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Title:

Body water and body composition of free-ranging Atlantic walruses (*Odobenus rosmarus rosmarus* L.) studied by isotope dilution

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Body water and body composition of walruses

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Abstract

Deuterium oxide was used to measure isotope dilution space 11 times in seven free-living, adult, male Atlantic walruses (*Odobenus rosmarus rosmarus* L.) (930 - 1597 kg) in N.E. Greenland, August 2000-2001. Equilibration of the intravenously injected isotope was complete after 2-3 hours. The application of a general correction factor developed for all pinniped species allowed the estimation of total body water content to 56.8 % (range: 36.6-73.3 %) of total body mass (TBM). Water turnover averaged 44.5 g/kg*day. For one animal it was possible to estimate water influx to 4.8 g/kg*day and water efflux to 21.8 g/kg*day. The estimates of body fat and body protein averaged respectively 24.7 % and 18.4 % of TBM. Similarly, for an "average" walrus with a TBM of 1300 kg body ash was calculated to average 26 kg and body gross energy to 18300 MJ. The assessment of body fat by isotope dilution did not differ significantly from the estimates of blubber content obtained from the dissection of walruses in the wild. This work demonstrates the feasibility of using the hydrogen isotope dilution technique for estimating body composition of walruses.

Introduction

The subcutaneous adipose tissue layer (blubber) is often used to assess body condition of pinnipeds (Gales and Renouf 1994; Gales, Renouf, and Noseworthy 1994) as it represents the main energy store for breeding and moulting (Bryden 1968). Hence, individuals with better body condition have greater chances of reproductive success (Bowen et al. 1994; Arnbom, Fedak, and Boyd 1997; Pomeroy et al. 1999). Proper estimation of fat content, hence body condition, is relevant for the evaluation of the responses at an individual level to major changes in the carrying capacity of a population (Fay, Kelly, and Sease 1989).

Direct chemical analysis of body composition of seals has been carried out only on five species (Reilly and Fedak 1990; Lydersen, Hammill, and Ryg 1992; Oftedal, Bowen, and Boness 1993; Gales, Renouf, and Noseworthy 1994; Arnould, Boyd, and Speakman 1996; Oftedal, Bowen, and Boness 1996), not including walrus. Walrus body composition has been investigated only by dissection and weighing of body components (Knutsen and Born 1994).

Blubber is the only body component measurable directly in field studies by non-destructive methods. To date the only direct, non-invasive, non-destructive technique available to measure the thickness of the blubber layer of live seals is ultrasonography (Slip, Burton, and Gales 1992; Webb et al. 1998). Alternatively, the amount of an animal's body fat can be indirectly estimated *in vivo* from measures of total body water (TBW) by hydrogen isotope dilution analysis (IDA) (Sheng and Huggins 1979; Nagy and Costa 1980; Costa 1987; Oftedal and Iverson 1987; Reilly and Fedak 1990; Slip, Burton, and Gales 1992; Arnould, Boyd, and Speakman 1996; Bowen and Iverson 1998; Beck, Bowen, and Iverson 2003) or by bioelectrical impedance analysis (BIA) (Gales, Renouf, and Worthy 1994; Arnould 1995; Bowen, Beck, and Iverson 1999).

The species of pinnipeds for which validation of isotope dilution studies exist to date are Antarctic fur seals (*Arctocephalus gazella*) (Arnould, Boyd, and Speakman 1996), Gray seals (*Halichoerus grypus*) (Reilly and Fedak 1990), Ringed seal (*Phoca hispida*) (Lydersen,

Hammill, and Ryg 1992), Hooded seal (*Cystophora cristata*) (Oftedal, Bowen, and Boness 1993) and Harp seal (*Phoca groenlandica*) (Oftedal, Bowen, and Boness 1996).

Bioelectrical impedance analysis has proven fast, inexpensive and reliable for humans (Lukaski et al. 1986; Kushner et al. 1990), bears (Farley and Robbins 1994), horses (Forro et al. 2000), pigs and sheep (Jenkins, Leymaster, and Turlington 1988; Swantek et al. 1992). This technique is generally practical for field studies due to the light experimental setup. But though BIA seems to perform well for some species of pinnipeds such as harp, ringed (Gales, Renouf, and Worthy 1994) and gray seals (Bowen, Beck, and Iverson 1999), it appears to be a poor predictor for TBW in others such as female Anctarctic fur seals (Arnould 1995) and in both female and male harbor seals (*Phoca vitulina*), though it performed better with suckling pups (Bowen, Boness, and Iverson 1998) of the same species. Bowen *et al.* (1998) reported that in spite of sedation, the immobilized seal reacted to handling with muscular contractions that possibly induced great variability in the BIA measures. They stated that (a) chemical anesthesia actually improves the precision of the technique and (b) that instead of using them independently, BIA measurements can be "a valuable adjunct" to the more precise IDA techniques (Bowen, Beck, and Iverson 1999).

