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PROLONGED FASTING IN PINNIPEDS

A

THESIS

Presented to the Faculty

of the University of Alaska Fairbanks

in Partial Fulfillment of the Requirements

for the Degree of

DOCTOR OF PHILOSOPHY

By

Lorrie Darlene Rea, B.S., M.S.

Fairbanks, Alaska

December 1995

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PROLONGED FASTING IN PINNIPEDS

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ABSTRACT

Marine mammals are capable of fasting for extremely long periods at different stages of their life cycle. The first objective of this thesis was to determine how plasma chemistry changed during fasting in large free-ranging phocids, northern elephant seal pups. Next, elephant seals of very low (LWM) and very high weaning mass (HWM) were examined to address how weaning mass impacts fasting chemistry. In the third section, blood chemistry was utilized to study the transition from suckling to weaning in Weddell seal pups, because behavioral verification of weaning is difficult in this species. Lastly, blood chemistry and body morphology of Steller sea lion pups were examined for indications of possible nutritional deficiency that could be associated with apparent declines in juvenile survival of sea lions in Alaska.

In average mass (AWM) elephant seals, changes in blood urea nitrogen (BUN), non-esterified fatty acid (NEFA) and beta-hydroxybutyrate (β-HBA) concentrations provide strong evidence that the pups effectively minimize protein loss through increased reliance on lipid metabolism and ketone body production early in the fast. Elephant seals maintain this phase of protein sparing for up to 11 weeks.

Size of elephant seal pups at weaning influenced how stored fuels were utilized during the fast. LWM pups showed higher NEFA and β-HBA levels than average or HWM pups but showed no indication of increasing protein mobilization before they left the beach. HWM pups showed evidence that they may be able spare more protein

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than average pups.

Plasma metabolite levels and the accompanying rates of mass change suggest that Weddell seal pups typically fast after weaning. High β-HBA concentrations seen within 1 to 3 weeks of weaning are similar to levels seen during the first 3 weeks of fasting in other phocid species.

Blood chemistry and body morphology data collected from 168 Steller sea lion pups showed no indication that young pups from areas of population decline were nutritionally compromised. The clinical plasma chemistry profiles showed no indication of general poor health in any of the areas studied.

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TABLE OF CONTENTS

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Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

LIST OF FIGURES

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LIST OF TABLES

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LIST OF ABBREVIATIONS

AG	- axillary girth
ANOVA	- analysis of variance
AWM	- average weaning mass
BUN	- blood urea nitrogen
CBC	- complete blood count
CI	- condition index
DI	- density index
Hb	- hemoglobin
Hct	- hematocrit
β-HBA	- β-hydroxybutyrate
HWM	- high weaning mass
LWM	- low weaning mass
MCHC	- mean corpuscular hemoglobin content
MCV	- mean cell volume
NEFA	- non-esterified fatty acid
RBC	- red blood cell
SD	- standard deviation
SL	- standard length
SG	- specific gravity

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Chapter 2, *"Plasma metabolite concentrations indicate metabolic adaptation to prolonged fasting in free-ranging northern elephant seal pups"*, will be submitted as a manuscript co-authored with M. Castellini to Physiological Zoology. Chapter 3, *"Weaning mass determines how body reserves are utilized during prolonged fasting in northern elephant seal pups"*, will also be submitted with M. Castellini for publication in the same issue of Physiological Zoology. Research on northern elephant seals was supported in part by a grant from the National Institutes of Health to M. Castellini. I would like to thank the Pinniped Physiology Group at University of California, Santa Cruz for access to the elephant seal colonies at Año Nuevo State Reserve and for use of laboratory and holding facilities at Long Marine Lab. Special thanks go to my co-workers Sean Adams, Jeannine Williams, Ayaka Amaha and John Sanders for collaboration on the field portion of this project. I would also like to thank Jeannine Williams and Dan Crocker for providing blood samples collected from suckling pups during their own research endeavors.

Chapter 4, *"Body condition and plasma metabolites as indicators of nutritional independence in Weddell seals"*, will be submitted after revision to the Journal of Experimental Biology as a manuscript co-authored with M. Castellini. This study was part of a larger research program supported through a grant to J.W. Testa and M. Castellini from the National Science Foundation, Office of Polar Programs. This manuscript is a product of the field efforts of the entire Antarctic research team. Many

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Chapter 5, *"Health status of young Alaskan Steller sea lion pups as assessed by blood chemistry and body condition"*, will be submitted to Canadian Journal of Zoology as a manuscript co-authored by M. Castellini and B. Fadely. This manuscript includes body condition relationships developed by B. Fadely in his doctoral research. This research was sponsored by Alaska Sea Grant with funds from the National Oceanic and Atmospheric Administration, Office of Sea Grant, Department of Commerce and from the University of Alaska with funds appropriated by the state. I would also like to acknowledge the logistical support and cooperative efforts of T. Loughlin, R. Merrick and other researchers from the Steller sea lion research group, NMML, NMFS, NOAA, Seattle, WA. I would like to thank R. Davis (Texas A&M University, Galviston) and D. Calkins (Alaska Department of Fish and Game, Anchorage, AK) for providing blood samples for pups captured in Southeast Alaska.

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Chapter 1. Introduction

Prolonged fasting is part of the natural annual cycle in several pinniped species, and animals abstain completely from food and fresh water for several weeks to months. In most terrestrial mammals long-term fasting is considered disadvantageous, resulting in mass loss and vital organ damage, and is usually associated with low food availability or disease. However, in pinnipeds, fasting is routinely linked to breeding, rearing young, and molting. Over the last two decades a considerable body of literature has developed on the behavioral, ecological and physiological aspects of fasting in pinnipeds and other fasting-adapted species such as penguins and bears. The main focus in much of this work has been to determine how these animals differ from species that do not tolerate long-term fasts (such as humans and rats). This dissertation will address how body mass and plasma metabolite concentrations change during the post-weaning fast in large body mass, free-ranging pinnipeds. Metabolic chemistry can then be used as a diagnostic tool to infer nutritional status of animals where we cannot observe their feeding behavior such as diving seals.

FASTING ADAPTATIONS

During periods of food deprivation animals must rely upon the mobilization of body stores to maintain homeostasis and provide an adequate glucose energy supply to the central nervous system. Hepatic glycogen stores (the body's main carbohydrate reserve) are typically depleted within the first couple of days of fasting. At this time, the breakdown of proteins (ie., skeletal tissue and internal organs) provides amino acid

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precursors for the neogenic production of glucose. Gradually, the mobilization of lipid stores (ie. adipose tissue) increases, with accompanying increases in ketone body production (primarily beta-hydroxybutyrate (β-HBA) in seals) as a by-product of lipid metabolism. Reliance on protein catabolism decreases as a result, and animals enter a period of protein sparing. Both β-HBA and glucose derived from amino acids and glycerol are used by the central nervous system at this time. Lipid mobilization supplies fatty acids and glucose (through gluconeogenesis, from glycerol) to the rest of the body. If fasting continues, animals face degradation of critical protein stores (loss of muscle mass and vital organ tissue continues at a decreased rate) and problems of acid/base balance (metabolic acidosis or ketosis) due to high concentrations of acidic ketone bodies. In species that routinely fast, adaptations have evolved to minimize these processes (see review in Castellini and Rea 1992).

A predictable pattern of three phases of metabolic adaptation have been shown in fasting-adapted species such as bears and penguins. In the emperor penguin (Aptenodytes forsteri), rapid changes in metabolism occur during Phase I as the bird defends body glucose, prepares to mobilize fat and decreases protein utilization. In captive emperor penguins Phase I lasts 5 ± 1 d (Robin et al. 1988). This phase begins as soon as the birds leave the open ocean and head "inland" onto the frozen sea ice to their breeding areas. By the time they reach their breeding sites, they have entered Phase II fasting. This protein sparing stage is extremely long and continues until the male is either relieved from chick rearing by the female, or abandons the young, at about 120 days. During this phase, there is a marked reduction in protein utilization,

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which accounts for less than 5% of the energy supply with fat making up the remainder. Ketone bodies and non-esterified fatty acids (NEFA) increase and the bird loses weight in a constant and linear manner. There is also evidence of selective mobilization of stored fatty acids by the penguins during this period (Groscolas 1990). Adipose stores exhibit preferential decrease of eicosapentaenoic acid (20:5n-3) and vaccenic acid (18:1n-7) but retention of long-chain monounsaturated NEFA (with chain lengths of 20 to 24 carbons). This change in adipose tissue composition results from a highly selective release of particular NEFA rather than from selective oxidation or redeposition into fat stores. The composition of released NEFA is high in 20:5n-3, 20:4n-6 and 18:1n-7 but is low in long-chain monounsaturated fatty acids.

If emperor penguins are forced to fast beyond their natural departure time they enter into Phase III fasting. At about 120 days, protein utilization increases rapidly, probably because the lipid stores have reached a critically low level. At this point, emperor penguins have only 20% of its initial fat reserves left. However, given the evidence for selective utilization of NEFA, the key point may not be the absolute amount of lipid reserve, but rather the type of lipids that remain. Circulating levels of NEFA and ketone bodies decrease. Thus, the increase in protein utilization is necessary not only to provide total body energy, but also to provide amino acids for gluconeogenesis so that the central nervous system will have a fuel source as ketone body levels decrease. The significant increase in protein utilization drives up circulating blood uric acid, a waste product of protein catabolism. Fasting-adapted species appear to end their fast prior to entering Phase III, although captive penguins

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have been shown to successfully resume feeding after entrance into this phase.

FASTING SEALS

Long-term fasting is an integral part of the natural history of phocid seals. Breeding, pup rearing, and the annual molt all require extended periods of time on land, and seals undergo voluntary periods of prolonged fasting two times per year to participate in these activities. In polygynous breeding systems, in which the breeding males compete for and hold territories, males may remain fasting on the rookery for up to 90 days (Le Boeuf 1981). Also, phocid seals are characterized as having a short but intensive lactation period during which the female remains on land and fasts while nursing her pup. The pups are weaned abruptly and in many species the young also undergo an extended post-weaning fast before leaving to feed (Riedman 1990). In most cases there is a considerable energy expenditure associated with these activities (ie., active combat to defend harems, breeding, molting or nursing a pup) and adults may lose 36 to 42% of their original body mass during this time (Costa et al. 1986; Deutsch et al. 1990).

Newly weaned pups have been the major focus in studies of adaptation to prolonged fasting in seals. Animals of this age group are easily handled and provide a model of body reserve utilization. Developing pups are active throughout their post-weaning fast (in some cases making daily forays to the water), but there are no additional energy demands associated with breeding, molting or milk production like other age classes. The length of the post-weaning fast varies among species. Those species known to undergo the longest periods of fasting (grey, harp and northern

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elephant seals) have been the most intensively studied to date. In the wild, grey seals (Halichoerus grypus) fast for 3 to 4 weeks and harp seals (Phoca groenlandicus) fast for 6 weeks immediately after weaning (Reilly 1991, Worthy and Lavigne 1987). Northern elephant seal pups (Mirounga angustirostris) undergo a particularly long post-weaning fast of 9 to 12 weeks (Reiter et al. 1978).

During this post-weaning period a wide range of behavioral and metabolic adaptations enable fasting pups to conserve water, energy and critical body reserves (such as skeletal mass and vital organ tissue). For example, decreased urinary output (Adams and Costa 1993, Reilly 1991, Nordøy et al. 1993), utilization of counter-current exchange in the nasal turbinates (Huntley et al. 1984) and the development of sleep apnea (Blackwell and Le Boeuf 1993, Castellini et al. 1994) conserve body water during prolonged fasting.

METABOLIC RATE AND BODY MASS LOSS

The rate of mass loss decreases during the first 2 to 4 weeks of the post-weaning fast in grey seal and elephant seal pups, and then remains relatively stable and low throughout the rest of the fast. This decrease in rate of mass loss is primarily due to a decrease in total body metabolism. Metabolic rate decreases as fasting progresses in many species, both on a whole body basis, and also when corrected for decreasing body size and changing body composition. Northern elephant seal pups lost mass at a faster rate during the first month of the post-weaning fast (0.87 ± 0.06 $kg \cdot d^{-1}$) than during the second, 4 week period (0.51 ± 0.03 $kg \cdot d^{-1}$; Rea and Costa 1992). This decrease in mass loss was accompanied by a 20% decline in mass specific

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metabolic rate seen in elephant seal weaners over the 10 week fast (Rea and Costa 1992). Grey seals decreased the rate of mass loss by 50% after 2 weeks of fasting (from approximately 0.8 $kg \cdot d^{-1}$ to 0.4 $kg \cdot d^{-1}$) while metabolic rate decreased by 45% during the same period (Nordøy et al. 1990). Nordøy et al. (1993) showed a similar exponential decrease in body mass during 32 days of fasting in harp seal pups. Both grey and harp seal pups decreased body mass to 70% of initial weaning mass within the first month of fasting (Nordøy et al. 1990, Nordøy et al. 1993). In contrast, it took elephant seals 8 weeks of fasting to accomplish this 30% mass loss (Kretzmann et al. 1993). Thus, while seals can increase their fasting time by decreasing metabolic rate, but they still face the problem of protein wasting and vital organ damage.

PROTEIN SPARING

In penguins and bears, prolonged Phase II fasting with sparing of body fat and particularly body protein, is the key to enduring extended periods of fasting. During Phase II the proportion of protein reserves being utilized decreases, both on an absolute and relative mass loss basis. Harp seals exhibited decreased nitrogen loss in the urine after 2 - 3 days of fasting which may represent a shift from Phase I fasting to a protein sparing stage (Nordøy et al. 1993). Reilly (1991) also showed a decline in urinary nitrogen loss during 3 weeks of fasting in grey seal pups. Similarly, a 69% decline in urine nitrogen excretion was documented in elephant seals during the 10 week post-weaning fast (Adams and Costa 1993). Harp seals were shown to decrease the relative contribution of protein oxidation to overall energy expenditure from 9% at weaning to 2 - 4% after 30 days of fasting (Nordøy et al. 1993). Adams and Costa

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(1993) showed low relative rates of amino acid oxidation in northern elephant seals throughout the fast (< 4%). In that study, Phase I fasting may have been missed, since urine collections did not begin until 3 days post-weaning. However, the low rate of amino acid oxidation agrees closely with the 1.5 to 2.7% measured in fasting elephant seal pups by Pernia et al. (1980). Grey seals show similar low percent contributions during the fast (6%, Nordøy and Blix 1985; 6%, Worthy and Lavigne 1987; 4-8%, Nordøy et al. 1990; 6%, Reilly 1991). These relative contributions of protein oxidation to metabolism increase significantly when animals enter Phase III fasting. Emperor penguins increased their contribution of protein oxidation to total energy expenditure from 4 to 23% during Phase III, at 21 kg body size, and to 56% when body mass had decreased to 13 kg (Robin et al. 1988). Similarly, the contribution of amino acid oxidation to overall energy expenditure increased from 6% to 16% in grey seals after 52 days of fasting (Nordøy et al. 1992), suggesting entrance into Phase III.

LIPID MOBILIZATION

Protein sparing is greatly facilitated by increased lipid mobilization during fasting. Studies of fatty acid metabolism (Castellini et al. 1987) and glucose regulation (Kirby 1992; Kirby and Ortiz 1994) suggest a strong reliance upon lipid metabolism in both feeding and fasting elephant seals. Kirby and Ortiz (1994) suggest that a high fat milk diet preadapts phocids to fasting before they are weaned, due to decreased insulin secretion and decreased sensitivity, making these animals hyperglycemic, hyperlipidemic, and hypoinsulinemic compared to terrestrial mammals. Insulin concentrations decreased further during fasting (from 11 ± 4 $\mu U \cdot mL^{-1}$ at weaning to 8

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± 2 $\mu U \cdot mL^{-1}$ at 8 weeks in fasting elephant seals) allowing gradually increased rates of lipolysis during the post-weaning fast (Kirby and Ortiz 1994). Keith and Ortiz (1989) also showed that fasting northern elephant seal pups have higher rates of glucose recycling than seen in other mammals.

There is strong evidence that lipid mobilization increases during the post-weaning fast in both grey seals and harp seals, indicating a prolonged Phase II. Fatty acid concentrations increased 2-fold during the 50 days of fasting in grey seals, as ketone body levels increased from 0.12 ± 0.05 mM at 3 days to 2.20 ± 1.12 mM at 52 days (with a peak of 3.12 ± 1.21 mM seen at 37 days fasting; Nordøy and Blix 1991). Similarly, harp seals showed a 2.5-fold increase in NEFA levels over 30 days of fasting while β-HBA increased from 0.06 ± 0.02 mM at 1 day to 1.62 ± 0.44 mM at 30 days (Nordøy et al. 1993). In all of these studies of fasting seals, metabolite levels are used as an indicator of fuel mobilization. There are limited data on the rates of turnover of these individual metabolites, and no indication of whether these rates change during fasting, therefore absolute metabolite concentrations can only infer the relative changes in the use of these energy stores. Metabolic rate and body mass loss data suggest that grey seals undergo Phase I fasting for 1 to 14 days, and then pass into Phase II that may last for 14 to 48 days (Nordøy et al. 1990). Phase III fasting was implicated by increased contribution of protein oxidation after 45 days of fasting (Nordøy et al. 1992). In harp seals, a shift in urinary nitrogen loss represents a move from Phase I to Phase II at 2 to 3 days post-weaning (Nordøy et al. 1993). High BUN levels seen at 30 days of fasting may suggest entrance into Phase III, although pups

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naturally fast for up to 6 weeks in the wild. In elephant seal pups, increasing β-HBA concentrations during the post-weaning fast (from less than 0.3 mM at weaning to over 1.3 mM by 8 weeks fasting) suggest that this species also shows increased lipid mobilization with fasting (Castellini and Costa 1990). However, it is difficult to determine precisely how body fuel utilization changes in elephant seals without monitoring both lipids and byproducts of protein catabolism simultaneously.

