Blood metabolites as indicators of nutrient utilization in fasting, lactating phocid seals: does depletion of nutrient reserves terminate lactation?

J.E. Mellish and S.J. Iverson

Abstract: Metabolites of lipid (free fatty acids (FFA) and β-hydroxybutyrate (βHBA)) and protein (blood urea nitrogen (BUN)) oxidation were measured during lactation in 18 female grey seals (*Halichoerus grypus*) and 6 female hooded seals (*Cystophora cristata*) as indicators of nutrient depletion and possible cues for pup weaning. FFA levels were high during lactation in both grey seals (51.2 ± 2.3 mg·dL^{-1}) and hooded seals (67.0 ± 8.1 mg·dL^{-1}), and levels were primarily related to the rapid lipid mobilization required for their high respective milk-fat outputs ($P = 0.002$). βHBA concentrations were negligible throughout lactation in both species (0.30 ± 0.14 and 0.03 ± 0.01 mg·dL^{-1}, respectively). Grey seals exhibited a decrease in BUN levels over the course of lactation (i.e., days 0–15, 39.3 ± 1.8 – 23.5 ± 3.3 mg·dL^{-1}, $P < 0.001$), which suggests protein sparing despite the added energetic cost of milk production over the 16-d lactation period. In contrast, hooded seals showed higher levels and no change in BUN levels (i.e., days 0–3, 43.2 ± 2.1 – 45.8 ± 2.1 mg·dL^{-1}, $P > 0.3$), suggesting that there is less need to spare protein in a species which lactates for only 3.6 d. Females of both species weaned their pups before entering stage III fasting, therefore metabolite levels do not appear to be a physiological cue for weaning.

Résumé : Les métabolites de l'oxydation des lipides (acides gras libres (FFA) et β-hydroxybutirate (βHBA)) et des protéines (azote uréique du sang (BUN)) ont été mesurés au cours de la période d'allaitement chez 18 femelles du Phoque gris (*Halichoerus grypus*) et 6 femelles du Phoque à capuchon (*Cystophora cristata*) en tant qu'indicateurs de l'épuisement des réserves de nutriments et déclencheurs possibles du sevrage des petits. Les concentrations de FFA sont élevées au cours de l'allaitement chez le Phoque gris (51,2 ± 2,3 mg·dL^{-1}) et le Phoque à capuchon (67,0 ± 8,1 mg·dL^{-1}) et elles sont reliées surtout à la mobilisation rapide des lipides nécessaires à une abondante production de lait ($P = 0{,}002$). La concentration de βHBA est négligeable chez les deux espèces au cours de toute la période d'allaitement (0,30 ± 0,14 et 0,03 ± 0,01 mg·dL^{-1}, respectivement). Les Phoques gris subissent une diminution de leur BUN durant l'allaitement (i.e., 39,3 ± 1,8 – 23,5 ± 3,3 mg·dL^{-1} du jour 0 au jour 15; $P < 0{,}001$) ce qui indique une économie de protéines malgré les coûts énergétiques additionnels de la production de lait au cours des 16 jours de l'allaitement. En revanche, chez le Phoque à capuchon, les concentrations de BUN, plus élevées, ne changent pas (i.e., 43,2 ± 2,1 – 45,8 ± 2,1 mg·dL^{-1} du jour 0 au jour 3; $P > 0{,}3$), probablement parce que l'économie des protéines est moins nécessaire chez une espèce dont l'allaitement ne dure que 3,6 jours. Les femelles des deux espèces sèvrent leurs petits avant d'entreprendre la phase III de leur jeûne et donc, la concentration des métabolites ne semble pas être le facteur physiologique déclencheur du sevrage.

[Traduit par la Rédaction]

Introduction

Fasting is characterized by three physiological phases. In phase I, the limited hepatic glycogen reserves are depleted, since glucose is the obligatory fuel for the central nervous system (CNS). Phase II rapidly follows phase I, with increased mobilization of stored fat (triacylglycerols (TG)) and subsequent increases in circulating free fatty acids (FFA). FFA are oxidized to fuel most tissues, while glycerol derived from TG is used for gluconeogenesis to fuel the CNS. Protein is partially spared, as indicated by low levels of blood urea nitrogen (BUN) (Castellini and Rea 1992), although protein degradation does provide limited amounts of amino acids for gluconeogenesis (Lehninger et al. 1993). Eventually, oxaloacetate is depleted as a result of gluconeogenesis, which prevents the complete oxidation of fatty acids. These factors lead to the production of ketone bodies (acetoacetate, βHBA, and acetone) by the liver, which can then be used by the CNS as an alternative energy source (Lehninger et al. 1993). The production and export of ketones facilitates the further breakdown of fatty acids (Nordøy and Blix 1991; Lehninger et al. 1993), but when production exceeds tissue capacity, ketone accumulation reduces the blood pH (Balasse and Fery 1989). This condition, which is known as acidosis or ketosis, is a predictable consequence of

Received March 9, 2000. Accepted November 6, 2000. Published on the NRC Research Press Web site on February 9, 2001.

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 DOI: 10.1139/cjz-79-2-303

fasting in most species and leads to coma and death if untreated (Castellini and Rea 1992; Lehninger et al. 1993). In phase III (terminal starvation), lipid utilization and ketone-body production fall, while protein degradation (and hence BUN level) increases, and this is followed by multiple organ failure and death (Castellini and Rea 1992).

