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Fatty acid metabolism in fasting elephant seal pups

M.A. Castellini[1]*, D.P. Costa[2], and A.C. Huntley[2]

[1] Zoology Department, University of British Columbia, Vancouver, B.C., Canada V6T 2A9
[2] Long Marine Laboratory, Institute of Marine Science, University of California, Santa Cruz, California 95064, USA

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Summary. The turnover of two plasma free fatty acids (FFA) were measured in 5 northern elephant seal pups (*Mirounga angustirostris*) after approximately 2 months of post-weaning fasting. Turnover was determined using simultaneous bolus injections of ^{14}C-palmitate, ^{3}H-oleate and Evans blue (EB) administered via an indwelling extradural intravertebral catheter. At this time in their natural fast, the seals exhibited plasma FFA levels and turnover values higher than reported for other marine mammals and most terrestrial carnivores. There was no consistent difference between plasma FFA turnover measured by palmitate or oleate tracers. The results imply that FFA metabolism is the primary source of energy during fasting. This is interesting in light of previous observations of minimal ketoacid accumulation and low levels of glucose and protein energy production during fasting in these juvenile seals.

Introduction

Northern elephant seals undergo prolonged fasts throughout their life cycle (Costa et al. 1986; Le Boeuf et al. 1972; Reiter et al. 1978). Pups usually fast from food and water on shore for 2–3 months after weaning (Ortiz et al. 1978). Past studies have documented the changes in blood chemistry and water/nitrogen balance that occur throughout the post-weaning fast (Costa and Ortiz 1980, 1982; Pernia et al. 1980). While there appears to be an increase in circulating FFA levels during the fasting period, ketoacids do not accumulate (Acetoacetate 0.04 mM; β-hydroxybutyrate 0.05 mM; Costa, unpublished data). Past studies on glucose and protein metabolism in fasting elephant seal pups have shown that both of these two fuel sources combined provide at most 10% of the seals' resting energy requirements (Pernia et al. 1980; Keith 1984). While it is likely that fat is the primary source of metabolic energy, no previous studies have quantified its role during fasting or looked at differential FFA utilization. Keith (1984) provided a value for the half life of labelled palmitate in a single fasting elephant seal pup and concluded that turnover must be rapid. Ortiz et al. (1978) measured water turnover and body mass changes in these seals and suggested that fat metabolism must be the primary source of energy based on calculations of its water content and necessary utilization to maintain water balance. Based on these observations, quantitative estimates of FFA turnover become critical to further understand fasting metabolism in these seals.

* To whom offprint requests should be sent at the following address: Long Marine Laboratory, University of California, Santa Cruz, CA 95064, USA

Materials and methods

Weaned, fasting northern elephant seal pups ($n=5$; average weight = 97.6 kg; approximate age = 2.5–3.0 months) captured at Ano Nuevo, California during late April and early May, were transported to and maintained at Long Marine Laboratory, University of California, Santa Cruz. The seals were held in an outside enclosure for 2–3 days prior to treatment. Under light ketamine anaesthesia and sterile conditions, venous catheters were placed percutaneously into the extra-dural intravertebral vein of the seals (Davis 1983; Davis et al. 1983; Castellini et al. 1986) and extension catheters attached. Experiments were carried out only on animals that were awake and breathing normally. The seals undergo periods of long duration sleep apnea, during which time they do not appear to be in steady state (Castellini et al. 1986; Huntley 1984). Each seal was used only once for these studies and was kept at the laboratory for about 1 week.

Radioactive ^{14}C-palmitate was prepared for injection by evaporating its organic carrier (toluene), then redissolving in ethanol and complexing the FFA to bovine serum albumin

