

Body Composition Changes, Metabolic Fuel Use, and Energy Expenditure during Extended Fasting in Subantarctic Fur Seal (*Arctocephalus tropicalis*) Pups at Amsterdam Island

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ABSTRACT

The fasting metabolism of 71- to 235-d-old subantarctic fur seal (*Arctocephalus tropicalis*) pups from Amsterdam Island, southern Indian Ocean, was investigated during the long foraging trips of their mothers. Body lipid reserves were proportionally greater in female than male pups and higher in post-moult (37%) than premoult (10%) animals. The mass-specific rate of mass loss did not differ between the sexes but was lower than observed in other species. Daily mass loss was estimated to 56% fat, 10% protein, and 34% water. The rate of protein catabolism (15 g d^{-1}) was negatively related to the size of initial lipid stores and accounted for 9% ($\pm 1\%$) of total energy expenditure. However, body composition changes during the fast were not equal between the sexes, with females relying more on protein catabolism than males (11% and 5% of total energy expenditure, respectively). Energy expenditure ($270 \text{ kJ kg}^{-1} \text{ d}^{-1}$) and metabolic water production ($11.5 \text{ mL kg}^{-1} \text{ d}^{-1}$) rates are the lowest reported for an otariid species. These results suggest that subantarctic fur seal pups greatly reduce activity levels to lower energy expenditure in addition to adopting protein-sparing metabolic pathways in order to survive the extreme fasts they must endure on Amsterdam Island.

Introduction

A large number of vertebrate species undergo extended periods of fasting throughout their life history (Nelson 1980; Cherel et

al. 1988; Oftedal 1993). In addition to behavioural reductions in energy expenditure, a common physiological response adopted by these species is the use of protein-sparing metabolic pathways and the utilisation of body lipid reserves as the primary metabolic fuel (Boyd and Duck 1991; Cherel et al. 1992, 1994, 1995; Atkinson et al. 1996). Although fasting episodes commonly occur during adulthood, a few species experience prolonged fasting during early developmental stages. Among these, young phocid seals, which undergo a single postweaning fast of up to 70 d (Ortiz et al. 1978; Reiter et al. 1978), and king penguin chicks, which endure a fast of up to 5 mo during winter when parents are unable to adequately provision them because of reduced local food availability (Cherel and Le Maho 1985; Cherel et al. 1987), have been shown to use similar strategies of energy conservation and metabolic fuel use as species adapted to fasting during adulthood (Ortiz et al. 1978; Worthy and Lavigne 1987; Reilly 1991; Nordøy et al. 1993).

Throughout lactation (4 mo–3 yr depending on species), female otariid seals (fur seals and sea lions) alternate between short suckling periods ashore and long foraging trips to sea (Gentry and Kooyman 1986). For the majority of lactation, otariid pups are entirely dependent on their mother for nutrition and, therefore, repeatedly fast (in contrast to phocid seal pups and king penguin chicks) on land during the maternal foraging trips and survive on stored body reserves accumulated during the previous suckling period (Gentry and Kooyman 1986; Costa 1991; Guinet and Georges 2000). In otariids, the duration of maternal foraging trips varies among species, location, and environmental conditions (Gentry and Kooyman 1986; Lea and Hindell 1997; Francis et al. 1998; Boyd 1999; Georges and Guinet 2000b; Arnould and Hindell 2002). In subantarctic fur seals (*Arctocephalus tropicalis*) located on Amsterdam Island ($35^{\circ}55'S$, $77^{\circ}30'E$), maternal foraging trips to sea vary throughout the 10-mo lactation period from an average of 10 d in summer, when pups are young, to 3–4 wk in winter just before the weaning period (Georges and Guinet 2000a, 2000b). Consequently, subantarctic fur seal pups have to face fasting durations that are among the most extreme repeated fasting durations, not just of otariid seals but infant vertebrates in general (Guinet and Georges 2000).

Several recent studies of fasting metabolism in young otariid seals have documented reductions in energy expenditure and have provided indirect evidence that these animals adopt the protein-conserving pathways of fasting-adapted species (Rea et

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al. 2000; Arnould et al. 2001; Donohue et al. 2002). These investigations, however, involved relatively short fasting periods in comparison with those experienced by subantarctic fur seal pups. In addition, changes in body composition, the relative importance of lipids and proteins as metabolic fuels, and the factors affecting their use have not been documented during fasting in otariid pups. Furthermore, numerous recent studies have documented sex-based differences in body composition and rate of fasting mass loss in otariid seal pups (Arnould et al. 1996a, 2001; Ono and Boness 1996; Guinet et al. 1999; Arnould and Hindell 2002; Donohue et al. 2002). While it has been hypothesised that these differences are associated with sex-based differences in metabolic fuel use (Arnould et al. 1996a, 2001), this has yet to be documented.

