Am J Physiol Regul Integr Comp Physiol 297: R1582–R1592, 2009.
First published September 23, 2009; doi:10.1152/ajpregu.90857.2008.

Physiological response to extreme fasting in subantarctic fur seal (*Arctocephalus tropicalis*) pups: metabolic rates, energy reserve utilization, and water fluxes

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Submitted 23 October 2008; accepted in final form 17 September 2009

Verrier D, Groscolas R, Guinet C, Arnould JPY. Physiological response to extreme fasting in subantarctic fur seal (*Arctocephalus tropicalis*) pups: metabolic rates, energy reserve utilization, and water fluxes. *Am J Physiol Regul Integr Comp Physiol* 297: R1582–R1592, 2009. First published September 23, 2009; doi:10.1152/ajpregu.90857.2008.— Surviving prolonged fasting requires various metabolic adaptations, such as energy and protein sparing, notably when animals are simultaneously engaged in energy-demanding processes such as growth. Due to the intermittent pattern of maternal attendance, subantarctic fur seal pups have to repeatedly endure exceptionally long fasting episodes throughout the 10-mo rearing period while preparing for nutritional independence. Their metabolic responses to natural prolonged fasting (33.4 ± 3.3 days) were investigated at 7 mo of age. Within 4–6 fasting days, pups shifted into a stage of metabolic economy characterized by a minimal rate of body mass loss (0.7%/day) and decreased resting metabolic rate (5.9 ± 0.1 ml $O_2 \cdot kg^{-1} \cdot day^{-1}$) that was only 10% above the level predicted for adult terrestrial mammals. Field metabolic rate (289 ± 10 $kJ \cdot kg^{-1} \cdot day^{-1}$) and water influx (7.9 ± 0.9 $ml \cdot kg^{-1} \cdot day^{-1}$) were also among the lowest reported for any young otariid, suggesting minimized energy allocation to behavioral activity and thermoregulation. Furthermore, lean tissue degradation was dramatically reduced. High initial adiposity (>48%) and predominant reliance on lipid catabolism likely contributed to the exceptional degree of protein sparing attained. Blood chemistry supported these findings and suggested utilization of alternative fuels, such as β-hydroxybutyrate and de novo synthesized glucose from fat-released glycerol. Regardless of sex and body condition, pups tended to adopt a convergent strategy of extreme energy and lean body mass conservation that appears highly adaptive for it allows some tissue growth during the repeated episodes of prolonged fasting they experience throughout their development.

starvation; metabolic rate reduction; protein sparing; lipid metabolism; β-hydroxybutyrate

ANIMALS RELY ON FOOD INTAKE to fuel the energetic costs of basal metabolism, thermoregulation, physical activity, immunity, growth, and reproduction. When food is not available, they must use body reserves to match their metabolic requirements. An animal's capability to resist starvation is, therefore, determined by its ability to store energy and control its allocation during periods of food restriction (78). Hence, accumulating large energy stores in anticipation of periods of food shortage is of high survival value (24). Correspondingly, obese individuals can tolerate fasts of longer duration than leaner individuals of the same species (25, 49, 51, 70). In addition, limiting energy expenditure can delay the depletion of energy reserves. In this regard, larger animals are at an advantage for they have lower metabolic rates per unit mass due to allometric scaling and thus can survive longer on finite body stores (46, 72). For instance, baleen whales can subsist with little or no food for > 4–6 mo (61) and large phocid seals can fast spontaneously up to several months each year (29). In animals with limited storage capacity and high metabolic rates, such as small mammals and birds, an alternative strategy to avoid lethal starvation is to switch to a hypometabolic state (e.g., daily torpor or seasonal hibernation) in response to stressful environmental conditions (39).

The nature of the substrate used is also of major importance (24). During complete abstinence from food, energy is mainly derived from the oxidation of body lipids and proteins, since carbohydrate stores (e.g., glycogen) are generally exhausted within 2–3 days (17, 26). However, body proteins can be only partly depleted (<40–50% before death occurs) due to their vital enzymatic, mechanical, and structural functions and thus represent the limiting factor to starvation survival (18, 49). Lipids, on the contrary, can be almost entirely depleted to cover maintenance requirements (24) and have a higher energy density (72). Consequently, the ideal situation for a fasting animal would be to be able to use only fat and totally spare body proteins (24). Indeed, animals adapted to long-term fasting, such as bears, seals, and penguins (21), achieve high levels of protein sparing during periods of aphagia, with protein catabolism contributing to only 2–10% of total energy expenditure (1, 10, 24, 69, 70), whereas these values can reach 20–40% in nonadapted species (25, 51). The necessity to conserve proteins might become even more crucial in species that are involved in nutrient-demanding processes such as lactation (e.g., bears and pinnipeds) (61) or growth and development (e.g., phocid pups after weaning and king penguin chicks during the austral winter) (29, 74) while fasting.

Otariids (fur seals and sea lions) have a characteristic protracted lactation pattern (4 mo to 3 yr depending on species) during which mothers alternate between brief nursing episodes ashore (1–4 days) and regular foraging trips to sea to gather the resources required for milk production leaving their pup on land (40). Consequently, otariid pups fast regularly throughout the period of maternal dependence, typically from 1–3 days in sea lions to 4–6 days in most fur seal species (40), but records beyond 3–4 wk have been reported. In particular, such pro-

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longed fasting durations have been recorded at Amsterdam Island (Southern Indian Ocean) where lactating subantarctic fur seals (*Arctocephalus tropicalis*) undertake the longest maternal foraging trips of any otariid seal due to the great distances they must travel at sea to reach food resources (up to 1,600 km away from breeding colonies) (11, 42). Hence, pups of this species have to repeatedly endure extended fasting periods averaging durations of 14 days in summer [from birth to 3 mo of age, for a body mass (BM) of 5–12 kg] to 28 days in winter (at 7–9 mo of age, for a BM of 8–18 kg), with regular records up to 2 mo (11, 42, 43).

Such fasting durations appear extreme among otariid seal pups in which capacity to resist sustained food deprivation generally remains quite limited in most species. For instance, in Antarctic fur seal (*A. gazella*) pups, mortality by starvation has been found to augment significantly with increasing maternal foraging trip duration, although maternal absences remain of moderate length (≤8 days) (53). Similarly, in years where environmental perturbations, such as El Niño events, affect food availability and the ability of otariid mothers to locate prey, catastrophic repercussions for breeding success can occur, with up to nearly 100% of pup mortality recorded in Galapagos fur seals (*A, galapagoensis*), Galapagos sea lions (*Zalophus californianus wollebaeki*), and South American sea lions (*Otaria flavescens*) (73, 77). In fact, in addition to facing the longest intersuckling intervals of any mammalian infant (42), on a mass-specific basis, subantarctic fur seal pups born at Amsterdam Island also endure one of the longest fast of any physically active mammal. Furthermore, they have to face these extreme fasting durations in alternation with short (2–4 days) suckling episodes repeatedly throughout the period of maternal dependence (42). Thus, although almost constantly fasting, subantarctic fur seal pups must optimize both their growth and the development of physiological capabilities (e.g., thermoregulation, oxygen storage) and behavioral skills (e.g., learning to swim and dive) to successfully meet the demands of independent living at the time of weaning.

Little is known of the physiological adaptations enabling subantarctic fur seal pups to cope with such conflicting demands. More generally, whereas fasting is a major component of otariids' life at any life history stage, their adaptations to starvation remain poorly documented. Recent studies of fasting metabolism in young otariid seals have suggested reductions in energy expenditure and adoption of protein-conserving pathways (7, 65), but these investigations were restricted to brief fasting episodes (2–5 days) and provided indirect evidence only. Preliminary estimates of energy expenditure over moderate fasting periods (10–18 days) at Amsterdam Island have suggested that subantarctic fur seal pups might develop low metabolic rates in response to low maternal provisioning (12). This needs, however, to be verified. How energy and resources are allocated and which parameters regulate these allocation patterns is also not known, in particular, throughout fasts of extreme durations. The aim of the present study, therefore, was to investigate the metabolic adaptations to extreme fasting in subantarctic fur seal pups. To do so, we determined: *1*) the changes in BM and resting metabolic rates (RMRs) throughout natural episodes of prolonged fasting in winter when periods of maternal absence are the longest; *2*) the associated changes in plasma metabolites, body composition, and pattern of fuel partitioning; *3*) the rates of total energy expenditure and water flux; and *4*) the influence of body composition and gender on these parameters.