Among the more precise methods, the hydrogen (tritium or deuterium) IDA technique has been widely used on several species of vertebrates in field situations where it can be utilized alone or coupled with other IDA techniques (e.g. ^{18}O) for the determination of energy expenditure (Nagy 1987; Speakman 1997; Nagy, Girard, and Brown 1999; Acquarone et al., *unpublished*), water flux (Depocas, Hart, and Fisher 1971; Nagy and Costa 1980; Lydersen, Hammill, and Ryg 1992; Boyd, Arnbom, and Fedak 1993; Lea et al. 2002) food consumption (Costa 1988; Costa, Croxall, and Duck 1989; Nagy, Girard, and Brown 1999) and milk transfer between mother and offspring (Lydersen et al. 1996; Carlini et al. 2000; Donohue et al. 2002). Several authors consider IDA to be a very precise technique for pinnipeds, provided sufficiently long immobilization time for isotope equilibration (up to 3 hours) (Bowen and Iverson 1998).

The hydrogen IDA technique has been chosen for this study because of its superior precision compared to other techniques to measure the hydrogen dilution space, and thus infer TBW and body fat, in free-living walruses. The body fat content thus derived has been compared with direct measures of walrus body composition from animals killed during the Inuit's traditional hunt in Greenland (Knutsen and Born 1994) and with one animal that was dissected *in situ* in NE Greenland in 2001 (this study).

The data presented in this work may form the basis for a deeper understanding of the physiology of this species and it provides essential data for future studies involving isotope dilution.

Materials and Methods

Study site

The study was carried out during August in 2000 and 2001 at Sandøen (74°15.7'N, 20°09.7'W) and at Lille Snenæs (76°52.7'N, 19°37.9'W) in North-East Greenland, which are the only two regularly-used, terrestrial haulouts for walruses in Greenland (Born, Gjertz, and Reeves 1995).

Choice of animals

Fourteen individual walruses were chosen among the all-male groups using the haulout. Within the study period some animals were captured several times within the same year and between the years (Table A) (Project approval of the Greenland Home Rule, file:28.40.10). Animal choice criteria were: sufficiently large tusk size for instrument attachment, quiet behaviour for effective immobilization, relative placement farthest from the shoreline to minimize risk of escape at sea in the anesthesia induction phase and relative placement clear of the other hauled-out individuals for safer remote injection of the immobilizing agent (Born and Knutsen 1992a).

Immobilization

Before handling the animals were completely immobilized by remote injection of etorphine HCl when hauled-out on land, using the protocol described in Born and Knutsen (1992a), Griffiths *et al.* (1993) and Acquarone et al. (*unpublished*). When effectively immobilized the animals were approached and rolled supine whereafter their length and girth were measured (American Society of Mammalogists 1967) for estimation of total body mass (TBM) (Born et al. 2003), the instruments were attached to the tusks and venous access for blood sampling and isotopic enrichment was gained by catheterization of the epidural vein in the lumbar region. After completion of this handling, the animals were usually allowed to regain consciousness and to escape to sea at will. In some cases the animals were kept sedated for up to 7 hours (Griffiths et al., *unpublished*) to investigate the dynamics of isotopic equilibration. Upon recapture a similar immobilization procedure was effectuated followed by somatic measuring, blood sampling and instrument data retrieval.

Water metabolism

At initial capture the venous blood was sampled through the epidural catheter for determination of background isotope concentration. An intravenous dose of either concentrated (99.8 %) deuterium oxide (98 to 148 ml) or of doubly labeled water (98 to 158 ml, containing 44 % deuterium oxide-99.8 %) or of a saline solution of deuterium oxide (629 ml, 5.3 % and 996 ml, 4.7 %) was subsequently administered. In all cases the source of deuterium oxide was spectroscopy grade heavy water (Merck 1.13366, E.Merck, D-6100 Darmstadt, Germany). In 7 cases it was possible to sample blood at regular intervals from the infusion of the isotope. This allowed the determination of isotope concentration in the blood for up to 6 hours from enrichment. The subjects returned to the beach and were re-immobilized after 3 to 19 days from isotopic enrichment, blood was sampled again either through an epidural vein catheter or in two cases by bleeding the hind flipper plexus. On one occasion it was possible to re-enrich the animal and subsequently to obtain a second series of

blood samples. At all times whole blood was directly poured in 2-ml standard glass vials immediately after sampling and flame sealed.

Seawater background samples were also collected at both study locations and immediately flame sealed in 2-ml glass vials to investigate fluctuations in ambient isotope concentrations.

All samples were stored at ambient temperature (max 12.9 °C, usually <5 °C) while in the field and were subsequently kept refrigerated at 5 °C prior to analysis.

Sample analysis

In preparation for mass spectroscopy, all blood samples were vacuum distilled into Pasteur pipettes (Speakman 1997) and the distillate was used for determination of ^{2}H concentration. $^{2}H_2$ gas was produced by reduction with excess $LiAlH_2$ as described in Ward et al. (2000) The isotopic composition of the injectate was measured by diluting a weighed quantity of the injectate (0.1 - 0.2 ml) into a weighed quantity of tap water (60 ml). This mixture was then treated in exactly the same manner as the distillate from the blood samples. In each batch of samples for analysis, laboratory standards were included to account for day-to-day variation in the analyzer. All isotope enrichments were measured in δ-units and converted to absolute ppm using the established ratios for reference materials.