The aims of this study were, therefore, to determine (1) changes in body composition, (2) metabolic fuel use, (3) energy expenditure, and (4) the factors influencing these parameters in subantarctic fur seal pups during 2–3 wk fasting periods at Amsterdam Island.

Material and Methods

Field Procedures

The study was conducted at La Mare aux Elephants breeding colony, on the northeast side of Amsterdam Island, where one of the largest breeding colonies can be found (Roux 1987; Guinet et al. 1994). During the 1998–1999 pupping period (November 28–January 8), 200 newborn pups were sexed, weighed (± 0.05 kg), and identified by a numbered piece of plastic tape glued to the fur on the top of the head (Georges and Guinet 2000a). At about 1 mo of age, each pup was tagged in the trailing edge of both foreflippers with an individually numbered plastic tag (Dalton Rototag, Nettlebed, U.K.). Of this known-age group of pups, premoult (age = 71–115 d) and postmoult (age = 156–235 d) individuals were selected at random during March and May–July, respectively.

Once selected, pups were captured 2 d after the mother's departure to sea (to allow sufficient time for complete voiding of ingested milk from the stomach; Oftedal and Iverson 1987; Arnould et al. 1996a; Donohue 1998) following a normal suckling period. Each pup was weighed (± 0.05 kg) before receiving a 1-mL intramuscular injection of tritiated water (HTO, 200 $\mu\text{Ci mL}^{-1}$). They were then kept in an enclosure for 3–4 h to allow the isotope to equilibrate with the total body water (TBW) pool (Costa 1987; Arnould et al. 1996a). A blood sample (2–5 mL) was subsequently collected by venipuncture from an interdigital vein in a hind flipper to determine the TBW by isotope dilution (Arnould et al. 1996a; Speakman 1997). The pup was then released, and the absence of the mother from the colony was monitored twice daily by visual inspection. Regular weighing (every 2–3 d) of the pups during the maternal absences confirmed no food was consumed during these fasting periods. For the purposes of this study, however, only the

masses measured when TBW was determined were considered. Foraging trips of individual adult females at Amsterdam Island are very regular, and their return to the colony can be predicted with some accuracy on the basis of previous trip durations (Georges and Guinet 2000a). Consequently, study pups were recaptured 1–2 d before the expected return of their mother, 11–19 d after initial capture. On final recapture, a body mass measurement and blood sample were again collected before a second 1-mL dose of HTO (200 $\mu\text{Ci mL}^{-1}$) was administered as was done previously. A final blood sample was collected after 3–4 h of equilibration to determine changes in body composition and water flux rate during the fasting period (Costa 1987; Speakman 1997).

All blood samples were kept cool (4°C) for several hours before the serum fraction was separated by centrifugation. Aliquots (1 mL) of serum were stored frozen (–20°C) in plastic vials until analysis in December 2001.

Laboratory Procedures

Thawed 0.1-mL aliquots of serum were distilled into pre-weighed scintillation vials following the procedures of Ortiz et al. (1978). The vials were again weighed, and the mass of the distilled water was determined by subtraction before 3.5-mL scintillant (Pico Fluor 40; Canberra Packard) was added to each vial. The specific activity of tritium in the distilled water was then analysed for 10 min using a Packard liquid scintillation 1600TR multipurpose counter. In order to determine the specific activity of the tritium administered to the pups, subsamples of the injectate (diluted by a factor of 5,000) were counted at the same time as the samples. Each sample was analysed in duplicate.

Calculations

Body composition was determined at the beginning and end of the fasting period. The TBW pool size of each pup was calculated from the HTO dilution space using the equation of Arnould et al. (1996b), determined empirically by desiccation of Antarctic fur seal (*Arctocephalus gazella*) pups: TBW (kg) = 0.11 + 0.97 HTO space (kg).