METHODS

Study site and animals. All procedures involved in the present study were approved by the Ethics Committee of the French Polar Institute and the Polar Environment Committee of Terres Australes et Antarctiques Françaises. They complied with the Agreed Measures for the Conservation of Antarctic and Subantarctic Fauna and current French laws.

The present study was carried out on the subantarctic fur seal breeding colony of La Mare aux Elephants, located on the northeast coast of Amsterdam Island, Southern Indian Ocean (37°55′S, 77°30′E). In this colony, adult females give birth to a single pup each year from late November to early January, and weaning takes place around mid-October. During the 2004–2005 pupping season, 160 pups of previously tagged females were marked at birth using temporary codes glued to the fur on the top of their head, as part of a long-term population-monitoring program. At ~1 mo of age, each marked pup was tagged in the trailing edge of both fore-flippers with an individually numbered plastic tag (Dalton Rototag, Nettlebed, UK) (42).

Between July and early September 2005, 20 tagged pups (10 males, 10 females) facing the prolonged winter fasts were selected at random out of the known-age cohort and serially sampled throughout one single period of maternal absence. To detect fasting bouts in the study animals, individual maternal attendance patterns were monitored at least twice daily by visual inspection of the colony. Study periods commenced at the end of a maternal attendance period ashore as the mother departed the colony on a foraging trip and continued until she returned to nurse again. At the time of first capture, pups were fully molted, aged 217 ± 2.6 days (range: 195–236 days), and weighed 16.1 ± 0.5 kg (range: 12.2–20.0 kg). Pups were captured on *days 0*, *1*, *2*, and *4* following maternal departure, and subsequently every 4 days until the end of the natural fast. Pups were also opportunistically weighed in the colony on *day 6*. Since the pups were left free ranging in the colony between sampling periods, not all animals could be located and captured on each sampling day. Furthermore, maternal absence durations varied between individuals, and the fasting periods covered were, therefore, of unequal durations between study animals.

Morphometric measurements and RMR determination. Upon capture, animals were weighed in a large Hessian bag by using a spring scale (±0.05 kg) after collection of a blood sample (5–10 ml, representing <1.5% of total blood volume) by venipuncture of either an interdigital vein in the hind-flipper or the brachial vein of the fore-flipper for measurement of plasma metabolites throughout fasting. Pups were then transported inside the bag to the nearby research station (300–600 m from pup location in the colony) where they were allowed to rest in an outdoor enclosure (2.5 m × 2 m, exposed to natural climatic conditions) for at least 1 h, prior to undergoing metabolic rate measurements. No pups struggled or panicked once placed in the enclosure, settling quickly and often falling asleep minutes after placement.

Rates of carbon dioxide production and oxygen consumption were determined by standard open-flow respirometry (79) at each sampling day (except *days 1* and *6*), to measure the RMR of the animals. The respirometry system used was similar to that described by Arnould et al. (9). Nitrogen dilution tests (36) were used to check for any leaks within the system. Prior to each trial, the O_2 analyzer was two-point calibrated using H_2O- and CO_2-free atmospheric air and pure nitrogen. The CO_2 analyzer was calibrated twice daily using H_2O- and CO_2-free atmospheric air and a 5% CO_2 in nitrogen mixture (Messer France, Saint Herblain, France). On the first day of capture (*day 0*), RMR measurement started after a minimum period of 12 h from maternal departure to allow pups to enter a "postabsorptive" state. Once introduced into the metabolic chamber, pups were allowed to

rest and acclimatize for 1 h. Pup behavior was checked regularly through a small Plexiglas window built on the chamber lid. Pups became calm within minutes, if not immediately after placement. Any change in activity such as signs of struggling, panic, or sleep was recorded. Measurements of O_2 and CO_2 concentrations, temperature, humidity, pressure, and flow were recorded continuously throughout the duration of the experiment (2–3 h). However, only the values corresponding to the 15 min of minimum O_2 consumption after the hour of acclimatization were used for the calculations of resting values of CO_2 production and O_2 consumption using the equations of Withers (79). Rates of O_2 consumption were designated as RMR, which corresponds to the maintenance requirements at thermoneutrality. Conversion from mililiters O_2 to kilojoules was made assuming a calorific equivalent of 20 kJ:l O_2 (72). The respiratory quotient, which yields information about the proportion of energy derived from the different types of nutrient, was calculated as the ratio of resting CO_2 production to resting O_2 consumption (35, 72). Upon completion of the respirometry trial, body length was measured from the tip of the nose to the extremity of the last caudal vertebrae with a rigid tape (±0.5 cm), and pups were finally released in the colony at the site of capture.

Body composition and water flux measurements. Body composition and water flux were measured by the hydrogen isotope dilution technique (28). This technique has been previously validated in young otariids using tritiated water (5). In addition to the routine procedure on *day 2* (i.e., after 2 days of fasting, to ensure complete stomach emptying) (6, 33), pups were given an intramuscular injection (~1 ml) of a weighed dose (±0.001 g) of tritiated water (7.40 MBq/ml) after the initial blood withdrawal and weighing. Three to four hours later, an equilibration blood sample (3–5 ml, representing < 0.5% of total blood volume) was collected to determine the total body water pool (TBW) (5, 28). To determine changes in body composition and water flux throughout fasting, pups were injected with a second weighed dose of tritium (3.70 MBq/ml) after collection of a background blood sample toward the expected end of the fast. Dates of mothers' return to the colony were predicted on the basis of pup age and previous trip durations (43). On an average, the second injection was performed at day 23.5 ± 1.3 of fasting. A final blood sample was collected after 3–4 h of equilibration. All blood samples were centrifuged, and the plasma fraction stored frozen (−20°C) until analysis within 6 mo.

The specific activity of tritium in plasma water was determined using the evaporative-freeze capture technique (63) as previously detailed by Beauplet et al. (12). Tritium dilution space (HTO; liters) was calculated using the following equation: $HTO = (A_i \cdot V)/(A_{eq} - A_0)$, where A_i represents the specific activity of the injectate (dpm/ml), V is the injection volume (ml), A_{eq} is the specific activity of the equilibration sample, and $A_{0\ is}$ the specific activity of the background sample. TBW (liters) was calculated from HTO as determined empirically in Antarctic fur seal pups: TBW = 0.11 + 0.97 HTO (5). Assuming water is exclusively distributed within lean BM (LBM), LBM was calculated from TBW using the equation: LBM = TBW/α, where α represents the fractional water content of LBM. The value of α was not determined empirically for this study, and the value of 0.747 reported for Antarctic fur seal pups was used for calculations (5, 12). Total body lipid (TBL) was then calculated as TBL = BM − LBM, where total body protein (TBP) = LBM − TBW.

Average field metabolic rate (FMR) represents an animal's rate of total energy expenditure in natural conditions. In the present study, FMR was calculated from the amounts of protein and lipid lost by the pups throughout the fasting period, assuming calorific equivalents of 18.0 kJ/g and 39.3 kJ/g, respectively (72). Contribution of lipids and proteins to FMR, respectively, were then calculated. Metabolic water production (MWP) resulting from the catabolism of lipid and protein was estimated assuming 107 g and 41 g, respectively, of water produced per 100 g of matter oxidized (12). Rates of water efflux and water influx were calculated from the decrease in specific activity of tritium in body water using *equations 5* and *6* of Nagy and Costa (56). Finally, the FMR/RMR ratio was used as an index of energy allocation to behavioral activity (in the absence of feeding and reproductive costs at thermoneutrality).