Some species, such as penguins, bears, large cetaceans, and pinnipeds, are capable of sustaining repeated periods of nonterminal fasting that can last for several weeks to several months by prolonging phase II (Nelson 1980; Derocher et al. 1990; Costa 1991; Adams and Costa 1993; Cherel et al. 1993; Atkinson and Ramsay 1995). In these species, fat is relied upon as the primary energy source, BUN levels remain low, indicating protein sparing for extended periods, and ketosis does not occur. Thus, it has been suggested that metabolic shifts associated with entry into phase III (decreased FFA and βHBA and increased BUN levels) may induce termination of the fast, although this has only been documented in penguins that were prevented from ending their fast naturally (Groscolas 1986; Robin et al. 1998). Most previous studies on fasting blood chemistry in phocid seals have focused on pups during their postweaning fast (Pernia et al. 1980; Costa and Ortiz 1982; Castellini et al. 1987; Castellini and Costa 1990; Nordøy and Blix 1991; Nordøy et al. 1992, 1993; Adams and Costa 1993). These and other studies have demonstrated that phase III (starvation) is not typically seen in naturally fasted animals. This is not unexpected, as a fasting animal should terminate its fast with enough reserves left to allow an appropriate margin for replenishment. However, temporal changes in the blood chemistry of fasting lactating phocids have not been examined, although some species incur extreme energetic losses during lactation.

In some large phocid seals, such as the grey seal (*Halichoerus grypus*) and hooded seal (*Cystophora cristata*), adult females fast completely during lactation. Females are separated from their marine food supply, as they give birth and nurse their young on land or ice. Therefore, they depend solely on body energy accumulated prior to the reproductive season. This energy is stored primarily in the form of subcutaneous blubber (up to 40% of body mass; Fedak and Anderson 1982; Costa et al. 1986; Mellish et al. 1999*a*, 1999*b*). Like other fasting-adapted species, female phocids appear to be capable of extending phase II through heavy reliance on lipid oxidation (90–92% of total energy loss and 98% of energy oxidation; Mellish et al. 1999*a*, 1999*b*). But unlike some other fasting-adapted species that conserve energy through reduced metabolic rates and (or) hibernation (Nelson 1980; LeNinan et al. 1988; Cherel et al. 1993; Atkinson and Ramsay 1995; Farley and Robbins 1995), lactating female phocids are active and have greatly increased total daily energy expenditures associated with both maintenance metabolism and the export of milk energy (Costa et al. 1986; Mellish et al. 2000).

On Sable Island, female grey seals lactate for about 16 d and lose 35% of their initial mass by the end of lactation, which represents, on average, 68 and 16% of their initial fat and protein reserves, respectively (Mellish et al. 1999*b*). It is thought that the loss of 90 and 30% of initial fat and protein reserves, respectively, represents a critical threshold at which some fasting-adapted animals initiate foraging behaviour (Cherel et al. 1988; Castellini and Rea 1992). However, some female grey seals appear to fast beyond these levels (Mellish et al. 1999*b*). Thus, it is unclear whether similar limits apply to lactating grey seals and (or) play a role in determining the length of the lactation period in general. In contrast, female hooded seals in the Gulf of St. Lawrence produce more than twice as much milk per day as grey seals, but lactate for only 3.6 d (Bowen et al. 1985). Hooded seals lose only 16% of their initial body mass by weaning (27 and 7% of body-fat and protein stores, respectively; Mellish et al. 1999*a*). Weaning is abrupt in both species, but the specific cue for weaning is unclear and may be related to the physiological consequences of prolonged fasting and rapid depletion of body energy reserves. However, with the exception of limited data obtained on northern elephant seals (*Mirounga angustirostris*; Castellini and Costa 1990; Williams 1995), the metabolites of tissue catabolism have not been examined in fasting, lactating phocids, particularly with concurrent measures of tissue loss and milk energy output.

The objectives of our study were threefold: (1) to characterize the temporal patterns of fasting metabolites (i.e., circulating FFA, βHBA, and BUN levels) in lactating grey seals and hooded seals over the course of lactation and to relate these to concurrent measures of tissue loss and nutrient output; (2) to explore the possibility that changes in fasting blood metabolites may be a physiological cue to terminate lactation (particularly in the longer fasting grey seal); and (3) to examine differences in fasting blood chemistry between the two species, which expend energy at an enormous rate but for considerably different periods of time, resulting in large differences in total body nutrient depletion.

Materials and methods

Field procedures

Eighteen grey seal females (body mass 196.9 ± 6.02 kg (mean ± SE), range 138.0–234.0 kg) were studied during the reproductive seasons of January 1996 (n = 6) and 1997 (n = 12) on Sable Island, Nova Scotia, Canada (43°55′N, 60°00′W). Females were captured on the day of parturition (day 0), as indicated by the presence of a freshly expelled placenta and the thin, wet appearance of the pup. A blood sample (10 mL) for metabolite analysis was taken via the extradural vein with a sterile 3.5-in. 18-gauge spinal needle immediately after female had been captured in a net. Females were weighed to the nearest 0.5 kg using a 300-kg Salter scale suspended from an aluminum tripod. Females and their pups were held in a large fenced enclosure (100 m^2) containing other mother–pup pairs and resident males at a density typical of the main breeding colony, to provide easy access throughout the study period. Females were reweighed and an additional blood sample was taken on days 5, 10, and 15 of the approximately 16-d lactation period. Pups were considered weaned on the day the female departed or when the female was consistently separated from her pup by >30 m.

Six hooded seal females (body mass 235.3 ± 10.55 kg, range 203.0–270.0 kg) were studied on the pack ice in the Gulf of St. Lawrence, Canada (46°34′–46°42′N, 61°51′–63°09′W) in March 1997. Females with newborns (day 0) were identified from a helicopter by the presence of a placenta and fresh blood on the ice. A blood sample (10 mL) was taken immediately after capture and females were weighed as described above. As the pack ice drifted

Table 1. Components of maternal mass loss and milk-nutrient output during lactation in grey seals (*Halichoerus grypus*) and hooded seals (*Cystophora cristata*).