SCOPE OF STUDY

The aim of this dissertation is to further our understanding of how pinnipeds are metabolically adapted to withstand bouts of prolonged fasting. Chapter 2, *"Plasma metabolite concentrations indicate metabolic adaptation to prolonged fasting in free-ranging northern elephant seal pups"*, investigates how plasma chemistry changes during 9 to 12 weeks of fasting in free-ranging elephant seal pups. This chapter shows how body resources are partitioned in comparison to smaller body size pinniped species that undergo much shorter post-weaning fasts like harp and grey seal pups. In particular, it examines whether free-ranging elephant seals leave the rookery prior to entering Phase III fasting, since captive fasting studies in other species have shown evidence of this metabolic shift after only 4 to 7 weeks.

Chapter 3, *"Weaning mass determines how body reserves are utilized during prolonged fasting in northern elephant seal pups"*, looks at how animals of different body size cope with a long-term fast. Here, the questions of whether initial body reserves influence fast duration or the way body stores are utilized are addressed. Kirby and Ortiz (1994) suggest that by the very nature of their high fat content (and

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high fat diet) phocid pups may be preadapted to fasting metabolism.

In Chapter 4, *"Body condition and plasma metabolites as indicators of nutritional independence in Weddell seal pups"*, the question of whether Weddell seal (Leptonychotes weddellii) pups undergo a post-weaning fast is addressed. These pups accompany their mothers into the water during the nursing period (at 1 to 2 weeks of age). However, it is unknown whether pups learn to forage during these forays, and are feeding on fish while still suckling, or whether they fast before hunting prey. In this study, the concentrations of plasma metabolites were examined during the nursing and post-weaning periods to identify blood chemistry shifts typical of fasting phocids.

In Chapter 5, *"Health status of young Alaskan Steller sea lion pups as assessed by blood chemistry and body condition"*, I propose that populations that are physiologically compromised can be identified by measuring body condition and metabolic chemistry. With declines of the Steller sea lion (Eumetopias jubatus) populations in Alaska come growing concern that pups are unhealthy or receive inadequate nutrition. In this chapter I utilized measures of metabolite levels (used in phocids to identify fasting biochemistry), hematology, and body condition to determine the health status of young sea lions.

Chapter 6, *"Prolonged fasting in young phocids; current knowledge and future research directions"* provides a synthesis of our current understanding of how phocid seals are adapted to prolonged fasting early in life. This chapter emphasizes the interspecific differences seen between body size and the duration of the post-weaning fast, and outlines the possible role of body composition in metabolic adaptation to

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fasting. This chapter summarizes plasma metabolite changes during the post-weaning fast in 4 phocid species and suggests avenues of future research.

Lastly, Chapter 7, *"Biochemistry of prolonged fasting and applications to assessing physiological state; Conclusion"* summarizes new findings on the biochemistry of prolonged fasting in northern elephant seal pups, and the influence of body mass on fasting adaptations. This chapter also evaluates the usefulness of blood chemistry changes in determining the ecophysiological state of other pinnipeds such as Weddell seal pups and suckling Steller sea lion pups.

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Chapter 2. Plasma metabolite concentrations indicate metabolic adaptation to prolonged fasting in free-ranging northern elephant seal pups.

INTRODUCTION

Prolonged fasting is an integral part of the natural history of the northern elephant seal (Mirounga angustirostris). Breeding, rearing pups and molting all require prolonged periods of time on land during which elephant seals have no access to food or fresh water. Adult males fast up to 90 days while defending breeding territories (Le Boeuf 1981), and adult females fast for 4 to 6 weeks while giving birth and nursing their pups (Reiter et al. 1978). Following a one month nursing period, weaned elephant seal pups remain on their natal beaches fasting for 9 to 12 weeks (Costa and Ortiz 1980). During this period a wide range of behavioral and metabolic adaptations enable fasting pups to conserve water and energy. Decreased urinary output (Adams and Costa 1993), utilization of counter-current exchange in the nasal turbinate (Huntley et al. 1984) and development of sleep apnea (Huntley et al. 1984; Castellini et al. 1994) help conserve body water. Metabolic rate decreases progressively during the post-weaning fast (Rea and Costa 1992) and sleep apnea increases metabolic savings (L. Rea unpubl. data).

Other species exhibit adaptations that allow for the progressive decrease of protein catabolism and sparing of critical muscle and organ tissues in exchange for the lipolysis of adipose stores during prolonged fasting (see review in Castellini and Rea (1992)). In grey and harp seals, metabolic rate decreases during the first 2 weeks of

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fasting, consistent with decreased rates of body mass loss (Worthy and Lavigne 1987; Nordøy et al. 1990; Reilly 1991). Plasma metabolite concentrations change to reflect decreasing reliance on protein reserves and increased utilization of lipid stores (Nordøy and Blix 1991; Nordøy et al. 1993). Evidence for these adaptations have also been shown in northern elephant seal pups by Adams and Costa (1993). They report a 60% reduction in urea production over the 10 week fast, which suggests protein sparing. Castellini et al. (1987) showed high NEFA concentrations and rapid rates of turnover in 5 elephant seal pups late in the post-weaning fast. They calculated that fasting pups oxidize as much as 81% of this turnover and could produce sufficient glucose to fuel the central nervous system if as little as 25% of the glycerol released from the high NEFA turnover were converted via gluconeogenesis. Lipid metabolism is particularly important for glucose regulation in a species such as the elephant seal which relies on food sources and energy stores with very low carbohydrate content (high fat milk, high fat fish, and adipose tissue stores during fasting (Kirby and Ortiz 1994)).

Northern elephant seal pups provide a unique opportunity to study metabolic adaptations to prolonged fasting under natural conditions. Weaned pups have high site fidelity to birth beaches, even when making daily forays into the water. Thus, individuals can be recaptured at frequent intervals while maintaining natural activity patterns and physical development. In this study, 19 free-ranging northern elephant seal pups were studied during the 2 to 3 month post-weaning fast to test the hypothesis that plasma metabolite concentrations change during prolonged fasting, in response to changes in fuel utilization.

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METHODS

Sample collection

Nineteen weaned elephant seal pups were studied during the 9 to 12 week post-weaning fast during three consecutive breeding seasons (1990 -1992) at Año Nuevo, California. In 1990, nine pups were studied through the entire post-weaning fast. In 1991, emphasis was placed on pups during the latter part of the fast, after 35 to 40 days of fasting (n=5). In 1992, studies included suckling pups and continued over the first 4 to 10 weeks of fasting (n=5). Only pups with weaning masses of 75 to 140 kg were included in this study since body mass at weaning, the length of the fast and levels of plasma metabolites were found to be significantly related (Chapter 3). When no mass data were available, pups were included if they were estimated to be within this weight range. With the exception of 2 pups housed at Long Marine Laboratory, University of California Santa Cruz (UCSC), in 1991, sampling was concluded when pups left the beach to begin foraging at sea.

Prospective study animals were distinctively marked during the suckling period with hair bleach (Lady Clairol®) and two numbered hind flipper tags (Rototag®). Thus an accurate age and weaning date were known for 17 pups. For 2 animals of unknown birth or weaning date, age was estimated based on condition of pelage and the degree of tooth eruption (Reiter et al. 1978). When accessible, weaned pups were captured weekly to monitor mass loss and changes in plasma metabolite concentrations. Pups were weighed using a hanging mechanical scale (±1 kg). While seals were manually restrained, a 20 mL blood sample was drawn into heparinized Vacutainer® collection

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tubes from the hind flipper plexus using an 18 or 20 gauge needle (Geraci 1971). Blood samples were held on ice until centrifuged (up to 4 hours later) and the plasma was removed and frozen. Samples collected on the mainland at Año Nuevo were processed at the Long Marine Lab and immediately stored at -20°C. Plasma samples collected during research excursions to Año Nuevo Island were frozen and stored (up to 10 days) in a liquid nitrogen cooled CryoPak shipper (Taylor-Wharton, -196°C). Plasma samples were transferred to a -20°C freezer at Long Marine Laboratory where they were stored for up to 1 month. Upon return to the laboratory at University of Alaska Fairbanks (UAF) samples were held in a -80°C freezer until analysis. Glucose concentrations of the plasma were measured using a YSI Model 2300 Stat glucose/L-lactate autoanalyzer. Methods for the determination of plasma concentrations of blood urea nitrogen (BUN), non-esterified fatty acids (NEFA) and β-hydroxybutyrate (β-HBA) were previously described by Castellini et al. (1993).

In addition, 20 elephant seal pups were studied at the beginning (week 1) and end (week 4) of the suckling period in 1991 and 1992 to establish levels of plasma metabolites seen in nursing animals. The majority of these animals (n=15) were not handled as weaned pups. However, 6 pups were studied in 1992 both at the end of the nursing period (4 weeks of age) and again within one week after weaning (5 weeks of age). These data illustrate changes in blood chemistry that occur during the transition from feeding to fasting. Pups were held for 2 to 5 hours and were successfully reunited with their mother within 6 hours. In some instances multiple blood samples were drawn within a single study period. Unless otherwise stated, metabolite values

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were obtained from blood samples taken within one hour of capture.

Five yearling elephant seals were also opportunistically sampled between May 4 and 9, 1991 at Año Nuevo. It is unknown how long these animals had been fasting, but none had yet begun to molt.

Mass calculations

Body mass was monitored in 11 of the 19 pups over the course of the post-weaning fast. The number of weights recorded for each pup ranged from 3 to 9 over the 4 to 12 weeks of study. Least squares linear regression analysis was used to estimate body mass at each capture and at weaning. Separate regression equations were produced for the first 4 to 6 weeks of fasting and the latter portion of the fast, where data were available, since rates of mass loss decrease significantly in this species after the first month of fasting (Rea and Costa 1992). R^2 values ranged from 0.978 to 0.999. The measured or estimated mass at each capture was then expressed as a percentage of calculated weaning mass to adjust for the wide range of individual body mass.

Statistical analyses

Paired t-tests were used to compare metabolite levels in suckling pups between weeks of capture, in pups studied during the transition period from suckling to fasting and in consecutive samples collected on the same day ($p \leq 0.05$; Statistix®). One-way analysis of variance (ANOVA) was used with Tukey's multiple range test to test differences among suckling pups, weaned pups and yearlings. Differences were considered significant if $p \leq 0.05$. All values were presented as mean ± standard

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deviation (SD). Inspection of this data set suggested that percent body mass and blood chemistry data were sufficiently independent over time that use of ANOVA was considered a valid substitute for a repeated measures test.

RESULTS

Suckling pups

Plasma metabolites

Mean concentrations of plasma metabolites measured in 20 pups during the suckling period are shown in Table 2.1. A paired t-test showed no significant difference in plasma levels of β-HBA and BUN, but plasma concentrations of NEFA and glucose decreased significantly from birth to the end of the suckling period (NEFA $p=0.0379$; glucose $p=0.0003$). There were no significant differences in plasma metabolite levels ($n=6$; BUN $p=0.8135$; NEFA $p=0.0629$ or β-HBA $p=0.0733$) seen in newly weaned pups compared to values determined during the last week of suckling for those individuals. When comparing consecutive samples collected on the same day, only BUN concentration was found to increase over the 2 to 4 hour interval between samples ($n=12$, $p=0.0332$).

Weaned pups

Mass loss

The mean mass of pups at weaning was 102.9 ± 15.1 kg and ranged from 85.4 to 127.0 kg. Over the first 4 to 6 weeks of fasting, mass loss averaged 0.7 ± 0.2 $kg \cdot d^{-1}$ ($n=9$; range 0.5 to 1.1 $kg \cdot d^{-1}$) which equated to a decline of 0.7 ± 0.2 $\% \cdot d^{-1}$ ($n=9$;

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range 0.4 to 1.0 %·d^{-1}; Figure 2.1). This resulted in a 21.0 ± 6.2% loss after 4 weeks of fasting. After 6 weeks, pup mass had declined to 73.8 ± 6.6% of the initial mass. The rate of mass loss decreased during the latter part of the post-weaning fast to 0.4 ±0.1 kg·d^{-1} (n=8; range 0.3 to 0.6 kg·d^{-1}). In pups weighed after 10 weeks of fasting, body mass had dropped to 62.6 ± 9.8% of calculated weaning mass.

Plasma metabolites

Plasma glucose levels ranged from 6.7 to 9.8 mM in fasting elephant seal pups. Mean glucose concentrations showed a slight but significant decrease during the first 8 weeks of fasting (p=0.0102; Figure 2.2). The circulating glucose levels in week 8 did not differ significantly from those seen during week 10 (p=0.2212). Five yearling seals sampled at the beginning of the molting fast had plasma glucose concentrations of 8.0 ± 0.3 mM, which was not significantly different from that of fasting pups.

Plasma concentrations of BUN decreased significantly over the first 4 weeks of the post-weaning fast (n=12, p=0.0001; Figure 2.3) and then remained relatively stable during the second month (n=12, p=0.0569). The average rate of decline measured in 12 individuals during the first 4 weeks was 0.6 ± 0.5 mM·wk^{-1}. A representative plasma profile (Figure 2.4) shows marked decline in BUN early in the fast with low stable concentrations maintained until the pups leave the beach. However, several other pups showed substantial fluctuations in BUN concentrations, particularly during the latter part of the fast. One pup sampled past 80 days of fasting (while held at Long Marine Lab) showed plasma BUN levels increase to 7.6 mM after sustaining concentrations of 3.3 to 5.4 mM for 5 weeks. Yearlings had BUN concentrations of

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7.9 ± 0.4 mM at the beginning of the fast (n=5).

Plasma concentrations of β-HBA gradually increased during the post-weaning fast (Figure 2.5). The rate of increase of β-HBA over the first 8 weeks of fasting was 0.34 ± 0.07 $mM \cdot wk^{-1}$. Smaller pups (75 to 100 kg at weaning) showed a faster rate of increase during the first 4 weeks (p=0.0465), but no differences were seen related to pup size during the latter post-weaning fast (p=0.2119). Figure 2.4 shows steady increases in β-HBA over the entire fast in one pup (#90-08), but individual patterns varied a great deal. Ketone bodies increased consistently in all animals during the first 4 weeks, but after this β-HBA patterns were variable among individuals both in the maximum concentrations reached (range 0.71 to 1.66 mM) and the timing and number of peak levels observed (range 31 to 73 days fasting at first peak). Ketone body concentrations measured in yearlings averaged 0.17 ± 0.06 mM (n=5).

The ratio of plasma HBA concentration to BUN concentration increased throughout the first 2 months of fasting (p=0.0002, Figure 2.6). This ratio declined significantly during week 10 (p=0.0402). The HBA:BUN ratio decreased further to 0.21 ± 0.08 (n=2) after 80 days of fasting. The same ratios ranged from 0.01 to 0.03 in yearlings (n=5).

Plasma NEFA concentrations increased significantly during the first 5 weeks of fasting (n=12, p=0.0031) but fluctuated widely during the rest of the fast (Figure 2.7). Thus, no significant differences were measured among pups sampled at week 4 and week 8. No pattern was discernable in the relationship of these fluctuations with the length of the fast or with plasma concentrations of other metabolites (Figure 2.4).

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Plasma concentrations of fatty acids ranged from 1.85 to 4.03 mM in fasting yearlings.

DISCUSSION

Changes in plasma metabolite levels measured over the suckling period were similar to those reported from the same study site in Kirby (1992), although absolute concentrations of NEFA were 2-fold higher and BUN levels were lower than previously measured. Kirby and Ortiz (1994) concluded that due to the high fat and low carbohydrate content of phocid milk, suckling pups do not develop the ability to secrete or to utilize insulin effectively. Pups are hyperglycemic and hyperlipidemic as compared with other mammals and thus are preadapted to long-term fasting.

Rates of mass loss observed during the first month of fasting in free-ranging elephant seal pups (0.7 ± 0.2 $kg \cdot d^{-1}$) were similar to those measured in captive grey seals (0.8 $kg \cdot d^{-1}$ decreasing to 0.4 $kg \cdot d^{-1}$ after 14 days (Nordøy et al. 1990) and an average of 0.44 $kg \cdot d^{-1}$ (Reilly 1991)). However, this rate of loss is a 1 to 2%$\cdot d^{-1}$ decline from weaning mass in grey seals compared with only 0.7%$\cdot d^{-1}$ loss in elephant seals, even though free-ranging elephant seals pups are presumably more active than captive grey seals held in cages. The decrease in rate of mass loss seen in both species (in grey seals after 14 days and in elephant seals after 4 weeks) is accomplished through a decrease in metabolic rate associated with fasting (Nordøy et al. 1990; Rea and Costa 1992).

Glucose concentrations remained above 6.5 mM throughout the 2 to 3 month fast suggesting that mobilization of body stores provided sufficient gluconeogenic

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precursors (ie. glucogenic amino acids and glycerol) and glucose substitutes (such as NEFA and β-HBA) to avoid depletion of circulating glucose levels. Grey and harp seal pups also maintain high plasma glucose concentrations through several weeks of fasting (Worthy and Lavigne 1982; Nordøy and Blix 1991; Nordøy et al. 1993).

Fasting elephant seals showed strong evidence of protein sparing throughout the post-weaning fast. During the first month of fasting, BUN concentrations decreased significantly followed by sustained low plasma levels until pups left to feed. Adams and Costa (1993) reported that protein catabolism contributed only 1.5% of the total energy needs in elephant seal pups at the end of the post-weaning fast versus 4% early in fasting. There was no indication of entrance into Phase III fasting (where ketone body and NEFA levels decline and reliance upon body protein stores increases substantially) in any pup free to leave the beach. The only pup to show increasing plasma BUN concentrations at the end of the fast was held at Long Marine Laboratory and unable to choose its time of departure. Interestingly, when BUN increased, this pup still maintained β-HBA concentrations of 1.1 mM and circulating NEFA close to 2 mM. There is strong evidence of entrance into Phase III in grey and harp seal pups that were captive during the post-weaning fast. Nordøy et al. (1992, 1993) report significant increases in BUN concentrations (over 50 mM) at the end of the captive studies.