Plasma metabolites. Plasma concentrations of triglycerides, β-hydroxybutyrate (β-OHB), nonesterified fatty acids (NEFA), and glycerol were measured as indicators of lipid metabolism (e.g., digestion, lipid oxidation, and lipolysis, respectively). Plasma urea was used as an indicator of protein catabolism and plasma creatinine for skeletal muscle catabolism. The plasma urea-to-creatinine ratio (U/C) was then used as a corrected index of whole body protein catabolism (7, 58). In addition, U/C has also been shown to accurately predict glomerular filtration rate in pinnipeds (32). Circulating concentrations of glucose, urea, creatinine, triglycerides, glycerol, and β-OHB were determined from heparinized plasma using enzymatic methods (kits GL3816, UR3825, CR3814, TR3823, GY105, and RB1007, respectively; Randox Laboratories, Crumlin, Antrim, UK) on a RX Daytona autoanalyzer (Randox Laboratories). Nonesterified fatty acid concentrations were determined manually from EDTA-treated plasma (kit FA115; Randox Laboratories).

Statistical analyses. Statistical analyses were performed using SPSS (version 12.0 for Windows and version 17.0 for MAC; SPSS, Chicago, IL). The Kolmogorov-Smirnov test was used to determine whether the data were normally distributed, and an *F*-test was used to confirm homogeneity of variances. Linear mixed models were used to analyze data with a repeated-measure pattern (52). Individuals were used as random effect, and fasting days as ranks for repeated measures. For each mixed-model analysis, the covariance structure was examined, and the best fit selected based on lowest Schwarz's Bayesian criterion. Gender and fasting phase-related effects were systematically tested by mixed ANCOVA and removed from the model if not significant ($P < 0.05$). Where means were compared by mixed ANOVA, Sidak adjustments were performed to allow for multiple pairwise comparisons. The proportion of variance attributable to the random variable "individual" was determined by variance component analysis based on restricted maximum likelihood estimation. Values are reported as means ± SE, and results were considered significantly different at $P < 0.05$.

RESULTS

BM loss and metabolic rates. The study pups fasted naturally for an average of 33.4 ± 3.3 days ($n = 20$, range: 15–73 days). There was no difference in fasting duration between male and female pups ($t_{18} = 0.27$, $P = 0.790$). BM, rate of BM loss, and body length did not differ between the sexes (Tables 1 and 2). Therefore, data for males and females were pooled for all subsequent analysis. Throughout the fast, pup body length increased significantly, while BM decreased exponentially by an average of 22% (range: 11–32%) (Table 1 and Fig. 1*A*). On the basis of changes in mass-specific rate of daily BM loss (dm/m·dt), two typical phases of BM changes were characterized. During *phase I*, dm/m·dt decreased significantly from 16.0 ± 0.9 $g \cdot kg^{-1} \cdot day^{-1}$ during the first 24 h of the fast to 9.2 ± 1.2 $g \cdot kg^{-1} \cdot day^{-1}$ on *day 6* ($F_{15,138} = 14.75$, $P < 0.001$). Thereafter, dm/m·dt was maintained at 7.3 ± 0.2 $g \cdot kg^{-1} \cdot day^{-1}$ until the end of the study period, defining *phase II* (Fig. 1*B*). The level of dm/m·dt reached in *phase II* was independent of initial total body lipid (TBL) mass and %TBL ($F_{1,18} = 0.00$, $P = 0.982$ and $F_{1,18} = 0.08$, $P = 0.779$, respectively).

RMR also decreased exponentially throughout fasting ($F_{1,39} = 111.83$, $P < 0.001$), with a 30% reduction occurring within 4 days of fasting (Fig. 2*A*). Mass-specific RMR decreased by nearly 25% within 4 fasting days ($F_{14,132} = 10.90$, $P < 0.001$)

Table 1. *Body length, body mass, and body composition of subantarctic fur seal pups at the beginning and the end of the fasting period studied at Amsterdam Island*

	Beginning of the Fast			End of the Fast			Fasting Effect		Sex Effect		
	Pooled	Males	Females	Pooled	Males	Females	*F* Statistics	*P*	*F* Statistics	*P*	Var_{ind}
Body length, cm	**81.5±0.9**	81.8±1.3	81.2±1.3	**82.1±0.9**	82.6±1.3	81.6±1.3	$F_{1,18}$ = 7.38	**0.014**	$F_{1,18}$ = 0.18	0.675	34.8
Body mass, kg	**16.1±0.5**	16.2±0.7	16.0±0.7	**12.7±0.5**	12.8±0.7	12.5±0.7	$F_{1,19}$ = 1145.42	**<0.001**	$F_{1,18}$ = 0.00	0.998	70.5
LBM, kg	**8.0±0.2**	8.3±0.4	7.8±0.4	**7.8±0.2**	8.0±0.4	7.6±0.4	$F_{1,12}$ = 5.39	**0.038**	$F_{1,18}$ = 0.77	0.393	40.0
TBL, kg	**7.6±0.3**	7.5±0.4	7.8±0.4	**4.9±0.3**	4.8±0.4	5.1±0.4	$F_{1,13}$ = 236.90	**<0.001**	$F_{1,18}$ = 0.24	0.630	20.1
TBL, %	**48.5±0.9**	47.0±1.3	50.1±1.3	**38.3±1.2**	36.7±1.4	39.8±1.4	$F_{1,16}$ = 80.76	**<0.001**	$F_{1,19}$ = 2.00	0.173	19.8

Differences for fixed effects (fasting and sex) were considered significant at $P < 0.05$ (in bold). LBM, lean body mass; TBL, total body lipid; Var_{ind}, %variance attributable to random individual effects.

and, thereafter, plateaued at 5.9 ± 0.1 ml $O_2 \cdot min^{-1} \cdot kg^{-1}$ throughout the second phase of the fast (Fig. 2*B*). The level of mass-specific RMR reached in *phase II* was independent of initial TBL and %TBL ($F_{1,18}$ = 1.83, P = 0.193 and $F_{1,18}$ = 0.11, P = 0.738, respectively). When all data were pooled, RMR was positively related to BM (Fig. 3*A*), regardless of pup sex ($F_{1,9}$ = 0.08, P = 0.785) but with a significant effect of fasting phase ($F_{1,84}$ = 49.05, $P < 0.001$). RMR scaled to $BM^{0.73}$ in *phase I* and $BM^{0.87}$ in *phase II*, at close but yet significantly higher levels than that predicted for basal metabolic rate in adult terrestrial mammals (46): 1.4 and 1.1 times above Kleiber's prediction in *phase I* and *phase II*, respectively (t_{160} = 11.23, $P < 0.001$) (Fig. 3*B*). At the onset of the fast

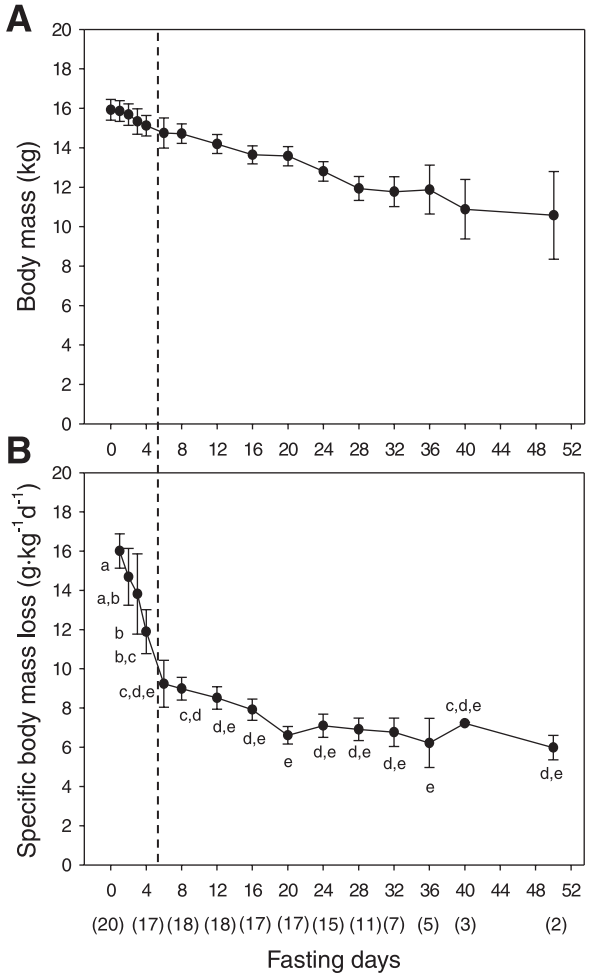

Fig. 1. Changes in body mass (BM) (*A*) and mass-specific rate of daily BM loss (*B*) during prolonged fasting in subantarctic fur seal pups at Amsterdam Island. Numbers in parentheses below fasting days denote the sample size for the corresponding sampling time. Vertical dash line indicates the transition between *phase I* and *phase II fasting*, as determined by changes in the mass-specific rate of daily BM loss. For mass-specific BM loss, symbols that do not share a common letter represent significantly different means (mixed ANOVA and Sidak: $P < 0.05$).

