Effects of mass and body composition on fasting fuel utilisation in grey seal pups (*Halichoerus grypus* Fabricius): an experimental study using supplementary feeding

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Summary

This study used supplementary feeding to test the hypothesis that fuel partitioning during the postweaning fast in grey seal pups is affected by size and composition of energy reserves at weaning, and by extra provisioning. Mass and body composition changes were measured during suckling and fasting to investigate the effect of natural differences in energy reserves at weaning on subsequent allocation of fat and protein to energy use. We fed seven pups for 5 days after weaning, to investigate the effect of increased fuel availability, and particularly protein, on fuel utilisation. After correcting for protein used during the moult, the proportional contribution of fat was 86–99% of total energy use. Pups with greater energy reserves, i.e. those that were heavier and fatter at weaning, had higher rates of fat and energy use. There was no significant relationship between adiposity at weaning and proportional contribution of fat to energy use, perhaps due to a limited sample size or range of body masses and adiposity. Supplemented individuals used energy, specifically fat, much faster and utilised proportionally less of their endogenous protein by departure than non-supplemented individuals. Fat metabolism contributed a similar percentage to daily energy use in both groups. These findings show that pups spare protein, even when energy use is dramatically increased. Pups that receive greater maternal provisioning and lay down more protein may have increased survival chances at sea. This study highlights the importance of protein reserves in first year survival of grey seal pups.

Key words: protein depletion, fat reserves, energy use, fuel allocation, energy partitioning, first year survival.

Introduction

The way that offspring allocate the resources provided by their mothers is likely to have important consequences for their future success. This balance is one of the most important and least understood elements in the links between foraging and life-history strategy (Boggs, 1992). Trade-offs between allocation of resources to fat storage, protein deposition (growth) and metabolism involve complex and subtle interactions, and can have immediate effects on the performance and survival of young animals as they become nutritionally independent. This study tested the hypothesis that fuel use during fasting in grey seal (*Halichoerus grypus* Fabricius) pups depends upon the size and composition of available energy sources. We investigated whether pups that receive more fuel, and in particular protein, utilise fat and protein differently while fasting from those that receive less. We examined the effects of the size of energy reserves at weaning on variation in fat and protein utilisation during the postweaning fast. We also supplemented a group of pups with fat and protein (as herring) immediately after weaning and compared their mass and body composition changes with unfed pups.

Female grey seals usually produce a single pup each year, which they feed on high-fat milk during a brief (~18 day), intensive nursing period (Fedak and Anderson, 1982). While suckling, pups lay down lean body mass and a substantial blubber layer (Anderson and Fedak, 1987; Fedak and Anderson, 1982; Mellish et al., 1999). The amount of fat and protein laid down by the pup depends on the degree of maternal provisioning and the pup's metabolism. Investing more resources to rear a pup that is heavier and fatter at weaning is costly to females, which experience reduced pupping success in the year following a large investment (Pomeroy et al., 1999). However, there are benefits to the offspring of being larger and in better condition. Probability of first year survival increases with body mass and size of energy reserves at weaning in a variety of pinniped species (Baker and Fowler, 1992; Biuw, 2003; Burns, 1999; Harding et al., 2005; Hindell, 1991; Hindell et al., 1999; Le Boeuf et al., 1994; McConnell et al., 2002;

McMahon et al., 2000; McMahon and Burton, 2005), including grey seals (Hall et al., 2001; Hall et al., 2002). Their survival depends not only on the degree of maternal provisioning, but also on allocation of fat and, perhaps more importantly, protein, to energetic and developmental needs after weaning.

Grey seal pups are weaned abruptly and undergo a land-based fast of 1–4 weeks before going to sea (Reilly, 1991). They are reliant on their endogenous fat and protein reserves while fasting on land, and during the first days or weeks at sea, until they begin to feed. Pups face a trade-off between the use of body stores for metabolic fuel and for tissue structure and function. In addition to its role as the major energy reserve, fat provides insulation against the cold water temperatures once the animals go to sea. Protein has a low energy density, but plays a small, significant role as a metabolic fuel, and is also a major structural and functional component of tissues (Caloin, 2004). The trade-off between the contribution of fat and protein to energetic needs and developmental requirements may be linked to body size and composition at weaning and will influence how long pups can fast before they succumb to starvation.

The ability of seal pups to undergo an extended land-based postweaning fast is attributed to their low physical activity levels, metabolic depression, protein sparing and heavy reliance on their fat reserves to meet energetic requirements (Nordoy and Blix, 1985; Nordoy et al., 1990; Reilly, 1991; Worthy and Lavigne, 1987). While some protein use is unavoidable, protein sparing delays depletion of protein to critical levels, when tissue structure and function are irreversibly compromised and terminal starvation begins (Caloin, 2004; Garrow, 1959; Garrow et al., 1965). In other mammals, differences in size and body composition lead to substantial variability in the proportional contribution of protein and lipid to energy demands while fasting, and an inverse relationship exists between adiposity and the contribution of protein to energy expenditure (Atkinson et al., 1996; Cherel et al., 1992; Caloin, 2004; Dulloo and Jacquet, 1999; Dunn et al., 1982; Goodman et al., 1980). Inter-individual variability in the proportional contribution of fat and protein to energy expenditure has been demonstrated in some phocids (Biuw, 2003; Crocker et al., 1998; Muelbert et al., 2003; Noren and Mangel, 2004). Elephant seal (*Mirounga* sp.) pups with greater fat reserves at weaning use fat catabolism to meet proportionally more of their energy costs than leaner pups (Biuw, 2003: Noren and Mangel, 2004). The contribution of protein to metabolism varies as a function of body composition in lactating adult northern elephant seal (*M. angustirostris*) females (Crocker et al., 1998). Muelbert and colleagues (Muelbert et al., 2003) have shown that body composition changes in harbour seal (*Phoca vitulina*) pups in the first 19 days after weaning are related to weaning body mass. The largest pups show no change in body composition as they lose mass, whereas percentage lipid content declines in the lightest pups (Muelbert et al., 2003).