Calculations of isotope dilution space and water turnover rate

Calculations of hydrogen isotope dilution space size were made according to the method described in Speakman (1997). In particular the values of dilution space and fractional turnover rate (K_d) were calculated using the iterative calculation method assuming steady state. TBW was estimated from the hydrogen isotope dilution space using a general regression equation developed to correct for the overestimation of TBW from dessication [TBW = 0.003 + 0.968 * (H-dilution space)] (Bowen and Iverson 1998). The equations (3), (4) and (6) from Nagy and Costa (1980) were used for determination of water flux (WF).

Body composition

Body composition was calculated from estimates of TBW derived from the hydrogen dilution space. All walruses for which a TBW estimate was available were included in the calculations for body composition except walrus M (Table A). This individual's estimated TBW content indicated an extremely lean animal when used in conjunction with the established allometric equations. For all other individuals total body fat (TBF) was calculated according to the general allometric equation for mammals [% TBF = 100 - (% TBW/0.732)] (Pace and Rathbun 1945). The values derived from these equations were compared to the similar results from the allometric equation for total body fat, total body protein (TBP), total body ash (TBA) and total body gross energy (TBGE) derived for gray seals [% TBF = 105.1 - 1.47 $*$ (% TBW); % TBP = 0.42 $*$ (% TBW) – 4.75; TBA (in kg) = 0.1 – 0.008 $*$ (TBM in kg) + 0.05 $*$ (TBW in kg); TBGE (MJ) = 40.8 $*$ (TBM in kg) – 0.4] (Reilly and Fedak 1990). These estimates of % TBF were compared using two-tailed t-tests for data with unequal variances (Zar 1999) to the body composition of walrus estimated by dissection (Knutsen and Born 1994, and this study).

On 21 August 2001, an adult male (H) with a TBM of 1550 kg was dissected after it had died in connection with handling. Skin, blubber, muscle and all internal organs were weighed separately following the methods in Knutsen and Born (1994).

Satellite-linked radio transmitter and time depth recorder data analysis

Either an ARGOS System SDR-T10 or SDR-SSC3 Satellite-linked radio-transmitter (SLRT) with Time at Depth Histograms (6 animals) or a SPOT2 SLRT with time-at-temperature histograms (7 animals) and a Mk7 time depth recorder (TDR) with 500 m range in 2000 (4 animals) and 1000 m in 2001 (3 animals) (all instruments: Wildlife Computers, Redmond WA, USA) were attached to the tusks of the walruses using the method in Born and Knutsen (1992b) and Gjertz *et al*. (2001). Six animals were equipped with a SLRT only and one with a TDR only (Table A).

In addition to providing data on location, the SDR-T10 and SDR-SSC3 transmitters were able to collect dive data to a depth of 250 or 500 m with a resolution of 1 or 2 m, respectively. Information on haul-out and at-sea time was collected by two of the SLRT in 2000 via ”timelines” (TIM) (Born, Teilmann, and Riget 2002). The satellite transmitters, their sampling protocols and data processing are described in Born *et al.* (2002) and Acquarone and Born (unpublished).

During August the walruses were immobilized and handled several times at the beach. It cannot be excluded that handling, and in particular the prolonged anesthesia, may have affected their natural behavior to an unknown extent. Immobilization can cause some post treatment drowsiness (Born and Knutsen 1992a; Griffiths, Wiig, and Gjertz 1993). Because the study animals were equipped with SLRT’s we were able to compare their overall behavior during August when disturbance occurred with their behavior during September when there was no disturbance. For that purpose the information obtained via the SDR-T10 SLRT’s on TIM, time-at-depth, daily-average-dive-depth, and daily-max-dive-depth (DMDD) was used.

The TDR data were analyzed using the software provided by the manufacturer (Zero-Offset-Correction and Dive-Analysis). Periods when the walruses were hauled out on land or ice were identified from the instrument’s temperature profile and excluded from the analysis of dive activity. Minimum depth for a dive to be considered a foraging dive was assumed to 6 m. The time spent at sea or out of the water was determined from the temperature records of the TDR (only temperatures below 2.5 °C were considered as originating from a submerged sensor). Number of dives per day, dive duration and surface times were also determined for each individual.

Correlation between Water Turnover Rate and Activity

Linear regression was employed to investigate correlation between activity levels and water turnover rate. The activity parameters chosen for comparison with water turnover rate during

the monitoring period included “% time hauled-out”, “% time > 6 m” (*i.e.* likely foraging dives), “number of dives”, “average dive depth” and “average dive duration”.