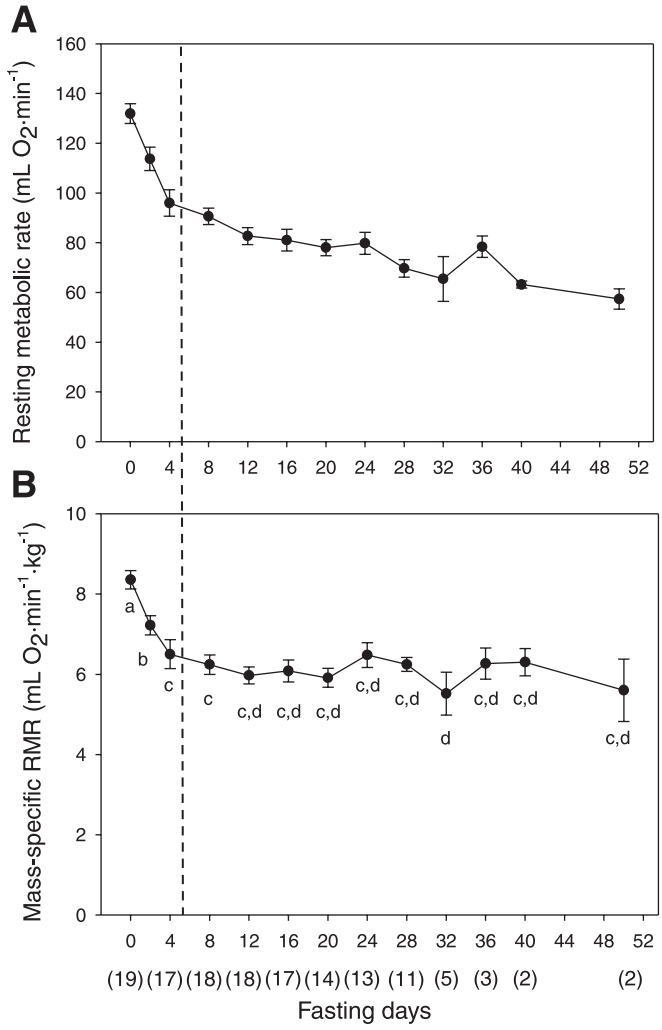

Fig. 2. Changes in resting metabolic rate (RMR) (*A*) and mass-specific RMR (*B*) during prolonged fasting in subantarctic fur seal pups at Amsterdam Island. Symbols that do not share a common letter represent significantly different means (mixed ANOVA and Sidak: $P < 0.05$).

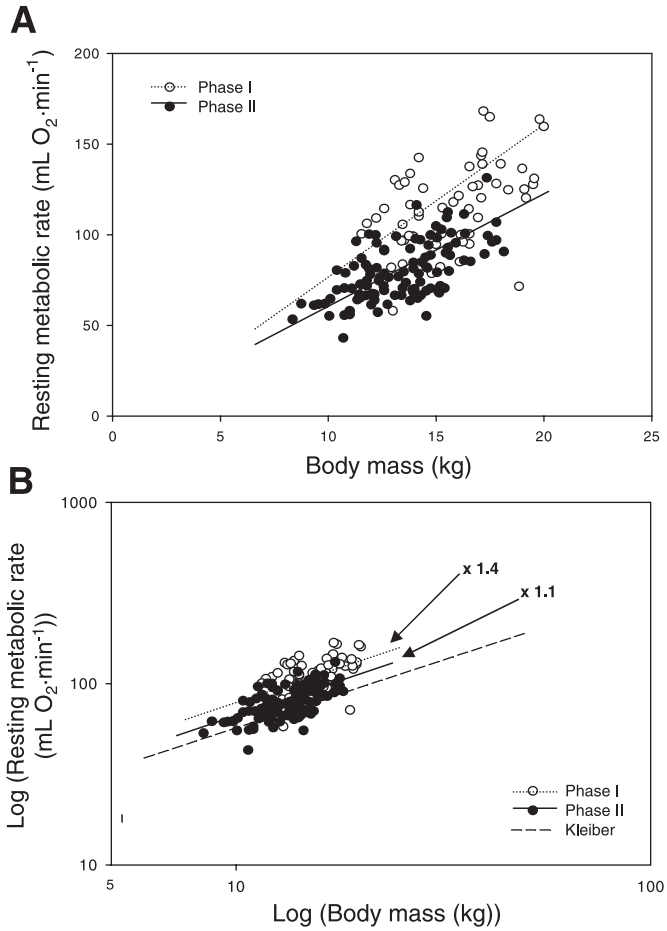

Fig. 3. Untransformed (*A*) and log-log (*B*) relationships between RMR and BM according to fasting phases in fasting subantarctic fur seal pups at Amsterdam Island. Predictive equations were: $y = 5.40 \times + 30.81$ in *phase I* ($n = 57$, $r^2 = 0.26$, $F_{1,21} = 13.95$, $P = 0.001$) and $y = 5.14 \times + 11.11$ in *phase II fasting* ($n = 105$, $r^2 = 0.77$, $F_{1,31} = 42.45$, $P < 0.001$) (*A*). The log-log regression of RMR on BM was: $y = 0.73 \times + 1.19$ in *phase I* ($n = 57$, $r^2 = 0.23$, $F_{1,19} = 11.63$, $P = 0.003$) and $y = 0.87 \times + 0.92$ in *phase II fasting* ($n = 105$, $r^2 = 0.48$, $F_{1,30} = 44.19$, $P < 0.001$). The dashed line represents the theoretical relationship for adult terrestrial mammals predicted by Kleiber (46) and the numbers with an arrow show the average level above this prediction (*B*).

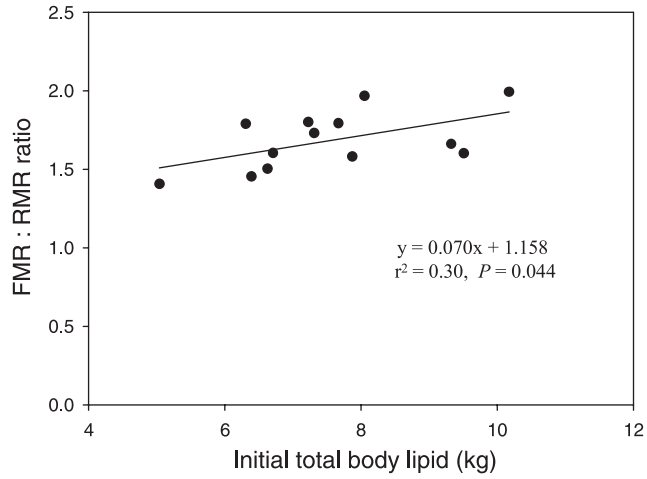

Fig. 4. Effect of initial body lipid mass on individual FMR/RMR ratios used as indices of energy allocation to behavioral activity in fasting subantarctic fur seal pups at Amsterdam Island. FMR, field metabolic rate. Each point represents an individual determination ($n = 13$).

(fed state), pup RMR (8.4 ± 0.2 ml $O_2 \cdot min^{-1} \cdot kg^{-1}$) was 1.6 times above Kleiber's level only.