Constant increases in β-HBA during the first month of fasting reflect an increased mobilization of lipid stores that facilitates protein sparing. However, even after 10 weeks of fasting with increasing β-HBA values, there was no evidence of

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ketosis (ie. excessive ketone body production leading to metabolic acidosis). Plasma concentrations of β-HBA always remained below 2 mM in elephant seal pups. A direct sparing effect on protein can be accomplished even with low ketone body levels. Infusion of β-HBA, so that the concentration goes from 0.5 to 2.0 mM, can decrease leucine oxidation in humans by 30% (Anonymous, 1989). Nordøy et al. (1993) reported maximum ketone body levels after 4 weeks of fasting in captive harp seal pups (1.6 ± 0.4 mM) that were within the range of those found for elephant seal pups in this study (0.7 to 1.7 mM between 4 and 10 weeks post-weaning). Nordøy and Blix (1991) showed a much higher range of maximum β-HBA levels (1.5 to 8.5 mM) in captive grey seals fasted 32 to 52 days. However, due to the high individual variability of β-HBA concentration seen after the first 4 to 6 weeks of fasting in all three species, it is difficult to judge if peak or maximum levels were overlooked between samples. Castellini and Costa (1990) reported a deflection point in β-HBA levels for 6 of 10 pups studied that occurred about 10 days before pups left the beach. In the present study, the degree of fluctuation of β-HBA concentrations (ie., the number of deflection points or peaks) varied considerably in pups as well as the maximum values reached. However, pups sampled by Castellini and Costa (1990) also showed a very narrow range of body size (Castellini, pers. comm.).

Non-esterified fatty acid concentration was the most variable of the metabolites measured, both among and within individuals studied. If NEFA's provide the major energy source to muscles later in the post-weaning fast, plasma concentrations would be expected to fluctuate with the activity level of the pup. Variability in the NEFA

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concentrations increased after 4 weeks post-weaning, at the time that pups begin to make daily forays into shallow water near the rookery. Mean plasma concentrations were similar to those seen in fasting grey and harp seals (Nordøy and Blix 1991; Nordøy et al. 1993) and in swimming harbor seals (Davis et al. 1993). Bailey et al. (1981) found fasting harp seal pups to have higher NEFA levels than in fed pups. Castellini et al. (1987) also found very high NEFA values and rapid turnover during the post-weaning fast in elephant seals.

A steady increase in the HBA:BUN ratio indicates a prolonged period of Phase II fasting during which pups showed steadily increasing ketone body levels with either stable or decreasing plasma BUN concentrations. This relationship would be expected to reverse when pups enter Phase III fasting due to increased BUN levels and depletion of lipid reserves resulting in decreased β-HBA concentrations. The gradual plateau of this relationship is another indication that elephant seal pups, when unrestrained, leave the natal beaches prior to the entrance into Phase III fasting.

Conclusions

Changes in plasma BUN, NEFA and β-HBA concentrations provide strong evidence that northern elephant seal pups effectively minimize protein loss through increased reliance on lipid metabolism and ketone body production early in the fast. Elephant seals maintain this phase of protein sparing (Phase II) for up to 11 weeks. This is in contrast to harp and grey seal pups which show evidence of Phase III after only 30 to 52 days of fasting in captivity. The ability of elephant seal pups to maintain Phase II longer than other phocid species may be related to differences in the initial

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energy reserves available (elephant seal pups are typically 2 to 3-fold larger than grey or harp seal pups at weaning) and to the low relative rate of mass loss seen in the larger elephant seal pups. Grey and harp seal pups decrease to 70% of the initial weaning mass within 4 weeks of fasting, but elephant seal pups do not reach this 70% level until after 6 to 8 weeks post-weaning.

Captive fasting studies have found harp and grey seal pups to enter Phase III fasting at the end of the fasting period, however, these studies typically held animals to the limit of their natural fasting durations or longer. Free-ranging elephant seal pups can be sampled at regular intervals during the post-weaning fast without altering the natural duration of the fast. The present study suggests that, when free to end the fast under natural conditions, elephant seals leave the beach prior to entering Phase III fasting.

Table 2.1. Mean plasma metabolite concentrations (± SD) during the suckling period. Similar letters (a or b) denote significant differences found between means ($p \leq 0.05$). * denotes a smaller sample size for glucose concentrations, n=6.

Week	n	BUN (mM)	NEFA (mM)	β-HBA (mM)	Glucose (mM)
1	20	8.25 ± 2.74	2.39 ± 1.11[a]	0.11 ± 0.05	8.9 ± 0.4[b,*]
4	20	7.82 ± 3.04	1.35 ± 0.79[a]	0.12 ± 0.04	7.3 ± 0.4[b,*]

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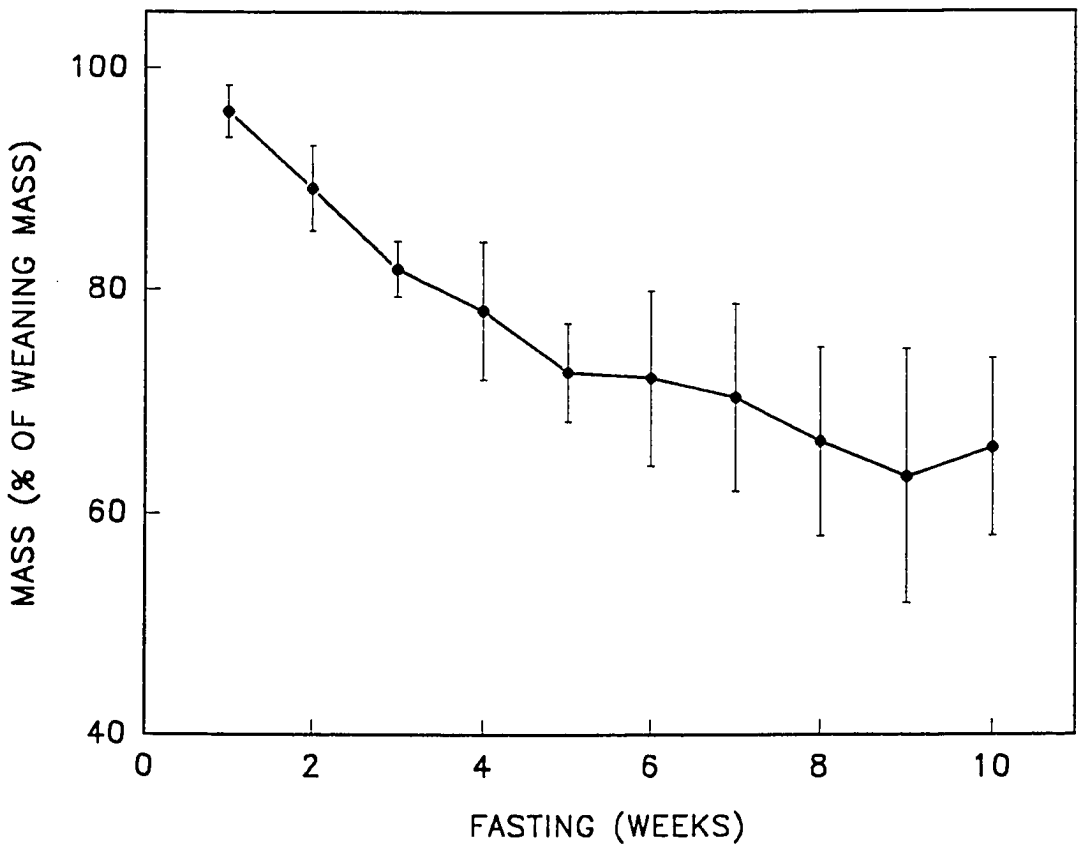

Figure 2.1. Relative body mass loss for 11 northern elephant seal pups during the post-weaning fast, represented as a percentage of calculated weaning mass. Error bars represent ± SD.

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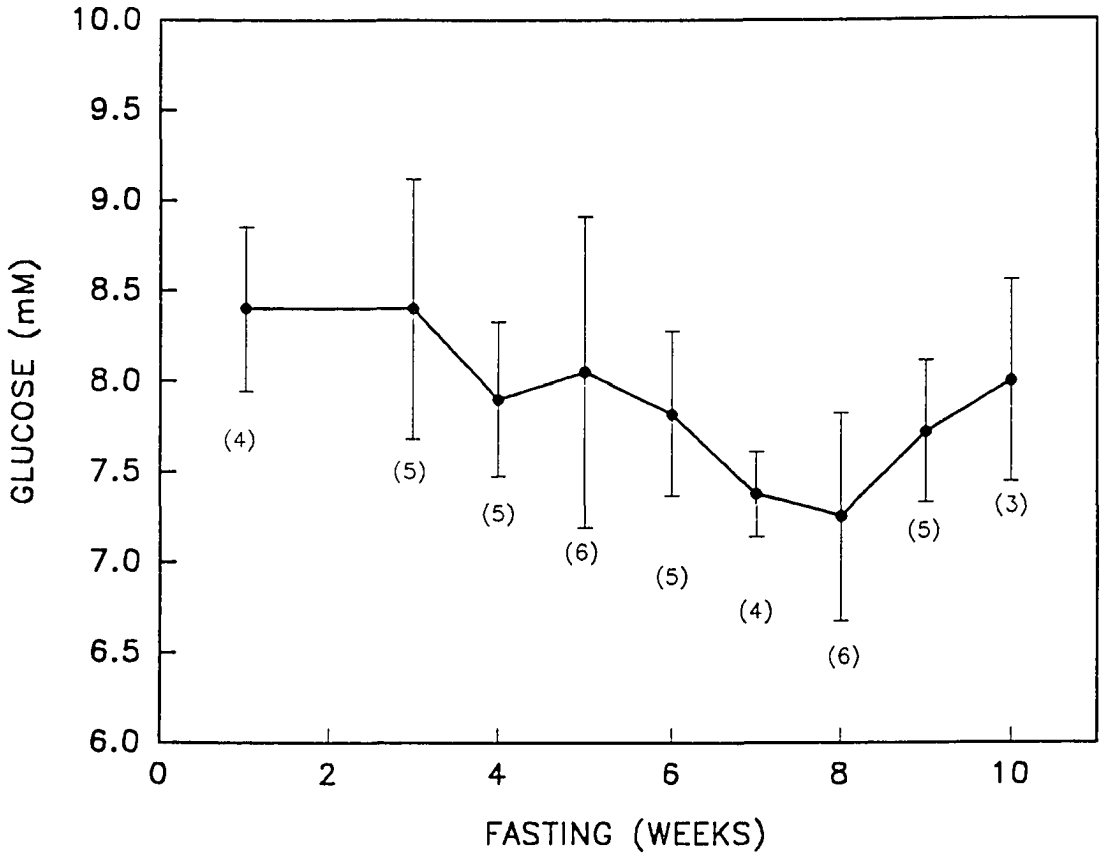

Figure 2.2. Mean plasma glucose concentrations for 11 northern elephant seal pups during the post-weaning fast. Error bars represent ± SD. Sample sizes are indicated in parentheses.

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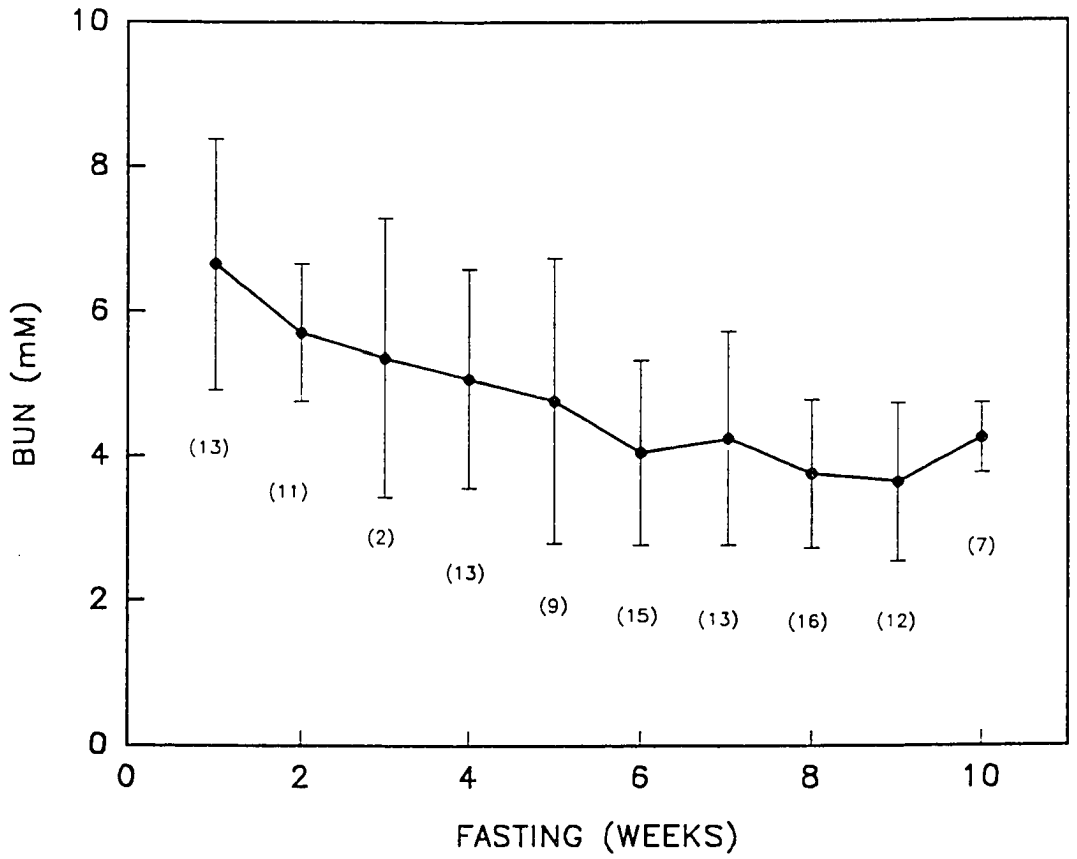

Figure 2.3. Mean plasma concentrations of blood urea nitrogen (BUN) for 19 northern elephant seal pups during the post-weaning fast. Error bars represent ± SD. Sample sizes are indicated in parentheses.

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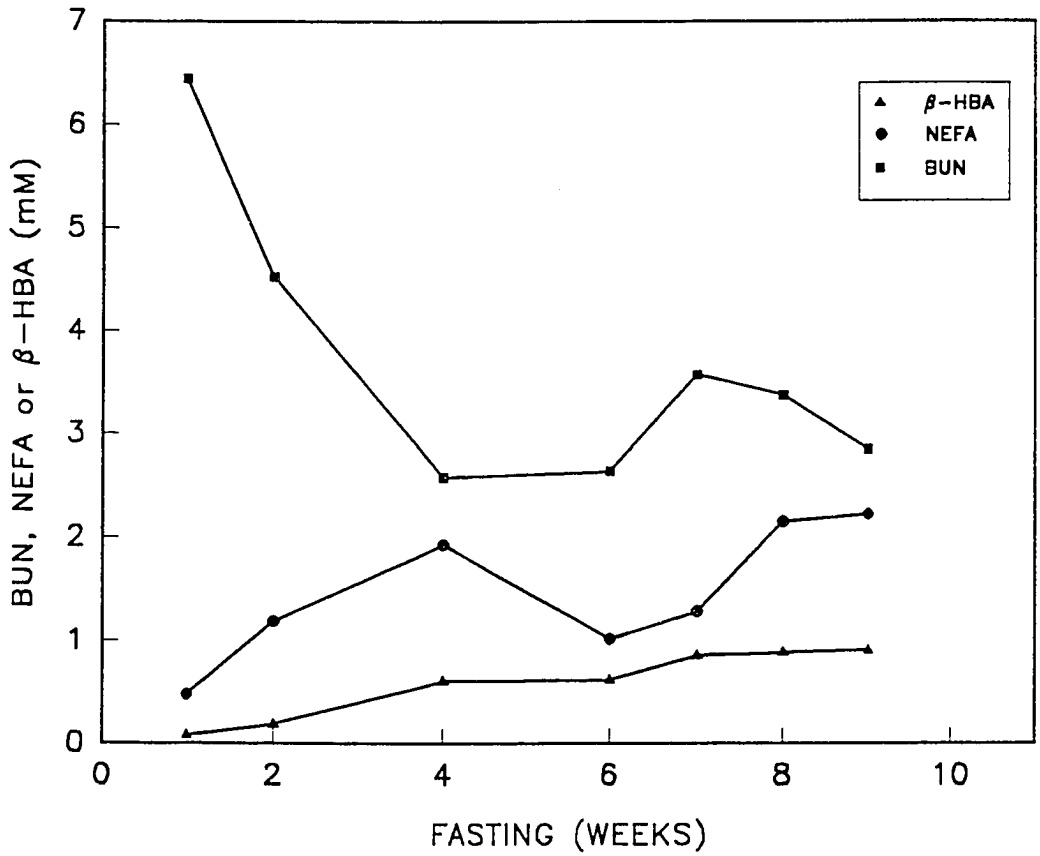

Figure 2.4. Pattern of metabolite changes in a representative elephant seal pup during the post-weaning fast.