(Castellini et al. 1985; Wolfe 1984). Tritiated oleate was first evaporated to eliminate any ^3H_2O and then dissolved in ethanol. Evans Blue was prepared in a stock solution and autoclaved. An aliquot of ^{14}C-palmitate was mixed with the ^3H-oleate, the solution filtered through a sterile micro-pore filter (Nalgene 0.20 micron) and a known amount of sterile Evans Blue added. The total volume of the injection dose was usually about 3–4 ml. Two 10 µl samples were taken to assess the radioactivity of the dose. The bolus was administered and the catheter flushed several times. Blood samples were collected by first withdrawing 2 to 3 times the catheter volume and then known volumes at time intervals up to several hours. Samples were drawn into heparinized Vacutainers, the plasma was separated by centrifugation and then frozen until analysis. This procedure has been used before on seals both in the laboratory (Castellini et al. 1985) and in the field (Guppy et al. 1986). Plasma free fatty acid concentration was measured enzymatically on the thawed samples (Miles et al. 1983; Ramirez 1984; Shimizu et al. 1979). The radioactivity of the FFA was measured by extracting the plasma sample into an organic layer (2-methoxyethanol: butyl ether) by standard methods (Bergmann et al. 1980). A 100 µl aliquot of the organic layer containing the FFA fraction was counted in 8.0 ml of ACS-II (Amersham) using standard liquid scintillation methods. Turnover was calculated by plotting the FFA specific activity vs time, integrating the area under the curve to a point when only 5% of the dose remained, and dividing the dose administered by the area of the curve (Hetenyi et al. 1983; Wolfe 1984; Castellini et al. 1985). Some fraction of the injected label will move into other pools such as triglycerides or adipose tissue and therefore we are measuring a whole body disappearance rate of plasma FFA. The relative turnover and oxidation of these other pools will then impact upon our estimates of energy balance and this is covered in detail in the discussion. Evans Blue concentration was measured by clearing the plasma of any flocculent material in an Eppendorf microfuge (13000 RCF) and then measuring absorbance of the cleared plasma at 624 nm in a Pye-Unicam spectrophotometer. Plasma volume was calculated by comparing the measured absorbance in equilibrated samples to a standard Evans Blue (EB) dilution curve made up in plasma. Upon completion of the experiments, the catheters were removed and the seals returned to the rookery site at Ano Nuevo, Ca.

Results and discussion

Examples of typical dilution curves for the triple injection of labelled palmitate, oleate and EB are shown in Fig. 1. The patterns show the rapid disappearance of labelled FFA and the slow loss of the plasma dye EB. Since there was no significant difference between the mean rate of palmitate and oleate turnover, their rates could be averaged (11.0 µmol/min·kg; Table 1). Based on Evans Blue dilution, plasma volume was about 4.5% of body mass in the pups. We also examined an apparently healthy, molting and fasting adult female elephant seal at Ano Nuevo and found plasma FFA levels that were significantly less (0.347 m*M*) than those seen in the pups and a plasma volume of about 7% body mass.

The elevated levels of plasma FFA in the fasting seal pups (Table 1) are interesting for several

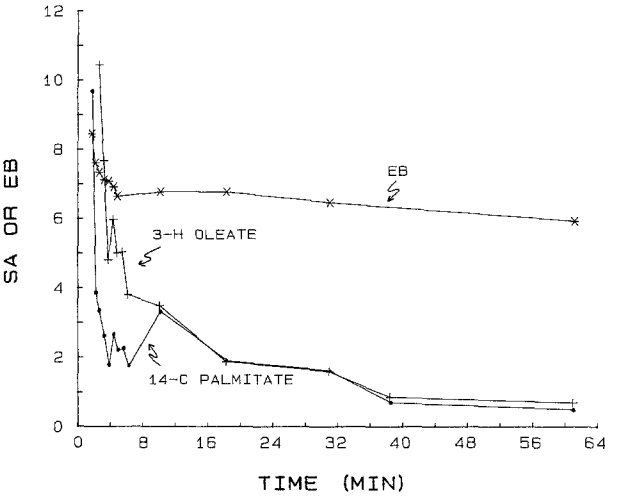

Fig. 1. Plasma specific activity (*SA*) decay curves of ^{14}C-palmitate and ^3H-oleate (DPM/µmol $\times 10^{-3}$), and Evans Blue (*EB*) equilibration curve (ABS $\times 10$) after a simultaneous triple label injection into the extradural vein of a resting elephant seal pup

reasons. As in many terrestrial mammals, FFA concentration increases during fasting (Cahill et al. 1966; Grande 1964; Nelson 1980; Steele et al. 1968). This suggests that there are increased rates of lipolysis to support decreased dependence on carbohydrate stores as the fast progresses (Steele et al. 1968). Data from newly weaned elephant seal pups suggest plasma FFA levels lower than those reported here (about 1.88 m*M*; Costa, unpublished data). The absolute levels of FFA in the plasma are the highest reported for any marine mammal (gray seal, 0.83 m*M*; Castellini et al. 1985; harbor seal, 1.75 m*M*; Davis 1983; Weddell seal, 1.18 m*M*; Guppy et al. 1986; harp seal, 1.21 m*M*; John et al. 1980) and are slightly lower than those reported for polar bears (2.73 m*M*; Fergusson and Folk 1971).