FMR throughout the fasting periods studied averaged 289 ± 10 $kJ \cdot kg^{-1} \cdot day^{-1}$, regardless of pup sex (Table 2). Fasting FMR was at 1.68 ± 0.05 times above the average RMR calculated for each pup over the period of time elapsed between two TBW measurements. The FMR/RMR ratio did not differ between the sexes ($t_{11} = -0.55$, $P = 0.592$) but was positively related to initial TBL (Fig. 4).

Utilization of body reserves. The metabolic changes observed throughout the fast were associated with significant changes in plasma metabolite concentrations (Fig. 5). *Phase I fasting* was characterized by a marked decrease in plasma levels of triglycerides (80%) ($F_{15,5} = 18.51$, $P < 0.001$), glycerol (50%) ($F_{14,123} = 6.44$, $P < 0.001$), urea (50%) (from 18.3 ± 0.8 to 9.6 ± 0.5 mmol/l; $F_{15,5} = 7.54$, $P = 0.019$), and U/C (50%) ($F_{15,7} = 16.40$, $P < 0.001$), which remained minimal throughout *phase II*. In contrast, there was a significant increase in plasma concentrations of NEFA (100%) ($F_{14,118} = 6.77$, $P < 0.001$), β-OHB (>200%) ($F_{15,110} = 110.12$, $P < 0.001$), and creatinine (25%) (from 64.2 ± 1.8 to 79.2 ± 2.5 μmol/l; $F_{15,147} = 18.65$, $P < 0.001$) between *phase I* and *phase II fasting*. Plasma glucose concentration remained elevated (>6 mmol/l) throughout the fast and only showed a slight linear decrease ($F_{15,138} = 2.63$, $P = 0.002$). In contrast with the BM and metabolic rate data, sex differences in blood chemistry were found (Fig. 5). In *phase I fasting*, plasma levels of triglycerides and glycerol were significantly higher ($F_{14,5} = 10.97$, $P = 0.010$ and $F_{12,110} = 2.26$, $P = 0.013$, respectively), and β-OHB concentrations were lower ($F_{14,91} = 1.96$, $P = 0.030$) in female than in male pups (Fig. 5) On the contrary,

Table 2. *Rate of mass loss, energy expenditure, and water fluxes in subantarctic fur seal pups throughout the fasting period studied at Amsterdam Island*

	Pooled	Males	Females	*F* Statistics	*P* Value	Var_{ind}
Body mass loss, %/day	0.78±0.02	0.77±0.03	0.80±0.04	$F_{1,18} = 4.37$	0.517	49.6
Rate of lipid loss, g/day	102±6	97±8	109±9	$F_{1,11} = 1.03$	0.333	50.0
Rate of protein loss, g/day	4.3±0.8	5.1±0.9	3.4±1.4	$F_{1,11} = 1.12$	0.313	50.0
FMR, $kJ \cdot kg^{-1} \cdot day^{-1}$	289±10	278±15	296±14	$F_{1,11} = 0.80$	0.393	50.0
Protein contribution to FMR, %	1.9±0.4	2.5±0.5	1.3±0.5	$F_{1,11} = 2.29$	0.161	29.8
Estimated MWP, $ml \cdot kg^{-1} \cdot day^{-1}$	7.8±0.3	7.5±0.4	8.0±0.4	$F_{1,11} = 0.82$	0.385	50.0
Water influx $ml \cdot kg^{-1} \cdot day^{-1}$	7.9±0.9	8.2±1.2	7.7±1.3	$F_{1,11} = 0.06$	0.808	50.0
Water efflux $ml \cdot kg^{-1} \cdot day^{-1}$	8.8±1.0	8.8±1.6	8.8±1.4	$F_{1,11} = 0.00$	0.989	50.0

No significant sex effects were observed on the reported variables ($P > 0.05$ in all cases). FMR, field metabolic rate; MWP, metabolic water production.

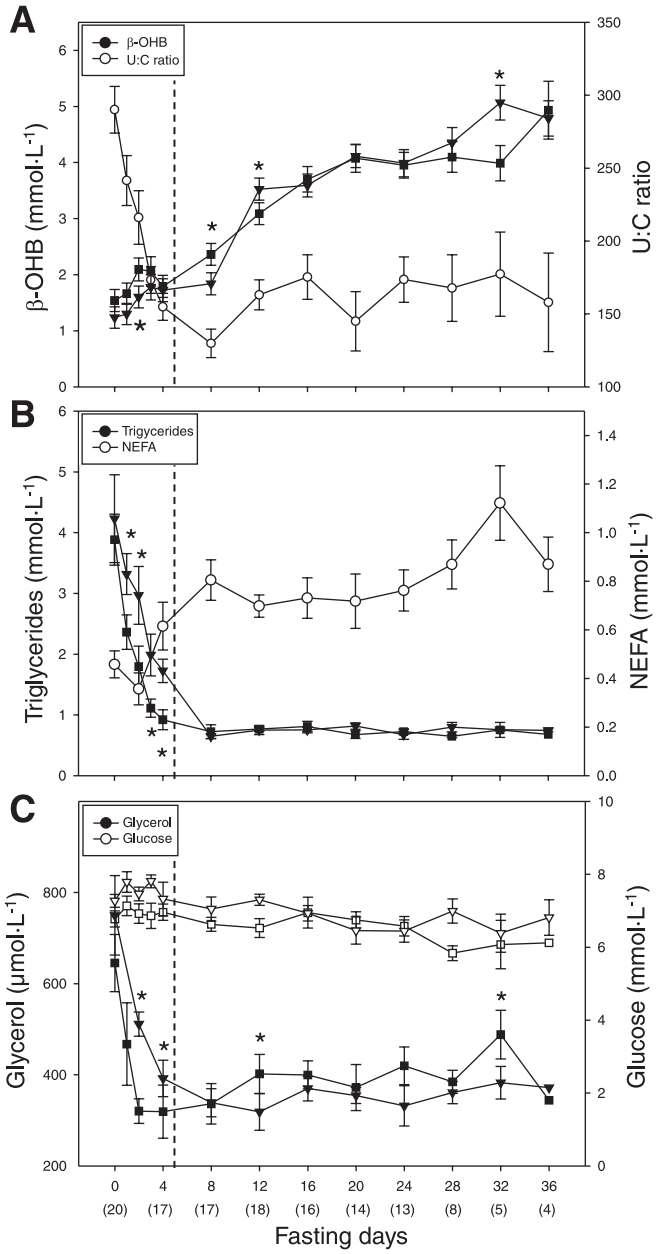

Fig. 5. Changes in plasma metabolite concentrations throughout prolonged fasting in subantarctic fur seal pups at Amsterdam Island. Vertical dash line indicates the transition between *phase I* and *phase II fasting*, as determined by changes in mass-specific rate of daily BM loss. Data for male (squares) and female (triangles) pups are presented separately when significant sex-based differences between means were detected (mixed ANOVA: $P < 0.05$). *Significant differences between the sexes at specific fasting days (Sidak: $P < 0.05$). In the absence of significant sex differences (mixed ANOVA: $P > 0.05$), data for pooled sexes are represented by circles. Numbers in parentheses below fasting days denote the sample size for pooled sexes for the corresponding sampling time. The variable U/C ratio represents plasma urea-to-creatinine ratio. NEFA, nonesterified fatty acids; β-OHB, β-hydroxybutyrate.

male pups tended to display lower β-OHB concentrations and greater glycerol levels than females in *phase II*. Overall, plasma glucose was significantly higher in females throughout the entire fast ($F_{1,30} = 7.82$, $P = 0.009$).