Lean body mass (LBM) was calculated from TBW assuming a constant proportion of water in lean tissue using the following equation: LBM (%) = TBW (%) / C_1 , where $C_1 = 0.747$ (determined by Arnould et al. 1996b from the proximate chemical composition of Antarctic fur seal pups). Percentage total body lipid (TBL) was then calculated by deduction:

$$\text{TBL (\%)} = 100 - \text{LBM (\%)}$$

Total body protein (TBP) mass was calculated from LBM (kg),

assuming a constant proportion of LBM is protein, using the following equation:

$$\text{TBP (kg)} = C_2 \times \text{LBM (kg)},$$

where $C_2 = 0.214$ (see Arnould et al. 1996b). Mass-specific rates of mass loss (MSRML) of pups were calculated using the following equation:

$$\text{MSRML (\% d}^{-1}\text{)} = 100 \times [\log(M_f) - \log(M_i)]/\Delta t,$$

where M_i is initial mass (kg, day 2), M_f is the final mass, and Δt is the time elapsed between the two mass measurements (see Guinet and Georges 2000).

The amounts of lipid and protein catabolised during fasting were used to calculate an average field metabolic rate (FMR) throughout the study period assuming calorific equivalents of 39.3 and 18.0 kJ g⁻¹ for lipid and protein, respectively (Schmidt-Nielsen 1983). Metabolic water production resulting from the catabolism of lipid and protein (MWP_C) was estimated assuming 107 and 41 g, respectively, of water produced per 100 g of tissue catabolised (Ferrando 1964).

Total water influx (TWI) and efflux rates of pups during fasting were calculated from the decrease in specific activity of tritium in body water using Nagy and Costa's (1980) equation 6 (for an exponentially decreasing TBW pool size) and equation 5, respectively. In fasting animals, if no preformed water is consumed, TWI represents the metabolic water production (MWP_{WF}) resulting from the catabolism of body lipid, protein, and carbohydrate stores (Speakman 1997). Consequently, by comparing the observed MWP_{WF} with MWP_C values determined from body composition changes, free water drinking by pups during fasting was investigated.

Because of logistical constraints and sample contamination during storage, not all individuals were sampled at both the start and end of the fasting period. When two TBW measurements were made in an individual, a single result was selected at random to investigate factors influencing body composition. All statistical analyses followed the methods of Sokal and Rohlf (1981) and were performed with the SYSTAT 9.0 statistical software (SYSTAT, 9.0 statistics, SPSS). The Kolmogorov-Smirnov test was used to determine whether the data were normally distributed, and an F -test was applied to confirm the homogeneity of variances. Differences between linear regressions were tested by ANCOVA. When multivariate analyses were used, correlations were statistically analysed using General Linear Model (GLM), and the contribution of significant factors was assessed using a backward stepwise analysis (level of rejection $P > 0.1$). Unless otherwise stated, values are reported as means \pm SE, and statistical significance was considered to be $P < 0.05$.

Results

Body Composition

A total of 43 single TBW measurements was obtained from 10 premoult (six females, four males) and 33 postmoult (17 females, 16 males) pups. Over the mass range analysed (6.5–18.5 kg), male pups were found to have a larger absolute and mass-specific TBW pool than did females (ANCOVA, $F_{1,41} = 7.745$, $P < 0.01$, and $F_{1,41} = 6.306$, $P < 0.02$, using pup mass as a co-factor and absolute and percentage TBW, respectively, as the dependent variable; Fig. 1a). This resulted in females having higher mass-specific TBL values than did males regardless of pup age (ANCOVA, $F_{1,41} = 4.306$, $P < 0.05$; Fig. 1b). Proportionally, however, body lipid reserves were significantly less in premoult than postmoult male ($7.7\% \pm 1.4\%$ and $35.6\% \pm 1.2\%$, respectively; $t_{18} = -12.924$, $P < 0.0005$) and female ($12.2\% \pm 3.2\%$ and $38.4\% \pm 0.9\%$, respectively; $t_{21} = -9.204$, $P < 0.0005$) pups.