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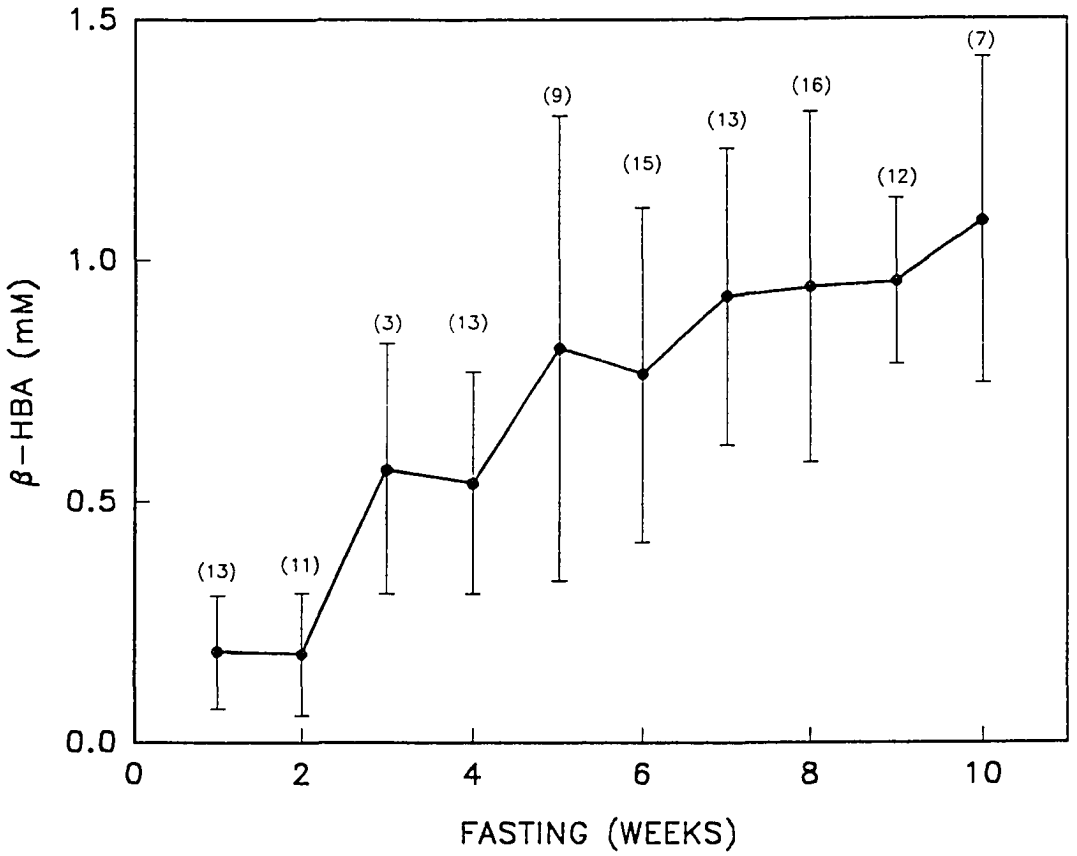

Figure 2.5. Mean plasma concentrations of β-hydroxybutyrate (β-HBA) for 19 northern elephant seal pups during the post-weaning fast. Error bars represent ± SD. Sample sizes are indicated in parentheses.

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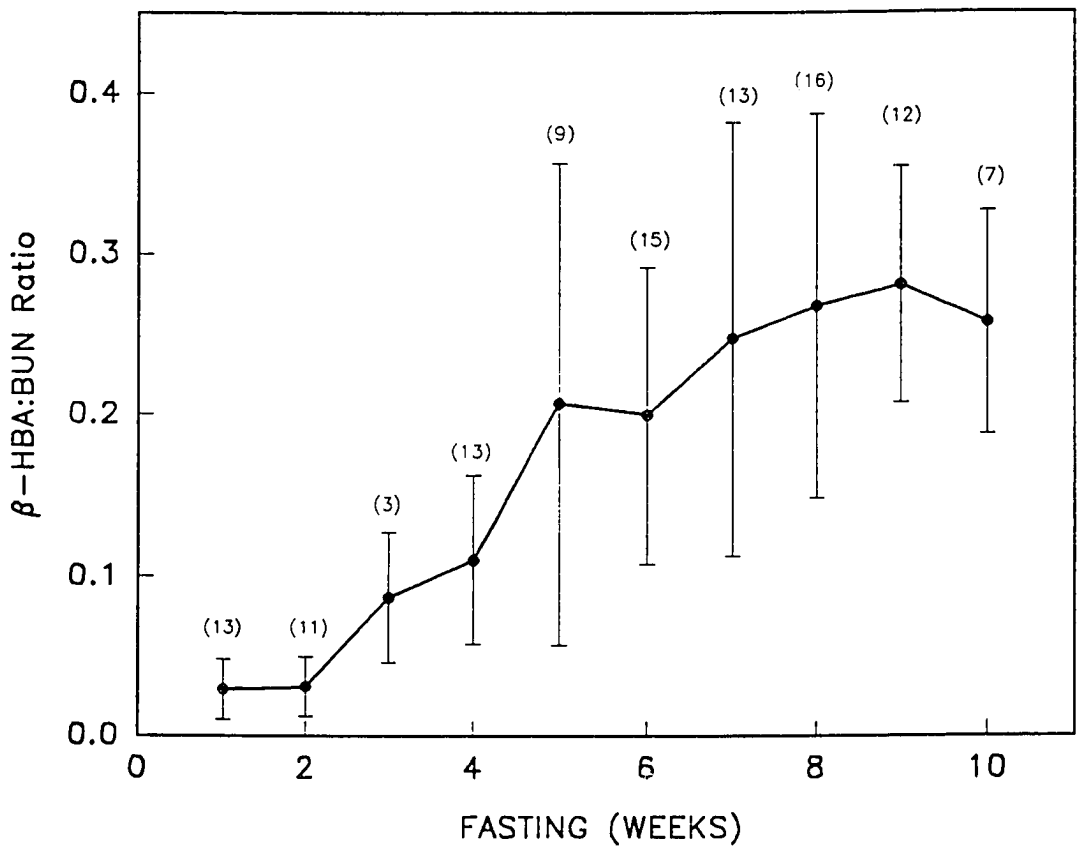

Figure 2.6. Changes in the β-HBA:BUN ratio for 19 northern elephant seal pups during the post-weaning fast. Error bars represent ± SD. Sample sizes are indicated in parentheses.

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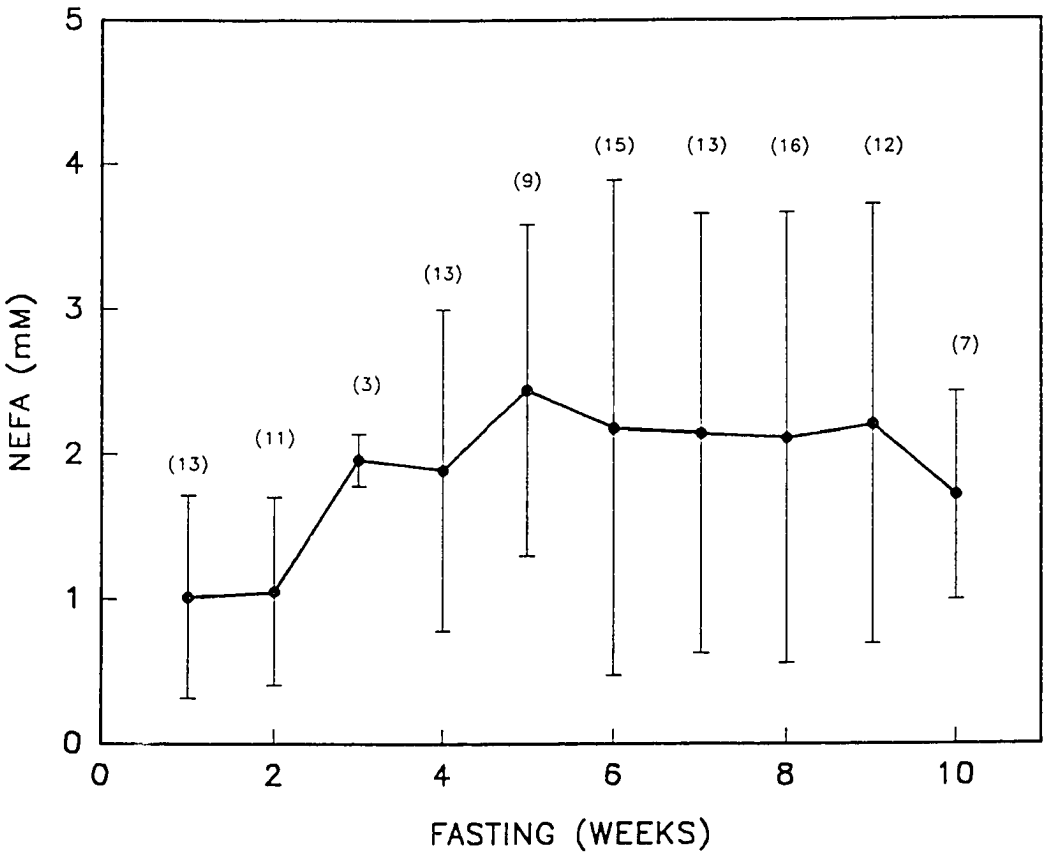

Figure 2.7. Mean plasma concentrations of non-esterified fatty acids (NEFA) for 19 northern elephant seal pups during the post-weaning fast. Error bars represent ± SD. Sample sizes are indicated in parentheses.

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Chapter 3. Weaning mass determines how body reserves are utilized during prolonged fasting in northern elephant seal pups.

INTRODUCTION

Northern elephant seal pups are typically nursed on land for 4 weeks before weaning. The pups gain significant lean and adipose mass, reaching 40 to 50% fat content before their mothers leave for sea (Ortiz et al. 1978). Each year, however, several pups are weaned 4 to 7 days earlier than the normal 25 to 28 days and are abandoned with less mass and body fat than average (Rea 1990; Kirby 1992). Conversely, larger than average pups may nurse longer and/or may obtain milk from unrelated lactating females after their mother's departure (Reiter et al. 1978).

Regardless of body size at weaning, all pups remain fasting on the beach after their mothers depart. In elephant seals, the duration of the post-weaning fast has been related to both absolute body mass at weaning (Arnbom et al. 1993; Kretzmann et al. 1993) and to body fat content (Kirby 1992). Smaller, leaner pups leave the rookery earlier than average weaners (Rea 1990; Kirby 1992). Morris et al. (1989) showed that small elephant seal pups had a first year survival rate of 19% versus 30 to 46% for medium sized weaners. The largest pups in their analysis (160 to 250 kg) showed the highest rates of survival (51%). Additional analysis of the same data by Le Boeuf et al. (1994) showed no differences in rate of survival to 1 year in pups weaned between 80 and 170 kg. Low weaning mass (LWM) pups were excluded from this analysis since small pups (usually less than 50 kg) were often found dead on the rookery, or

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washed up dead nearby after departure from the beach.

Although the reasons for prolonged fasting in newly weaned elephant seal pups are not fully understood, there is evidence to suggest that this post-weaning period is important for the physiological and behavioral development of diving skills. Within 4 weeks of weaning pups venture into shallow water and spend increasing amounts of time away from the beach as the fast progresses (Reiter et al. 1978). Thorson and Le Beouf (1994) documented increased dive depth and duration and increased oxygen carrying capacity aided by increases in hematocrit, hemoglobin and myoglobin concentrations during the fast. Pups increased the length of voluntary breath-hold (apnea) during sleep on land during this two month period (Blackwell and Le Boeuf 1993, Castellini et al. 1994). If LWM pups leave the rookery before the development of these necessary foraging abilities are complete, a lower rate of survival during the first year at sea might be expected.

There are some data from other species that relate body fat to fasting ability. Cherel et al. (1992) found that in lean rats the duration of fasting was limited by the availability of lipid reserves, but in obese rats fasting was limited by the depletion of body proteins long before lipid stores declined. Obese rats maintained fasting much longer, relying on maintained lipid mobilization to limit proteolysis and spare critical tissue proteins.

In this study, small and large elephant seal pups were studied to test the hypothesis that weaning mass determines how stored fuels are utilized during prolonged fasting. This study examined whether small pups showed the same patterns

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of fuel mobilization as large pups, simply at a faster pace, or whether fasting metabolism follows set patterns in each species regardless of body reserves. Low weaning mass pups in particular have the potential to demonstrate what factors might limit the ability to fast in pinnipeds and what metabolic shifts may trigger the animals to end the fast.

METHODS

Sample collection

Eleven weaned northern elephant seal pups studied at Año Nuevo, California were separated into 2 categories depending upon body mass at weaning. Low weaning mass pups (n=5) weighed less than 75 kg at weaning and high weaning mass pups (HWM, n=6) were greater than 140 kg at the beginning of the post-weaning fast. Data for average weaning mass pups (AWM, n=19), weighing 75 to 140 kg at weaning, have been presented in Chapter 2.

Prospective study animals were distinctively marked during the suckling period with hair bleach (Lady Clairol®) and two numbered hind flipper tags (Rototag®), ensuring an accurate age and weaning date. Weaned pups were captured weekly, when they could be found, to monitor mass loss and changes in plasma metabolite concentrations. Pups were weighed using a hanging mechanical scale (±1 kg). Methods for blood collection and sample analyses have been described in Chapter 2.

Mass calculations

Multiple mass measurements were recorded for all LWM pups and for 3 of the

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6 HWM animals. Each of these 8 pups was weighed 3 to 8 times during the 4 to 12 week study periods. Least squares linear regression analysis was used to estimate body mass at the time of each capture and at weaning (described in Chapter 2). R^2 values ranged from 0.9681 to 0.9964. The measured or estimated mass was then expressed as a percentage of calculated weaning mass. For the HWM pups with only 1 or 2 mass measurements, weaning mass was calculated based on the average rate of mass loss in the three other HWM pups (0.65 $kg \cdot d^{-1}$, n=3).

Statistical Analysis

One-way analysis of variance (ANOVA, Statistix®) was used to determine significant differences in plasma concentrations of glucose, blood urea nitrogen, β-hydroxybutyrate, and non-esterified fatty acids between LWM and HWM pups. Paired Student's t-tests were used to test for significant changes in metabolite levels within each weight class. Differences were considered significant if $p \leq 0.05$. All values were presented as mean ± standard deviation (SD).

RESULTS

Mass loss

Low weaning mass pups departed the rookery after an average of 31.6 ± 5.3 d. In contrast, the final samples from HWM pups were collected between 56 and 84 d post-weaning. However, these dates were not an accurate measure of the length of the post-weaning fast since five HWM animals remained on the beach at the end of sampling.

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LWM pups decreased mass from a mean of 66.1 ± 4.0 kg at weaning to 49.2 ± 2.4 kg at the time of departure, 4 to 5 weeks later. This represented a 25.0 ± 4.3% decrease from mass at weaning (Figure 3.1). HWM pups lost only 15.3 ± 1.3% of initial body mass over the first 5 weeks (n=2), but by the end of the study had lost 24.6 ± 3.5% of weaning mass. Over 8 to 10 weeks of fasting, HWM pups decreased from 149.6 ± 9.2 kg to 109.7 ± 7.8 kg . Thus, both groups had a similar absolute rate of mass loss; 0.54 $kg \cdot d^{-1}$ and 0.65 $kg \cdot d^{-1}$ in LWM and HWM pups respectively (p= 0.2943). However, small pups reached 75% of their weaning mass in half the time of very large pups.

Plasma metabolites

The decline in glucose suggested in Figure 3.2 for LWM pups was not significant. Individual pups did not show decreasing glucose concentration over time. HWM pups also showed no significant change in circulating glucose levels. Glucose concentrations were not significantly different between the two weight classes.

Blood urea nitrogen concentration declined significantly during 5 weeks of fasting in LWM animals (p=0.0408; Figure 3.3). HWM pups also showed a significant decline in BUN levels between 3 and 7 weeks of fasting, after which concentrations remained stable and low. LWM pups showed significantly higher BUN than HWM pups only during the first week of fasting (p=0.0296).

Ketone body levels increased significantly during the first 4 weeks of fasting in LWM pups, followed by a consistent drop in concentration in all animals sampled at 5 weeks post-weaning (n=3; Figure 3.4). HWM pups showed similar increases in β-

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HBA during the first 5 weeks (p=0.0008). However, LWM pups reached higher β-HBA levels during this first month of fasting (p=0.0430). Small pups also showed a faster rate of β-HBA increase (0.064 ± 0.028 $mM \cdot d^{-1}$) than did HWM pups (0.017 ± 0.011 $mM \cdot d^{-1}$). After 5 weeks of fasting there was increased variability in β-HBA values for HWM pups (Figure 3.4). Pups weaned at 180 kg or more maintained low β-HBA concentrations throughout the 10 week fast (Figure 3.5), with one pup showing an obvious increase only after 12 weeks of fasting. Pups weaned between 140 and 160 kg reached higher ketone body concentrations earlier in the fast than seen in the largest pups.

The ratio of β-HBA:BUN showed no significant change during the post-weaning fast in either LWM or HWM pups. There were no significant differences seen between LWM and HWM pups and ratios were highly variable in both groups.

Plasma fatty acid concentrations increased significantly in LWM pups between weeks 2 and 5 (p=0.0342; Figure 3.6). In contrast, fasting HWM animals showed no significant trend in NEFA levels. By 5 weeks LWM pups reach significantly higher fatty acid levels than seen in HWM pups.

DISCUSSION

Mass loss

LWM pups terminated the post-weaning fast at least 5 weeks earlier than larger pups, presumably due to insufficient body reserves required to complete the customary 3 month fast. Kirby (1992) showed a strong relationship between the fat content and

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the length of the post-weaning fast, with lean pups departing early. Kretzmann et al. (1993) and Arnbom et al. (1993) also showed positive relationships between length of the fast and mass at weaning in northern and southern elephant seal pups respectively. As critical changes in oxygen carrying capacity (increased hematocrit, hemoglobin, myoglobin and blood volume; Castellini et al. 1990, Thorson and Le Boeuf 1994) and breath-hold ability (Blackwell and Le Boeuf 1993, Castellini et al. 1994) occur during these first few months on land, early departure could adversely effect diving performance and survival of underweight pups.