	Grey seals				Hooded seals
	Early lactation (n = 18)	Mid-lactation (n = 17)	Late lactation (n = 16)	Overall (n = 16)	Overall (n = 6)
Fat loss ($kg \cdot d^{-1}$)	2.5±0.2	2.5±0.2	2.6±0.1	2.6±0.2	5.1±0.9
Milk-fat output ($kg \cdot d^{-1}$)	1.0±0.1	1.5±0.1	1.7±0.1	1.4±0.1	3.8±0.5
Protein loss ($kg \cdot d^{-1}$)	0.3±0.1	0.3±0.0	0.3±0.1	0.3±0.1	0.8±0.3
Milk-protein output ($kg \cdot d^{-1}$)	0.2±0.0	0.3±0.0	0.3±0.0	0.3±0.0	0.4±0.1

Note: All data are from Mellish et al. (1999*a*, 1999*b*) for the individuals used in this study (see Methods). For grey seals, early, mid, and late lactation refer to days 0–5, 5–10, and 10–15, respectively. Lactation could not be split into stages for female hooded seals because of the use of two sampling points. Milk output in hooded seals was estimated on the basis of pup growth rates (Oftedal et al. 1993; Mellish et al. 1999*a*). Values are given as the mean ± SE.

Table 2. Circulating FFA, βHBA, and BUN levels ($mg \cdot dL^{-1}$) during lactation in fasting female grey seals.

	Day 0 (n = 18)	Day 5 (n = 18)	Day 10 (n = 17)	Day 15 (n = 16)	P
FFA	35.1±2.8	47.2±4.3	63.1±4.9	58.6±5.2	<0.001
βHBA	0.19±0.07	0.10±0.03	0.27±0.11	0.65±0.47	ns
BUN	39.3±1.8	37.3±2.8	33.5±3.2	23.5±3.3	<0.001

Note: Values are given as the mean ± SE; ns, not significant. Differences in metabolites over time were tested by repeated-measures ANOVA.

more or less continuously, VHF radio transmitters and green fluorescent dye were placed on the ice pan with each pair to facilitate relocation. Females were again sampled and weighed on day 3 of the 3.6-d lactation period. We were not able to determine exact weaning dates for these mother–pup pairs, owing to field constraints.

Blood samples were kept on ice in a cooler until return to the field laboratory (<4 h), after which samples were centrifuged at 2000 rpm for 20 min. Serum aliquots were stored at –20°C until analysis (1–4 months later). All animal-handling procedures were approved by the Dalhousie University Animal Care Committee and the Canada Department of Fisheries and Oceans.

Sample analysis

FFA were analyzed in duplicate using a WAKO NEFA-C enzymatic kit (WAKO Chemicals U.S.A., Inc., Baltimore, Md.). βHBA was measured using Sigma Technical kit No. 310-UV (Sigma-Aldrich Canada Ltd., Oakville, Ont.) modified for phocid seals by Castellini and Costa (1990). BUN was analyzed using Sigma Technical Kit No. 535 (Sigma-Aldrich Canada Ltd.). We compared patterns of fasting metabolites with data on body nutrient loss and milk output obtained concurrently during the course of other studies using isotope-dilution methodology and milk-composition analysis (Mellish et al. 1999*a*, 1999*b*). These data are summarized in Table 1.

Data for all females were used in all analyses, with the exception of one female grey seal that ceased lactation after day 5 and was therefore not included in day 10, day 15, and overall average analyses. Another female grey seal was extremely small in body mass (134.0 kg) and terminated lactation on day 12, but was held until day 15 sampling. Data for this female were not included in day 15 averages, but the average of her data for days 0–10 was used for overall averages, as this period constituted approximately 83% of her lactation period. All females were sampled on schedule, except for one grey seal resampled on day 6 and one hooded seal resampled on day 2 because of severe winter storms. Levels of all three metabolites are expressed, as analyzed, in milligrams per decilitre (mean ± SE) unless otherwise stated. Grey seal data were also divided into early (days 0–5), mid (days 5–10), and late (days 10–15) lactation. Hooded seal data could not be similarly separated into stages, owing to the limitations of two sampling points.

Most statistical analyses were performed using StatView 4.1 for Macintosh. Changes in grey seal metabolites over the course of lactation were analyzed using repeated-measures ANOVA. Changes in hooded seal metabolites over the course of lactation and relationships between variables were analyzed using Wilcoxon's signed-rank test and Spearman's rank correlation to account for departure from normality, given our small sample size. Relationships between serially correlated variables (i.e., FFA level and daily fat loss and milk-fat output and BUN level and daily protein loss and milk-protein output tested over early, mid, and late lactation) were tested using mixed-effects repeated-measures regressions in S-Plus 4.0 for Windows. Differences in metabolite levels between species were tested using unpaired t tests. Because all female grey seals were observed on a daily basis, behavioural observations relevant to the study (e.g., abnormal suckling behaviour, estrous, and pup weaning) are included as additional information.

Results

Grey seals

Circulating FFA levels in grey seals increased over the course of lactation ($P < 0.001$; Table 2). FFA levels declined between days 10 and 15 (>15 $mg \cdot dL^{-1}$) in four females, which may have been associated with the termination of lactation; three of these females weaned their pups on day 15 and the fourth weaned her pup within 48 h of day 15 sampling. Another female who weaned her pup on day 12 exhibited a drop in FFA level between days 5 and 10. There was no relationship between overall average FFA levels and length of the lactation period ($P > 0.5$).

We compared FFA levels with concurrent rates of body-fat loss and milk-fat output obtained using isotope-dilution methodology (Table 1), since circulating FFA are a result of fat mobilization and are largely used for milk-fat production.

Fig. 1. Relationship between serum FFA level and daily fat loss (*a*) and daily milk-fat output (*b*) ($y = 17.956 + 24.802x$) throughout lactation (early, mid, and late) in grey seals (*Halichoerus grypus*). FFA values represent the average for each period for each female. Data were tested using mixed-effects repeated-measures regressions.