Results

Background concentration of deuterium

In 2001 background deuterium concentration in seawater at Lille Snenæs averaged 148.2 ppm (SD = 8.1, range 131.2 - 168.6 ppm, n = 20). Background deuterium concentration in walrus blood averaged 152.9 ppm (SD = 6.5, range 138.5 - 165.0 ppm, n = 17). The walrus blood values did not show significant differences between the two sampling locations: Sandøen and Lille Snenæs. However, there were significant differences between the two sampling years ($t = -4.48$, $p < 0.05$, $df = 15$). The mean values for 2000 and 2001 were 148.4 ppm (SD = 4.4, range 138.5 - 152.4 ppm, n = 9) and 158.0 ppm (SD = 4.5, range 152.8 - 165.0 ppm, n = 8) respectively.

In 2001, when deuterium concentration was measured simultaneously in sea water and walrus blood, background enrichment levels in the blood of the animals did not differ significantly from sea water enrichment ($t = 0.82$, $p > 0.05$, $df = 7$).

Isotope equilibration time and dilution space

The time required for complete equilibration of the deuterium bolus within the body water pool was estimated visually to 2-3 hr from the graphs of decline in isotope concentration for seven enrichments (Figure 1).

The mean TBW content was 56.8 % of TBM (SD = 9.3, range 36.6 – 73.3 %; n = 11) (Table B). In 2000, the isotope dilution space size for animal C was measured twice, 19 days apart. An increase in TBW from 50 % to 60 % of TBM was seen between the first and the second measure. Three animals were captured both in 2000 and 2001. Between the two years, the estimates of TBW had decreased to 37 % of TBM for animal C, while for animal G and H, TBW decreased from 63 % to 55 % and from 60 % to 56 % of TBM, respectively.

Water Turnover Rate and Water Flux

Mean water turnover rate (WTR) was 44.5 g/kg*day (SD = 29.6; range 14.0 – 96.2 g/kg*day; n = 8) (Table C). For walrus C it was possible to measure TBW for twice within the same season which allowed to estimate water flux (WF) according to equations (4), (5) and (6) in Nagy and Costa (1980). There was no difference between the water efflux calculated for body water volume changing linearly or exponentially with time respectively with equation (4) and (5) and therefore only the results of equation (4) are presented.

Body Composition

Total body fat calculated from the equation of Pace and Rathbun (1945) averaged 24.7 % of TBM (SD = 10.8, range 12.0 - 50.0 %, n = 10). The corresponding value calculated from the equation for gray seals of Reilly and Fedak (1990) was 24.1 % of TBM (SD = 11.6, range 10.4 - 51.3 %, n = 10). There was no significant difference ($t = 0.12$, $p > 0.05$, $df = 18$) between the two estimates. Neither of the calculations of TBF (Pace and Rathbun 1945; Reilly and Fedak 1990) differed significantly from the mean value of 18 % (SD = 4.3, range 11 - 26 %, n = 15) for the measures of blubber content in relation to TBM for North West Greenland walruses of all ages and both sexes (Knutsen and Born 1994) (two tailed *t*-test - unequal variances, $t = 1.88$, $p > 0.05$, $df = 10$ for Pace and Rathbun (1945) and $t = 1.59$, $p > 0.05$, $df = 10$ for Reilly and Fedak (1990). However, there was a significant difference between these estimates of TBF and the mean value of 15 % (SD = 2.8, range 11 - 19 %, n = 7) reported in the same study for adult males only (two tailed *t*-test - unequal variances, $t = 2.72$, $p < 0.05$, $df = 10$ for Pace and Rathbun (1945) and $t = 2.38$, $p < 0.05$, $df = 10$ for Reilly and Fedak (1990).

The dissection of animal H, produced a value for blubber content in relation with body mass of 21 %. Some 15 days previously, and after an equilibration time of 0.85 hr its TBF was estimated to 23 and 24 % by the equation of Pace and Rathbun (1945) and Reilly and Fedak (1990) respectively.

The animals in this study weighed on average 1300 kg (SD = 155, range 1086 – 1554 kg, n = 10). Total body protein averaged 18.4 % (SD = 3.3, range 10.6 – 22.3 %), TBA averaged 25.6 kg (SD = 6.0, range 13.0 – 34.0 kg) and TBGE averaged 18300 MJ (SD = 5044, range 10384 – 28887 MJ) (method from Reilly and Fedak 1990).

A comparison of disturbed and undisturbed behaviour

First it was explored to what extent SLRT and TDR data on haul out behavior were similar during the period of deuterium oxide dilution experiments, as the two instruments data recording method differs considerably in resolution. For only two animals in 2000 (B and G: Table A) simultanoeus TIM data were available from both instruments in August. For B the total time hauled out during the experimental period was 30 and 20 % recorded by the SLRT and the TDR, respectively. The corresponding values for G were 51 and 55 %, respectively. This indicates that data from the two instruments reflected the haul-out activity reasonably similarly.

This conclusion may also be valid if single haulouts are compared. During the experimental period both instruments registered five full haulout events. The duration of these haulout periods recorded in both instruments were well correlated ($r = 0.97$, $Z = 3.06$, $p < 0.05$, $n = 5$).