Even though LWM pups depart the rookery weighing little more than newborn pups (40 - 50 kg), lipid stores are probably not exhausted. Pups weaned between 50 and 75 kg typically have fat contents of 30 - 40% (Rea 1990; Kirby 1992). Since body mass declines are equally distributed between the lean and fat compartments during fasting (Rea and Costa 1992), pups would be expected to retain at least 15 to 20 kg of fat when leaving for sea. This is, however, much lower than the 40 to 50 kg (40 - 50%) expected for HWM pups at departure and may effect thermoregulation in LWM pups.

Plasma metabolites

Blood glucose concentrations in LWM and HWM groups were similar to those seen in AWM pups and several other pinniped species (see Chapter 2). Even very small pups were capable of maintaining high circulating glucose levels throughout the fast. Most mammals, including humans and rats, maintain relatively stable circulating glucose concentrations during fasting (Cahill et al. 1966; Goodman et al. 1980; Tallas

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and White 1988). Similar results have been shown in fasting ptarmigan (Lindgård et al. 1992). The combination of ketosis and gluconeogenesis from protein precursors enabled lean birds to maintain elevated glucose concentrations throughout fasting, even during Phase III starvation.

Animals from both mass groups showed evidence of a protein sparing mechanism reflected in decreasing BUN concentrations as the fast progressed. However, LWM pups initiated fasting with much higher circulating BUN levels and never reached the sustained low values seen in AWM (Chapter 2) and HWM pups. In fasting birds and rats, the effectiveness of protein sparing during fasting has been linked to initial fatness (Goodman and Ruderman 1980; Lowell and Goodman 1987; Le Maho et al. 1988; Lindgård et al. 1992; Cherel et al. 1992). Lean ptarmigan (2 - 3% fat mass) show a much higher contribution of protein catabolism to total energy expenditure (22 to 41%) than do birds with 16 to 23% body fat (< 10% of energy expended; Lindgård et al. 1992). High weaning mass pups showed a further decline in plasma BUN levels compared with AWM profiles (Chapter 2) after 7 weeks of fasting, suggesting that larger pups may be able to spare more protein than small and average-size weaners. However, studies on rats and ptarmigan both suggest that there is a limit of fatness above which there is no further improvement in protein sparing ability and contributions of nitrogen to daily energy expenditure could not be reduced below 3 to 7% (Cherel et al. 1992; Lindgård et al. 1992). Pinnipeds, in general, exhibit a relatively low contribution of protein catabolism to energy expenditure during fasting (1.5 to 8% in northern elephant and grey seals; Pernia et al. 1980, Nordøy and Blix

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1985, Worthy and Lavigne 1987, Nordøy et al. 1990, Reilly 1991, Adams and Costa 1993). It is unknown how much this percent contribution could be decreased by improved protein sparing in the HWM northern elephant seal pups.

The rate of increase of plasma β-HBA concentration was strongly associated with the individuals' mass at weaning. Smaller, leaner pups reached peak ketone body levels earlier in the fast and showed the expected secondary decline in concentration after only 5 weeks. Peak β-HBA levels in LWM pups were similar to maximum concentrations seen in AWM pups (Chapter 2) and those previously reported for northern elephant seal weaners by Castellini and Costa (1990). In contrast only one HWM pup reached β-HBA levels greater than 1 mM during 10 weeks of fasting, and the largest pups maintained concentrations below 0.5 mM. However, the lower β-HBA levels seen during every week of the study in HWM pups compared with AWM pups were not significant differences ($p > 0.05$).

It is not known why LWM and HWM pups did not show a significant increase in the β-HBA:BUN ratio as seen in AWM pups in Chapter 2. It is possible that the sample sizes of these two groups (n=5 and n=6 respectively) were not adequate to address the question of the significance of this relationship.

Higher concentrations of circulating β-HBA and NEFA seen in LWM pups may be an indication of higher rates of lipolysis during early fasting than are seen in HWM animals. Klein et al. (1988) found rates of lipolysis to be similar in lean and obese men when expressed as a function of lean body mass. A similar relationship may be anticipated when HWM and LWM pups are compared. Thus, HWM pups

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would be expected to show a lower rate of lipolysis when expressed as a function of total body mass. Depressed insulin levels during fasting in humans allow increased lipolysis of adipose stores and the release of NEFAs that fuel hepatic ketogenesis (Cahill et al. 1966). Kirby (1992) found significantly lower plasma insulin concentrations in LWM pups (termed "runts" in her study; 7.1 ± 1.6 $\mu U \cdot mL^{-1}$) than in AWM pups (termed "normals" in her study; 7.8 ± 0.6 $\mu U \cdot mL^{-1}$). Higher rates of ketogenesis would be expected to accompany these lower insulin levels shown in LWM pups. Lean human subjects studied by Klein et al. (1988) also showed lower plasma insulin concentrations than their obese counterparts.

Conclusions

Even though pups less than 75 kg at weaning showed higher lipid mobilization than AWM or HWM pups and showed the expected peak in ketone bodies earlier in the fast, no LWM pup showed evidence of entrance into Phase III or terminal starvation before leaving the beach. Fatty acid levels remained high, β-HBA concentrations were still higher than those seen in HWM weaners and there was no indication of increasing protein mobilization with increased plasma BUN. There was also no evidence of increased metabolic rate prior to departure from the rookery in LWM pups studied by Rea and Costa (1992). Therefore, low weaning mass pups probably left the natal beaches before lipid reserves were depleted and were able to maintain a protein sparing metabolism throughout the 4 to 5 week post-weaning fast. High weaning mass pups also gave no indication of entrance into Phase III fasting, but showed evidence that they may be able to sustain a protein sparing metabolism for

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longer than seen in the AWM pups discussed in Chapter 2.

Not only do HWM pups accumulate additional body reserves that ensure a minimum length of the post-weaning fast, but the composition of these additional body stores may also influence metabolism. Since body fatness usually increases with increasing total body mass, larger pups also have the advantage of a higher percent body fat component. High fat content seems to be an effective physiological adaptation for fasting since it influences hormone levels (insulin, in particular) that regulate metabolism. The results of this study support the idea that there may be a threshold body or fat mass which allows for the regulation of fasting metabolism to sustain lipolysis of adipose stores while minimizing lean tissue degradation during prolonged fasting. Small pups showed higher relative rates of mass loss and lipolysis than HWM pups, and they never reached the low levels of circulating BUN seen in HWM. Thus, LWM pups showed indications of metabolic adaptation to fasting, but not to the same degree as AWM and HWM pups, suggesting that their ability to conserve energy stores were limited.

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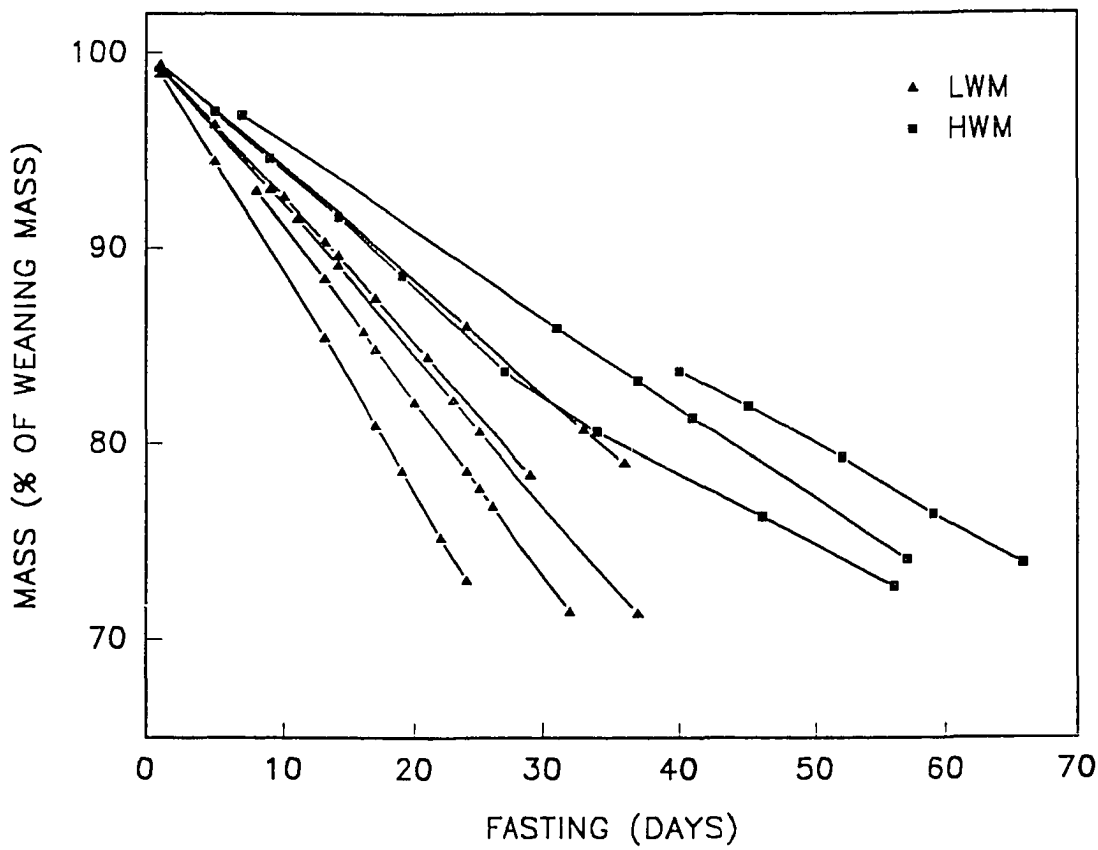

Figure 3.1. Relative body mass loss for 5 low weaning mass (LWM) and 3 high weaning mass (HWM) northern elephant seal pups during the post-weaning fast, represented as a percentage of calculated weaning mass.

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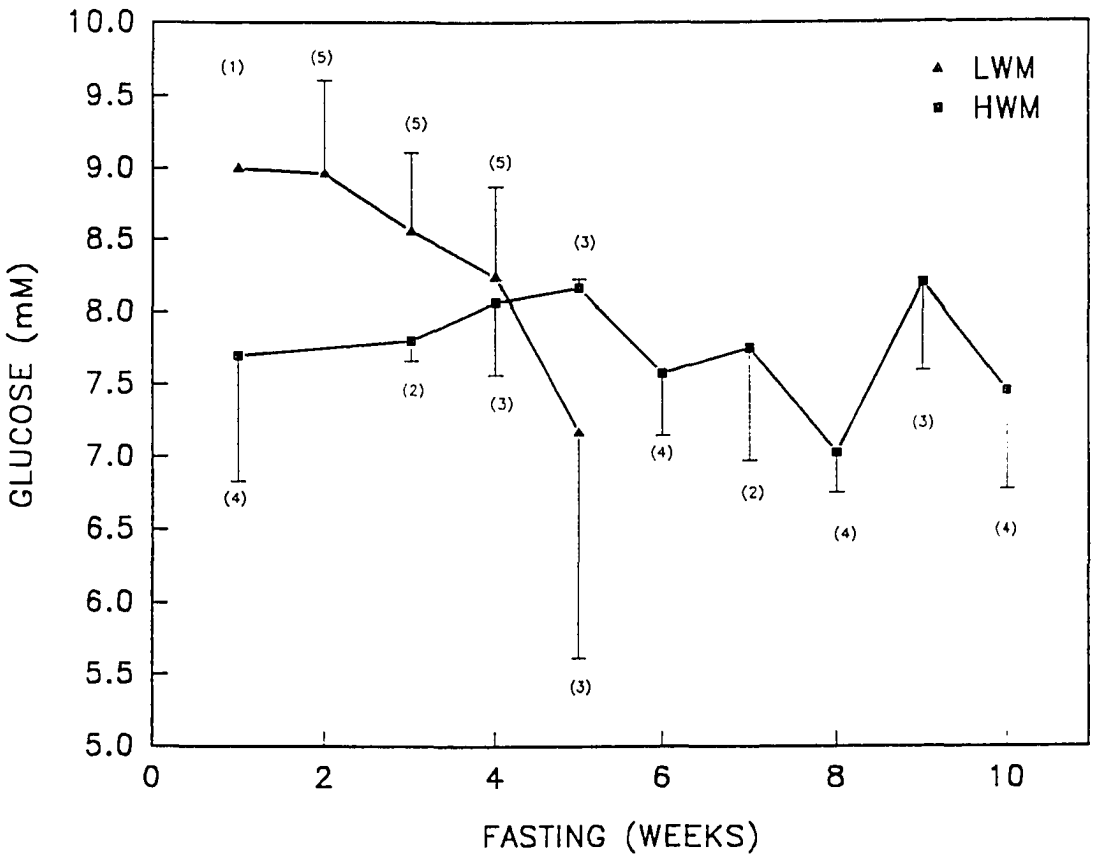

Figure 3.2. Mean plasma glucose concentrations for 5 low weaning mass (LWM) and 6 high weaning mass (HWM) northern elephant seal pups during the post-weaning fast. Error bars represent ± SD. Sample sizes are indicated in parentheses.

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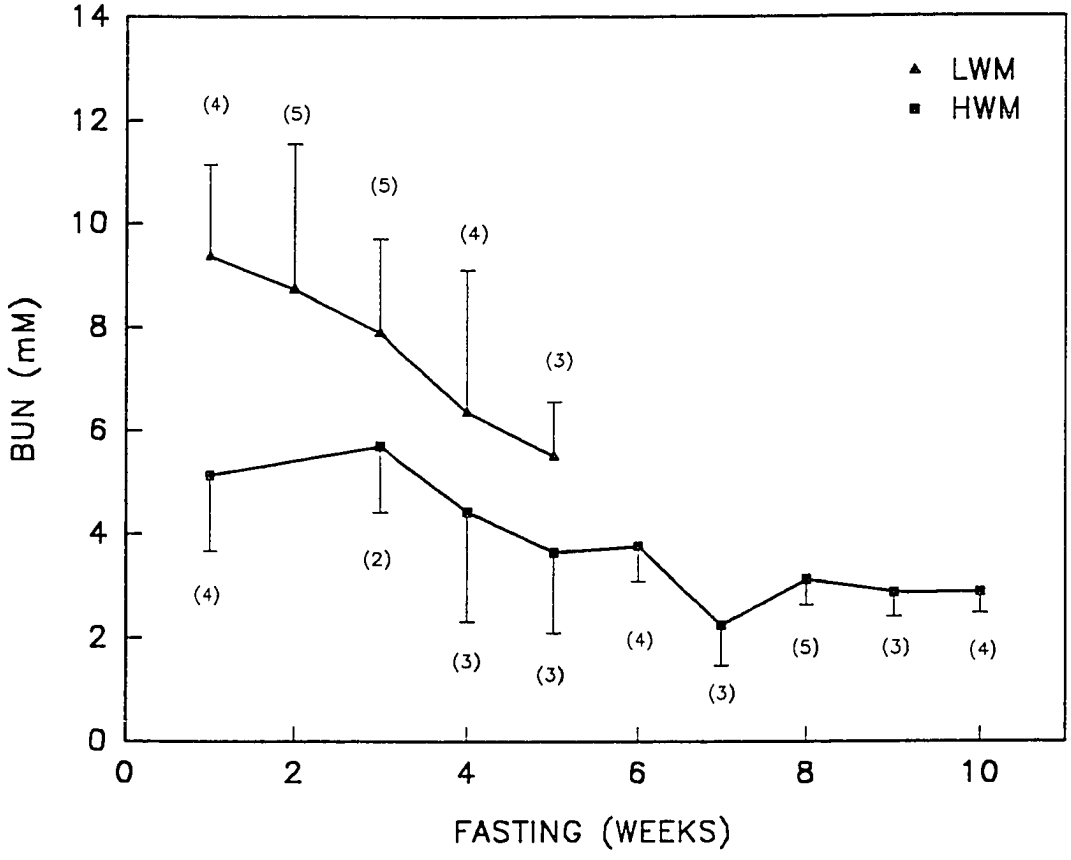

Figure 3.3. Mean plasma concentrations of blood urea nitrogen (BUN) for 5 low weaning mass (LWM) and 6 high weaning mass (HWM) northern elephant seal pups during the post-weaning fast. Error bars represent ± SD. Sample sizes are indicated in parentheses.

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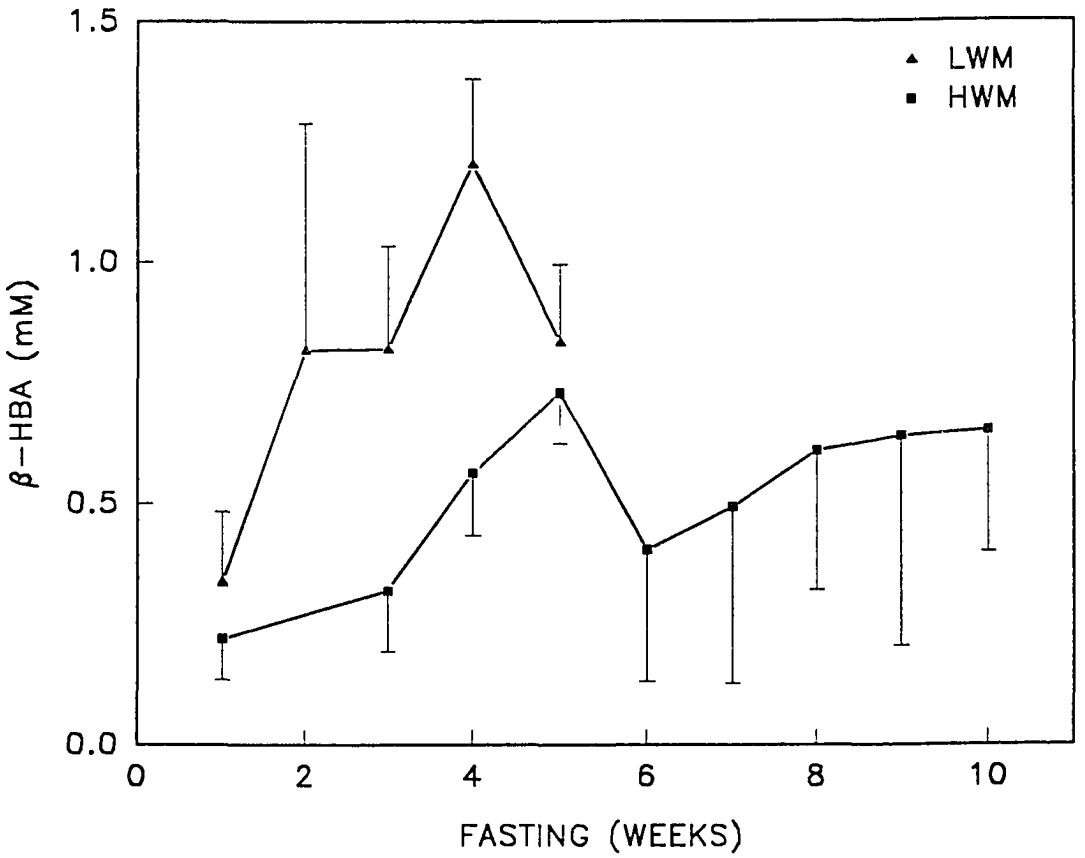

Figure 3.4. Mean plasma concentrations of β-hydroxybutyrate (β-HBA) for 5 low weaning mass (LWM) and 6 high weaning mass (HWM) northern elephant seal pups during the post-weaning fast. Error bars represent ± SD. Sample sizes are as shown in Figure 3.3.