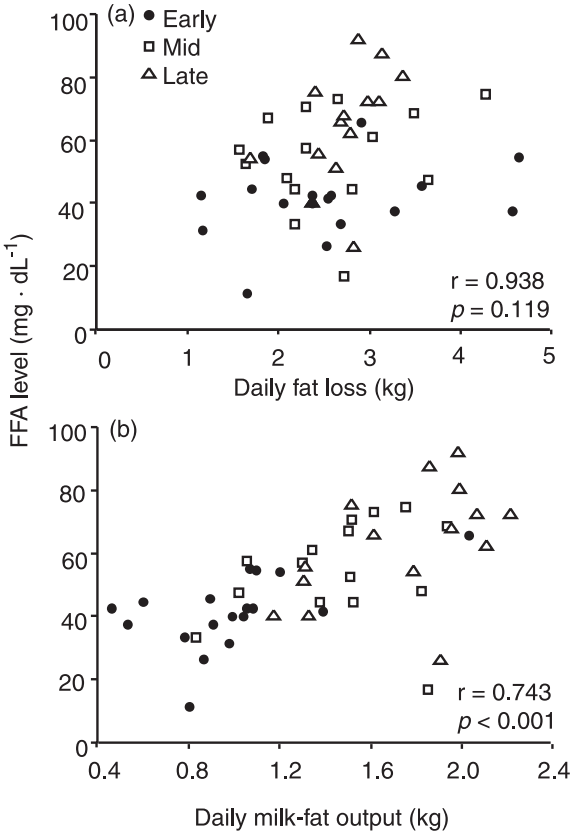
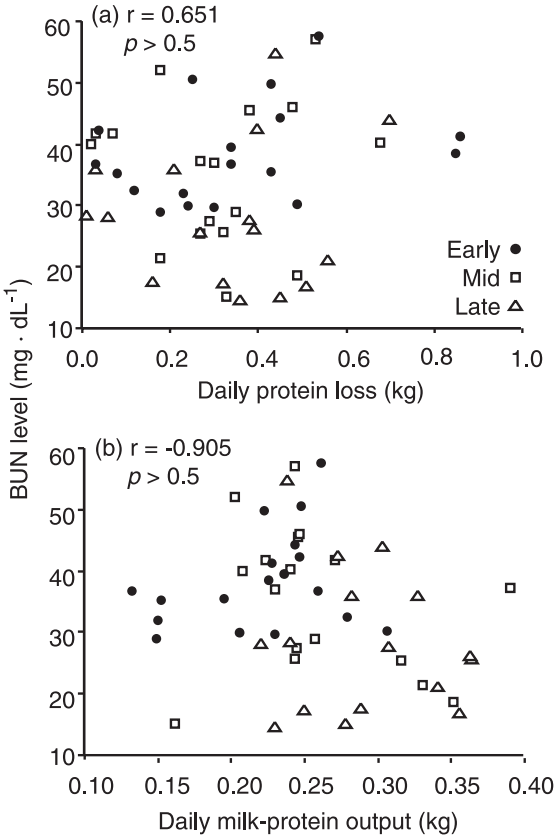


Fig. 2. Relationship between serum BUN levels and daily protein loss (*a*) and daily milk-protein output (*b*) throughout lactation (early, mid, and late) in grey seals. BUN levels represent the average for each period for each female. Data were tested using mixed-effects repeated-measures regressions.



There was no significant correlation between FFA and daily fat loss across the three stages of lactation (early, mid, and late; Fig. 1*a*). There was also no correlation between FFA levels at day 15 and the relative amount of fat lost ($P = 0.256$). However, FFA levels were strongly correlated with rates of milk-fat output, both among females and within and across stages of lactation (Fig. 1*b*).

Circulating βHBA levels in grey seals were low throughout lactation (averaging 0.30 mg·dL^{-1}; Table 2), although there was some fluctuation among individuals. Two females exhibited exceptionally high βHBA levels on day 15 (8.02 and 1.46 mg·dL^{-1}) and weaned their pups shortly thereafter. However, one of these females had high βHBA levels throughout lactation (0.94–1.56 mg·dL^{-1}). βHBA levels were unrelated to either concurrent FFA levels (i.e., days 0, 5, 10, and 15; $r = 0.091$, $P = 0.486$, $n = 15$) or rates of fat loss (i.e., early, mid, and late lactation; $r = 0.050$, $P = 0.747$, $n = 15$). There was no relationship between the total amount of fat lost or the relative amount of fat lost and circulating βHBA levels at day 15 ($r^2 = 0.001$, $P > 0.5$, and $r^2 = 0.089$, $P = 0.262$, respectively, $n = 17$). The length of the lactation period was also unrelated to average circulating βHBA levels ($P > 0.5$).

BUN levels in grey seals decreased over the course of lactation ($P < 0.001$; Table 2), but did not change uniformly in all females. Four females exhibited slight increases in BUN levels on day 15; two of these weaned their pups on day 16, while the other two did not wean their pups until day 18. However, in general, relative increases or decreases in BUN levels among females did not appear to relate to weaning date. The length of the lactation period also was not significantly related to average BUN levels ($P = 0.404$).

Protein lost by lactating grey seals represents a combination of protein that is oxidized for maternal metabolism and protein that is mobilized and exported (but not oxidized) during milk-protein synthesis and secretion. Of total protein loss in females, little (<13%) is due to oxidation (Mellish et al. 1999*b*). Nevertheless, overall protein loss may represent a proxy for relative oxidation rates. There was no correlation between BUN levels and daily protein loss (Fig. 2*a*) or between BUN and daily milk-protein output (Fig. 2*b*). There was also no correlation between BUN levels and the ratio of protein loss to milk-protein output ($r = 0.194$, $P = 0.173$). There was, however, a positive relationship between BUN levels on day 15 and both the total amount of protein lost ($P = 0.022$; Fig. 3) and the percentage of protein lost at that point ($r^2 = 0.358$, $P = 0.014$). Nevertheless, all females appeared to maintain relatively consistent BUN levels throughout lactation, as even the females that were smallest at parturition (138.0–148.0 kg) maintained BUN levels (10.2–

Fig. 3. Relationship between circulating BUN levels in female grey seals on day 15 of lactation and total amount of body protein lost.