Among the four animals enriched with deuterium oxide (B, C, E, G), that transmitted information during August 2000 on time spent at different depths (TAD), three spent between 25 and 37 % of the “at sea” time below 6 m depth (i.e. they were possibly diving for food) (Table D). However, animal E only spent ca. 8 % of the “at sea” time below 6 m. The activity of B and E was also followed during September. Both animals used more time (B = 68 %, E = 16 %) below 6 m in September than in August (Table D). In comparison, walrus H, which in 2000 was not enriched with deuterium oxide, spent 53 and 66 % of its time below 6 m in August and September respectively (Table D).

During August 2000, the percentage of dives below 6 m varied between 46 and 56 % in the enriched animals, whereas it was 36 % in the one animal that was not enriched that year (H) (Table E). In September both of the enriched animals (B, E) dived comparatively more below 6 m than in August (93 and 64 % of dives respectively) (Table E). Animal (H), not enriched in 2000, also dived markedly more below 6 m in September (53 %) than in August (36 %) (Table E).

In three walruses (B, E and G in 2000) it was possible to compare DMDD between deuterium enrichment and blood sampling with maximum depths recorded after the last blood sampling (Table F). Only one of the animals presented a near significant difference ($p = 0.05$) between dive depths during the two periods (G). However, for this animal only two days with a record of maximum dive depth during the deuterium dilution measurement period were available.

These data indicates that in August both the enriched and the not-enriched animals spent less time below 6 m than in September and that in August they seem to dive more frequently to depths above 6 m. However, the lack of significant difference in mean daily maximum dive depth between the experimental period for the isotope dilution study and the following period, indicates that they were not influenced in their behavior by the experimental procedures.

Effect of activity on water turn-over

Both water turnover rate and dive activity information were available for 6 animals. In none of the animals was there a clear correlation between water turnover rate (WTR) and average dive duration ($R^2 = 0.45$, $p > 0.05$), % of time spent below 6 m ($R^2 = 0.35$, $p > 0.05$) and with the number of dives deeper than 6 m ($R^2 = 0.22$, $p > 0.05$). Furthermore, there was no correlation between WTR and either % of time hauled out ($R^2 = 0.02$, $p > 0.05$) or average dive depth ($R^2 = 0.01$, $p > 0.05$).

Discussion

This study was a field investigation of the water metabolism of walruses in view of future IDA studies (Doubly Labeled Water and Body Composition) where isotope dosage depends heavily on the size of the dilution space and on the importance of the water turnover.

Total Body Mass

The precision of the TBM values for each study subject is essential for the accuracy of the body water and body composition calculations. The values of TBM were calculated from direct measures of standard length and axillary girth (American Society of Mammalogists 1967) according to the allometric equations for Atlantic walruses of Born *et al.* (2003) which revised and improved the equations of Knutsen and Born (1994) by including additional three directly weighed animals. The equations of Born *et al.* (2003) have a $r^2 = 0.99$ and are therefore considered a good predictor of body weight from measures of body size.

Background enrichment of the isotope

Variations of backgound levels of the isotopes used for enrichment can influence the results of the analysis and have to be taken into careful consideration (Speakman 1997). In this study it was chosen to sample venous blood of each animal immobilized in order to obtain an individual reference point for further calculation. During the 2001 season, seawater was also sampled regularly simultaneously with walrus blood. The results of the analysis indicate small and statistically non-significant daily fluctuations of both the environmental and the animal background levels. However, there was a significant difference between blood background levels between the two sampling years. This observation confirms the importance of measuring the background isotope concentration during the experimental period.

Isotopic equilibration

If the isotope is administered intravenously and blood is sampled before complete equilibration there is a risk to underestimate TBW. Generally in pinnipeds equilibration times

range from 0.5 – 3.0 hr (Bowen and Iverson 1998). Prior to the current study an isotope equilibration experiment for a single walrus has been conducted (Lydersen et al. 1992). A 960-kg walrus was enriched with an intravenous injection of tritium oxide and venous blood was sampled regularly for 5 hr following the bolus injection. Observations indicated that equilibration was reached already after 1 hr after the injection of the isotope. Speakman (1997) suggested that the short equilibration time observed by Lydersen *et al.* (1992) was caused by the intravenous injection of the isotope followed by blood sampling from the same body water pool in a multi-pool model and therefore that complete isotopic equilibrium in the body was not achieved. An equilibration time of 1 hr is surprisingly short as equilibration times longer than 1 hr have generally been observed in large animals (Bowen and Iverson 1998). Differences in blood flow distribution in the different body compartments have been identified as the possible cause of this long equilibration time (Coleman et al. 1972). In the present study, venous blood was sampled regularly for up to 6 hr post intravenous bolus injection. The results indicate that the equilibration time for walruses administered an intravenous bolus of isotope is approximately 2-3 hr and this value was adopted in the calculations of TBW.