Given the evidence for a positive correlation between FFA concentration and turnover (Steele et al. 1968), the high FFA levels in fasting elephant seal pups imply a rapid turnover in the plasma and, in fact, the absolute levels of turnover are the highest reported for any marine mammal. Even if the results are corrected for mass specific metabolic rate (Table 1), the elephant seal pups still show significantly elevated turnover rates compared to Weddell seals (13 µmol/min·$kg^{0.75}$; Guppy et al. 1986), harbor seals (18.8; Davis et al. 1985) and gray seals (25.0; Castellini et al. 1985). In addition, no significant difference was found in estimates of total plasma FFA turnover using either palmitate or oleate as the tracer. This result is similar to previous findings in humans (Havel et al. 1964). Therefore, for at least these 2 major

Table 1. Body mass, plasma volume and plasma FFA values in elephant seal pups

Seal	Body mass (kg)	Plasma volume (l)	Plasma [FFA] (m*M*)	Palmitate turnover (μmol/min·kg)	Oleate turnover (μmol/min·kg)	Mean FFA turnover μmol/min·$kg^{0.75}$
1	102.5	3.72	2.119	9.1	11.6	32.8
2	102.5	4.75	1.82	9.1	7.2	25.7
3	102.9	5.64	2.322	14.6	16.1	48.7
4	93.1	3.69	3.133	7.4	6.7	21.7
5	87.1	4.17	2.028	14.0	14.3	43.1
Mean	97.6	4.39	2.284	10.8	11.2	34.4
±SD	7.2	0.82	0.507	3.2	4.2	11.4

fractions of the total FFA plasma pool, differential utilization does not appear to be significant in the fasting elephant seal pup.

As mentioned earlier, the role of fat as the primary energy source during fasting in seal pups was based on the relatively minor roles of other fuel sources (Ortiz et al. 1978; Pernia et al. 1980). Based on the values from the present study, we can now directly estimate the contribution of circulating FFA to daily energy metabolism.

The resting metabolic rate of fasting elephant seal pups is about 5.8 ml O_2/min·kg (Huntley 1984). Since protein and glucose turnover and oxidation together make up at most 10% of this rate (Pernia et al. 1980; Keith 1984), then roughly 5.2 ml O_2/min·kg must be accounted for by the oxidation of other fuel sources. Given the length of the fast (2–3 months), it is unlikely that internal glycogen stores would be large enough to support such a rate. In other mammals, glycogen depletes rapidly during starvation (Grande 1964). By elimination, this leaves only the mobilization of adipose tissue as the primary fuel source, a conclusion confirming previous work (Ortiz et al. 1978; Pernia et al. 1980; Costa and Ortiz 1982). It should be noted however, that seals primarily metabolize fat at all times, even when not fasting, and thus the transition to long term fasting may not necessarily involve major modifications in metabolic fuel recruitment. This is of some concern in determining the effect of fasting in these elephant seal pups. The pups initiate their long fast at weaning and data from Costa (pers. comm.) show a gradual increase in plasma NEFA from 1360 μ*M* at 1 week fasted to 2300 μ*M* at 2–3 months fasted. At the end of the fast the pups go to sea. Thus, it is not possible to measure FFA turnover on 'feeding' pups. To force feed the seals would be difficult and questionable in terms of the effects of such a treatment on the pups' lipid utilization pattern. The gradual increase in FFA concentration implies a gradual increase in FFA turnover but it must be emphasized that these seals usually derive most of their energy from fat and thus the fasting period in effect increases their lipid utilization rate from high to even higher.