Body composition was determined in all pups at the beginning of the fast (on *day 2*) and in 13 of the 20 pups (7 males, 6 females) near the end of the fasting period, after an average of 26.5 ± 1.3 fasting days (Table 1). No significant sex differences were observed in body composition or lipid and protein catabolism (Tables 1 and 2). Lean BM (LBM; range: 6.4–9.7 kg) and TBL (range: 2.4–10.2 kg) were linearly related to pup BM, with BM loss comprising 86.2 ± 2.4% TBL ($r^2 = 0.96$, $F_{1,16} = 663.98$, $P < 0.001$) and 13.7 ± 2.4% LBM ($r^2 = 0.88$, $F_{1,15} = 13.66$, $P = 0.002$) (Fig. 6). Whereas LBM decreased modestly (3%) throughout the fast, TBL and %TBL were substantially reduced (by 36% and 21%, respectively) (Table 1). The relative contribution of lipids and proteins to FMR did not differ significantly with sex (Table 2) and was not related to initial %TBL (range: 42.4–52.0% with CV = 6%; $F_{1,11} = 1.04$, $P = 0.330$). Lipid catabolism (102.4 ± 5.8 g/day) was found to fuel 98.1 ± 0.4% of total energy expenditure, while protein loss (4.3 ± 0.8 g/day) contributed to an average of 1.9 ± 0.4% only. The respiratory quotient was also independent of pup sex ($F_{1,125} = 0.023$, $P = 0.880$), and remained stable at an average value of 0.80 ± 0.5 throughout the fast ($F_{8,135} = 0.815$, $P = 0.591$).

Water fluxes. There was no sex difference in MWP calculated from the amounts of lipid and protein catabolized during fasting, as well as in water efflux and water influx (Table 2). Overall, water influx and MWP were positively correlated ($n = 13$, $r^2 = 0.37$, $F_{1,11} = 6.35$, $P = 0.028$) and did not differ significantly ($t_{12} = -0.67$, $P = 0.516$). The water influx-to-MWP ratio was 1.05 ± 0.13. Water loss, as measured from the difference between water efflux and influx, averaged 13.7 ± 3.0 ml/day and, thus, represented 11.4 ± 3.0% of average daily BM loss.

DISCUSSION

The results of the present study demonstrate a remarkable tolerance in growing subantarctic fur seal pups to prolonged fasting. The metabolic responses displayed by these animals characterized the early adoption of an efficient strategy for energy conservation and protein sparing.

Phase I–phase II fasting in subantarctic fur seal pups. In fasting birds and mammals, three consecutive phases of fasting have been described with *phase I* being characterized by an initially high but rapidly decreasing daily BM loss and protein

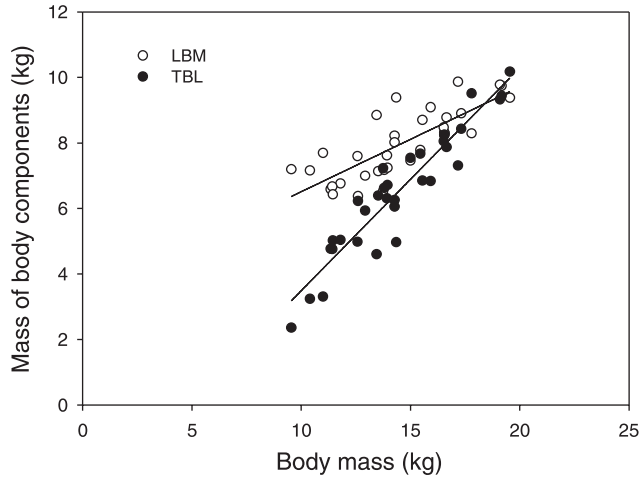

Fig. 6. Lean BM (LBM) and total body lipid (TBL) in relation to total BM in fasting subantarctic fur seal pups at Amsterdam Island.

catabolism; *phase II* with a low daily mass loss, minimal protein catabolism, and high level of lipid mobilization providing the majority of energy expenditure; and *phase III* with increasing rates of mass loss and protein catabolism (21, 25, 26). The ability to prolong the metabolic state of *phase II* determines an animal's resistance to extended fasting (24). In the present study, subantarctic fur seal pups exhibited metabolic and biochemical adjustments consistent with a transition from *phase I* to *phase II fasting* within 4–6 days, as soon as digestion and assimilation processes were achieved. Considered as a phase of adaptation, *phase I* was typically marked by significant reductions in specific rate of BM loss (55%), RMR (30%), and mass-specific RMR (25%), concomitant with a shift in blood chemistry, suggesting the activation of metabolic pathways promoting protein sparing and preferential reliance on body lipids. The near-steady state of *phase II*, typically characterized by minimal protein loss and high level of lipid utilization, as indicated by the maintenance of low plasma U/C and urea concentrations vs. elevated NEFA concentrations (2-fold) and continuous increase in plasma β-OHB (>3-fold), was maintained for the remainder of the fast.

No indication of a transition from *phase II* to *phase III fasting*, such as increasing rates of BM loss and protein catabolism with concomitant decrease in ketone levels late in the fast (25, 70), could be identified. Although only a few pups actually underwent fasting durations > 30 days in the present study, we calculated that, given pup fat content at the onset of winter fasting bouts (48.5%; cf. Table 1) and the rate of body lipid depletion measured throughout the fasts (102 g/day; cf. Table 2), a minimal fasting duration of 67 to 91 days would be required for the pups to approach a critical level of adiposity similar to the threshold of 3–9% suspected to trigger the shift to *phase III* in rats and penguins, respectively (24). This is two- to threefold the average natural fasting duration observed in the study cohort. Hence, these findings suggest that subantarctic fur seal pups are able to maintain the metabolic state of *phase II fasting* throughout the prolonged periods of natural fasting they experience repeatedly in winter.

An efficient strategy of energy conservation. When scaling to $BM^{0.75}$, the RMR of otariid pups are generally 2–4 times the level predicted by Kleiber's equation (46) for terrestrial adult mammals of similar size (7, 33, 71, 76). Such elevated RMR are consistent with the greater metabolic demands of rapidly growing infants (15) and more generally with the elevated metabolic rates exhibited by marine mammals (27). In contrast, in the present study subantarctic fur seal pups exhibited consistently reduced metabolic rates regardless of their feeding state (1.1 or 1.6 times Kleiber's level in the prolonged fasted state or fed state, respectively). The average rate of BM loss throughout the study period (0.78%/day over the entire fast and 0.70%/day in *phase II fasting*) was consistent with the lowest range of data previously reported for this species in the same colony (0.7–3.0%/day) (12, 43) but substantially low compared with those found in other otariid pups of similar body size during maternal absences (2.8–5.0%/day) (6, 7, 33, 76). Accordingly, and although slightly overestimated because obtained during a fasting period partly including *phase I* (*days 2* to *6*), the rates of energy expenditure (RMR and FMR) and MWP were also among the lowest recorded for otariid pups to date (7, 12, 33).

For the majority of the study period (*phase II fasting*), pup RMR was reduced to a minimal level, similar to values reported for large, weaned phocid pups (up to 200 kg) (59, 64, 80) and close to that of terrestrial adult mammals of similar size (46). During fasting, animals can decrease their metabolic rate through a reduction in gastrointestinal organ mass, reduction in body temperature, and/or diminution in behavioral activity (19, 26, 78). Ultimately, some species are capable of eliciting a deep metabolic depression that is a typical feature of mammalian hibernators (19, 39). In the present study, the RMR reduction in response to sudden food deprivation occurred within 4 days only. It is likely that the suppression of specific dynamic action, the increase in energy expenditure following feeding (54), with the completion of digestion and assimilation processes participated to this abrupt decrease in RMR throughout *phase I fasting*. The rapidity of this decrease reveals the early adoption of an energy-saving strategy, which might represent an adaptation to the repeated fasting pattern characterizing otariid infants (7, 65). In contrast, a period of 2–4 wk is needed in phocid seal pups before similar levels of RMR reduction are observed (59, 64). Furthermore, opportunistic observations at the study site suggested that subantarctic fur seal pups spend considerable time sleeping on land during winter maternal absences. Previous studies have shown that O_2 consumption can be decreased by 16–50% in pinniped pups, while sleeping in air (34, 80). In addition, significant reductions in metabolic rate linked to increasing time spent in breath hold during development, in particular while sleeping, have been observed in young phocids and otariids (47). Hence, substantial amounts of time spent sleeping on land and/or in apnea could significantly contribute to the low metabolic rate reported in the present study.