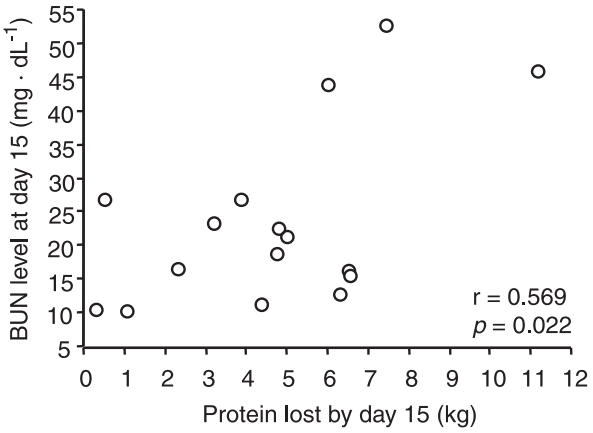


Table 3. Circulating FFA, βHBA and BUN levels (mg·dL^{-1}) during lactation in fasting female hooded seals.

	Day 0 (n = 6)	Day 3 (n = 6)	P
FFA	55.5±7.0	78.6±10.7	0.05
βHBA	0.03±0.01	0.04±0.02	ns
BUN	43.2±2.1	45.8±2.1	ns

Note: Values are given as the mean ± SE; ns, not significant. Differences in metabolites over time were tested by Wilcoxon's signed-rank test.

51.2 mg·dL^{-1}) similar to or lower than those of the females that were largest (224–234.0 kg, 12.9–48.1 mg·dL^{-1}).

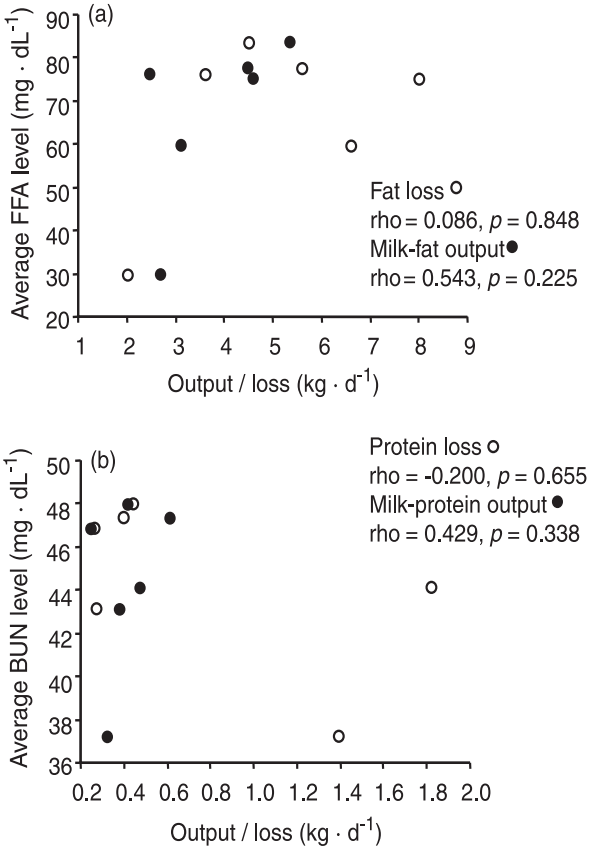
Hooded seals

Circulating FFA levels in hooded seals were elevated near parturition and increased significantly by day 3 (P = 0.034; Table 3). As in female grey seals, circulating FFA levels were not significantly related to daily fat-loss rates (Fig. 4*a*). In contrast to grey seals, there was also no significant relationship between estimated daily milk-fat output and FFA levels (Fig. 4*a*). However, one female had a particularly low day 0 FFA level (31.5 mg·dL^{-1}), which declined further by day 3 (28.1 mg·dL^{-1}). This female gave birth to an exceptionally small pup (50% of normal birth mass) and had very low rates of fat loss and estimates of milk-fat output (Mellish et al. 1999*a*).

Circulating βHBA levels were negligible in female hooded seals throughout lactation (Table 3). βHBA levels were unrelated to concurrent measures of circulating FFA (i.e., days 0 and 3; rho = –0.305, P = 0.311) or daily fat loss (i.e., average βHBA level; rho = 0.086, P = 0.848).

BUN levels did not change over lactation in female hooded seals, but remained at approximately 43–46 mg·dL^{-1} (Table 3). There was considerable variation in daily rates of protein loss among females (0.3–1.8 kg·d^{-1}; Table 1), but this was not significantly associated with BUN levels (Fig. 4*b*). BUN levels were also not related to estimated daily milk-protein output (Fig. 4*b*) or to the ratio between

Fig. 4. (*a*) Relationship between average serum FFA level, daily fat loss, and daily milk-fat output in lactating hooded seals (*Cystophora cristata*). (*b*) Relationship between average serum BUN level, daily protein loss, and daily milk-protein output during lactation in hooded seals. FFA and BUN values represent the average for each female.

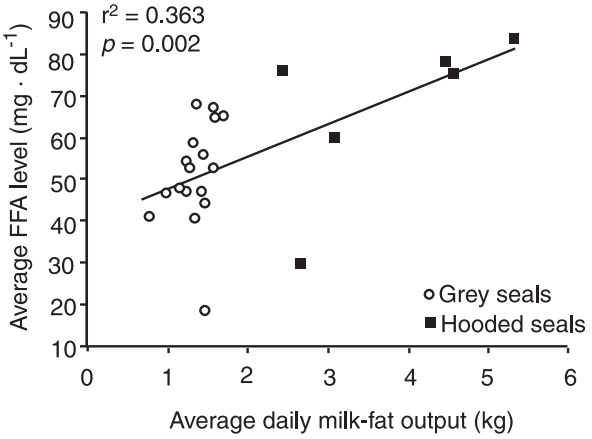


daily protein loss and milk-protein output (rho = –0.314, P = 0.482).

