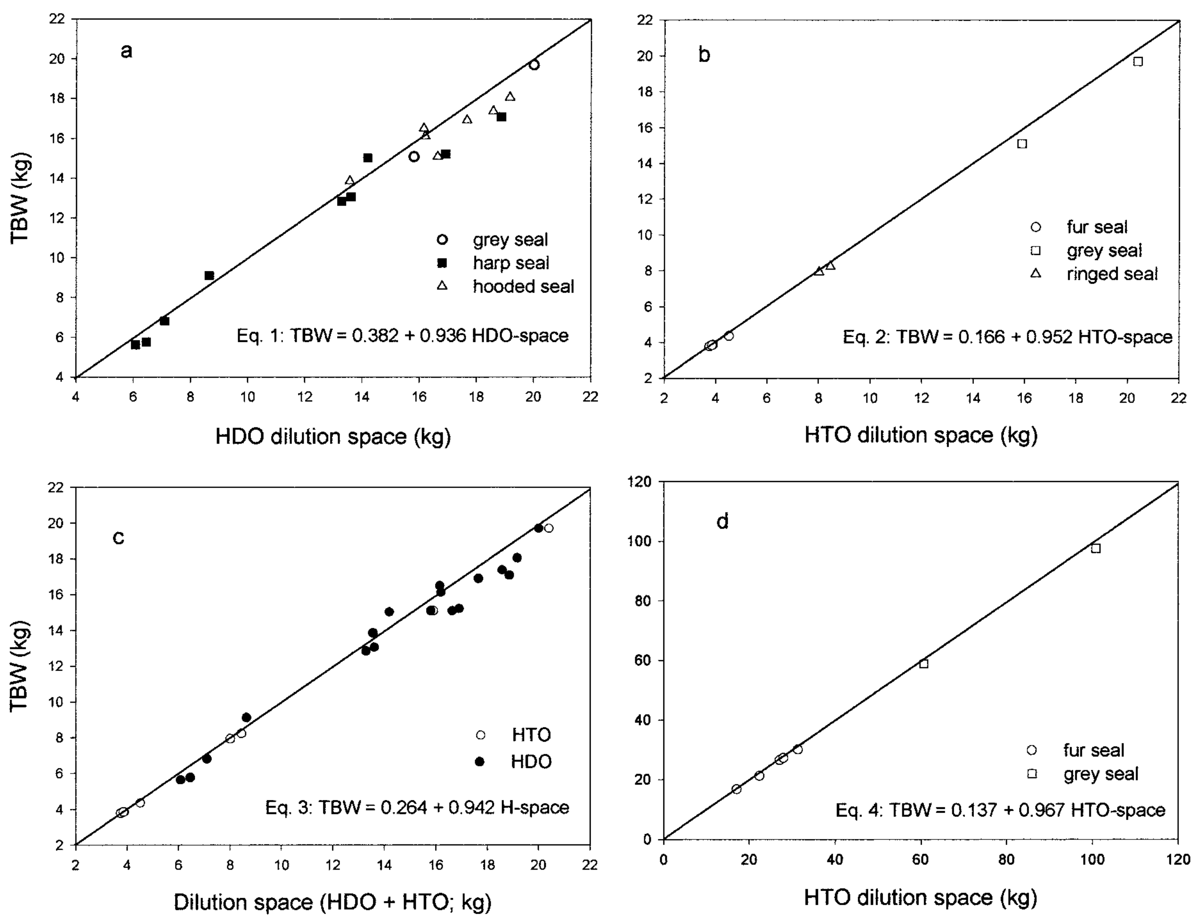

Figure 1. Relationships between TBW estimated by carcass desiccation and D_2O (HDO) dilution space in neonates (*a;* $n = 18$, standard error of the slope [se_{b1}] = 0.038, $r^2 = 0.972$, $P_{(slope = 1.0)} = 0.12$) among species; HTO dilution space in neonates (*b;* $n = 8$; $se_{b1} = 0.007$, $r^2 = 0.999$, $P_{(slope = 1.0)} < 0.001$) among species; D_2O (HDO) dilution space and HTO dilution space in pinniped neonates (*c;* $n = 22$, $se_{b1} = 0.024$, $r^2 = 0.987$, $P_{(slope = 1.0)} < 0.001$); and HTO dilution space in adults (*d;* $n = 7$, $se_{b1} = 0.003$, $r^2 = 0.999$, $P_{(slope = 1.0)} = 0.03$). One-to-one reference lines are also plotted.

mated TBW by an average of 1.6% in two ringed seal (*Phoca hispida*) pups. Oftedal et al. (1993) found no significant difference between D_2O dilution space and TBW by desiccation in seven hooded seal (*Cystophora cristata*) pups, although the mean difference between the two estimates was +3.4% ± 1.82%. D_2O dilution space was 4.7% ± 2.16% greater than TBW by desiccation in nine harp seal (*Phoca groenlandica*) pups, but again this difference was not significantly different from zero (Oftedal et al. 1996).