Can the turnover of plasma non-esterified FFA (NEFA) provide enough energy to meet the resting metabolic requirements of the seal or must other lipid pools be utilized? This calculation involves knowing the average turnover of NEFA (11.0 μmol/min·kg from Table 1) and the amount of oxygen required to completely oxidize 1 μmol of FFA (for palmitate, 0.584 ml oxygen/μmol or 6.4 ml O_2 for the complete oxidation of 11 μmol). Therefore, to supply the lipid based oxygen utilization of 5.2 ml O_2/min·kg, almost 81% of the NEFA turned over must be oxidized, as shown:

Where RMR O_2 = (FFA O_2) × (% oxidized)
therefore fraction oxidized = RMR O_2/FFA O_2
This equals (5.2 ml O_2/min·kg)/(6.4 ml O_2/min·kg) = 0.81

While it is unlikely that such a high FFA oxidation fraction would be routinely utilized, values in this range have been found in exercising seals (84%; Davis et al. 1985) and humans (73%; Havel et al. 1964).

It is reasonable to assume that lipid fuel sources are better modeled using a complex pool including NEFA, VLDL triglyceride bound fatty acids, adipose triglycerides, etc. Thus, the dependence on NEFA oxidation would go down the more these other pools became utilized. In dogs and rats for instance, it has been shown that NEFA and VLDL-FA are both utilized equally (Wolfe 1982; Wolfe and Durkot 1985). This would suggest that the NEFA fraction oxidized would only have to be about 40% in fasting seal pups. This is close to known values of 33% in resting harbor seals (Davis 1985). Unfortunately, no lipid pools other than NEFA have ever been examined for fuel re-

cruitment rates in marine mammals and therefore there is no way to estimate the balance between NEFA and other sources.

It is informative to calculate the impact of high FFA metabolism on ketone body utilization in the fasting seal. Repeated observations have shown that elephant seal pups do not appear to accumulate ketoacids in the blood during fasting (Costa, unpublished data). A similar lack of apparent ketosis is seen in the fasting hibernating bear (Nelson 1980). In the bear, it has been suggested that the glycerol byproduct of fat metabolism provides an adequate source of carbohydrate for CNS requirements via hepatic gluconeogenesis (Cahill, pers. comm.). Thus, fuel for CNS metabolism of the bear can be supplied without the production of ketoacids. In the fasting seal, a similar scenario is possible. If we again assume that turnover of plasma free fatty acids and VLDL triglyceride bound fatty acids are equivalent, then the seal would metabolize about 22 μmol of fatty acids/min·kg or 2156 μmol/min for a 98 kg animal. Such a rate would produce approximately 686 μmol of glycerol/min. If the liver converted merely 25% of this glycerol to glucose, which is not unreasonable given the high metabolic demand for gluconeogenesis during fasting, then hepatic tissue would provide about 86 μmol of glucose/min under these conditions for CNS metabolism. The measured in vitro metabolic rate for seal brain tissue is about 83 μmol glucose/min (Hochachka 1981). Thus, even minimal (25%) recycling of glycerol to glucose is sufficient to maintain cerebral metabolism in fasting elephant seal pups. The bulk of the remaining glycerol pool could then be cycled back into lipogenesis, a process thought to be important in preventing fasting ketosis in hibernating bears (Nelson 1980), or could simply be used by other glycolytic dependent tissues in the seal pups.

In summary, by late in their post-weaned fasting stage, northern elephant seal pups exhibit elevated plasma FFA concentrations and increased FFA turnover. Depending on the oxidation rate of other lipid pools, it is possible that less than 50% of the FFA turnover would have to be oxidized to maintain daily energy requirements. As has been suggested for hibernating bears, the hepatic conversion of the glycerol liberated by lipolysis to glucose units could easily provide the carbohydrate supply necessary for CNS metabolism thus bypassing the demand for ketogenesis. Unfortunately, the biochemical mechanisms involved in regulating the interaction of the FFA and ketogenic pathways have not yet been studied in these seals. Likewise, the role that VLDL triglyceride bound fatty acids play in energy metabolism is not known in marine mammals. However, the processes described here suggest that the fasting seal pup constitutes a marine equivalent to the fasting, hibernating bear. Given the availability and tractable nature of the elephant seal pups, this species should provide an important model for the study of fasting metabolism in large mammals.

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