Species comparisons

FFA levels in female grey seals were lower than in female hooded seals at parturition (i.e., day 0; P = 0.003) and overall (P = 0.020), but there was no significant difference between species near weaning (i.e., days 15 and 3, respectively; P = 0.076). Although there were no consistent relationships between circulating FFA levels and rates of fat loss or milk-fat output within each species (Figs. 1 and 4), the large differences in fat loss and milk-fat output between species accounted for much of the species differences in circulating FFA levels (fat loss: r^2 = 0.332, P = 0.004; milk-fat output: Fig. 5). βHBA levels did not differ significantly between the two species at the beginning (P = 0.185) or the end of lactation (P = 0.452), or overall (P = 0.273). While BUN levels did not differ significantly between grey seals and hooded seals at parturition (P = 0.263), they were significantly lower in grey seals than in hooded seals near weaning (P < 0.001) and overall (P = 0.013). There was no significant relationship between average BUN levels and daily protein loss when the two species were combined (P = 0.262), nor was there a significant relationship between

Fig. 5. Relationship between average serum FFA level and average daily milk-fat output in lactating grey seals and hooded seals ($y = 37.126 + 5.67x$).



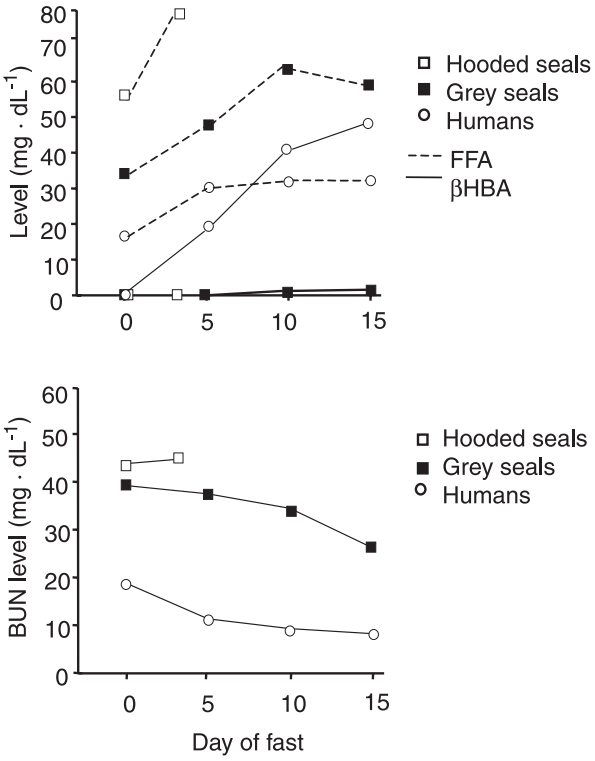
average BUN levels and rates of daily milk-protein output ($P = 0.120$).

Discussion

Metabolites of lipid oxidation

In a fasting or starving animal, there are predictable changes in blood chemistry associated with the catabolism of body fat. An increase in lipolysis for maintenance energy requirements results in elevated FFA levels as the fast progresses (Castellini and Rea 1992). This elevation has been demonstrated in king penguin (*Aptenodytes patagonica*) chicks (LeNinan et al. 1988), adult quails (*Coturnix coturnix*; Sartori et al. 1995), hedgehogs (*Erinaceus europaeus*; Cherel et al. 1995), hibernating black bears (*Ursus americanus*; Nelson et al. 1975), phocid pups (Castellini et al. 1987; Nordøy et al. 1993), and lactating female phocids (Williams 1995; Tables 1 and 2). However, FFA levels in fasting, lactating females reflect the need to mobilize stored lipid for both maintenance metabolic requirements and milk-fat production. Given the high rates of milk-fat output in grey seals and hooded seals (representing 52 and 82% of their body fat loss, respectively), it is not surprising that their FFA levels were proportional to rates of milk-fat output and twice those in fasted, nonlactating rats (3–17 mg·dL^{-1}; Goodman et al. 1980) and humans (28 mg·dL^{-1}; Cahill 1976; Fig. 6). Phocid seals also have a much greater blood volume per unit body mass than most other mammalian species (1.5–2.0 times; Riedman 1990), which suggests that these differences may represent even greater absolute levels of FFA mobilized. Perhaps surprisingly, FFA levels in lactating females of both species did not exceed the ranges reported for fasting northern elephant seal pups (65 mg·dL^{-1}; Castellini et al. 1987) and harp seal (*Phoca groenlandica*) pups (40–96 mg·dL^{-1}; Nordøy et al. 1993). However, given the greatly elevated levels of energy expenditure and export during lactation, FFA uptake by the mammary gland and other organs (e.g., liver, muscle) may be rapid enough to maintain lower overall concentrations in the blood. Our measurements of FFA for both lactating grey seals and hooded seals (35–79 mg·dL^{-1}) were within the range for lactating northern elephant seals (43–88 mg·dL^{-1}; Williams 1995). And from the current study, it

Fig. 6. Changes in serum FFA, βHBA, and BUN levels during prolonged fasting in humans (modified from Cahill 1976) and lactating grey seals and hooded seals (this study).



appears that higher rates of daily milk-fat output (and therefore greater rates of lipid mobilization) are associated with correspondingly higher FFA levels (Fig. 5).