Overall, total energy expenditure was recorded at 1.7 times above RMR only, which is less than that reported for postmolt northern fur seal pups (33) but similar to that of phocid seals during their postweaning fast when they remain mainly inactive on land (60, 69). This suggests minimized energy allocation to behavioral activity and thermoregulation in our study pups. Actually, it has been suggested that, in contrast to other otariid infants, subantarctic fur seal pups at Amsterdam Island spend relatively little time in costly aquatic activities (i.e., playing in water, learning to swim and dive) (44). In particular, the proportion of time spent bathing during winter (~7%), when facing the longest maternal absences, is substantially lower than at less advanced stages of pup development (Verrier D, unpublished data). Such a strategy of limiting behavioral activity is likely to play a crucial part in the high level of energy saving attained. In addition, the subtropical climate experienced at Amsterdam Island and the thick insulative layer provided by abundant subcutaneous adipose tissue likely contribute to limit needs for regulatory thermogenesis in those animals (72).

Primary reliance on body fat stores and protein sparing. The average adiposities measured in the present study (>48% at maternal departure and >38% by the end of the prolonged fasting episode) were by far the highest values yet reported for an otariid (8, 33, 71) and also above the average %TBL reported for most phocid pups at weaning (37–42%) (20, 60, 69). More generally, such adiposities (>45–50%) are rare in the wild and rather found in obese people and laboratory or domestic animals (24). In addition to the exceptionally high-fat

content of maternal milk in this species (42% on average and >52% in winter; the greatest ever reported among otariids) (41), a strategy of limiting energy expenditure and directing nutritional resources to adipose tissue could contribute to such lipid accumulation. Alterations in endocrine factors known to play an important role in the regulation of adipose tissue mass and/or fat deposition, such as leptin, thyroid hormones, insulin, and cortisol (3, 38, 75), could, for instance, mediate the metabolic pathways improving pup efficiency for calorie storage. In addition, the very high-fat content of pup diet should contribute to limit the metabolic cost associated with feeding (specific dynamic action), which is dependent upon the nature of the nutrients absorbed (e.g., maximal for protein-rich meals, while fat and carbohydrates elicit almost no metabolic response) (54, 78) and, hence, promote the storage of the energy ingurgitated. With the greatest fat contents among otariid infants, subantarctic fur seal pups are able to survive the longest periods of food deprivation. This is in agreement with the positive relationship between the amount of fat stores at the beginning of the fast and the duration of fasting observed in many vertebrate species (18, 24, 25, 51, 60, 70).

Furthermore, adiposity seems to determine the degree of protein sparing during fasting, which constitutes the limiting factor to starvation survival: the more lipids available at the onset of the fast, the more efficiently body proteins are spared (24, 25). As expected, with their exceptionally high body fat content, subantarctic fur seal pups were found to display exceptionally low rates of protein catabolism (4 g/day) and contribution of proteins to total energy expenditure (≤2%) among other fasting species. This contribution was about 2–3 times lower than for species with a comparable (≥40%) initial adiposity (average contribution = 5%, $n = 6$) (Fig. 7). In total, proteins contributed to only 3.5% of BM loss over the entire fast, which is twofold less than in fasting emperor penguins (70). Yet, the level of protein sparing actually reached might be underestimated in the present study, for the first set of water pool measurements were taken on *day 2*, i.e., 4 days before the shift into the true stage of minimal protein utilization (*phase II fasting* starting at *day 6*) had actually occurred.

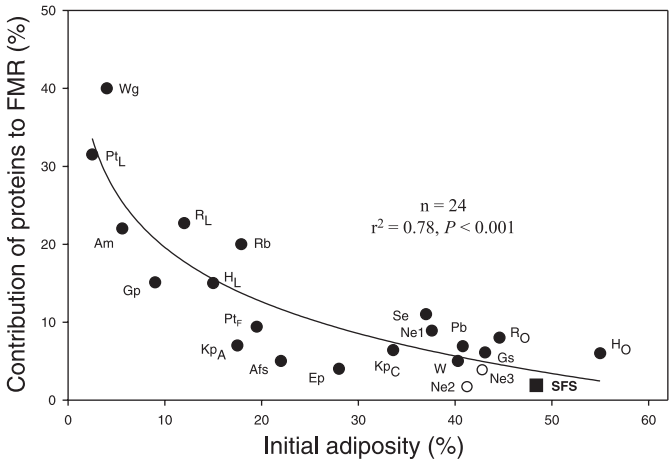

Fig. 7. Relationship between initial adiposity and contribution of proteins to total energy expenditure (FMR) during fasting in different bird and mammal species. Wg, William grouse (24); Pt_L, lean ptarmigan (51); Am, American marten (24); R_L, lean rat (25); Rb, rabbit (68); Gp, gentoo penguin (24); H_L, lean human (49); Pt_F, fat ptarmigan (51); Kp_A, adult king penguin (24); Afs, Antarctic fur seal pup (7); Ep, emperor penguin (70); Kp_C, king penguin chick (24); Ne, Northern elephant seal pup [Ne1 (●); mass balance method (60); Ne2 and Ne3 (○), urinary urea turnover method (1, 45)]; Se, Southern elephant seal pup (mass balance method) (20); Pb, polar bear (10); W, woodchuck (68); Gs, grey seal pup (59, 69); R_O, obese rat (25); H_O, obese human (49); SFS, subantarctic fur seal pup (present study).

These findings are consistent with an optimal strategy for LBM conservation at the expense of TBL in fasting subantarctic fur seal pups. Beyond the immediate survival to prolonged fasting episodes, the degree of protein sparing attained might still enable them to deposit LBM for growth despite the low rate of nutrient delivery in winter. This idea is supported by the moderate, yet significant, increase in body length found across the study period, showing that growth was not arrested throughout winter fasts in contrast with king penguin chicks (74). This could be achieved through a process of protein recycling, in particular from gut proteins, to ensure the growth of other tissues, such as muscles and skeleton during prolonged fasting. Recycling of gut protein during prolonged fasting has been observed without impairing rapid gut functional recovery and digestion efficiency at refeeding in aestivating anurans (30, 31) and upon rewarming and arousal in small seasonal mammals (19). Hence, as described in refeeding long-term fasted frogs (30, 31), a reduction in digesta passage rates to maximize nutrient assimilation efficiency, while rapid gut restructuring occurs, could explain the slow triglycerides clearance observed in the study pups following cessation of suckling. Conserving and recycling body proteins is also essential for tissue maturation and the development of physiological abilities, in particular those crucially involved in the successful transition to nutritional independence. For example, O_2 storage capacity determines diving ability and is acquired through the synthesis and accumulation of hemoglobin and myoglobin in animals' tissues (48).

In fact, during winter, fat appears to cover most of pup metabolic costs: from > 85% during suckling bouts (based on the contribution of lipids to milk gross energy) (41) to > 98% during maternal absences (present study). This suggests the existence of an extremely efficient enzymatic machinery for lipid catabolism and, in particular, to ensure energy production from lipids in these animals, independently of their nutritional status. In comparison, their proteolytic machinery should be little active (e.g., either by lessened availability, inhibition, and/or modulated efficiency of proteolytic enzymes), thus limiting the level of protein oxidation. Conversely, protein breakdown for energy production without signs of lipolysis activation has been shown to be a prominent feature of intermediary metabolism during fasting in animals on high-protein diets (e.g., carnivorous birds) (55). Furthermore, protein conservation has been shown to depend upon the level of fatty acid oxidation (13), a metabolic pathway that remains predominant regardless of nutritional transitions in subantarctic fur seal pups. In addition, NEFAs and their metabolites constitute endogenous ligands for peroxysome proliferators-activated receptors (14). In particular, NEFA-activated peroxysome proliferators-activated receptor-α increase fatty acid oxidation and ketone body production, and thus represent a "fasting-lipid-oxidation-glucose-sparing" regulator at the expense of muscle protein catabolism (37).