Total Body Water

One of the important assumptions when using IDA is that the administered isotope mixes completely and exclusively with the body water pool of the subject studied. In practice some of the isotope is lost to other body components such as the rapidly exchangeable hydrogen atoms in the organic constituents of the body (Ussing 1935). The dilution space can be overestimated if the experimental animal has a full digestive tract because the water molecules contained in the intestinal lumen are highly exchangeable with the body water molecules (Speakman 1997). However, most of the animals in this study had hauled out prior to enrichment and presumably their digestive tract was empty. Hauled out walrus usually have empty stomach and intestine (Fay 1982). Only a proper validation study involving isotopic enrichment followed by carcass desiccation of the same individual can fully assess the

magnitude of this overestimate for the species in question. Based on several studies Bowen and Iverson (1998) developed a general regression equation for the overestimation of TBW from desiccation by hydrogen IDA. This equation can be used to estimate TBW from H-dilution space for those pinnipeds for which validation data are not available. In absence of a true validation study, the present work adopted the above approach to estimate TBW. The TBW estimate by desiccation is also an approximation that might underestimate the "real" TBW because of evaporation or incomplete drying, but it is generally assumed as the reference value. Relating all measures to TBW by desiccation is useful because it provides a consistent basis for comparative studies (Bowen and Iverson 1998).

Water Turnover Rate

In this study it was possible to measure WTR in 8 cases (the WTR for two individuals was measured twice over the two seasons). Nagy and Costa (1980) have critically reviewed the relative importance of the assumptions listed by Lifson and McClintock (1966) which have to be valid in order to obtain reliable estimates of water flux from IDA methods. One of the assumptions is that body water remains constant during the measurement period (Nagy and Costa 1980).

In this study it was assumed that the body water pool remained constant throughout the measurement period. However, one animal was subjected to a second enrichment followed by a sufficient equilibration period at final recapture. The double equilibration allowed an estimate of TBW both at the beginning (630 kg) and at the end (746 kg) of the measurement period. This represents an increase in TBW from 50% to 60% of TBM and indicates that the TBW content of a walrus may actually change considerably with time. Changes in TBW for a free living and freely foraging animal can be due to exercise and foraging activity with consequent change in body condition/composition. For this animal it was possible to improve the calculation of WF using the equations in Nagy and Costa (1980).

Body Composition

The blubber layer is the major energy store used by pinnipeds (Bryden 1968) and it is often used to assess body condition in seals (Gales and Renouf 1994; Gales, Renouf, and Noseworthy 1994). Non-destructive methods for assessing body composition are obviously desirable in conservation studies. IDA provides the possibility of estimating TBW and hence TBF according to relationships established for other mammalian species (Pace and Rathbun 1945; Reilly and Fedak 1990). However, an initial validation of the general allometric equations is necessary. In the case of field studies of pinnipeds it is often logistically difficult to carry out accurate proximate body composition analysis. For this reason in this study, the body composition estimated from IDA has been compared with the data obtained from *in situ* dissection of the Atlantic walrus that were killed during the traditional walrus hunt by the Greenland Inuit (Knutsen and Born 1994). The comparison of the two data sets suggests that both the simple model of Pace and Rathbun (1945) and the specific model of Reilly and Fedak (1990) provide a useful approximation for walruses. The statistically non-significant small difference between the mean blubber content obtained in Knutsen and Born (1994) and the mean value from IDA could be ascribed either of two factors. Firstly blubber content obtained from dissection is necessarily an underestimate of the actual TBF value as blubber represents the most important but not the only body fat deposit. Furthermore, the method of physically separating blubber from the carcass used by Knutsen and Born (1994) implies a certain, though presumably minimal, loss of material. Secondly the values in Knutsen and Born (1994) refer to animals sampled in spring when the animals are leanest (Fay 1982).

The correspondence of the estimates of TBF in animal H as obtained from IDA and dissection confirmed of the validity of IDA estimates compared to direct weighing. It was not possible to compare the results of the application of the allometric equations for gray seals on the other body components, but the values are presented here as reference.

It has been mentioned previously that a full digestive tract can lead to overestimation of TBW. This could be one explanation for walrus M's apparently high hydration level.

However, it must be pointed out that a correction of the allometric equations for TBF in gray seals might be necessary in order to apply them to walrus as gray seal blubber has been analyzed to contain 9.1 – 35.9 % water (Reilly and Fedak 1990), while walrus blubber has been reported to contain 23 - 32 % water (Kuhnlein et al. 2002).

Finally, the results of a study on southern elephant seals indicate that morphometric modeling derived from surface area, mass, length and girth combined with IDA can accurately estimate TBW (Tierney et al. 2001). These results suggest that a similar relationship might be valid for other pinnipeds, hence walruses. Further concurrent measures of body composition by IDA and morphometric measures would in this case allow the development of a reliable predictor for body condition for this species to be used in field studies without the need of long immobilization times necessary for IDA studies.

Activity levels in the experimental period

Although clearly some individual variability in overall activity existed, it is likely that the diving activity of the treated animals had not been abnormally influenced by the handling procedures to which the animals had been subjected. Dive depth was not markedly different during the experimental period compared with the inshore situation in September when the animals were not subjected to any immobilization procedure (Table F). Studies of walrus activity by use of SLRT indicate that the individual time allocation between resting and diving of walrus during August and September may vary with a gradual decrease in hauled out time in September (Born and Acquarone unpublished). In this study it was not possible to study haulout behavior comparatively between the two periods, however, there is an indication of increased dive depth and of the duration of the dives in September compared to August (Tables D and E).