Owing to the large size of pinnipeds, validation studies on them are difficult to conduct, and it is unlikely that predictive equations can be developed for all species. Furthermore, as sample size is small in existing studies, failure to reject the null hypothesis of a slope of 1.0 may often simply reflect low statistical power. One way to approach this problem is to ask whether the existing data on pinnipeds fit a single predictive equation. We began by comparing, for both isotopes, the relationship between TBW by desiccation and hydrogen-dilution space among pinniped species. Validation studies using D_2O and HTO have been conducted with pups of five species (Fig. 1*a*, *b*). The data from these species fit a single regression well in the case of both isotopes (eqq. [1] and [2], Fig. 1*a*, *b*). D_2O overestimated TBW of pups by an average of 5.8%, whereas HTO did so by 4.7%. There was no significant difference in either the slopes or intercepts of the regressions of D_2O and HTO dilution spaces on TBW of pups, such that a single relationship described the data with good precision (eq. [3], Fig. 1*c*). However, mean differences between D_2O dilution space and TBW by desiccation were more variable (CV = 142.4%, $n = 18$) than differences between HTO dilution space and TBW (CV = 69.2%, $n = 8$). Adults of only two species were used in a comparison of TBW and HTO dilution space. Although the data are more limited than that on pups, again a single equation (eq. [4], Fig. 1*d*) described the data for both Antarctic fur seals and grey seals quite well. HTO overestimated TBW by 3.1% in adults.

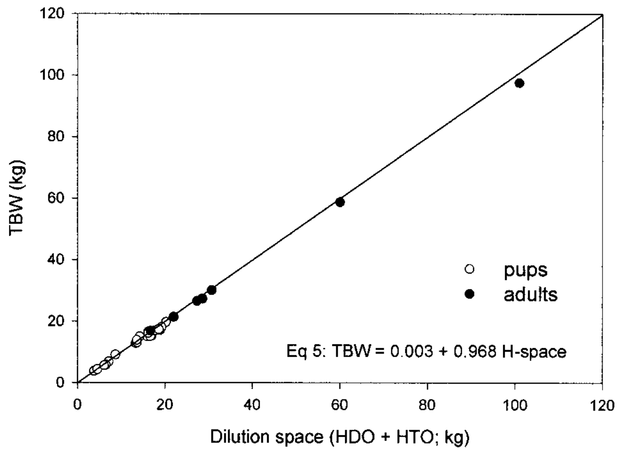

Figure 2. Relationships between TBW estimated by carcass desiccation and dilution space (D_2O [HDO] and HTO combined) over all ages and species ($n = 29$; $se_{b1} = 0.005$, $r^2 = 0.999$, $P_{(slope = 1.0)} < 0.001$). The one-to-one reference line is also plotted.

Given that there were no significant differences either among species or between isotopes, we fit a regression model to all the available pinniped data. The resulting regression equation (eq. [5], Fig. 2) shows that hydrogen-dilution space overestimated TBW by 3.3% ± 0.005%.

There are a number of reasons why TBW by desiccation and hydrogen-isotope dilution space may not be the same. Errors in isotope-dilution methods are generally overestimates and are systematic due to loss of isotope. By contrast, errors in the measurement of TBW by desiccation due to evaporation or incomplete drying result in underestimates. Compounding of these two potential errors widens the gap between estimates from isotope-dilution and desiccation. Nevertheless, it is usual to assume that TBW by desiccation is a measure of the true value.

Lack of equilibration of the isotope will result in different systematic errors depending on the route of isotope administration. Four routes have been used in pinnipeds: intravenous injection, intramuscular injection, intraperitoneal injection, and gastric intubation. In the case of intravenous administration, blood samples taken before equilibration has occurred will result in an underestimation of TBW. For the other routes of administration, lack of equilibration will result in overestimation of TBW. Error associated with lack of equilibration is easily avoided by determining isotope equilibration curves and then taking two serial samples after equilibration is most likely to have occurred.

Distribution of hydrogen isotopes following a bolus injection is a function of both circulation and diffusion of the isotope into the intercellular space (Hevesy and Jacobsen 1940; Edelman 1952). As both D_2O and HTO appear to be highly diffusible in water and across cell membranes (Hevesy and Jacobsen 1940), it appears that low perfusion rate in a large compartment of the body may be responsible for the rather long time (ca. 1–2 h; Pinson 1952; Houseman et al. 1973) to equilibration that is commonly observed in large mammals (Coleman et al. 1972).

Equilibration times in pinnipeds (i.e., 0.5–3.0 h) appear to be generally consistent with those found in other large mammals. Although equilibration curves have been measured in both pups and adults via several routes of administration (Costa 1987; Oftedal et al. 1987; Lydersen et al. 1992; Arnould et al. 1996; J. E. Mellish, C. A. Beck, S. J. Iverson, and W. D. Bowen, unpublished data), insufficient data are available to draw firm conclusions about the factors affecting equilibration times in pinnipeds. Milk in the stomach of suckling pups can significantly extend equilibration time (Costa 1987; Oftedal et al. 1987). There is evidence also from harbour seals that equilibration time of D_2O may be significantly delayed during late-term pregnancy (Glazebrook 1991).