Previous studies on fasting grey seal pups have shown that more than 90% of their energy costs are met by fat catabolism (Nordoy and Blix, 1985; Nordoy et al., 1990; Reilly, 1991; Worthy and Lavigne, 1987). Variation in fuel allocation between individuals, most notably with regard to body mass or the size of energy reserves, has not been reported. In the present study we investigated whether mass and composition at weaning influences fuel use in fasting grey seal pups. To determine whether providing extra resources, and particularly protein, can alter subsequent fasting changes in mass and body composition, we fed a group of pups after weaning, and compared their energy use and fuel allocation with pups that did not receive extra food.

Materials and methods
Study site and animals

This study was performed on the Isle of May, in the Firth of Forth, Scotland (56°11′N, 2°33′W) during October–December 2001 and 2002. All capture and handling procedures were performed under Home Office project licence #60/2589 and conformed to the Animals (Scientific Procedures) Act 1986. Twenty five healthy, wild grey seal pups were included in the study (Table 1). At first capture [at early lactation (age ~4 days) for 23 animals and within 2 days after weaning for the remaining two pups], the sex of each pup was recorded and the animal was fitted with a rototag (Dalton ID Systems, Henley on Thames, Oxon, UK) in the interdigital webbing of one rear flipper. The 23 pups handled in early lactation were captured again at late lactation (age ~15 days) to measure changes in mass and body composition during suckling. Pups were assumed to have

Table 1. *Number of animals of each sex in each group captured during lactation and for which mass change and body composition data were available and for which fast duration was known*

Group (N)	Sex	Lactation captures	Mass change	Body composition	Fast duration known
Fed (7)	M	4	4	3	3
	F	3	3	3	3
	Total	7	7	6	6
Control (12)	M	6	6	6	6
	F	6	6	5	6
	Total	12	12	11	12
Low (6)	M	2	2	2	2
	F	2	4	4	4
	Total	4	6	6	6

Fed pups were compared to Control pups only, to investigate the effects of supplementary feeding. Control and Low pups only were used to investigate the effects of mass and body composition on fasting fuel utilisation.

weaned when the female was not observed in attendance for 1 day. All pups were penned in a large (~115 m×80 m) outdoor enclosure within 2 days after weaning and remained there until reaching 70% of their weaning mass or 30 kg, whichever happened first. On release, pups were painted with unique large and highly visible symbols on their backs, and their presence/absence on the colony was noted daily. Pups present after release were re-weighed every 3 days until the study was ended (18/12/2001 or 16/12/2002), when most animals had left. Date of departure was assumed to be the day after the last sighting of the animal and was known for 24 of the 25 study animals. One Fed (see definition below) male was still on the colony after the researchers had left.

Effects of supplementary feeding on fuel use were investigated in 19 pups. Seven (Fed) pups received supplementary food on each of the first 5 days after penning. On each of these 5 days, after they had been weighed, Fed pups were given a single meal of 1–1.5 kg defrosted whole herring (*Clupea harengus*; Lunar Freezing, Peterhead, UK). Herring was used to deliver high energy in a single, short meal (~7 min per feed), without the need for stomach intubation, which is needed for feeding milk and can damage the oesophagus. Herring is readily available and is fed to newly weaned pups without adverse consequences, as well as juveniles and adults, in the seal holding facility at the Sea Mammal Research Unit (SMRU), University of St Andrews. The Control group consisted of 12 pups that experienced a similar handling frequency to Fed pups. The remaining six pups were handled only three times during fasting as part of a separate study (Low). They were used to investigate effects of weaning mass and body composition on fasting fuel use, but were excluded from direct comparisons with Fed animals due to differences in handling regime.

Analysis of body composition

Animals were weighed using a Salter spring balance (accuracy ±0.2 kg) each time they were handled (every three days after weaning for the Fed and Control groups, and three times after weaning for the Low group). Body composition estimates, using deuterium oxide (^2H_2O) dilution as described (Reilly and Fedak, 1990), were performed early and late in lactation for the 23 pups handled during suckling, on entry into the pen for the remaining two, and between 14 and 26 days after weaning (mean=21±3.5 days) for all study pups. Mass, but not body composition, was measured after the end of the feeding period in Fed pups. Thus all postweaning body composition changes incorporate the 5-day feeding period, as well as fasting, in Fed pups. After the animal was weighed, a plasma sample was taken from the extradural vein into a sterile 10 ml heparin treated vacutainer (Becton Dickinson, Cowley, Oxon, UK) to measure background ^2H_2O enrichment in body fluids [method D (Speakman and Racey, 1987)]. A pre-weighed dose of 3–5 ml ^2H_2O (99.9%; Sigma-Aldrich Chemicals, Gillingham, Dorset, UK) was then injected and a second plasma sample was taken 3–4.5 h later to determine ^2H_2O enrichment after equilibration with body water compartments (Costa et al., 1986; Reilly, 1991). After centrifugation, 4× 50 μl aliquots of each sample were flame-sealed into capillary tubes, and stored at room temperature until analysis. ^2H_2O enrichment in parts per million in two sub-samples of the background and enriched plasma

samples and standards was measured in duplicate in a pyrolysis inlet mass spectrometer (Micromass isoprime, GV Instruments, Manchester, UK) (Speakman and Krol, 2005). Samples were reanalysed if variation between replicates was over 2%. Dilution space was calculated as described (Krol and Speakman, 1999). Absolute mass and percentage of each of the body components (fat, protein, water and ash) were determined from body water content, using equations derived by comparison of ^2H_2O dilution with chemical composition of grey seal carcasses (Reilly and Fedak, 1990). This method has been validated and shown to be accurate by comparison to chemical analysis in Antarctic fur seals (*Arctocephalus gazella*) (Arnould et al., 1996a). Energy available for metabolism was calculated assuming energy densities of 39.33 kJ g^{-1} and 17.99 kJ g^{-1} for fat and protein, respectively (Schmidt-Nielsen, 1997). Table 1 summarises the number of pups in each group for which mass and body composition information was available.