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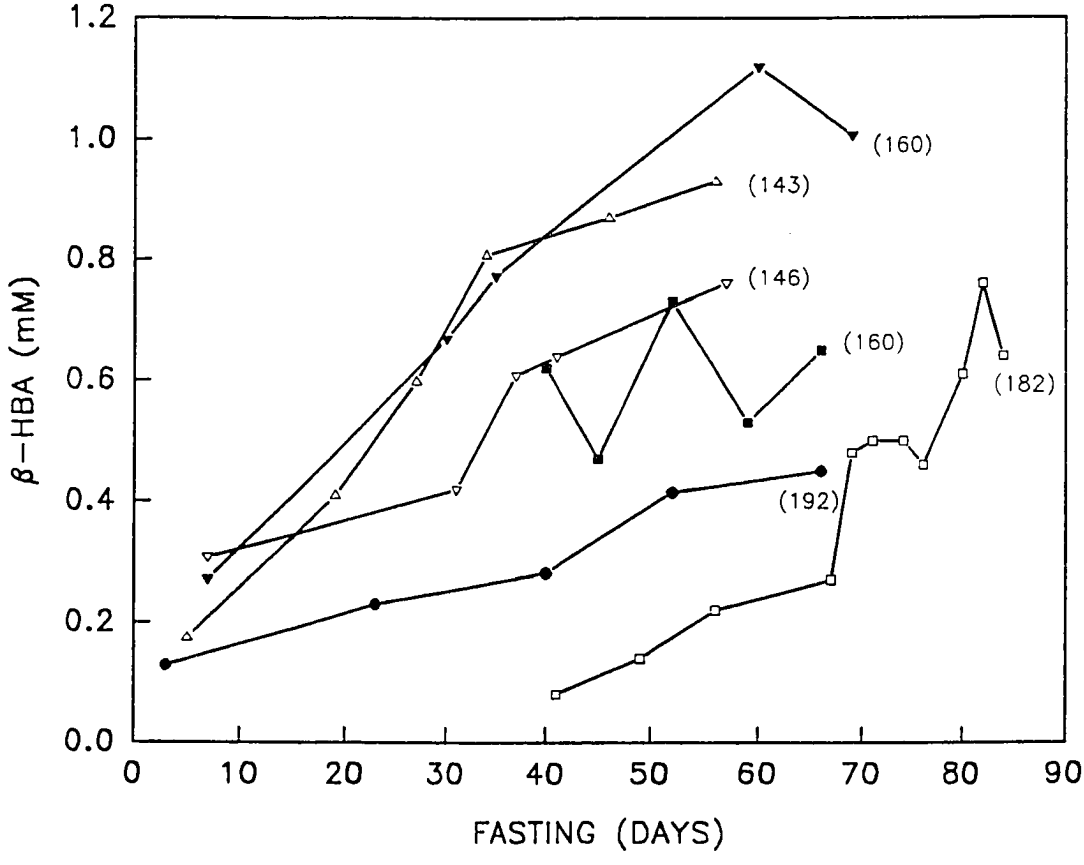

Figure 3.5. Changes in β-hydroxybutyrate (β-HBA) concentrations in 6 high weaning mass pups during the post-weaning fast. Estimated weaning mass of each pup is indicated in parentheses.

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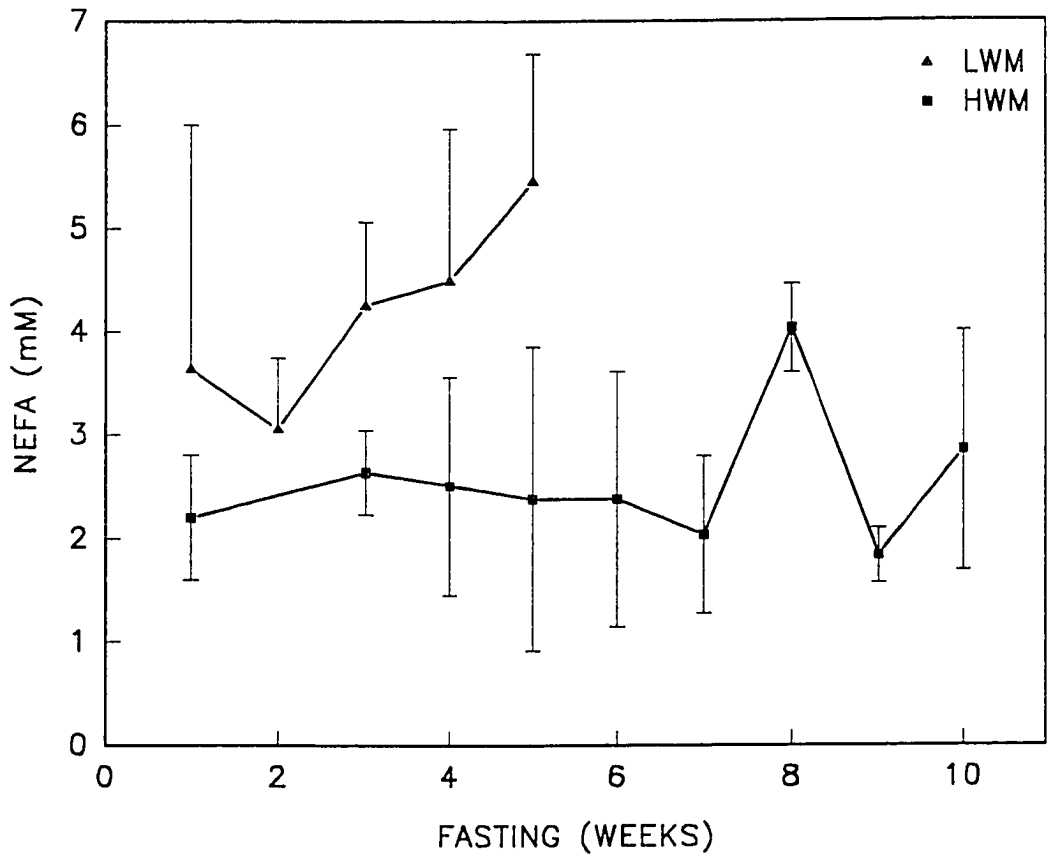

Figure 3.6. Mean plasma concentrations of non-esterified fatty acids (NEFA) for 5 low weaning mass (LWM) and 6 high weaning mass (HWM) northern elephant seal pups during the post-weaning fast. Error bars represent ± SD. Sample sizes are as shown in Figure 3.3.

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Chapter 4. Body condition and plasma metabolites as indicators of nutritional independence in Weddell seal pups.

INTRODUCTION

Weddell seal pups are born on the snow-covered annual shore-fast ice in Antarctica between early October and mid-November (Kaufman et al. 1975; Tedman and Bryden 1979; Thomas and DeMaster 1983a). Pups first enter the water with their mothers at age 8 to 20 days (Lindsey 1937; Stirling 1969) and spend increasing amounts of time in the water as lactation progresses. Tedman and Bryden (1979) report that 3 to 5 week old pups spend between 8 to 12 hours per day swimming. Haul-out patterns of mothers and 4 to 5 week old pups are synchronous (Thomas and DeMaster 1983b) but soon after, adult females spend more time in the water, presumably mating. Tedman (1985) showed that late in the lactation period both suckling frequency and duration decreased, and pups showed a decline in growth (Bryden et al. 1984). Concurrently, lactating females decrease their rate of mass loss (Hill 1987). Thomas and DeMaster (1985a) suggest that weaning is probably a gradual process resulting from the adult female leaving the pup for increasingly longer periods and then finally not returning.

There are several estimates of weaning age in the literature, most based on the last day that the mother was sighted with the pup or on the last observed suckling bout. Thomas and DeMaster (1983b) suggested that pups are weaned at 53 ± 5 days of age, and Lindsey (1937), Stirling (1969), Kaufman et al. (1975) and Hill (1987) give

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estimated weaning ages of 33 to 55 days. Several authors have reported significant weight loss following weaning in Weddell seal pups. In particular, Lindsey (1937) documented a 0.75 $kg \cdot d^{-1}$ decrease in body mass after a mean weaning age of 50.3 days in 18 pups. However, since pups spend a significant portion of their time in the water, it is difficult to judge whether this weight loss is due to complete fasting or to infrequent feeding, such that body mass can not be maintained.

Comparatively little is known about the nutritional development of Weddell seal pups after weaning. Stomach contents of older pups suggest they feed on different prey species than adults (Lindsey 1937) but we know little about when pups reach nutritional independence, if their milk diet is opportunistically supplemented with prey items during the suckling period, or how pups learn to forage. Of particular interest in this study is whether Weddell seal pups, like other phocids (such as the grey, harp and elephant seals) undergo a post-weaning fasting period. There are three ways in which Weddell seal pups could handle this transition to nutritional independence: firstly, pups may begin to feed on solid food during the suckling period, secondly, pups may begin foraging for prey species at weaning, or thirdly, pups may undergo a short post-weaning fast before foraging independently for crustaceans and small fish. It is unlikely that pups would fast post-weaning if they had already learned to secure live prey while suckling.

The biology of long-term fasting has been studied in three phocid species; northern elephant seals (Chapters 2 and 3), harp seals (Nordøy et al. 1993) and grey seals (Nordøy et al. 1990; Nordøy and Blix 1991; Nordøy et al. 1992). A predictable

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pattern of blood chemistry changes have been found to accompany decreasing body mass in these species during the post-weaning fast. If Weddell seal pups fast completely following weaning, blood metabolite levels should change in a similar pattern to those shown for fasting-adapted species.

METHODS

Sample Collection

Thirty-one Weddell seal pups were studied during the austral summers of 1992 (n=14) and 1993 (n=17) in McMurdo Sound, Antarctica. All pups studied were born at the pupping colony at Hutton Cliffs on the western coast of Ross Island (77°44′ S, 160°30′ E). Pups were free-ranging throughout the study, thus natural behavior (ie. diving) was unrestricted during the suckling and post-weaning periods. Research huts were positioned within walking distance of the colony and facilitated frequent observation of pregnant females and mother-pup pairs. Thus time of birth was known within 24 hours for all pups studied. Each pup was first examined within 2 days of birth. Thereafter, pups were sampled every 2 weeks to minimize disturbance of the mother-pup bond. Sixteen pups were sampled at weeks 2, 4, 6, 8 etc. of age and the remaining 15 pups during odd weeks (1, 3, 5, 7 etc.). During the two year study, subjects were evenly distributed between the two sexes (16F:15M). Pups were consistently located during the first 8 to 10 weeks of age. However, later in the season not all animals were located. As pups grow older their diving ability improves and they spend more time in the water. In addition, ice conditions progressively

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deteriorate, inhibiting search and allowing pups to move away. For these reasons, only 2 pups were sampled past 13 weeks of age, one at 15 weeks and the last at 17 weeks of age. Only pups that were captured for blood samples 4 or more times and only pups that reached a minimum of 75 kg during the nursing period were included in these analyses. Hill (1987) showed that Weddell seal pups weaned at less than 80 kg were not resighted in following years, and were termed non-survivors. The purpose of this study is to compare blood chemistry and body condition of average weaning mass Weddell seal pups with those levels seen in average weaning mass northern elephant seal pups which we know to be fasting (Chapter 2) and to determine if Weddell seal pups show similar evidence of fasting metabolism.

When in attendance, adult females were held off a short distance from the pup for the duration of sampling (maximum 40 min). When first captured, pups were tagged with two numbered rear flipper tags for identification. Pups were weighed in a mesh or vinyl restraint bag using an electronic hanging load cell (Ohaus Model I-20W, capacity of 500 kg ± 0.1 kg). They were then manually restrained so that a 20 mL blood sample could be drawn either from the extradural vein in young pups (up to 2 weeks of age, Geraci and Smith 1975) or from the pelvic venous plexus in older pups (Geraci 1971). Blood was collected directly into heparinized Vacutainer® collection tubes using 18 or 20 gauge needles. Samples were held in an insulated container to avoid freezing, and processed within one hour. All pups were reunited with their mothers immediately after handling. There were no instances of the study pup being rejected by its mother.

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Upon return to the research huts, heparinized whole blood was rewarmed and gently mixed. Hematocrit (Hct) was determined in duplicate using a battery operated field microhematocrit centrifuge (Compur M1100, samples spun at 5400 g (11500 rpm) for 3.5 min) and hemoglobin (Hb) concentration was measured spectrophotometrically using methanocyanide (Sigma Chemicals Kit 525-A). The remaining blood was centrifuged and the plasma was removed and frozen for later analysis. Samples were held in a liquid nitrogen cooled CryoPac shipper (Taylor-Wharton, -196° C) while at Hutton Cliffs, and stored at -80° C at McMurdo Station and at University of Alaska Fairbanks (UAF) until analysis. Methods for the analysis of glucose, BUN, NEFA, and β-HBA concentrations and for the determination of water content and specific gravity (SG) of the plasma have been previously described by Castellini et al. (1993). All values are presented as mean ± standard deviation (SD).

Mass calculations

Mass change was calculated as the difference between mass measurements at 2 consecutive captures (approximately a 2 week interval) divided by the number of days between measurements. Since pups were captured only every 2 weeks, mass changes represent the average result of mass gains and losses during that period. In the case of missing mass measurements (n=2), this created missing data for mass change over 2 consecutive captures. No attempt was made to calculate mass change over the respective 4 week periods.

Statistical analyses

Tukey's multiple range test was used with one-way analysis of variance

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(ANOVA) to identify significant differences among means at each week of age (Statistix®). Data for β-HBA:BUN ratio was arcsin transformed for statistical analysis. Differences were considered significant when $p \leq 0.05$.

RESULTS

Mass change

Weddell seal pups weighed 30.3 ± 3.7 kg at birth and ranged from 22.6 to 39.9 kg (n=31). Pups gained an average of 1.8 ± 0.4 $kg \cdot d^{-1}$ (range 1.1 to 2.8 $kg \cdot d^{-1}$; n=31) during the suckling period and obtained a mean maximum suckling mass of 105.8 ± 16.1 kg (n=29) between 5 and 7 weeks of age. Due to the wide range of body size at weaning (76.5 to 134.1 kg), body mass was expressed as a percentage of the maximum mass measured during the suckling period (Figure 4.1). Since exact weaning dates for the pups were unknown, this period of maximum suckling mass was estimated to be the weaning period and is represented on figures as a shaded area. Mass decreased significantly between the weaning period and 8 weeks of age (Figure 4.1) but the mean mass of 8 to 11 week old pups was still significantly higher than seen in 3 week old suckling pups. Although mean mass continued to decrease, such that 12 to 13 week old pups were only significantly heavier than 2 week olds, post-weaning body mass never dropped below 77% of the individuals' maximum suckling mass. By 15 to 17 weeks of age, the only 2 animals sampled had regained all mass losses, and reached greater than 100% of their maximum suckling mass (Figure 4.1).

The rate of mass change was constant and positive during the first 4 weeks of

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study (Figure 4.2), followed by a significant decline in the rate of mass change from 5 to 8 weeks of age. Overall rates of body mass loss (up to 0.7 $kg \cdot d^{-1}$) were seen in some individuals by 7 weeks of age. In the interval from week 6 to 8, the greatest mean rate of mass loss seen in this study was observed (0.7 ± 0.3 $kg \cdot d^{-1}$), with maximum rates of mass loss reaching 1.0 $kg \cdot d^{-1}$. Between weeks 8 and 13, mean rate of mass change remained constant with most pups showing overall mass loss during this period.

Hematology

Hematocrit increased significantly from 45.3 ± 3.1% (n=13) at 1 week of age to 51.9 ± 3.9% (n=16) at 3 weeks (Figure 4.3). Further increases were evident during the weaning period (59.8 ± 2.1% at 6 weeks of age; n=18), but Hct remained relatively stable after weaning. There was no relationship between Hct and the rate of mass loss seen in individual pups during the study.

Hemoglobin showed a significant increase in concentration between 1 and 3 weeks of age, from 19.0 ± 1.7 $mg \cdot dL^{-1}$ (n=13) to 21.7 ± 1.5 $mg \cdot dL^{-1}$ (n=16), and continued to increase into the weaning period (24.3 ± 1.8 $mg \cdot dL^{-1}$; n=11; Figure 4.4). Unlike Hct, further increases in Hb level were seen during weeks 11 and 12 (25.8 ± 1.7 $mg \cdot dL^{-1}$; n=18) over those seen during weaning (week 5). There was also no relationship seen between Hb content and the rate of mass loss in individuals.