Fasting Metabolism

Mass-loss rates and changes in body composition during fasting (11–19 d) were measured in 39 (20 female, 19 male) and 23

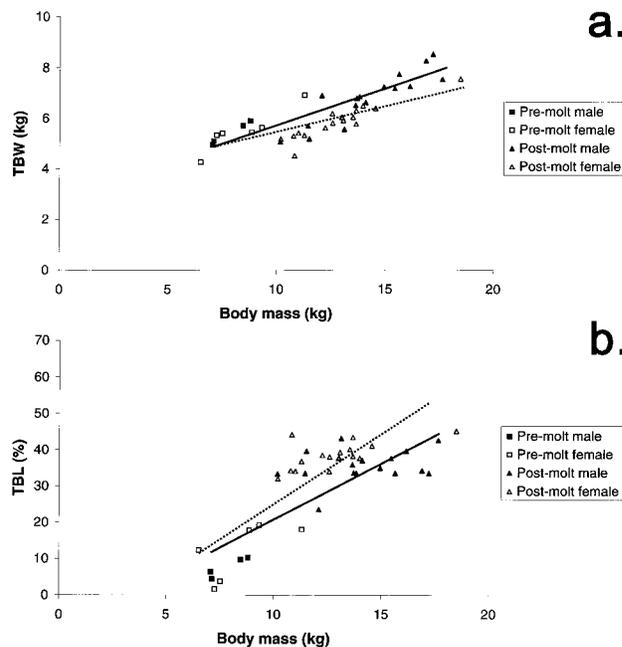


Figure 1. The relationship between body mass (kg) and (a) TBW (kg) content for male ($y = 0.294x + 2.726$, $r^2 = 0.781$, $P < 0.01$) and female ($y = 0.208x + 3.342$, $r^2 = 0.611$, $P < 0.01$) and (b) TBL (%) for male ($y = 3.026x - 9.152$, $r^2 = 0.660$, $P < 0.01$) and female ($y = 3.934x - 14.499$, $r^2 = 0.692$, $P < 0.01$) subantarctic fur seal pups at Amsterdam Island. Male and female pups are represented by filled and open symbols (and solid and dashed lines), respectively, and pre- and postmoult pups are represented by squares and triangles, respectively.

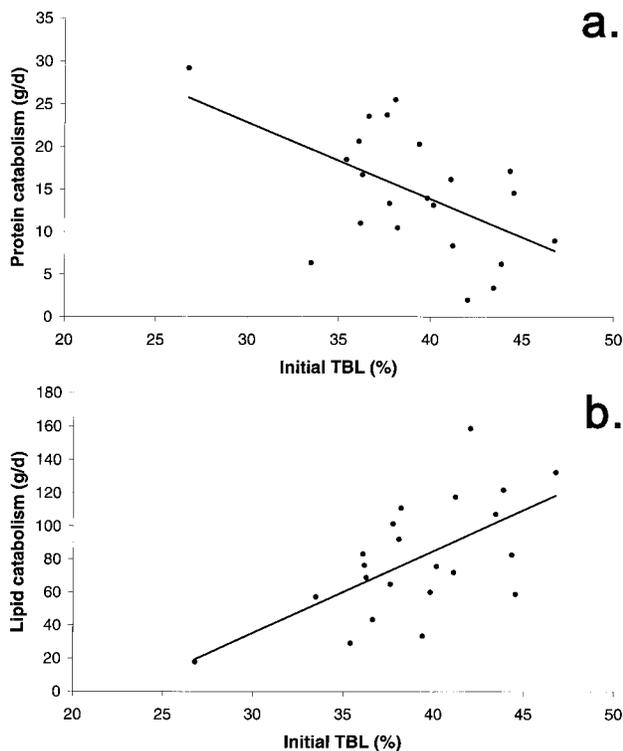


Figure 2. The relationship between initial TBL (%) and (a) protein catabolism rate (grams per day; $y = -0.902x + 49.922$, $r^2 = 0.298$, $P < 0.01$) and (b) lipid catabolism rate (grams per day; $y = 4.969x - 113.778$, $r^2 = 0.388$, $P < 0.01$) in fasting subantarctic fur seal pups at Amsterdam Island. The regressions were still significant following the omission of the apparent outlier (27% initial TBL; $r^2 = 0.195$, $P < 0.05$, for protein and $r^2 = 0.275$, $P < 0.02$, for lipid catabolism rate, respectively).

(15 female, 8 male) pups, respectively. Average absolute daily mass loss ($149 \pm 5 \text{ g d}^{-1}$) did not differ between the sexes ($t_{37} = 0.872$, $P > 0.3$). Similarly, the mass-specific rate of mass loss (MSRML) was not related to pup sex ($F_{1,21} = 0.597$, $P > 0.4$) but was, however, significantly ($t_{37} = 6.743$, $P < 0.0005$) higher in premoult ($1.80\% \pm 0.13\% \text{ d}^{-1}$) than postmoult pups ($1.14\% \pm 0.04\% \text{ d}^{-1}$). The relationship between MSRML and initial pup mass also approached significance ($F_{1,21} = 4.256$, $P = 0.052$).