Overall daily rate of mass gain during suckling and mass loss during fasting (kg^{-1} day^{-1}) and body composition changes during those periods were calculated for all pups. Increases in mass, water, energy, fat, protein and ash during suckling were calculated from the difference between measured values at first and last capture during lactation. Overall average daily rates of loss of mass, water, energy, fat, protein and ash during fasting were calculated from the difference between measured values at first and last capture after weaning, divided by the number of days between these two captures. As in northern elephant seal pups, 17.8% of their weaning protein mass was assumed to be utilised during the moult, which begins around the time of weaning in grey seal pups (Noren et al., 2003). This protein was not available for metabolism and was subtracted from weaning protein mass before protein mass loss, energy content and relative contribution of fat and protein to energy use were calculated.

The mass, energy, protein, fat and ash contents of the meals for the Fed group were included in the calculations, as follows. The herring contained 16–18% fat (Lunar Freezing, supplier's unpublished information) and therefore its composition was assumed to be similar to published values (Gallivan and Ronald, 1981) of 63% water; 18.5% fat; 15.5% protein. These values were used to calculate the total energy, water, fat and protein available to the seals by multiplying by the total amount of fish consumed by each animal (mean=5.87 kg±0.58 kg). The amount of energy required to digest the fish, termed the heat increment of feeding (HIF), was assumed to be 15.74% of its gross energetic content, as reported (Gallivan and Ronald, 1981) for a similar sized meal of herring fed to a harp seal (*Pagophilus groenlandicus*). Gross energetic content of fat and protein were assumed to be 39.5 and 23.5 kJ g^{-1} for this calculation (Schmidt-Nielsen, 1997). HIF was then subtracted from the energy available for metabolism. The protein, fat, water and ash mass of the fish was corrected for an assumed assimilation efficiency of 91%, determined from another experiment on harp seals fed a herring diet (Lawson et al., 1997). These values were divided by fast duration and added to the appropriate value for daily tissue loss already calculated for each of the Fed animals, i.e. animals assimilated then subsequently lost the tissue components from the fish. Faecal and urinary losses were not measured.

Weaning mass and body composition were extrapolated from mass changes during suckling and/or fasting, assuming animals continued to gain mass at the same rate between late lactation and weaning, or lose mass at the same rate from weaning to first capture postweaning. Departure mass and body composition were extrapolated from fasting loss rates. There is no evidence that healthy fasting seal pups undergo a change in fuel utilisation towards the end of fasting (Nordoy et al., 1990; Houser and Costa, 2003), as occurs in other fasting animals (Cherel et al., 1988a; Cherel et al., 1988b; Cherel et al., 1988c), unless held in captivity for substantially longer than the normal fast duration (Nordoy et al., 1992). Animals were thus assumed to continue to lose body components at the same rate between last postweaning capture and departure. The amounts of energy, fat and protein and % of protein at weaning that had been utilised by departure, daily change in % fat content and % contribution of fat to daily energy use were calculated.

Statistical analyses

Statistical analyses were performed using MINITAB (Minitab 13.32, Minitab Inc, 2000) or R (R 1.9.1, R Development Core Team, 2003) (Ihaka and Gentleman, 1996). Differences between Control and Fed groups in their pattern of mass loss over 3-day intervals from the start of fasting (i.e. date of natural weaning for Controls and end of feeding for Fed pups) were investigated using linear mixed-effects models (LMEs), which incorporate the covariance between successive repeated-measures data points in the model structure, thus accounting for individual differences in the response without over-parameterising the model (Chatfield, 1989; Crawley, 2002). Individual was included as a random term. Group and day after the end of feeding (i.e. days after weaning for Controls and days after the end of feeding for Fed pups) were included as fixed effects. The model was fitted using a maximum likelihood estimate. Differences between Fed and Control groups in body mass and composition variables at weaning and departure, and their average rate of loss after weaning were investigated. Body composition variables were derived from mass and body water measurements, thus multiple analyses of variance (MANOVAs) were used to determine whether the effect of group on these two variables was sufficiently different for them to be examined separately. Where there was a significant group difference in body water, MANOVA was used to investigate whether the effect was due to differences in fat or protein content.

The effect of natural variability in body size and composition on fasting fuel utilisation was examined using information from Control and Low pups for which body composition estimates were available for both weaning and departure (Table 1). Fed pups were excluded from this analysis because they were unlikely to be representative of naturally fasting pups. We performed forward and backward stepwise linear regression to investigate the effects of energy reserves at weaning on changes in mass and body composition parameters. Similar stepwise regression analysis has been used previously in studies investigating relationships between body composition parameters and measures of energy expenditure (e.g. Rosenbaum et al., 1997). Explanatory variables in the saturated models to explain fasting changes in fat, protein and total body masses, daily energy use, % change in fat and % contribution of fat to daily energy use were weaning body mass, absolute fat and protein masses, energy available for metabolism and % fat. Analysis of variance (ANOVA) was used to compare the fit between models each time a parameter was added or removed. Bonferonni corrections were applied ($P<0.008$ considered significant) to account for potential interdependence between dependent variables, since all body composition values were derived from mass and body water.