Interestingly, the average respiratory quotient of 0.80 maintained throughout the fast differs from the typical value of 0.71 expected for exclusive fat catabolism and indicates the oxida-

tion of a mixture of two-thirds lipids and one-third carbohydrates (35). Such a high proportion of glucose oxidation was unexpected, given that glucose oxidation has been shown to remain very low (<1%), despite high rates of endogenous glucose production and glucose recycling in fasting elephant seals (22, 23). The fasting durations relative to the small body size and developing status of subantarctic fur seal pups could, in fact, require slightly different physiological adjustments in the patterns of metabolic fuel use, which need to be clearly identified by further research. For instance, requirements of some glucose-dependent tissues in actively growing animals [such as the highly energy-demanding central nervous system of small-bodied, large-brained growing pups (17)] could account for a substantial proportion of carbohydrate oxidation. Substantial levels of gluconeogenesis from nonprotein precursors could permit the maintenance of elevated blood glucose concomitant with minimal LBM loss throughout fasting. Active use of fat-released glycerol as substrate for de novo glucose production (17) could explain the low-maintained plasma glycerol concentrations reported here. Other possible metabolic pathways, such as lipogenesis or nitrogen recycling (through the provisioning of carbon atoms for amino acid formation), have also been demonstrated for glycerol in hibernating fasting bears (2, 57). In addition, conversion of β-oxidation byproducts to glucose could contribute to maintain pup glycemia throughout fasting. In fasting humans, for instance, acetone has been shown to be responsible for up to 11% of plasma glucose production (67). Furthermore, surprisingly elevated concentrations of β-OHB (>4 mmol/l and up to nearly 8 mmol/l in *phase II*), compared with other fasting-adapted vertebrates, could further contribute to protein sparing by displacing glucose as central nervous system major fuel and allowing brain use of β-OHB (17). Such strategic utilization of β-OHB during arousal from torpor has recently been highlighted as a key mechanism in the metabolic adaptation to hibernation in small seasonal mammals (4). The discrepancies between the observed respiratory quotient and low protein loss reported in the present study could also represent evidence for nitrogen recycling as seen in bears (2, 57); the actual rate of protein oxidation would be compensated for by protein synthesis. Further investigation is necessary to elucidate the intricate metabolic pathways involved.

Water conservation. In addition to promoting resistance to starvation by protecting vital lean tissues, low rates of protein catabolism also limit the need to eliminate toxic by-products of nitrogen oxidation through urine production. This contributes to water conservation and the prevention of dehydration throughout prolonged food deprivation. Important reductions in urine excretion and urinary nitrogen output have been reported in phocid seal pups during their postweaning fast (1, 59). In the present study, the dramatic decrease in U/C throughout *phase I fasting* could reflect a reduction in glomerular filtration rate (32), which might represent a key mechanism for reducing urine production and maintaining water balance in long-term-fasting fur seal pups. Rates of water efflux were consistently particularly low, indicating that water was efficiently conserved throughout the winter fasts. Indeed, water loss represented only 10–11% of mass loss in fasting subantarctic fur seal pups, which is three- to fourfold less than in weaned elephant seal pups (20, 60).

Furthermore, the accordance between MWP and water influx estimates in the present study suggests a lack of substantial water drinking, which is in contrast to previous results in short-term fasting fur seal pups (12, 50). This indicates that fasting subantarctic fur seal pups were able to efficiently maintain water homeostasis during their winter fasts without an exogenous source of water, deriving all necessary water purely from body fat oxidation and implementing striking mechanisms of LBM conservation.

A convergent energetic strategy? In the present study, subantarctic fur seal pups at Amsterdam Island were found to adopt an impressive strategy of energy and LBM conservation at the expenses of TBL in response to the extreme fasts experienced in winter. Interestingly, whereas previous studies of metabolism in fasting-adapted species have shown relationships between BM loss and initial BM and/or adiposity (20, 60, 66, 70), we found no effect of initial BM and body composition on pup fasting energetics in the present study. Only the energetic investment into behavioral activity increased with increasing initial TBL (Fig. 4), indicating that the level of energy reserves available at the onset of the fast rather conditions flexibility in pups' activity budget and allocation into behavioral development.

Furthermore, in otariid species, male pups are typically heavier and leaner and grow faster than females. Previous studies have shown this to be due to differences in energy expenditure and/or metabolic fuel use and, thus, growth efficiency between the sexes (6–8, 33, 62). In the present study, however, we found no sex differences in body composition and fasting energetics. Furthermore, whereas sex-based differences in plasma metabolite concentrations during *phase I fasting* indicate that subantarctic fur seal pups could follow the "typical" otariid pattern of divergent fuel utilization between the sexes (e.g., with females relying more on protein catabolism than males that tend to favor LBM protection and growth upon fat storage) (7), in the "normal" postabsorptive state, both sexes shifted to a common metabolic strategy (e.g., based on primary body fat reliance and protein sparing, regardless of gender and body composition) as they entered *phase II* and prepared to face prolonged fasting. These observations are consistent with the global lack of sex difference in fuel partitioning observed over the whole fasting periods.

The relatively narrow range of pup adiposity at the onset of the fast (CV = 6%) could suggest that only the pups that were optimally prepared in terms of body condition could have survived up to and throughout winter and, thus, display the optimal physiological responses observed in the present study. Our results could, therefore, reflect how pups switch to a common strategy aiming to best enhance their survival in response to the evolutionary pressures exerted through: *1*) a high level of energy saving promoting pup survival in both the short (e.g., resistance to winter fasts, compensation for the unpredictability of maternal provisioning) and long (e.g., accumulation and preservation of body fat stores crucial for subsequent fasts and transition to nutritional independence) terms; and *2*) a high adiposity and predominant reliance on body fat permitting an exceptional degree of protein sparing, which in turn extend fasting abilities and might even allow pups to allocate nutrients into growth, tissue maturation, and development while almost constantly fasting.

Perspectives and Significance

The results of the present study suggest that subantarctic fur seal pups represent one of the most advanced evolutionary adaptations of any mammal to conditions of no food and no water during development. The extraordinarily efficient strategy of energy and LBM conservation they are able to adopt in a period of low maternal provisioning, based on the metabolic and biochemical results obtained, evokes striking similarities with a hibernation-like metabolism while maintaining some degree of activity, as previously insinuated for nonhibernating adult polar bears (57). Whether these extreme adaptations could partly be achieved through a reduction in body temperature (16, 57) should be examined in the future by using internal data loggers ensuring continuous monitoring of pup body core temperature. In addition, whether a certain degree of nitrogen recycling as demonstrated in hibernating black bears (2, 57) could explain the low rate of protein use reported in the present study and contribute to solving the trade-off between growth and survival in these animals also deserves further investigation. Overall, subantarctic fur seal pups represent a fascinating model to investigate the relationship between behavior, metabolic rate, body temperature, fuel selection, and adipose tissue development in mammals. Our current understanding of the physiological factors controlling intense fat deposition, in particular when the necessity to conserve and/or store fat is of crucial importance but competing with other metabolic requirements (e.g., fasting, growth, and development), could clearly benefit from it. Furthermore, deciphering the endocrine regulations governing the extreme adaptations presented in the present study, as well as the impressive anabolic machinery involved during the intense feeding periods bracketing the repeated fasting episodes would provide new insights into the mechanisms controlling nutritional transitions.

ACKNOWLEDGMENTS

We thank Alexandre Allag, Bruno Dauteloup, Matthieu Authier, and Hervé Conseil for their help in the field and Hélène Corbel for her assistance with mixed-model analysis. We are also grateful to Dan Crocker and two anonymous reviewers whose helpful comments greatly improved the present manuscript.

GRANTS

The present work was carried out at the Martin du Viviès research station and was financially and logistically supported by the French Polar Institute through Programs 109 and 119, the Terres Australes et Antarctiques Françaises, and Sea World Research and Rescue Foundation. D. Verrier was the recipient of an International Postgraduate Research Scholarship and Melbourne International Research Scholarship awarded by the University of Melbourne, Australia.

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