On average, daily mass loss comprised $56.3\% \pm 4.0\%$ lipids ($83.3 \pm 6.6 \text{ g d}^{-1}$) and $43.7\% \pm 3.9\%$ of LBM ($68.1 \pm 6.6 \text{ g d}^{-1}$). Protein catabolism ($14.6 \pm 1.4 \text{ g d}^{-1}$), representing $9.6\% \pm 0.8\%$ of daily mass loss, was negatively correlated with initial TBL (%) ($F_{1,21} = 53.973$, $P < 0.04$; Fig. 2a) but not with sex ($F_{1,21} = 2.013$, $P > 0.1$), age ($F_{1,21} = 1.519$, $P > 0.2$), or initial mass ($F_{1,21} = 0.237$, $P > 0.6$). Moreover, mass-specific protein use ($1.15 \pm 0.12 \text{ g kg}^{-1} \text{ d}^{-1}$) tended to be negatively related to initial TBL (%) ($F_{1,21} = 3.948$, $P = 0.06$), with a trend for females to have greater values ($F_{1,21} = 4.340$, $P = 0.051$) than

males. However, no relation was detected with pup age or initial mass ($P > 0.1$ in both cases). Lipid catabolism was positively related to initial TBL (%) ($F_{1,21} = 4.804$, $P < 0.05$; Fig. 2b) and negatively related to age ($F_{1,21} = 5.108$, $P < 0.04$), with males using more lipids ($F_{1,21} = 9.340$, $P < 0.01$) than females. However, no relation was detected with initial mass ($F_{1,21} = 0.526$, $P > 0.4$), and mass-specific lipid use ($6.34 \pm 0.46 \text{ g kg}^{-1} \text{ d}^{-1}$) was related with none of these parameters ($P > 0.1$ in both cases).

Mean FMR during fasting ($270 \pm 18 \text{ kJ kg}^{-1} \text{ d}^{-1}$) was significantly negatively related to pup age ($F_{1,21} = 6.062$, $P < 0.03$), with premoult pups ($416 \pm 31 \text{ kJ kg}^{-1} \text{ d}^{-1}$) having greater metabolic rates than postmoult ones ($262 \pm 16 \text{ kJ kg}^{-1} \text{ d}^{-1}$). There were no relationships, however, among FMR and pup sex, initial mass, or initial TBL (%) ($P > 0.1$ in all cases). For the sexes combined, $91.0\% \pm 1.3\%$ of energy expenditure during fasting was fuelled by lipid catabolism (the remainder from protein catabolism). Body composition changes during fasting, however, were not uniform between the sexes (Fig. 3). The proportion of mass loss comprised of lipids was significantly greater ($t_{21} = 2.099$, $P < 0.05$) in males ($67.0\% \pm 6.3\%$) than females ($50.6\% \pm 4.6\%$). Consequently, the calculated contribution of lipids to energy expenditure was significantly greater ($t_{21} = 2.624$, $P < 0.02$) in males ($94.6\% \pm 1.4\%$) than females ($89.1\% \pm 1.6\%$). The relative contribution of lipids to energy expenditure, however, was not related to pup age or initial TBL (%) ($P > 0.3$ in both cases).

Metabolic Water Production and Water Flux during Fasting

Metabolic water production ($11.5 \pm 0.7 \text{ mL kg}^{-1} \text{ d}^{-1}$) calculated from changes in body composition during fasting (MWP_C) was negatively related to pup age ($F_{1,21} = 46.98$, $y = -0.095x + 29.57$, $r^2 = 0.718$, $P < 0.0005$) but not to fasting duration, pup sex, or mass ($P > 0.1$ in all cases). Metabolic water production calculated from the observed water flux

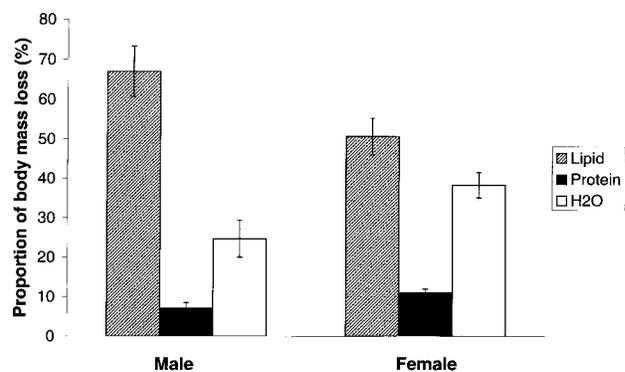


Figure 3. Composition of body mass loss during fasting in male and female subantarctic fur seal pups at Amsterdam Island. Data are presented as mean \pm SE.