Results

Impact of supplementary feeding

The average rate of mass loss during the first 7 days after weaning, which incorporated the feeding period for Fed pups, was significantly lower in Fed animals than in Controls (0.13 ± 0.09 kg day^{-1} vs 0.54 ± 0.04 kg day^{-1}; t-test: $T_{(17)}$=4.93; $P<0.0001$). Two of the Fed pups gained 0.15 kg during the feeding period. The changes in rate of mass loss over 3-day intervals from weaning (Controls) or the end of feeding (Fed) were compared and were significantly lower in Fed pups during the first 7 days after the end of feeding [LME: Akaike Information Criterion (AIC)=39.05, log-likelihood=31.53, N (individuals)=19, n (observations)=89; Fig. 1]. The rate of mass loss decreased over the first 10 days after weaning in the Control group, and then remained between 0.3 and 0.4 kg day^{-1}. In the Fed group the rate of mass loss was consistently lower than that of Controls and declined less steeply during the first 10 days after the end of feeding. It then increased significantly to levels comparable with those in the Control group by day 13, and remained similar to Control values thereafter. Note that mass loss values reported here are based only on the measured mass of the animals and do not include the mass of the fish consumed. There were no significant differences between Control and Fed pups in either the total duration of the postweaning period (Fed=27.8 ± 7.7 days vs Control=24.7 ± 5.2 days; t-test: $T_{(16)}$=1.04; P=0.315) or the duration of the fast, from the end of
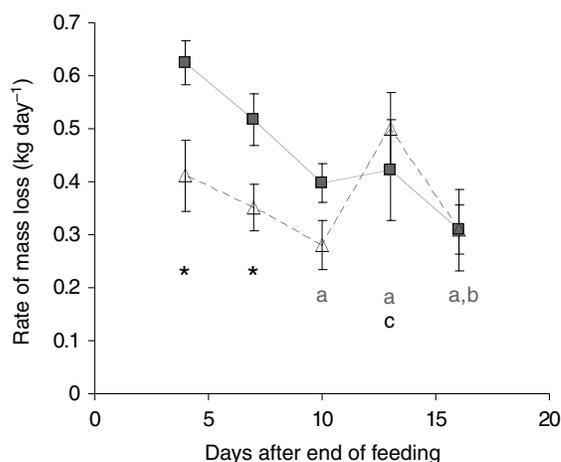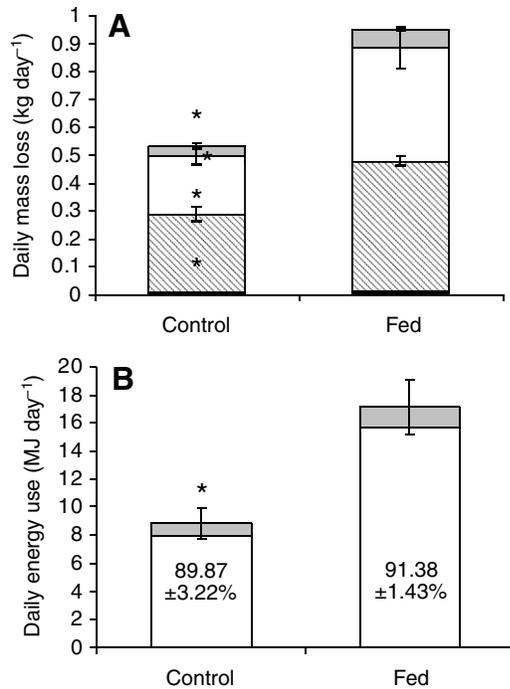

Fig. 1. Daily rate of mass loss (means ± s.e.m.) at 3-day intervals, excluding the additional mass of the fed fish, in Fed (open triangles; N=7) and Control (closed squares; N=12) pups. *Significant difference between groups ($P<0.05$). a, b, c (black, Fed; grey, Control) denote a significant difference ($P<0.05$) from day 4, 7 and 10, respectively, within group.

Fig. 2. (A) Mean daily rate of mass loss (total bar height), comprising ash loss (black), water loss (diagonals), fat loss (white) and protein loss (grey) during the postweaning period, including assimilated fish tissue components. (B) Daily energy use (total bar height), divided into contributions from fat (white: % of total shown on bar) and protein (grey) based on calculated changes in body composition, including fish tissue components. Values are means ± s.e.m. *Control group values that were significantly lower ($P<0.05$) than those from the Fed animals.

difference in the loss of mass and body water (MANOVA: $F_{(2,15)}=10.10$, $P=0.002$). The daily rates of mass loss, averaged over the whole postweaning period (ANOVA: $F_{(1,17)}=19.23$, $P<0.001$), water loss (ANOVA: $F_{(1,17)}=20.80$, $P<0.001$), fat loss (ANOVA: $F_{(1,17)}=9.47$, $P=0.007$), protein loss (ANOVA: $F_{(1,17)}=8.75$, $P=0.009$) and energy use (ANOVA: $F_{(1,17)}=11.19$, $P=0.004$) were significantly higher in Fed pups than in Controls. The percentage contribution of fat catabolism to daily energy use (Mann–Whitney: $W=116.0$, $P=0.888$) and the daily change in percentage body fat (Mann–Whitney: $W=126.0$, $P=0.281$) were not significantly different between groups.

Group differences in body composition at departure and total fuel used are shown in Fig. 3. Body mass and body water at departure responded differently to treatment group (MANOVA: $F_{(2,15)}=3.712$, $P=0.049$). Body mass at departure was not significantly different between the groups (ANOVA: $F_{(1,17)}=0.19$, $P=0.672$), whereas the difference in body water content at departure between groups was approaching significance (ANOVA: $F_{(1,17)}=4.02$, $P=0.062$) and was higher in the Fed group than in the Control group. This indicated a difference in departure body composition, which was investigated further. Fat mass at departure was not significantly different between groups (ANOVA: $F_{(1,17)}=2.95$, $P=0.105$). However, percentage fat at departure was significantly lower (ANOVA: $F_{(1,17)}=8.51$, $P=0.010$) and protein mass was higher (ANOVA: $F_{(1,17)}=4.55$, $P=0.049$) in the Fed group than in the Control group. The percentage of initial endogenous protein mass available at weaning for metabolism that was utilised by departure was significantly lower in the Fed group (ANOVA: $F_{(1,17)}=6.40$, $P=0.022$). They did not lose less protein mass overall (ANOVA: $F_{(1,17)}=1.10$, $P=0.309$), because the additional protein was derived from the fish. The Fed group used significantly more

the feeding period in the Fed group (Fed=22.8±7.7 days *vs* Control=24.7±5.2 days; *t*-test: $T_{(16)}=0.6$; $P=0.556$).