At birth, plasma samples showed low specific gravity (1.015 ± 0.012 $g \cdot mL^{-1}$; n=31), but increased significantly to 1.024 ± 0.004 $g \cdot mL^{-1}$ (n=16) by 4 weeks of age (Figure 4.5). Specific gravity decreased to 1.021 ± 0.005 $g \cdot mL^{-1}$ after a peak of 1.025

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± 0.009 $g \cdot mL^{-1}$ at 8 weeks of age, and levels were not significantly different than those seen in suckling pups. Water content of the plasma was high at birth (93.5 ± 1.2 %; n=31) but decreased significantly to 92.3 ± 0.7 % (n=16) by 3 weeks of age and remained stable for the duration of the study (Figure 4.6).

Plasma metabolites

Plasma concentrations of BUN were high at birth (7.6 ± 2.4 mM) but decreased significantly over the first week (Figure 4.7). Low, stable BUN concentrations were seen during the rest of the suckling period, with means ranging from 2.6 to 3.6 mM during weeks 1 to 5. Blood urea nitrogen concentrations then gradually increased from 3 to 11 mM between 8 and 17 weeks of age. By 13 weeks of age BUN levels had increased significantly above concentrations measured during the first 9 weeks (Figure 4.7). Low BUN concentrations were seen in pups during the weaning period, and in pups that exhibited high rates of body mass loss after weaning (Figure 4.8). The highest BUN levels were measured in pups that showed post-weaning body mass gains of greater than 0.3 $kg \cdot d^{-1}$.

Ketone body concentration ranged from 0.04 to 0.29 mM during suckling and increased gradually during the weaning period (Figure 4.9) to a maximum mean concentration seen at 8 weeks of age. This peak was significantly higher than β-HBA levels measured between 2 to 6 weeks of age. At week 9, β-HBA levels remained significantly elevated above those seen at week 5 but, by 11 weeks, levels had significantly declined from maximum values. Higher concentrations of β-HBA were seen in pups during periods of mass loss than during mass gain (Figure 4.10). Of pups

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which gained mass at a rate greater than 1 $kg \cdot d^{-1}$, 77% had ketone body concentrations less than 0.2 mM. Thus, only 23% of growing pups showed β-HBA levels greater than 0.2 mM. Samples with the highest β-HBA values for each pup were collected at 54 ± 8.6 days of age and showed a mean concentration of 0.30 ± 0.12 mM (range 0.12 to 0.60 mM, n=30). Thus the timing and the magnitude of individual peak β-HBA concentrations were different in each pup, but in all but 1 individual, peak β-HBA levels were seen during or immediately prior to the 2-week period of greatest mass loss (-0.6 ± 0.4 $kg \cdot d^{-1}$, n=30). Of these individual peak concentrations, 87% were greater than 0.2 mM.

Samples collected at 8 weeks of age showed a higher ratio of β-HBA:BUN concentrations than at birth (Figure 4.11). No other significant changes were measured in these pups during their first 13 weeks. Higher ratios were seen in pups during post-weaning mass loss than during subsequent periods of mass gain (Figure 4.12). Ratios seen during post-weaning periods of growth were in the same range as those seen in pups at the beginning of the weaning period (when at maximum suckling mass).

Fatty acid levels gradually declined during the suckling period and concentrations measured at 5 weeks of age (1.4 ± 0.7 mM) were significantly lower than those at 1 week old (3.3 ± 1.7 mM, Figure 4.13). NEFA concentrations seen in pups sampled at 9, 10, 12 and 13 weeks of age were not significantly different from those of nursing pups. There was a wide range of NEFA concentrations seen during the period of post-weaning mass loss with no discernable relationship to rate of mass loss (Figure 4.14). Concentrations seen during post-weaning mass gain were within the

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range of those levels measured during the weaning period.

Glucose concentration ranged from 3.1 to 9.6 mM and showed no significant change throughout the first 13 weeks of study (Figure 4.15). Variability about the mean values was high during the weaning period but decreased after 9 weeks of age.

DISCUSSION

Mass Change

Significant decreases in body mass following the weaning period could reflect either total fasting or inefficient foraging such that body mass could not be maintained. Since body mass changes were measured only over 2 week intervals, rates of mass change may represent a combination of mass gains and losses during that period. It is therefore difficult to determine precisely when the maximum rate of mass loss occurred or the magnitude. However, rates of mass loss seen from week 6 to 8 (0.66 ± 0.32 kg·d^{-1}; maximum rate of 1.03 kg·d^{-1}) are very similar to those reported by Lindsey (1937) for post-weaning mass loss when pups were weighed every 3 days.

Rates of mass loss in post-weaning Weddell seal pups are similar to those seen in other phocid species during the post-weaning fast. Fasting northern elephant seals lose from 0.5 to 1.1 kg·d^{-1} (mean 0.7 ± 0.2 kg·d^{-1}) during the post weaning fast (Chapter 2). Mass loss in fasting grey seals decreases from 0.8 kg·d^{-1} during the first week of fasting to 0.4 kg·d^{-1} during the second week (Nordøy et al. 1990) and fasting harp seals show similar rates of 0.4 kg·d^{-1} (Worthy and Lavigne 1983) during the post-weaning fast.

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The decrease in rate of mass loss seen in Weddell seal pups after 9 weeks of age suggests that many pups begin to forage at this time, although body mass losses of up to 0.5 $kg \cdot d^{-1}$ can be found as late as 12 weeks of age. The gradual decrease in rate of mass loss from 8 to 13 weeks of age suggests that pups may take weeks to gain sufficient skill at foraging to maintain body mass, but are successful at catching enough prey to lower the initial high rates of mass loss. Muelbert and Bowen (1993) reported that the daily rate of body mass loss decreased in weaned harbor seal pups after the onset of feeding (at 3 to >26 days post-weaning, mean 15 to 17 days). They found that the average rate of body mass loss for fasting pups was 0.28 ± 0.06 $kg \cdot d^{-1}$. Following the first evidence of solid food in their stomachs, pups continued to decrease mass at a rate of 0.11 ± 0.04 $kg \cdot d^{-1}$ for the next 3 weeks. At the conclusion of that study (at 5 to 6 weeks post-weaning) some pups maintained a stable body mass, but no pups had entirely regained mass lost during the fast.

Lindsey (1937) reported that at least one pup sampled at Bay of Whales in 1934 had both milk and a few crustaceans (euphasids and isopods) in its stomach. He also documented older pups with isopods, amphipods and fish eyes in stomach samples and suggested that crustaceans and shallow water fish would be easier prey for young animals to capture. Considering the significant rate of body mass loss following weaning, it is unlikely that pups forage successfully during the suckling period. However, since body mass data was collected at 2 week intervals in this study, it is difficult to rule out the possibility that some pups catch occasional prey during the suckling period.

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Hematology

Although pups spend time in the water from the age of 2 weeks, limited diving ability may not give pups access to prey immediately. Physiological parameters that affect diving ability change rapidly during the suckling period and are maximized only after pups reach 8 to 9 weeks of age. Increases in Hct and Hb seen in older Weddell seal pups during this study would allow an increase in oxygen carrying capacity of the blood, in turn allowing for increased dive capacity. This conclusion is supported by results of a simultaneous study on the diving behavior of a subset of the Weddell seal pups sampled in 1992 (Moss and Testa, in press). Average depth of dive was less than 30 m in pups during the suckling period with accompanying average durations of less than 3 min. Both average dive depth and average dive duration increased during the weaning period but appeared to remain relatively stable from 9 to 13 weeks of age, with durations from 4.5 to 6 min and depths of 70 to 185 m. However, slight increases continued in the daily maximum dive depth and duration for each individual between 9 to 13 weeks of age (Moss and Testa, in press). Until we have a better understanding of the prey of young Weddell seals, and where they are distributed in the water column, it is difficult to comment on whether this limitation on dive depth is important.

Changes in water content and specific gravity of the plasma were similar to those seen in other mammalian neonates. Northern elephant seal pups show decreases in water content of the plasma shortly after birth and show no signs of dehydration at any time during the post-weaning fast (Castellini et al. 1990). In both species the

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lowest contents are seen at or about the end of the suckling period.

Plasma metabolites

Study of mass changes gives us insight into what the pup has experienced over the preceding 2 week period but tells us little about the current metabolic status of the animal (ie. feeding or fasting). Key plasma metabolites show the most recent metabolic status, but in northern elephant seal pups (Chapter 2) metabolite concentrations can change significantly within hours or days. Thus if pups had undergone a short post-weaning fast but had begun feeding before the biweekly blood sample was drawn, no indication of fasting would be found in the blood chemistry profile. Similarly, if plasma metabolite levels are consistent with those seen in other fasting seals, it cannot be assumed that pups were fasting over the entire 2 week interval preceding that sample.

All species of phocid seals studied show low concentrations of BUN during the early portions of the post-weaning fast. Increases in BUN during fasting have only been shown to occur when pups are held in captivity and thus can not initiate independent feeding. Since Weddell seal pups here are free-ranging there is no reason to expect any increase in circulating BUN due to enforced fasting. The gradual increase in BUN levels seen after 8 weeks of age is consistent with this being a period of increasing food intake, particularly since rates of body mass loss are decreasing at the same time. The highest concentrations of BUN are measured following periods of post-weaning mass gain, suggesting that pups are feeding on high protein prey species.

It is unclear why plasma BUN levels were lower in suckling Weddell seal pups

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than in suckling elephant seal pups. The protein content of Weddell seal milk (8.9%, Tedman 1985) falls within the range of 7.6 to 11.7% reported for elephant seals (Reidman and Ortiz 1979).

All fasting phocids studied show a characteristic increase in β-HBA concentrations during the post-weaning fast (Castellini and Costa 1990; Nordøy and Blix 1991; Nordøy et al. 1993). Suckling northern elephant seal pups show a range of weekly mean values from 0.11 to 0.15 mM during the nursing period (Chapter 2). Within a week of weaning, β-HBA levels increase to 0.30 ± 0.16 mM and are in the range of 0.5 mM by 3 weeks post-weaning (Chapter 2). Ketone body concentrations measured in 8 and 9 week old Weddell seal pups fell within the range for elephant seal pups fasting 1 to 3 weeks. Mean β-HBA levels declined after 10 weeks of age in Weddell seals to levels seen in suckling pups. However, one pup showed β-HBA concentration as high as 0.60 mM at 12 weeks of age. This animal also lost mass at a rate of 0.5 $kg \cdot d^{-1}$ over the preceding 2 week interval suggesting that it was not foraging effectively. All samples that showed ketone body levels above 0.30 mM were seen following periods of overall body mass loss, and all samples collected after intervals of mass gain showed β-HBA concentrations within the same range as pups sampled during the weaning period.

Northern elephant seal pups show an increase in the ratio of β-HBA to BUN concentrations during the post-weaning fast (Chapter 2). By 3 to 4 weeks of fasting, they increase this ratio to about 0.1. Weddell seal pups also showed a peak in the ratio of these two metabolites at 8 weeks of age, but values were highly variable during

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both the suckling and post-weaning periods with values of up to 0.3 seen in both groups.

Fatty acid concentrations decreased during the suckling period, but were highly variable in Weddell seal pups. However, the gradual increase seen in NEFA from the weaning period up to 9 weeks of age was in the same range as those seen over the first 4 weeks of fasting in northern elephant seal pups (Chapter 2).

Weddell seal pups showed mean plasma glucose levels of approximately 6 to 8 mM throughout the suckling and post-weaning periods. These concentrations are slightly lower than those seen in northern elephant seal pups (6.7 to 9.8 mM, Chapter 2; Kirby 1992), and considerably lower than those reported for fasting harp (10.2 ± 0.3 mM, Nordøy et al. 1993) and grey seals (means of 10.4 to 12.6 mM, Nordøy and Blix 1991). Glucose levels showed no significant change with age of Weddell seal pups. Similarly, glucose concentrations are reported to remain stable during the first month of fasting in all of the above studies.

Conclusions

Blood chemistry levels and the accompanying rate of mass change together suggest that Weddell seal pups typically fast for 1 to 3 weeks following permanent separation from their mothers. Increasing BUN values after 8 weeks of age along with decreasing rates of mass loss suggest improved success foraging on high protein prey items. High ketone body concentrations seen in Weddell seal pups within 1 to 3 weeks of the weaning period are similar to levels seen during the first 3 weeks of fasting in other phocid species. These peak β-HBA levels directly coincide with the highest rates

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of mass loss in each individual. There is also some indication of a rise in the β-HBA:BUN ratio at week 8 which is consistent with short-term fasting. Weddell seal pups show concentrations and rates of increase of NEFA immediately following the weaning period similar to those seen in newly weaned elephant seals during the post-weaning fast. Pups show significant increases in Hct and Hb concentrations after 8 weeks of age that coincide with improved diving ability, suggesting that older pups would be more effective foraging in deeper waters. Although location and capture of pups is difficult after 13 weeks of age, there is some suggestion that pups of this age have successfully regained mass lost during the post-weaning period indicating that foraging is successful enough to allow for compensatory growth.

Taken together, data from metabolic patterns determined from fasting northern elephant seal pups suggest that in Weddell seal pups the post-weaning fast lasts only 2 weeks. Collection of morphometric (ie., mass) and biochemical (ie., metabolite concentration) data appears to be a powerful and complimentary union of techniques for the study of fasting biology in the field.

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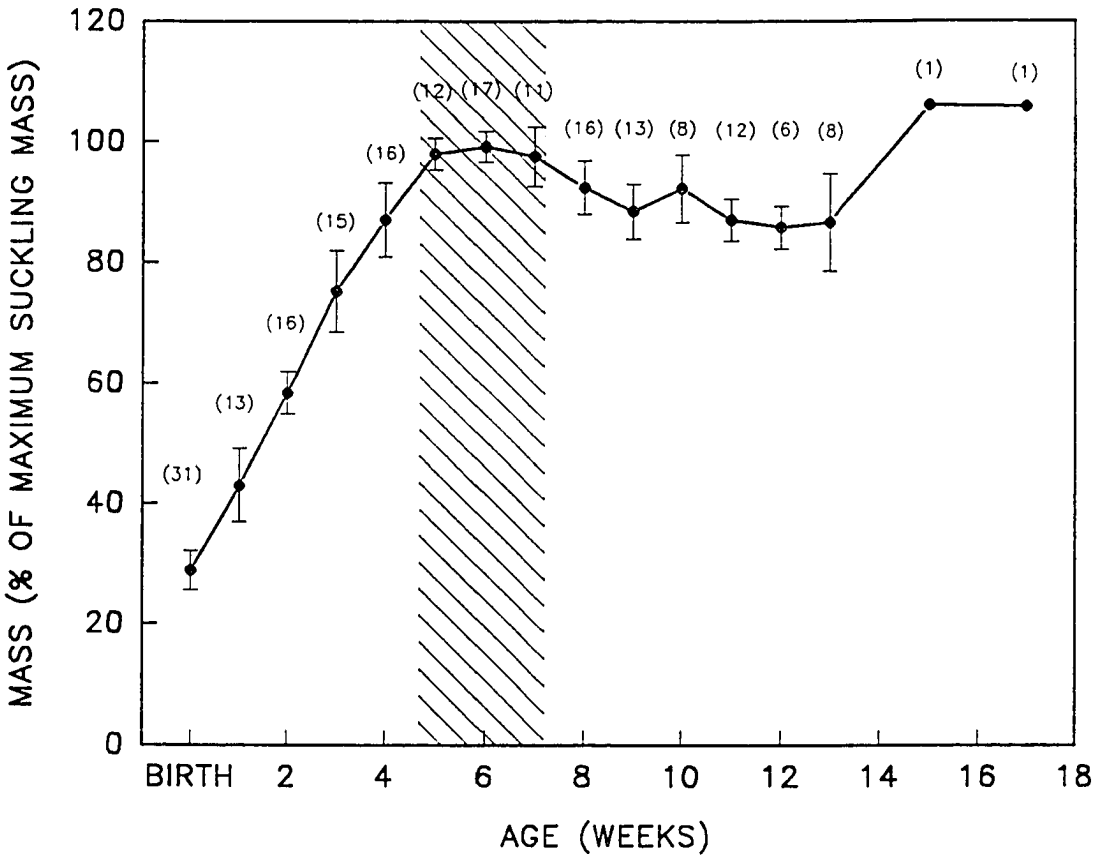

Figure 4.1. Changes in relative mass (mass expressed as a percentage of maximum suckling mass) seen during the suckling, weaning and post-weaning periods in Weddell seal pups. The shaded area represents the estimated weaning period. Sample sizes are indicated in parentheses.