The data available in this study don't indicate any correlation between the observed changes in activity levels and the body water flux. It is possible that the lack of correlation is due to the paucity and hetereogeneity of data, however, it is more likely that the behavior of

the studied animals was not markedly different during the two periods (e.g. feeding activity and energy expenditure).

It is suggested to assume the values of water content, turnover and flux in this study as a general measure for summering male walruses and that they be utilized as a basis for a proper validation study of the water and energy physiology of walruses.

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Year	Animal ID	TBM (kg)	No. of Captures	Locality	TBW	WTR	TDR	SLRT
2000	B	1280	4	LSN	x	x	Mk7	SDR-T10
	C	1250	3	LSN	x	x		SDR-T10
	D	1050	2	LSN				
	E	1120	3	LSN	x	x	Mk7	SDR-T10
	F	1090	3	LSN	x	x	Mk7	
	G	1310	2	LSN	x	x	Mk7	SDR-T10
	H	1550	1	LSN				SDR-SSC3
	A	930	1	SND				
2001	A	1230	1	SND				SDR-T10
	I	880	1	SND				
	J	1410	1	SND				SPOT2
	K	1070	1	SND				SPOT2
	L	840	1	SND				
	M	1170	1	LSN				SPOT2
	H	1550	2	LSN	x	x	Mk7	SPOT2
	C	1250	3	LSN	x	x	Mk7	SPOT2
	G	1370	3	LSN	x	x	Mk7	SPOT2
	N	1600	2	LSN				SPOT2

Table A: List of male Atlantic walruses included in this study of total body water (TBW) and water turnover rate (WTR) in NE Greenland in 2000 and 2001. Blood from all animals was sampled to estimate isotope background enrichment. The localities where the study was conducted were Lille Snenæs (LSN) and Sandøen (SND). The model of the instruments attached is shown (TDR = Time Depth Recorder, SLRT = Satellite Linked Radio Transmitter, Wildlife Computers, Redmond WA, USA).

ID	Date	Eq.time (h)	Water Dilution Space				TBW (kg)	TBW (% TBM)
			Mean (kg)	Min (kg)	Max (kg)	SD		
C	3-Aug-00	2.83	651	635	668	14	630	50.3
E	7-Aug-00	1.10	614	589	641	22	595	53.3
F	9-Aug-00	2.00	723	712	734	9	699	64.4
G	15-Aug-00	2.65	851	830	872	21	824	63.0
H	18-Aug-00	1.22	957	927	987	31	926	59.6
B	19-Aug-00	2.65	707	675	739	33	684	53.3
C	22-Aug-00	2.52	770	748	793	19	746	59.5
M	3-Aug-01	2.27	888	886	890	1	860	73.3
H	6-Aug-01	0.85	889	885	894	5	861	55.7
C	7-Aug-01	2.28	474	474	540	25	458	36.6
G	16-Aug-01	2.82	783	760	822	26	758	55.2

Table B: Measures of dilution space by deuterium dilution and estimated total body water (TBW) expressed in kg and % of body mass in Atlantic walruses in NE Greenland, 2000-2001. The estimate of TBW was derived from deuterium dilution according to the allometric equation for pinnipeds in Bowen and Iverson (1998).

Year	ID	Measure Period (d)	Kd (/d)	T½ (d)	TBW (kg)	WTR (g/kg*day)	WI (eq.6) (g/kg*day)	WE (lin.eq.4) (g/kg*day)
2000	B	12.0	0.09778	7.1	684	52.1		
	C	19.0	0.04867	14.2	630	24.5	4.8	21.8
	E	11.0	0.18030	3.8	595	96.2		
	F	13.1	0.11906	5.8	699	76.7		
	G	2.8	0.02218	31.3	824	14.0		
2001	C	8.7	0.05699	12.2	458	20.9		
	G	4.9	0.04153	16.7	758	22.9		
	H	14.9	0.08782	7.9	861	48.9		

Table C: Fractional turnover rate (K_d), half life ($T_{½}$), total body water (TBW), water turnover rate (WTR) for 6 different male Atlantic walruses and water efflux (WE) and water influx (WI) for one of these animals (walrus C) during a study in NE Greenland, 2000-2001. Water influx and water efflux were calculated according to (eq.4) and (eq.6) in Nagy and Costa (1980) respectively.

ID	Month	% time > 6 m	Dates	Hours monitored
B	Aug	37	3-27	51 * 6h
C		26	21-31	13 * 6h
E		8	11-28	23 * 6h
G		25	15-29	34 * 6h
H		*53*	*18-31*	*30 * 6h*
B	Sep	68	13-30	26 * 6h
E		16	1-19	13 * 6h
H		*66*	*1-30*	*91 * 6h*

Table D: Time (%) at sea by month spent below 6 m depth during August and September 2000 by 5 adult male Atlantic walruses in NE Greenland (2000) calculated using SLRT data alone (animals C, H) and integrating time at depth (TAD) data from SLRT and time depth recorder (TDR) data (animals B, E, G). The dates in August and September and the number of hours monitored are shown. All animals except walrus H were administered deuterium oxide.