Weaning body composition was not different between groups (MANOVA: $F_{(2,15)}=0.384$, $P=0.688$). However, there were group differences in body composition changes during the postweaning period, which included the duration of feeding for the Fed group (Fig. 2). Note that, in contrast to the 3-day interval mass loss data, these values include the resources provided by the supplementary food because they represent the use of the total amount of body components available to the animals over the whole postweaning period. There was a significant
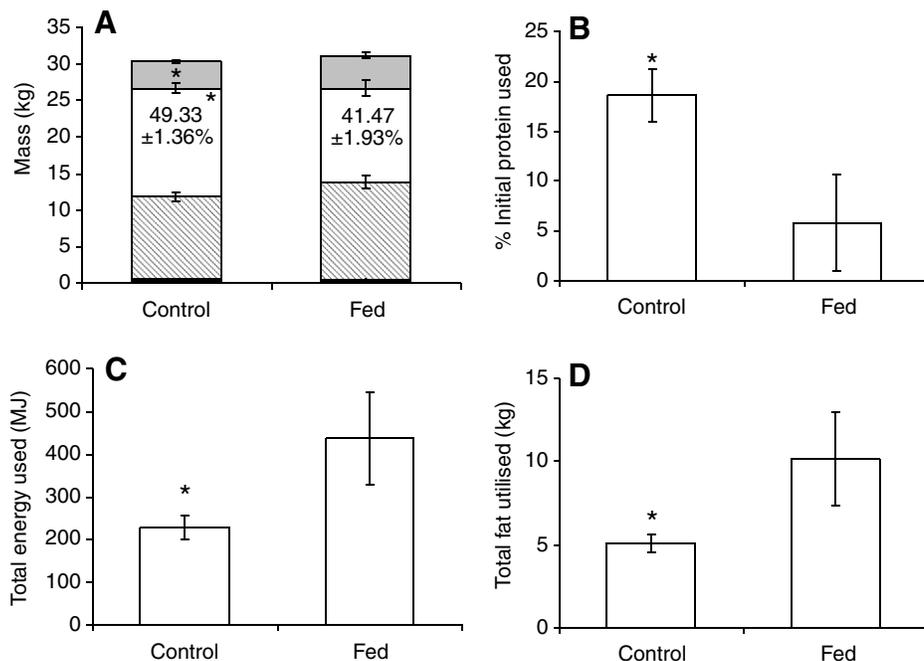

Fig. 3. Mean body composition and fuel used by departure in Fed and Control pups, including assimilated fish tissue components. (A) Mass at departure (total bar height), showing ash (black), water (diagonals), fat (white) and protein (grey) and % fat (value shown on bar). (B) Percent of initial protein mass, (C) energy and (D) fat mass used by departure. Values are means ± s.e.m. *Significant difference between groups ($P<0.05$).

Table 2. *Mean mass and body composition parameters for animals in Fed and Control groups for which body composition was estimated at weaning and departure*

	Fed	Control
Weaning		
Mass (kg)	47.51±10.38	44.40±5.10
Fat (kg)	22.04±6.06	20.34±3.00
Protein (kg)	5.71±0.93	5.4±0.6
% Fat	46.01±2.64	44.97±3.16
Energy (MJ)	951.44±251.45	867.48±126.55
Fasting		
Mass loss (kg day^{-1})	**1.00±0.20**	**0.60±0.18**
Fat loss (kg day^{-1})	**0.41±0.18**	**0.21±0.09**
Protein loss* (kg day^{-1})	**0.08±0.02**	**0.04±0.02**
DEU (MJ day^{-1})	**17.14±6.80**	**8.87±3.82**
% DEU fat	91.39±4.94	89.87±11.16
Daily change in % fat	−1.68±8.31	0.19±0.19
Departure		
Mass (kg)	30.91±3.76	30.09±3.80
Fat (kg)	12.84±2.636	14.91±2.30
Protein (kg)	**4.43±0.98**	**3.66±0.57**
% Initial protein used in metabolism*	**5.81±11.81**	**18.61±9.25**
% Fat at departure	**41.47±6.68**	**49.33±4.69**
Energy (MJ)	585.30±97.64	647.65±86.52
Total energy used (MJ)*	**437.47±267.20**	**228.46±93.72**
Total fat used (kg)	**10.17±6.83**	**5.09±1.93**
Total protein used (kg)*	1.09±0.56	0.84±0.45

Values are means ± standard deviation (s.d.). For *N* values, see Table 1.
DEU=daily energy use; % DEU fat=% contribution of fat to DEU.
Bold type indicates a significant difference between groups (*P*<0.05).
*Refers to protein used in metabolism, i.e. values after protein used in moulting was removed from calculations.

energy (Mann–Whitney: W=88.0, P=0.0169) and fat (Mann–Whitney: W=90, P=0.0277) than the Control group.

Computational tests to investigate the sensitivity of our findings to variation in assumed fish composition and digestibility were performed. The fat content of the fish was varied by ±3% to incorporate a range of body compositions from mature North Sea herring caught in summer. The assumed digestibility of the protein component of the herring was also reduced to 65% (Trumble et al., 2003). These tests did not produce substantial changes in the calculated rates of tissue or energy use (data not shown). For example, if only 65% of protein was usable by the animals, calculated rates of protein loss fell by ~10 g per day, and a 1% average increase in the relative contribution of fat to energy use was observed. Thus our findings are robust to potential errors in energy content and assimilation efficiency assumptions. Values for mass and body composition at weaning and departure and average daily changes in mass and body composition parameters during fasting are shown in Table 2.