Decreased FFA levels after prolonged fasting indicate reduced lipolysis and have been suggested as a potential signal of entry into phase III in fasting animals (Castellini and Rea 1992; Cherel et al. 1992). However, in fasting, lactating females, a drop in circulating FFA levels could signal an inability to continue fat-based metabolism and spare protein or could simply be a function of reduced milk output (and hence reduced fat mobilization) in advance of weaning. FFA levels fluctuated throughout lactation in female grey seals, but primarily as a function of the variation in milk-fat output (Fig. 1). This was also the case in the comparison between species (Fig. 5). In grey seals there was no clear effect of body-fat depletion on circulating FFA levels overall or at the end of lactation, even after fat losses of 60 kg or more. As stated previously, decreases in FFA levels prior to weaning were observed in only five females. Unfortunately, it is not possible to discern whether the drop in circulating FFA levels was due solely to the termination of maternal investment or could signal a shift in fasting phase. However, it would seem unlikely that a female would extend lactation to the critical stage where she has used all essential fat stores and would thus reduce her chances of survival or compromise future reproduction. Other cues may interact with the effects of body-nutrient depletion. For instance, while all five females that displayed a decline in FFA levels were among the smallest in terms of body size, concurrent hormone profiles for two of these females suggested that they had entered estrous (Mellish 1999), which is associated with mating and

leaving the colony. There was no accompanying evidence of elevation of BUN levels in these females, which might have signaled entry into phase III. Female hooded seals did not deplete nutrient stores severely during their brief lactation period, and maintained or increased FFA levels as lactation progressed.

During fasting, increases in FFA levels are typically accompanied by elevated βHBA levels, partially as a function of incomplete lipid oxidation as well as to serve as a glucose-sparing mechanism (Robinson and Williamson 1980; Krilowicz 1985). Elevation in βHBA levels during fasting has been observed in many fasting-adapted species, including penguins (5.4–24.9 mg·dL^{-1}; LeNinan et al. 1988; Robin et al. 1998), hedgehogs (5.3–17.5 mg·dL^{-1}; Cherel et al. 1995), black bears (0.2–1.3 mg·dL^{-1}; Nelson 1980), and phocid pups (0.6–32.5 mg·dL^{-1}; Castellini and Costa 1990; Nordøy and Blix 1991; Nordøy et al. 1993). However, these levels do not represent a state of ketosis. For instance, ketosis develops rapidly in fasting humans, with levels reaching 50 mg·dL^{-1} or more (Owen et al. 1969; Fig. 6). In contrast, little or no accumulation of βHBA is associated with prolonged fasting in domestic geese (LeMaho et al. 1981), dogs (de Bruijne and van den Brom 1986), or adult phocids (Tables 2 and 3; Castellini and Costa 1990; Williams 1995). The high availability and rapid turnover of blubber TG in fasting phocids may avoid the need for the increase in ketone production found in other fasting animals (Castellini and Costa 1990), as glycerol released from TG breakdown is likely directly shunted to gluconeogenesis, as in hibernating black bears (Ahlquist et al. 1984). Furthermore, βHBA may be an important energy source for the CNS, as has been suggested for fasting phocid pups (Nordøy and Blix 1991; Nordøy et al. 1993) and hibernating ground squirrels (genus *Spermatophilus*) (Hochachka and Guppy 1987). Thus, the rate of βHBA clearance may be sufficient to prevent accumulation in the bloodstream, as in fasting dogs (de Bruijne and van den Brom 1986).

At or near the onset of phase III starvation, βHBA levels have been shown to decrease concurrently with an abrupt decline in lipid oxidation and circulating FFA levels. Both fasting northern elephant seal pups and male emperor penguins, *Aptenodytes forsteri*, terminate their fasts and return to their feeding grounds immediately after such a drop in ketone levels (Castellini and Costa 1990; Castellini and Rea 1992; Robin et al. 1998). Female grey seals and hooded seals had considerably lower βHBA levels than were found in one study of lactating female northern elephant seals (0.31 mg·dL^{-1} in early lactation and 1.35 mg·dL^{-1} in late lactation; Williams 1995), but were well within the range reported in another study of female northern elephant seals at the end of lactation (6 weeks, 0–1.77 mg·dL^{-1}; Castellini and Costa 1990). Although two female grey seals had elevated βHBA levels at day 15, these values did not approach that of a ketotic human (see above). If the elevation and (or) decline in βHBA levels was acting as a cue in these females, it was neither exclusive nor consistent.

Products of protein degradation

The BUN level typically falls at the onset of food deprivation as the body attempts to spare protein, and accordingly, protein catabolism can remain low for prolonged periods in fasting-adapted species. However, in fasting, lactating phocids, lean body mass must be mobilized to provide material for producing milk proteins. Although female grey seals lose approximately 4.6 kg protein in 15 d, only about 0.6 kg of this appears to be oxidized (Mellish et al. 1999*b*). Thus, daily protein loss in lactating grey seals was approximately equivalent to estimates of daily milk-protein output (Table 1) and indicates a considerable ability to spare body protein over long periods. In contrast, lactating hooded seals appear to lose double the amount of protein required for milk-protein output (Table 1), but over a much shorter time. It has been suggested that in penguins, longer fasting species may be more efficient in protein sparing than those that fast for shorter periods (Cherel et al. 1993). Although we do not know the extent to which this may be true for other species, it is reasonable to assume that a female hooded seal, which loses only 7% of total body protein during lactation, would not need to be as effective at daily protein conservation as a female grey seal, which lactates for far longer and loses 16% of initial protein stores (Mellish et al. 1999*a*, 1999*b*). Consistent with this, circulating BUN levels were significantly higher in hooded seals than in grey seals. However, levels in both species were still within the range reported for other fasting-adapted species (12–39 mg·dL^{-1} for northern elephant seal pups (Costa and Ortiz 1982); 25–27 mg·dL^{-1} for lactating female northern elephant seals (Williams 1995); 17–48 mg·dL^{-1} for polar bears, *Ursus maritimus* (Derocher et al. 1990; Ramsay et al. 1991)).