(MWP_{WF}), however, was on average significantly greater ($14.6 \pm 1.0 \text{ mL kg}^{-1} \text{ d}^{-1}$; paired t -test, $t_{22} = 5.341$, $P < 0.0005$) than, and positively correlated to ($r^2 = 0.26$, $P < 0.05$; Fig. 4), MWP_C . It was also negatively related to pup age ($F_{1,21} = -6.185$, $P < 0.0005$) and positively related to fasting duration ($F_{1,21} = 3.078$, $P < 0.01$) but not to pup sex or mass ($P > 0.2$ in both cases). The mean difference between MWP_{WF} and MWP_C ($4.2 \pm 0.5 \text{ mL kg}^{-1} \text{ d}^{-1}$) represented an overestimation in metabolic water production of $55 \pm 7 \text{ mL d}^{-1}$ and was positively related to initial LBM (%) ($F_{1,21} = 9.769$, $P < 0.01$) but not with mass-specific protein catabolism or with pup sex ($P > 0.1$ in both cases).

Discussion

Body Composition

In contrast to phocid seal pups, which quickly develop a thick, insulative blubber layer (Bryden 1964; Blix et al. 1979), fur seal pups rely primarily on their dense fur for insulation (Irving et al. 1962; Trites 1990). The importance of this fur, which does not develop fully until pups moult their natal coat (Scheffer 1962), for reducing thermoregulatory energy costs is exemplified by the significantly lower basal and field metabolic rates of postmoult compared with premoult northern fur seal (*Callorhinus ursinus*) pups. Indeed, Donohue et al. (2002) found northern fur seal pups to have higher body lipid reserves postmoult (34%–39%) than premoult (14%–18%) and attribute this to an increased ability to divert nutritional resources into storage because of reduced thermoregulatory costs once the new pelage is attained. The resulting increased deposition of subcutaneous adipose tissue would then further enhance thermoregulatory savings (Donohue et al. 2002). In this study, an increase in body lipid reserves was also found between pre- (10.4%) and postmoult (37.1%) pups.

In addition to furnishing supplementary thermoregulatory benefits, stored lipids provide an important source of energy to fur seal pups during fasting periods while their mothers are at sea (Donohue et al. 2000; Arnould et al. 2001; Georges et al. 2001). The TBL (%) values observed in this study are similar to those of northern fur seal pups and within the range previously reported in Australian fur seal (*Arctocephalus pusillus doriferus*), Antarctic fur seal, and California sea lion (*Zalophus californianus*) pups (Ofstedal et al. 1987; Arnould et al. 1996a, 1996b; Donohue et al. 2000, 2002; Arnould and Hindell 2002). However, as subantarctic fur seal pups at Amsterdam Island experience a subtropical climate (Frenot et al. 2001), their thermoregulatory costs would be considerably lower than those of Antarctic, northern, and Australian fur seals. Consequently, the magnitude of their lipid stores would constitute a relatively greater energy reserve and may reflect an adaptation to the extreme fasting durations they must endure (11–23 d in comparison to 1–12 d of the other species; Gentry and Kooyman

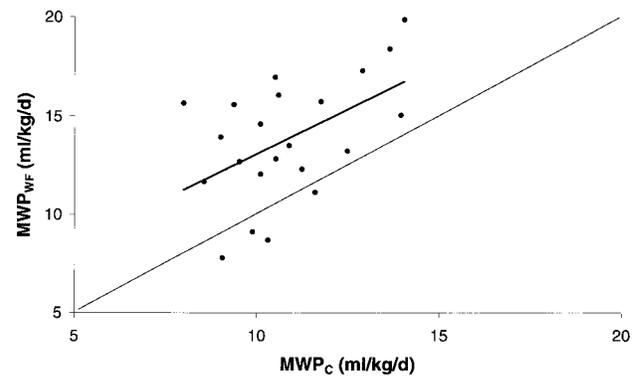


Figure 4. The relationship ($y = 0.912x + 3.966$, $r^2 = 0.258$, $P < 0.05$) between metabolic water production estimated from water flux rates (MWP_{WF}) and body composition changes (MWP_C) during fasting in subantarctic fur seal pups at Amsterdam Island. A line of equality represents the theoretical ideal correspondence between these two methods.