Impact of body composition on postweaning fuel use

The models that best explained variability in energy use and fuel allocation parameters in unfed (Control and Low) pups are shown in Fig. 4. The daily average fasting rate of mass loss increased as a function of protein mass at weaning, corrected for protein used in the moult (y=1.51x–0.09; r^2=0.503; $F_{(1,16)}$; P=0.0006). The daily average fasting rate of energy use

(y=0.02x–10.51; r^2=0.553; $F_{(1,16)}$; P=0.0002) and fat loss (y=0.0005x–0.27; r^2=0.551; $F_{(1,16)}$; P<0.0001) increased as a function of the energy available for metabolism at weaning. The apparent positive relationship between rate of protein loss and protein mass at weaning was not significant (y=0.018x–0.047; r^2=0.154; $F_{(1,16)}$; P=0.0598). Interestingly, there was no significant linear relationship between adiposity at weaning and % contribution of fat to energy use (y=–0.25x+63.13; r^2=0.03; $F_{(1,16)}$; P=0.510; Fig. 4C). Although the absolute fat content decreased markedly in all pups while fasting, percentage body fat increased significantly by ~4.07% from 45.84±3.31% at weaning to 49.93±4.01% at departure (paired *t*-test: $T_{(18)}$=4.95, P<0.0001), and this increase was negatively correlated with percentage contribution of fat to energy use (y=–0.02x+1.59; r^2=0.790; $F_{(1,16)}$; P<0.0001).

Discussion

Supplementary feeding altered fuel use dramatically during the postweaning period. Feeding increased both fat use and protein sparing. Additional food increased daily energy use during fasting by amounts greater than the energy content of the food. The availability of extra fuel caused animals to use more endogenous fat, indicating that extra metabolic costs of feeding were met by fat catabolism. Fed pups used protein at a higher rate than Control animals, but had used less endogenous protein by departure as a proportion of their initial reserves. They were thus able to preferentially utilise exogenous protein or lay it
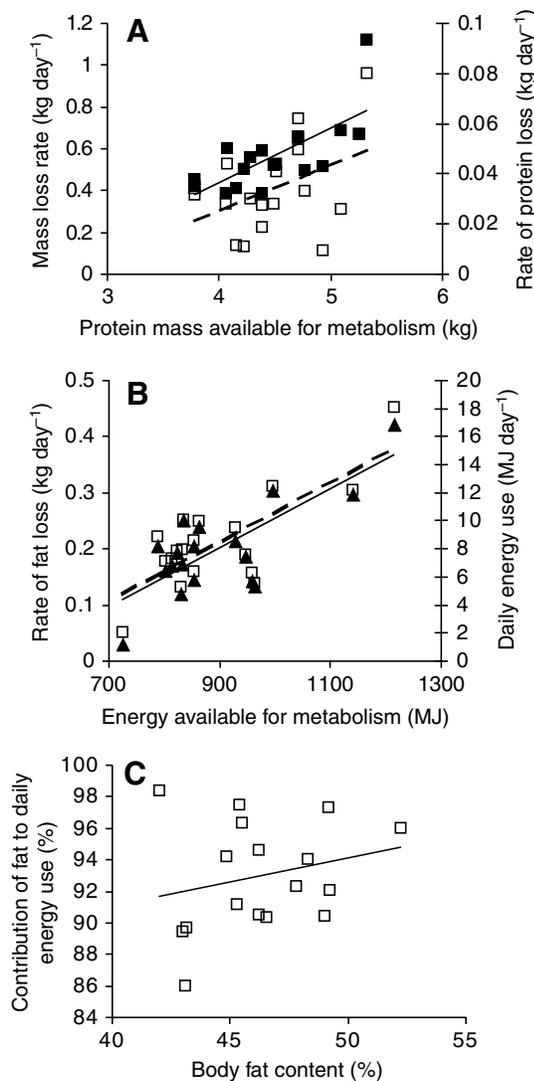
Fig. 4. (A) Relationships between protein available at weaning for metabolism and average rate of mass loss (solid squares, solid line) and rate of protein loss (open squares, broken line). (B) Relationship between energy available for metabolism at weaning and daily energy use (open squares, broken line) and daily fat loss (triangles, solid line). (C) Relationship between % contribution of fat to energy use and % fat content at weaning in Control and Low animals.

down as lean tissue to replace loss of their own reserves. These results are consistent with findings from northern elephant seal pups refed after the postweaning fast, which laid down protein mass despite being maintained on a fish diet that provided substantially less energy than their energetic costs (Rea and Costa, 1992). This study highlights the ability of seal pups to conserve protein while experiencing large changes in metabolic rate and fat utilisation. Pups that received extra food would have benefited largely as a result of greater protein availability, which should extend the amount of time available in which to find food before death from starvation (Caloin, 2004; Garrow, 1959; Garrow et al., 1965).

Although not comparable with the mass gained during suckling (~1.5–2 kg day^{-1}) (Anderson and Fedak, 1987; Bowen

et al., 1992; Fedak and Anderson, 1982; Mellish et al., 1999; Pomeroy et al., 1999), supplementation slowed the rate of mass loss and resulted in a small mass gain in some of the Fed pups, probably due to the presence of food in the gut, assimilation of the food into body stores, or both. The fall in mass loss rate early in the first 10 days after the onset of fasting in both Control and Fed pups was likely due to a reduction in metabolic rate that has been well documented in fasting animals (Arnould et al., 2001; Bowen et al., 1992; Castellini and Rea, 1992; Cherel et al., 1988a; Cherel et al., 1988b; Cherel et al., 1988c; Dunn et al., 1982; Hambly and Speakman, 2005; Le Maho et al., 1981; Nordoy et al., 1990; Rea and Costa, 1992).