ID	Month	% of all dives		Total no. dives	Dates	No. hours monitored
		> 3 m < 6 m	> 6 m			
B	Aug	49	51	2999	1-31	57 * 6h
C		44	56	357	28-31	4 * 6h
E		49	51	738	7-31	29 * 6h
G		54	46	1512	16-27	33 * 6h
H		*64*	*36*	*2036*	*18-31*	*27 * 6h*
B	Sep	7	93	1044	13-30	20 * 6h
E		36	64	243	1-19	9 * 6h
H		*47*	*53*	*1688*	*1-30*	*91 * 6h*

Table E: Percentage of dives at two depth intervals in August and September 2000 by 5 adult male Atlantic walruses in NE Greenland (2000) calculated using SLRT data alone (animals C, H) and integrating daily average dive (DAD) data from SLRT and time depth recorder (TDR) data (animals B, E, G). The dates in August and September and hours monitored are shown. All animals except walrus H were administered deuterium oxide.

ID	Period 1				Period 2				t-value	p	df
	Mean (m)	SD	min-max (m)	n	Mean (m)	SD	min-max (m)	n			
B	47	22	12-72	7	74	60	6-170	10	-0.24	0.81	14
E	75	48	36-128	3	57	50	24-132	4	0.69	0.52	5
G	8	0	8-8	2	36	26	12-84	9	2.32	0.05	9

Table F: Comparison of the means of the "daily maximum dive depth" values of 3 adult male Atlantic walruses in August and September 2000 in NE Greenland. **Period 1** corresponds to the duration of the deuterium dilution measures in August (from first enrichment to final sampling). **Period 2** corresponds to the following period in August and September 2000 where the animals were undisturbed. Values for dives during the deuterium oxide experimental period and outside this in August and September are presented. Data were Ln-transformed for the t-tests.

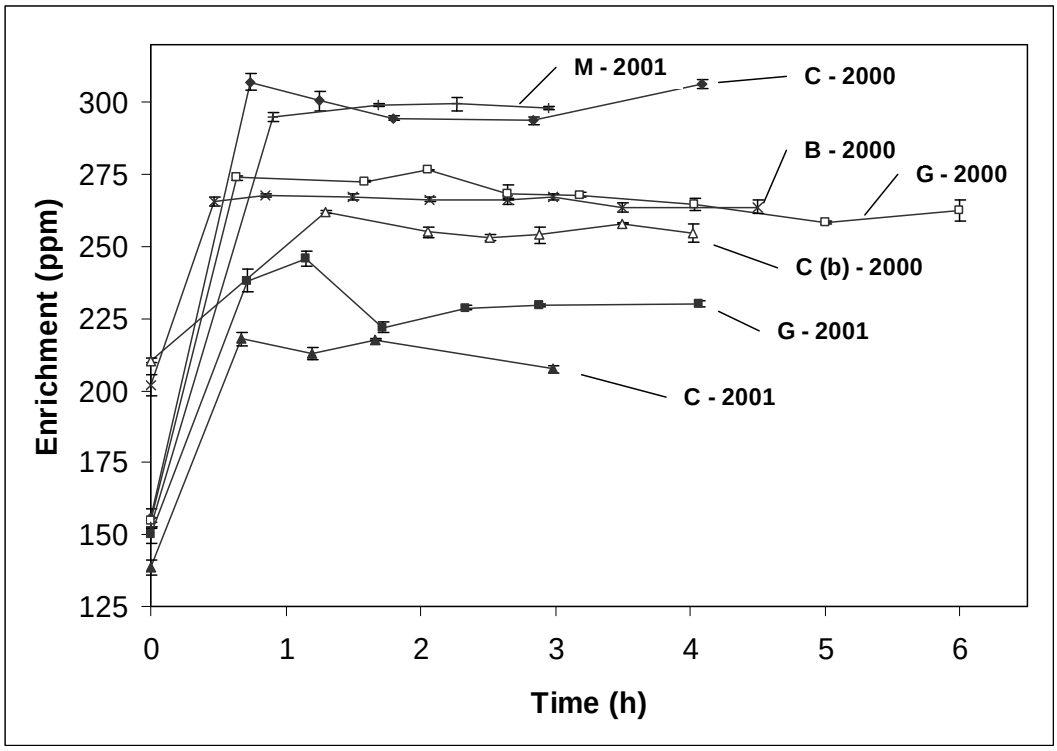

Figure 1: Absolute blood deuterium concentration (in ppm) in Atlantic walruses in NE Greenland (2000-2001) after each animal was enriched with a bolus of deuterium oxide at time = 0. The time axis represents time from enrichment. The concentration axis represents absolute concentration of deuterium. The enrichment year is shown after each animal ID. In 2000 animals B and C were enriched twice, for B only the second enrichment is shown, for C both enrichments are shown (C and C(b)). Animals C and G were enriched with deuterium both in 2000 and 2001.