The similarity between estimates of protein loss and milk-protein output in grey seals suggests that despite a net loss of lean body mass while fasting, lactating females may also undergo some protein synthesis. Indeed, subtraction of estimates of total milk-protein output from total protein loss produced a negative result in 41% of the females studied here. While error could have occurred in some of our isotope estimates, hibernating black bears also incur no net loss of lean body mass during extended periods of fasting, and in some cases even show an increase in lean body mass (Lundberg et al. 1976). This conservation of lean body mass is thought to be facilitated by efficient degradation of urea, the nitrogen recovered from which couples with glycerol to yield new amino acids (Nelson 1980). It has been speculated that this process occurs at a faster rate than urea production, as blood urea levels decreased during the hibernation period. BUN levels in female grey seals similarly fall during lactation (Table 2), while circulating glycerol levels remain constant (45.2 ± 2.6 mg·dL^{-1}; J.E. Mellish and S.J. Iverson, unpublished data). However, whether or not fasting lactating female phocids are truly capable of limited nitrogen recycling and (or) protein synthesis to offset losses due to milk-protein export and metabolic oxidation is not clear. Unlike products of lipid mobilization, BUN is not a product of protein mobilization and redistribution within the body (e.g., to the mammary gland for secretion in milk). Furthermore, it has been shown that in northern elephant seals, the glomerular filtration rate increases over the course of lactation, causing an increase in urea production (from increased protein turnover) to be matched by increases in urea excretion, with the result that the BUN level remains constant (Crocker et al. 1998). Therefore, comparisons of BUN levels with rates of protein

loss and (or) the ratio of protein loss to milk-protein output are likely to provide limited information. In addition, a considerable amount of urea may be secreted in some milks (e.g., 30–50% of acid-soluble nitrogen in human milk; Atkinson and Lönnerdal 1995), at levels that do not necessarily reflect those in the blood, and it may in fact be produced within the gland itself (Atkinson 1995; Atkinson and Lönnerdal 1995). Unfortunately, urea levels in phocid milks have not yet been measured. Thus, the BUN level alone is likely not a good indicator of protein oxidation.

In fasting-adapted species, protein catabolism typically remains at a low but constant rate throughout phase II regardless of the amount of lipid available (Castellini and Rea 1992). In penguins, rapid increases in BUN levels indicate a loss of homeostatic control and entry into phase III (Cherel et al. 1988, 1993). In emperor penguins the elevation in nitrogenous wastes in the bloodstream is accompanied by increased spontaneous locomotor activity and attempts to leave an artificial compound (Robin et al. 1998). In fasting grey seal pups, phase III is indicated by a fourfold increase in BUN levels (64–275 $mg \cdot dL^{-1}$; Nordøy et al. 1992). Despite the fact that four female grey seals exhibited a minimal increase in BUN levels at the end of the study (<4 $mg \cdot dL^{-1}$), and two of these weaned their pups within 24 h of final sampling, their BUN levels were low and likely were not a contributing factor in the termination of lactation. Owing to the constraints of our field study, only 12 of the 18 female grey seals were sampled within 48 h of weaning their pups. However, based on the present data and the similar lack of BUN accumulation in the longer fasting (26 d) northern elephant seal (Williams 1995; Crocker et al. 1998), it is unlikely that BUN accumulation is a primary factor determining lactation length in these species. The mechanisms by which fasting, lactating phocids prevent BUN accumulation are unclear. However, both decreased amino-acid turnover and decreased urine output may be involved, as has been found in fasting phocid seal pups (Pernia et al. 1980; Nordøy et al. 1992; Adams and Costa 1993) and black bears (Lundberg et al. 1976). Alternatively, it is possible that BUN levels do not increase in fasting, lactating phocids, owing to an increase in the glomerular filtration rate and urea excretion, as has been found in northern elephant seals (Crocker et al. 1998). However, there is also a concurrent increase in the rate of protein loss between mid and late lactation in northern elephant seals, which is not observed in grey seals (Table 1; Mellish et al. 1999*b*).

Despite the lack of significant metabolite accumulation in either grey seals or hooded seals, body-composition data show that tissue loss is considerable, at least in grey seals (Mellish et al. 1999*b*), and the magnitude may serve as a trigger for terminating lactation in some females. For instance, our lightest female grey seal began lactation at 138.0 kg body mass and with 31.2 kg of body fat. By the end of lactation she weighed only 90.0 kg, with an estimated 9.0 kg of fat remaining. It is unlikely that she would have been physically capable of maintaining milk secretion for any longer than we observed without compromising her ability to return to sea and feed again. Yet there was no evidence of a shift in her blood metabolite levels. In addition, females that weaned their pups within 48 h of our final sampling did so without any noticeable shift in blood chemistry. Clearly, female grey seals weaned their pups before entering stage III fasting as defined by the characteristics of blood metabolites. This indicates that females are capable of prolonging phase II while also coping with the energetic demands of lactation, and suggests that other factors, such as body energy depletion and (or) hormonal influences, are more likely to trigger weaning in these species.

Acknowledgments

We thank W.D. Bowen, M. Hammill, S. Lang, V. Lesage, J. McMillan, and R. Pelletier for their assistance in the field. We also thank G. Forbes, Officer-In-Charge of the Atmospheric Environmental Service, Environment Canada, for providing logistical support on Sable Island. We thank W.D. Bowen and H. Koopman for very helpful comments on an earlier version of the manuscript, and we especially thank D. Costa and M. Castellini for further improving the manuscript. This research was supported by Natural Sciences and Engineering Research Council operating and equipment grants to S.J.I., and by a Dalhousie Graduate Studies Fellowship and New Brunswick Women's Doctoral Scholarship to J.E.M. Additional support was provided by the Canada Department of Fisheries and Oceans.

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