Feeding either stimulated metabolic rate, or prevented a fast-induced metabolic depression. Although daily energy use after weaning in Fed pups was extremely variable and values lay within the range of previously reported fasting values (Reilly, 1991), the mean value was 17.41 MJ day^{-1}, almost double that of the Controls. The disparity was even greater if increased energy use only occurred during and immediately after feeding, instead of throughout the fast as the average daily energy use values imply. This feeding-stimulated increase in metabolism may, in part, represent greater energetic costs of digestion than were accounted for here. We used a high value for HIF (15.74% of the gross energetic content of the food), which was reported in captive seals fed similar quantities of herring (1–2 kg) to the amounts used in the current study (Gallivan and Ronald, 1981). However, animals that have previously been fasting experience greater metabolic costs of feeding because they must rapidly reverse the gut atrophy that occurs during starvation (Boza et al., 1999; Dunel-Erb et al., 2001; Karasov and Diamond, 1983; Secor et al., 1994). Feeding-induced increases in metabolic rate after starvation or fasting tend to be relatively modest in endotherms because their basal metabolic rate is already high. A 20% increase in metabolic rate, which is seen in adult harbor seals and northern elephant seal pups refed after fasting (Markussen et al., 1992; Rea and Costa, 1992) is not sufficient to account for the doubling in energy use in Fed pups compared to Controls. Although Fed pups had only recently weaned, in addition to the normal costs of digestion, it is likely that they required some restructuring and upregulation of the digestive machinery and other organs involved in nutrient processing to cope with the sudden presence of solid food in their previously empty gut and the novel diet not normally encountered so soon after weaning. The specific causes and timing of the alterations to metabolism could not be identified, partly because the direct effects of feeding on body composition were not known. The immediate effects of feeding after weaning on energy expenditure and longer term postabsorptive effects on body composition changes and energy use need to be teased apart to better understand the processes observed here. If a similar increase in metabolic rate occurs at the onset of feeding at sea, it could have important consequences for continued survival of pups if their intake does not match their increased expenditure.

This study highlighted the degree of variability in the use of fat and protein by fasting grey seal pups. Lean mass at weaning was positively correlated with mass loss and protein loss rates, although this latter relationship was not significant. Those pups with greater energy reserves at weaning had higher rates of energy use and fat loss than smaller, leaner animals. These

effects are likely to be largely due to the increase in metabolic costs that occurs with increasing body size (Kleiber, 1975).

Our results do not show that fuel use in fasting grey seal pups shifts towards a greater proportional contribution of fat to energy use with increasing adiposity at weaning. This is at odds with findings from a wide range of other species. Fasting humans (Dulloo and Jacquet, 1999), rats (Cherel et al., 1992; Dunn et al., 1982; Goodman et al., 1980), brown bears (*Ursus arctos*) (Hilderbrand et al., 2000), Gentoo (*Pycoscelis papua*) and king penguins (*Aptenodytes patagonicus*) (Cherel et al., 1993) and Svalbard ptarmigan (*Lagopus mutus hyperboreaus*) (Lindgard et al., 1992) allocate fat and protein to meet energetic costs based on their available reserves; fatter animals show a greater proportional contribution of fat to energy expenditure and spare protein more effectively. Fasting marine mammals, including elephant seal pups, subantarctic fur seal pups (*A. tropicalis*) and polar bears (*U. maritimus*), also show an increase in energy expenditure, contribution of fat to energy costs and the loss of fat tissue as a function of the size of initial fat reserves (Atkinson et al., 1996; Beauplet et al., 2003; Biuw, 2003; Carlini et al., 2001; Noren et al., 2003; Noren and Mangel, 2004). The proportional contribution of fat to energetic costs in grey seal pups is not a simple function of body fatness, and may depend on other factors such as activity levels and the requirements for fat and protein in developmental processes. Those studies on other phocid species that have demonstrated a significant positive relationship between adiposity and reliance on fat to meet energetic costs have included larger numbers of animals with much larger ranges of sizes and body compositions than used here and previously in grey seals (Biuw, 2003; Carlini et al., 2001; Muelbert et al., 2003; Noren et al., 2003; Noren and Mangel, 2004). The proportional contribution of fat to total energetic costs in grey seals clearly requires further investigation with a much larger sample of animals, including pups at the extremes of lean and fat body compositions.

In previous studies, fat contributed 94% to energy expenditure in wild and captive grey seal pups (Nordoy and Blix, 1985; Nordoy and Blix, 1991; Reilly, 1991; Worthy and Lavigne, 1987). Once the protein used for hair growth was removed from the calculations the values of proportional contribution of fat breakdown to energy use seen here (86–99%) were comparable with, but more variable than, these and other previously reported values in fasting seals. These previous studies employed methods that quantified protein losses more directly, such as measurement of urinary nitrogen production, and urea turnover and computed tomography (Adams and Costa, 1993; Houser and Costa, 2001; Nordoy and Blix, 1985; Pernia et al., 1980). The use of deuterium dilution to estimate body composition can overestimate protein catabolism if water is lost to inexchangeable pools, during, for example, *de novo* lipogenesis (Speakman, 1997). This is unlikely to be the cause of the lower values of proportional contribution of fat to energy use seen here. Some of the variability in the proportional contribution of fat to energy use may be due to individual differences in the percentage of weaning protein reserves utilised in replacing lost pelage, which may in turn relate to the timing of the moult with respect to weaning. The single value of 17.8% of weaning protein mass used in hair regeneration (Noren et al., 2003) is likely to be a particularly high estimate

for grey seals, which, unlike northern elephant seals, do not lose the underlying epidermis as well as the lanugo during moulting, and thus do not need to regenerate skin as well as hair. If a similar correction for protein incorporated into new hair growth was applied to the data from a previous study on fuel use in grey seal pups (Reilly, 1991), which used otherwise apparently identical methods to those used here, the proportional contribution of fat to energy use in those seals would be much greater than 94%. The discrepancy in protein use and proportional contribution of fat to energy use between our study and this previous work (Reilly, 1991) cannot be accounted for by methodology and the reasons for the difference between studies remains unclear.

Although healthy pups have substantial fat reserves, fat cannot be completely depleted because it is vital for both metabolic fuel and insulation at sea (Worthy and Lavigne, 1987). There is a remarkable degree of consistency in relative fat content between weaning and departure in the grey seal pups studied here, similar to findings in other phocid seal pups (Biuw, 2003; Carlini et al., 2001; Muelbert and Bowen, 1993; Nordoy and Blix, 1985; Noren et al., 2003; Rea and Costa, 1992). The mechanisms underlying this consistency in relative fat content, despite considerable mass loss, are likely to be consequences of a stochastic metabolic process observed in most animals in which fat is used and mobilised relative to its availability. However, these processes must require some degree of regulation, perhaps by hormones involved in fuel use in other animals (Mercer and Speakman, 2001).