1986; Lea and Hindell 1997; Francis et al. 1998; Boyd 1999; Georges and Guinet 2000b; Arnould and Hindell 2002).

Female subantarctic fur seal pups were found to have significantly greater body lipid stores than did male pups. This is consistent with the findings of previous studies on Antarctic, Australian, and northern fur seal pups, which have shown that females accumulate greater adipose tissue stores despite parity in milk consumption between the sexes (Arnould et al. 1996a, 1996b, 2001; Donohue 1998; Arnould and Hindell 2002; Donohue et al. 2002).

Fasting Metabolism

The MSRML of pups observed in this study is within the range previously reported at this colony (0.7%–2.8% d^{-1} ; Guinet and Georges 2000), which is substantially less than that reported in free-ranging Antarctic fur seal pups (2.8%–3.8% d^{-1} ; Arnould et al. 1996a, 2001; Guinet et al. 1999, 2000) except for one study (1.2%–1.5% d^{-1} ; Lea et al. 2002). As with previous studies at Amsterdam Island (Georges and Guinet 2000a; Guinet and Georges 2000), there was no sex difference in MSRML, which is consistent with the observed lack of sex difference in FMR. The significant decrease between pre- (1.8% d^{-1}) and postmoult (1.1% d^{-1}) MSRML was associated with a corresponding decrease in FMR (416–262 $\text{kJ kg}^{-1} \text{ d}^{-1}$). Donohue et al. (2002) also found a decrease in FMR following the moult in northern fur seal pups, despite them having increased levels of activity, and suggested this was largely because of reduced resting metabolic rates resulting from the increased insulative properties of the new pelage.

The mean fasting FMR observed in this study (270 $\text{kJ kg}^{-1} \text{ d}^{-1}$) is 60%–70% and 50% less than that reported in Antarctic and northern fur seal pups, respectively (Arnould et al. 2001;

Donohue et al. 2002). In this study, FMR was directly determined from the energetic equivalents of the lipid and protein lost, which may account for some of the discrepancies found between the studies. Nevertheless, whereas the substantially warmer ambient and water temperatures experienced by subantarctic fur seals at Amsterdam Island are likely to contribute to reduced thermoregulatory costs, this is unlikely to account fully for the extremely low levels of energy expenditure observed. Species adapted to long-term fasting (not associated with reproductive or migratory events) considerably reduce their behavioural activity levels and physiological functions during periods of food deprivation (Schmidt-Nielsen 1983). Previous studies have shown pinniped pups may reduce their metabolic expenditure by 16%–41% while sleeping in air (Worthy 1987; Donohue et al. 2000). While we have no data on the activity levels of subantarctic fur seal pups at Amsterdam Island, preliminary observations suggest these animals spend considerably more time sleeping than do pups of other fur seal species (C. Guinet, personal observation). Such a strategy is likely to delay the development of swimming and diving abilities (Baker and Donohue 2000). However, the energetic savings would minimize the depletion of body reserves, and it may be that subantarctic fur seal pups at Amsterdam Island trade off the early acquisition of foraging skills to survive the long fasts they must endure. Alternatively, such a low metabolic rate may also reflect lower metabolic demands of a relatively slow-growing pup (Guinet and Georges 2000).

As has been found using hydrogen isotope dilution techniques or gross chemical composition analysis in other fasting-adapted species (e.g., Belkhou et al. 1991; Castellini and Rea 1992; Cherel et al. 1994, 1995; Atkinson et al. 1996), the majority (56%) of mass lost during fasting was comprised of lipids. Protein catabolism (15 g d^{-1}) accounted for only 10% of total mass loss and 9% of total energy expenditure. Similarly, labeled-urea dilution techniques used in Arnould et al. (2001) found fasting Antarctic fur seal pups catabolised 19 g d^{-1} of protein, which represented only 5% of the total energy expenditure. A low contribution to total energy expenditure by protein catabolism has also been reported in grey (6%; Reilly 1991) and harp (4%–9%; Nordøy et al. 1993) seal pups. Arnould et al. (2001) suggested that in view of parity in milk consumption, the sex-based body composition differences observed in pups of Antarctic fur seals and other species may be due to differences in metabolic fuel use. In this study, male pups lost proportionally more lipid mass than did females during fasting. Furthermore, the contribution of protein catabolism to total energy expenditure in females (11%) was twice that observed in males (5%), supporting Arnould et al.'s (2001) hypothesis.