Current pinniped data suggest that HTO and D_2O occupy the similar dilution spaces and overestimate TBW by an average of 3.3%. There is evidence that D_2O and HTO also become distributed in the same equilibration dilution space in dogs (Robinson 1951), rabbits (Anbar and Lewitus 1958), and humans (Leibman et al. 1960). The level of overestimation in pinnipeds is similar to that found in a number of other mammalian taxa (Nagy and Costa 1980).

Although validation studies have been conducted in only five species, the available data indicate that TBW in pinnipeds can be estimated rather well from hydrogen-isotope dilution spaces. Clearly, more data are needed on adult pinnipeds. Also, as data are available in only one otariid species, other studies are needed to confirm that a single equation is adequate for both phocid and otariid pinnipeds. Nevertheless, it is clear from existing data that hydrogen-dilution space does overestimate TBW in pinnipeds in a predictable way. Thus, hydrogen-dilution space should not be used as a direct estimate of TBW. We suggest the use of equation (5) given in Figure 2 to estimate TBW in species for which data are not available. The adoption of this approach should provide a firmer basis for interspecific comparisons of total body fat, energy content, and energy expenditure derived from changes in body composition.

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Literature Cited

Anbar M. and Z. Lewitus. 1958. Rate of body-water distribution studied with triple labeled water. Nature 161:344.

Arnould J.P.Y., I.L. Boyd, and J.R. Speakman. 1996. Measuring the body composition of Antarctic fur seals (*Arctocephalus gazella*): validation of hydrogen isotope dilution. Physiol. Zool. 69:93–116.

Coleman T.G., R.D. Manning, Jr., R.A. Norman, Jr., and A.C. Guyton. 1972. Dynamics of water-isotope distribution. Am. J. Physiol. 223:1371–1375.

Costa D.P. 1987. Isotopic methods for quantifying material and energy balance of free-ranging marine mammals. Pp. 43–66 in A.D. Huntley, D.P. Costa, G.A.J. Worthy, and M.A. Castellini, eds. Approaches to Marine Mammal Energetics. Allen, Lawrence, Kans.

Edelman I.S. 1952. Exchange of water between blood and tissues. Characteristics of deuterium oxide equilibration in body water. Am. J. Physiol. 171:279–296.

Glazebrook K. 1991. The Use of Ultrasound and Isotope Dilution to Estimate Body Composition and Food Intake in Pregnant and Non-Pregnant Captive Harbour Seals (*Phoca vitulina concolor*). MSc thesis. Dalhousie University, Halifax, Canada.

Hevesy G. and C.F. Jacobsen. 1940. Rate of passage of water through capillary and cell walls. Acta Physiol. Scand. 1:11–18.

Houseman R.A., I. McDonald, and K. Pennie. 1973. The measurement of total body water in living pigs by deuterium oxide dilution and its relation to body composition. Br. J. Nutr. 30:149–156.

Leibman J., F.A. Gotch, and I.S. Edelman. 1960. Tritium assay by liquid scintillation spectrometry. Comparison of tritium and deuterium oxides as tracers for body water. Circ. Res. 8:907–912.

Lydersen C., M.O. Hammill, and M.S. Ryg. 1992. Water flux and mass gain during lactation in free-living ringed seal (*Phoca hispida*) pups. J. Zool. (Lond.) 228:361–369.

Nagy K.A. 1987. Field metabolic rate and food requirement scaling in mammals and birds. Ecol. Monogr. 57:111–128.

Nagy K. and D.P. Costa. 1980. Water flux in animals: analysis of potential errors in the tritiated water technique. Am. J. Physiol. 238:R454–R465.

Oftedal O.T., W.D. Bowen, and D.J. Boness. 1993. Energy transfer by lactating hooded seals and nutrient deposition in their pups during the four days of lactation. Physiol. Zool. 66:412–436.

———. 1996. Lactation performance and nutrient deposition in pups of the harp seal, *Phoca groenlandica*, on ice floes off southeast Labrador. Physiol. Zool. 69:635–657.

Oftedal O.T. and S.J. Iverson. 1987. Hydrogen isotope methodology for measurement of milk intake and energetics of growth in suckling young. Pp. 67–96 in A.D. Huntley, D.P. Costa, G.A.J. Worthy, and M.A. Castellini, eds. Approaches to Marine Mammal Energetics. Allen, Lawrence, Kans.

Oftedal O.T., S.J. Iverson, and D.J. Boness. 1987. Milk and energy intakes of suckling California sea lions, *Zalophus californianus*, pups in relation to sex, growth, and predicted maintenance requirements. Physiol. Zool. 60:560–575.

Pinson E.A. 1952. Water exchanges and barriers as studied by the use of hydrogen isotopes. Physiol. Rev. 32:123–134.

Reilly J.J. and M.A. Fedak. 1990. Measurement of the body composition of living gray seals by hydrogen isotope dilution. J. Appl. Physiol. 69:885–891.

Robinson C.V. 1951. A methane, proportional counting method for the assay of tritium. Rev. Sci. Instrum. 22:353–355.

Ussing H.H. 1935. Exchange of H and D atoms between water and protein in vivo and in vitro. Skand. Arch. Physiol. 78:225.