The size of fuel reserves at weaning, the rate at which those reserves are used and the amount of fat and protein depletion that can be tolerated, place immediate constraints on how long grey seal pups can survive before they begin to feed independently. Variability in fuel use and allocation can have a substantial impact on the ability to fast for extended periods (Biuw, 2003; Caloin, 2004; Cherel et al., 1992; Dulloo and Jacquet, 1999; Dunn et al., 1982; Goodman et al., 1980). The size of protein reserves and the rate at which they are used are often more limiting to survival than fat stores, especially in fat or obese animals (Caloin, 2004; Cherel, et al., 1992). Animals can starve to death while they possess substantial fat stores if body protein reaches levels at which tissue function and integrity is compromised (Cherel et al., 1992). This occurs when body protein stores are reduced by 30–50% in children (Garrow et al., 1965) and dogs (Garrow, 1959), irrespective of remaining fat depots. We predicted how long the pups in this study could survive after departure before their fat and protein reserves were depleted, assuming that death occurred when fat was depleted by 70–95% (Caloin, 2004), and protein mass was reduced by 30–50% of weaning values (Garrow, 1959; Garrow et al., 1965).

As in similar studies on southern elephant seal pups (Biuw, 2003; McConnell et al., 2002), it was assumed that pups did not begin to feed and continued to lose tissue components at the same rate at sea as they had on land. Intuitively one might anticipate that expenditure would be greater at sea because of elevated activity, in which case the pups would die sooner than estimated. However, empirical data from adult seals indicates that at sea metabolic rates may actually be lower than when they are on land (Arnould et al., 1996b). This is supported by captive studies in grey seals that have shown lower metabolic rates in

diving animals *versus* those resting at the surface (Sparling and Fedak, 2004), and would make the survival estimates conservative if this also applies to pups.

Similar to other studies on seals, we found that animals are predicted to die from protein depletion earlier than from fat depletion (77.6±59.2 *vs* 80.8±70.2 days after weaning), even assuming animals can tolerate as much as 50% protein loss and only 70% fat loss. Our predictions thus suggest that protein stores, rather than fat reserves, limit survival time in fasting grey seal pups. From these predictions we estimated that wild pups may be able to fast for an average of 6 weeks before protein is depleted. This contrasts with earlier studies on captive pups that suggested wild pups may be able to fast for only 3–4 weeks in total (Nordoy and Blix, 1985). If grey seal pups could only withstand a 30% reduction in protein, similar to that tolerated by terrestrial animals (Cherel et al., 1992; Garrow, 1959; Garrow et al., 1965), 25% of the animals in the current study would have starved to death before they left the colony. However, none of the pups died while fasting on land and several of those that had approached or reached what would normally be considered critical protein levels while ashore survived for several months at sea (SMRU, unpublished). This suggests that grey seal pups, like southern elephant seal pups (Biuw, 2003), have a greater tolerance to protein depletion than reported in terrestrial mammals. If pups can tolerate a 60% reduction in their protein reserves that can be mobilised for metabolism, they have an average of 36 days in which to find food before their protein reserves become critically depleted. For grey seal pups in the North Sea, this should be adequate time in which to locate food and learn to forage, given their travel rates and dispersion on leaving the colony (K.A.B., unpublished) and their proximity to feeding grounds, such as the Marr Bank and Wee Bankie. These predictions suggest that higher rates of protein use while fasting could compromise the ability of the pups to survive at sea by reducing the time available to find food before the onset of terminal starvation, especially during years when prey is scarce or hard to catch.

This study suggests a possible mechanism through which female grey seals that invest more in their offspring enhance their pups' first year survival probability. Pups that were given extra food spared protein reserves at the expense of fat stores and had more protein available to them. Pups that receive more milk from their mother may thus have a greater time margin in which to find food after leaving the natal colony before they deplete their protein reserves. The observed increased chance of first year survival in pups that wean heavier and fatter (Hall et al., 2001; Hall et al., 2002) may be due to greater protein stores and increased protein sparing capacity, rather than greater fat reserves, *per se*. Seal milk and herring have similar protein contents (~12% *vs* ~15.5%, respectively), and the amounts of protein received by the pups in this study were similar to or slightly lower than those that they may have received from extra provisioning over the same period by their mother. However, there are clear differences between feeding on high fat liquid milk and lower fat solid fish, despite similar relative protein contents of these two foods, and pups may assimilate and utilise them in radically different ways. The effects of extra maternal provisioning on body composition and fasting fuel use need further attention.

In summary, there is considerable variation in daily energy use and the contribution of fat to energy use in fasting grey seal pups, but this is not a function of percentage body fat. Feeding immediately after weaning seems to stimulate energy use, but the causes and possible consequences for wild pups are unclear. While this study leaves some important questions unanswered, it serves to highlight directions for further work. Additional studies are needed to investigate both the effect of initial adiposity on the proportional contribution of fat to energy use, and the effects of body fuel dynamics during fasting on first year survival of grey seals. Supplements of both protein and fat prior to fasting, separately and in combination, would help to clarify the regulatory mechanisms and information flow underlying decisions about energy partitioning in fasting pups. More studies like this are required if we are to integrate ecological and physiological studies of resource allocation, understand the role of environmental variability in shaping maternal strategies and life histories, and create models that allow us to link resource availability to population trajectories.

List of abbreviations

Control	group of pups handled with a comparable frequency to Fed pups
DEU	daily energy use
% DEU fat	percentage of daily energy use met by fat metabolism
Fed	pups that received supplementary food in the first 5 days after weaning
HIF	heat increment of feeding
LME	linear mixed-effects model
Low	group of pups handled only three times during the fast and not included in direct comparisons with Fed pups
SMRU	Sea Mammal Research Unit

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