The rates of protein and lipid catabolism were negatively and positively, respectively, related to the magnitude of the initial body lipid reserves. This is consistent with findings in other fasting-adapted species (e.g., polar bear, king penguin) where the larger the initial lipid reserves, the greater the ability to

conserve lean body mass (Cherel et al. 1992; Atkinson et al. 1996; Cherel and Groscolas 1999). Interestingly, however, while female pups had greater lipid reserves, they also had higher rates of protein catabolism, contrary to what might be expected from the general relationship between body lipid reserves and metabolic substrate use (Le Maho et al. 1988; Cherel et al. 1992). Previous studies on rats and humans have documented sex-based differences in metabolic fuel use and attributed these to the influence of sex hormones (Wade et al. 1985; Wade and Schneider 1992). These studies, however, have generally involved sexually mature individuals. Since subantarctic fur seals do not reach puberty until >4 yr of age (Bester 1990, 1995), the results of this study suggest the presence of a physiological mechanism enabling male pups to preferentially catabolise lipids, which is independent of reproductive status.

Metabolic Water Production and Water Flux during Fasting

Concomitant with the observed low levels of FMR, MWP_C ($15 \text{ mL kg}^{-1} \text{ d}^{-1}$) was lower than has been reported in pups of other fur seal species (20 – $27 \text{ mL kg}^{-1} \text{ d}^{-1}$; Costa and Gentry 1986; Arnould et al. 1996a; Lea et al. 2002). The observation that estimates of MWP_{WF} derived from fasting water flux were significantly greater than MWP_C values determined from body composition changes indicates that subantarctic fur seal pups at Amsterdam Island consume preformed water during fasting. Several studies of marine mammals have concluded that mariposia increases urinary osmotic space, which may increase the excretion rate of urea and help to eliminate the by-products of protein catabolism during phase one of fasting (Hui 1981; Costa 1982; Storeheier and Nordøy 2001). However, while the overestimation in MWP was positively correlated with initial TBP %, it was not related to the mass-specific protein catabolism.

Gentry (1981) observed that the incidence of mariposia was significantly higher in otariid seal species that inhabit warm climates. Ambient temperatures at Amsterdam Island can reach 24°C (Meteo France, unpublished data), and, while mariposia was observed on few occasions during this study, it was recorded during periods of high temperatures (G. Beauplet, personal observation). It is possible, therefore, that subantarctic fur seal pups at Amsterdam Island consume seawater during the extremely long fasting periods they must endure in order to compensate for high evaporative water loss. The observation that the overestimation in MWP was correlated with the fasting duration would tend to support this hypothesis. A consequence of mariposia by subantarctic fur seal pups at Amsterdam Island is that the use of hydrogen isotope dilution to measure milk consumption (Costa 1987; Oftedal and Iverson 1987) will not be accurate because the assumption that milk is the only source of preformed water will not be valid.

In summary, fasting subantarctic fur seal pups on Amsterdam Island exhibited one of the lowest FMR reported for an otariid

species, and catabolism of body lipids contributed the greatest proportion of metabolic energy. Hence, in addition to adopting the protein-sparing metabolic pathways observed in other species adapted to prolonged fasting, these pups appear to substantially reduce energy expenditure to further conserve body reserves in order to survive the long fasting durations they must endure while their mother is at sea on extended foraging trips. Metabolic fuel use, however, differed between the sexes, with female pups relying more on protein catabolism than males. This finding confirms previous suggestions (Arnould et al. 2001) that the higher body lipid levels observed in female otariid pups is caused by preferential protein catabolism. Concomitant with the low energy expenditure was a low rate of metabolic water production compared with that reported in other species. However, discrepancies between the metabolic water production values estimated from water flux rates and those calculated from body composition changes suggest the occurrence of water drinking during fasting.

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