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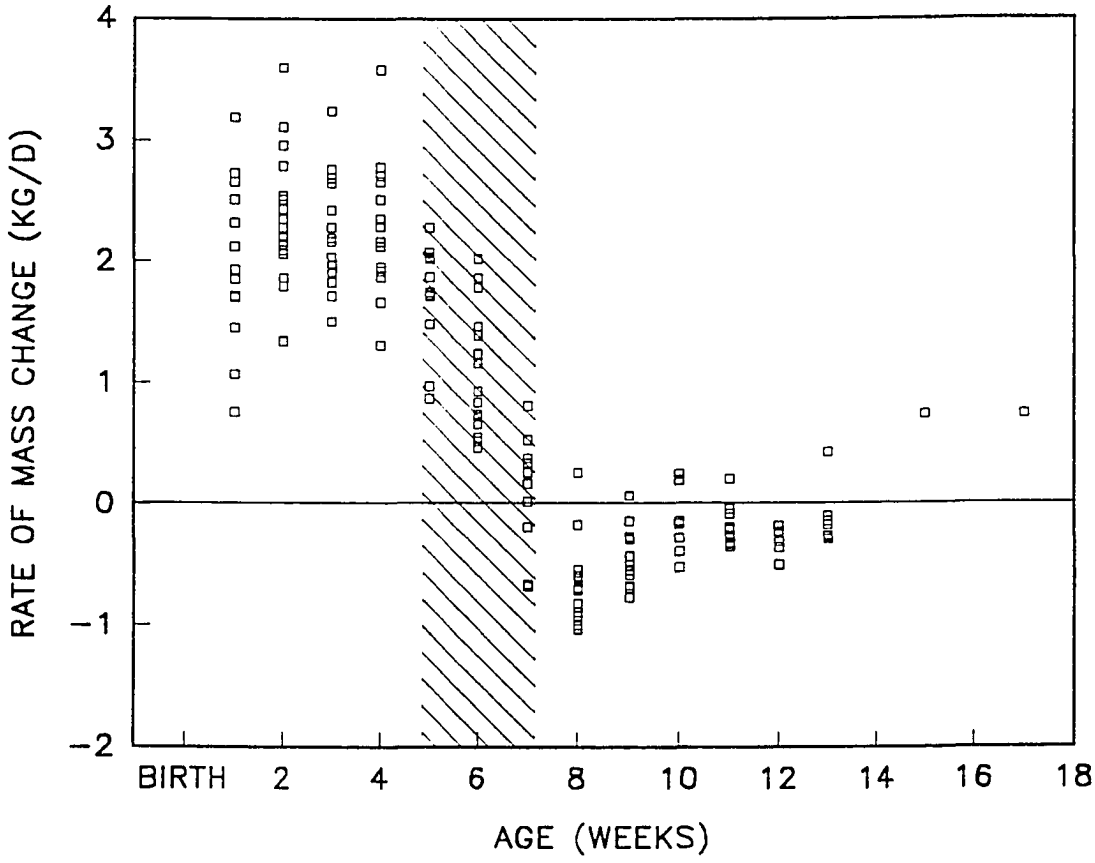

Figure 4.2. Changes in rate of mass change ($kg \cdot d^{-1}$) seen during the suckling, weaning and post-weaning periods in Weddell seal pups. The shaded area represents the estimated weaning period.

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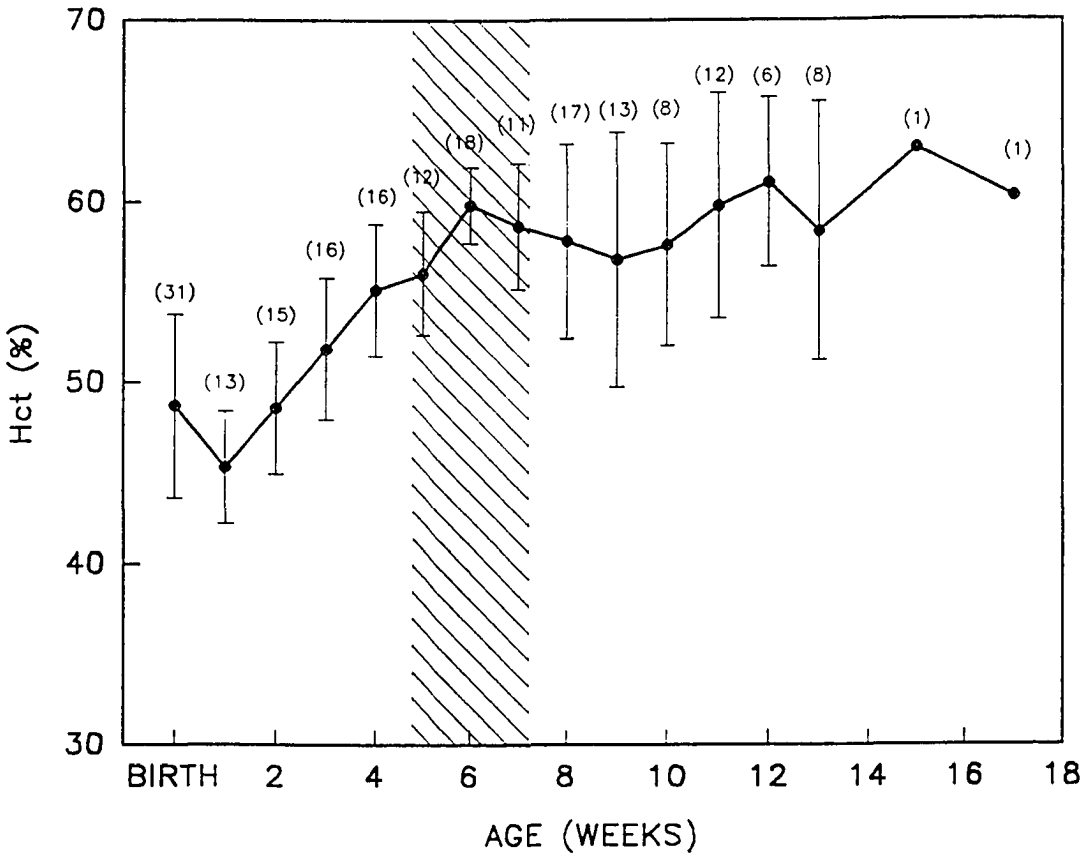

Figure 4.3. Changes in hematocrit (Hct) seen during the suckling, weaning and post-weaning periods in Weddell seal pups. The shaded area represents the estimated weaning period.

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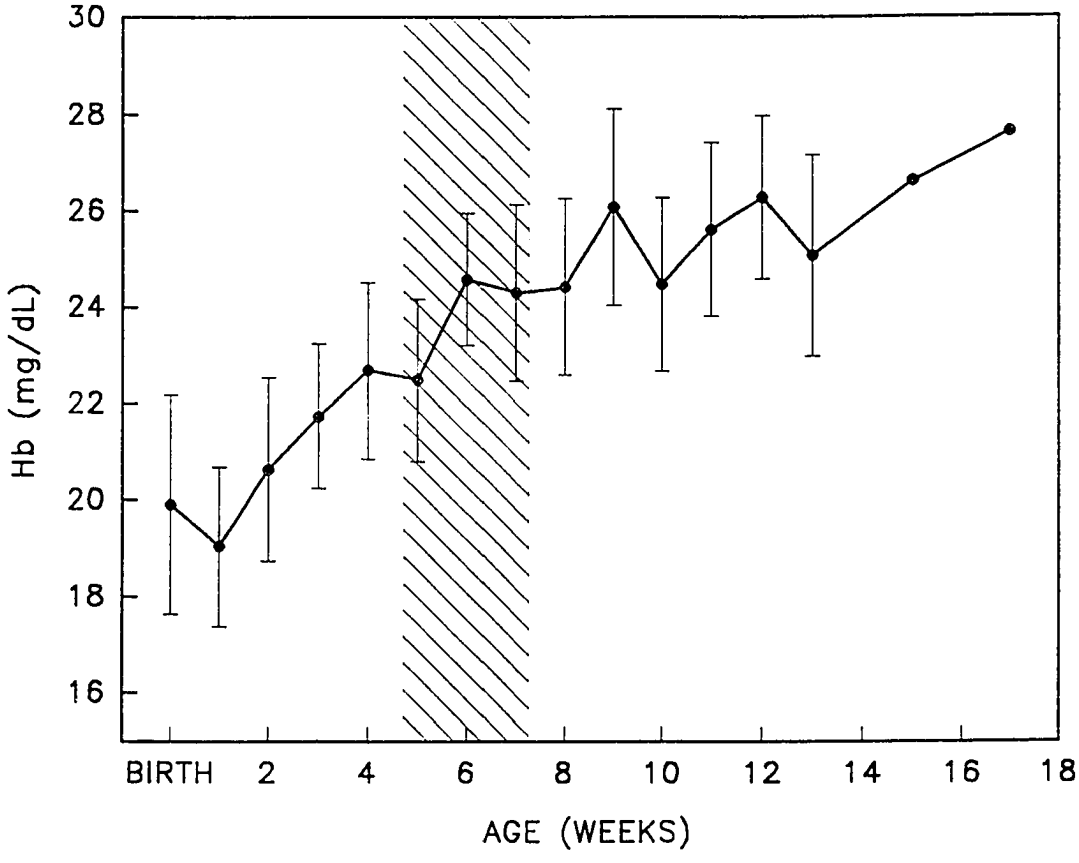

Figure 4.4. Changes in hemoglobin (Hb) concentrations seen during the suckling, weaning and post-weaning periods in Weddell seal pups. The shaded area represents the estimated weaning period.

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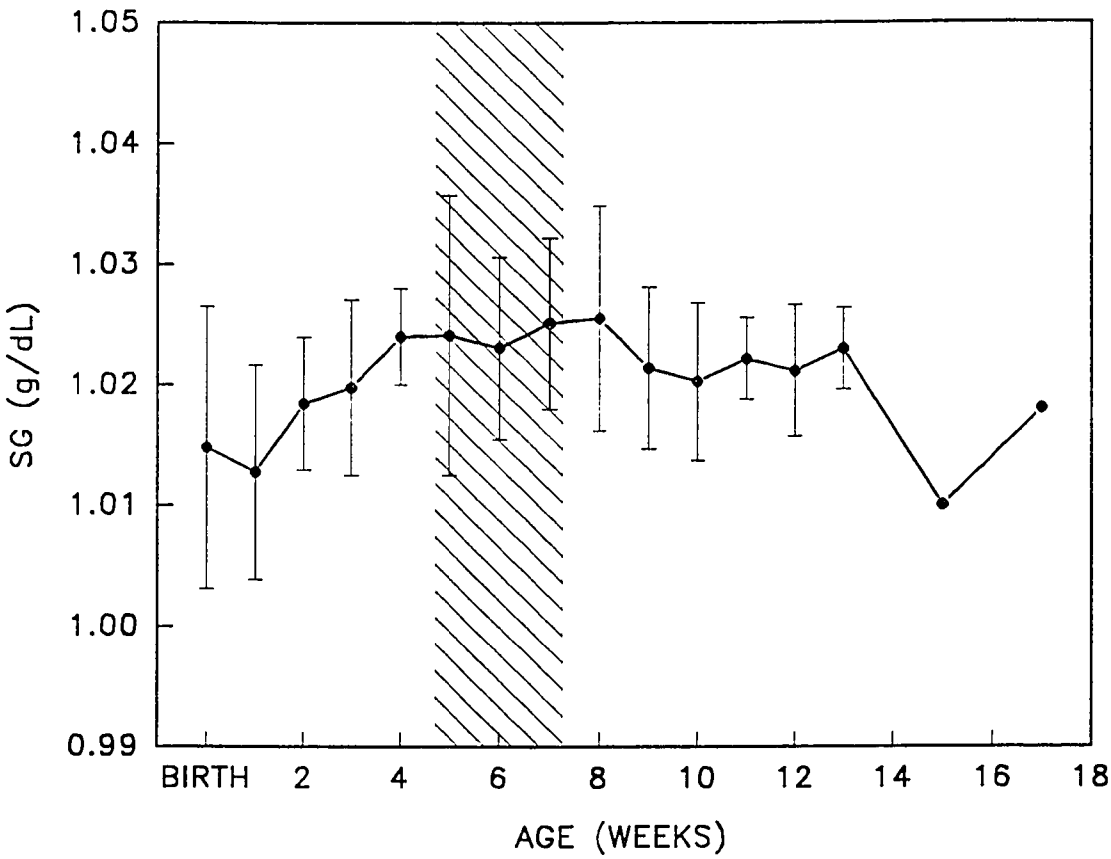

Figure 4.5. Changes in specific gravity (SG) of the plasma seen during the suckling, weaning and post-weaning periods in Weddell seal pups. The shaded area represents the estimated weaning period.

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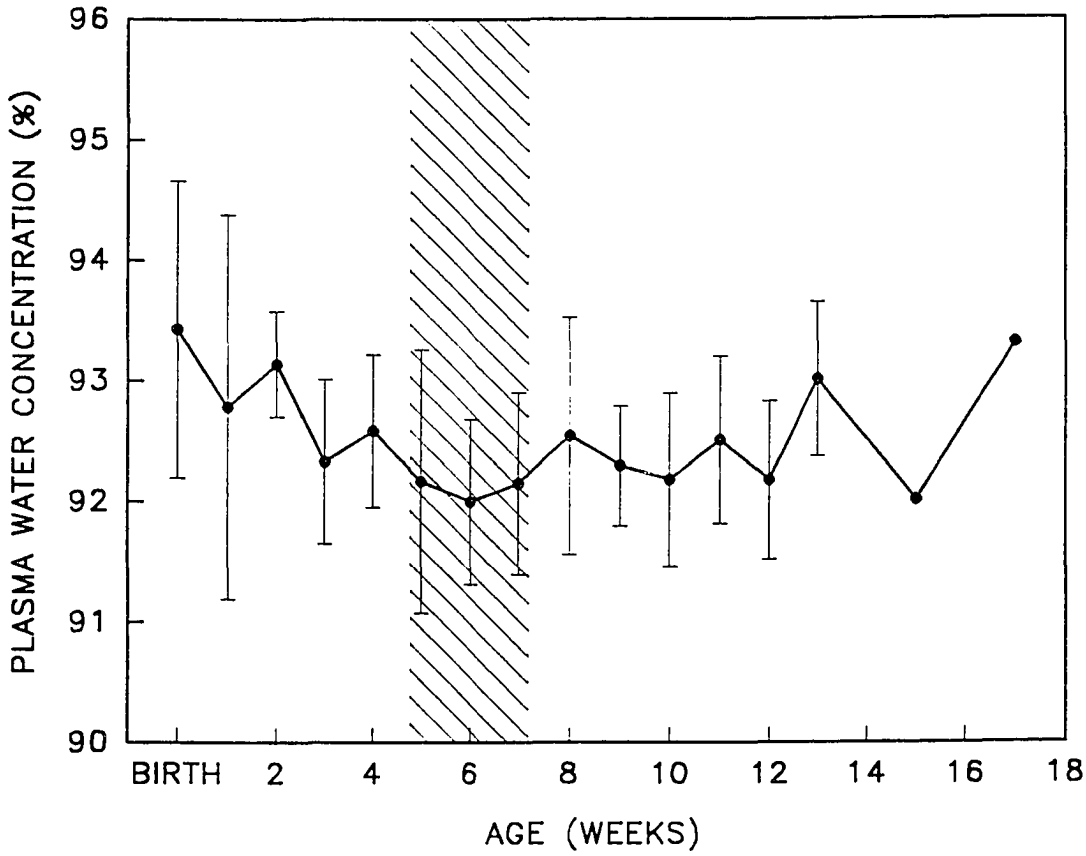

Figure 4.6. Changes in plasma water concentrations seen during the suckling, weaning and post-weaning periods in Weddell seal pups. The shaded area represents the estimated weaning period.

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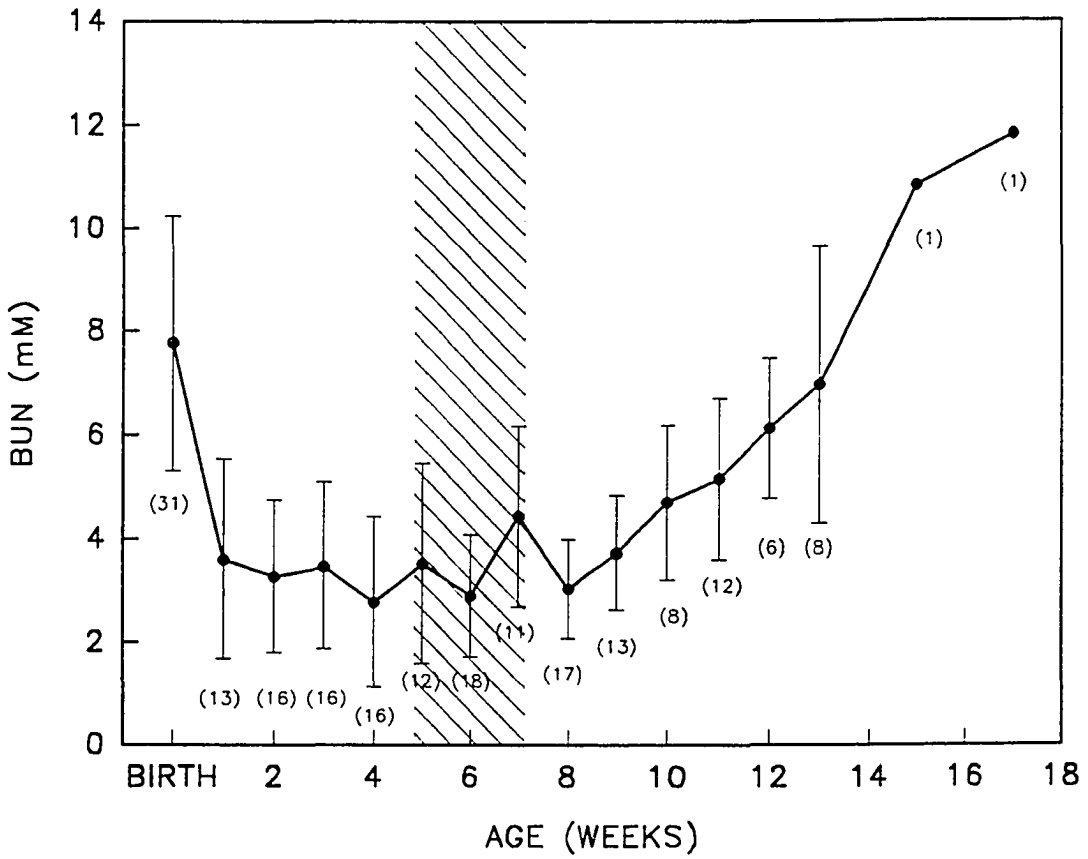

Figure 4.7. Changes in plasma concentrations of blood urea nitrogen (BUN) seen during the suckling, weaning and post-weaning periods in Weddell seal pups. The shaded area represents the estimated weaning period. Sample sizes are indicated in parentheses.

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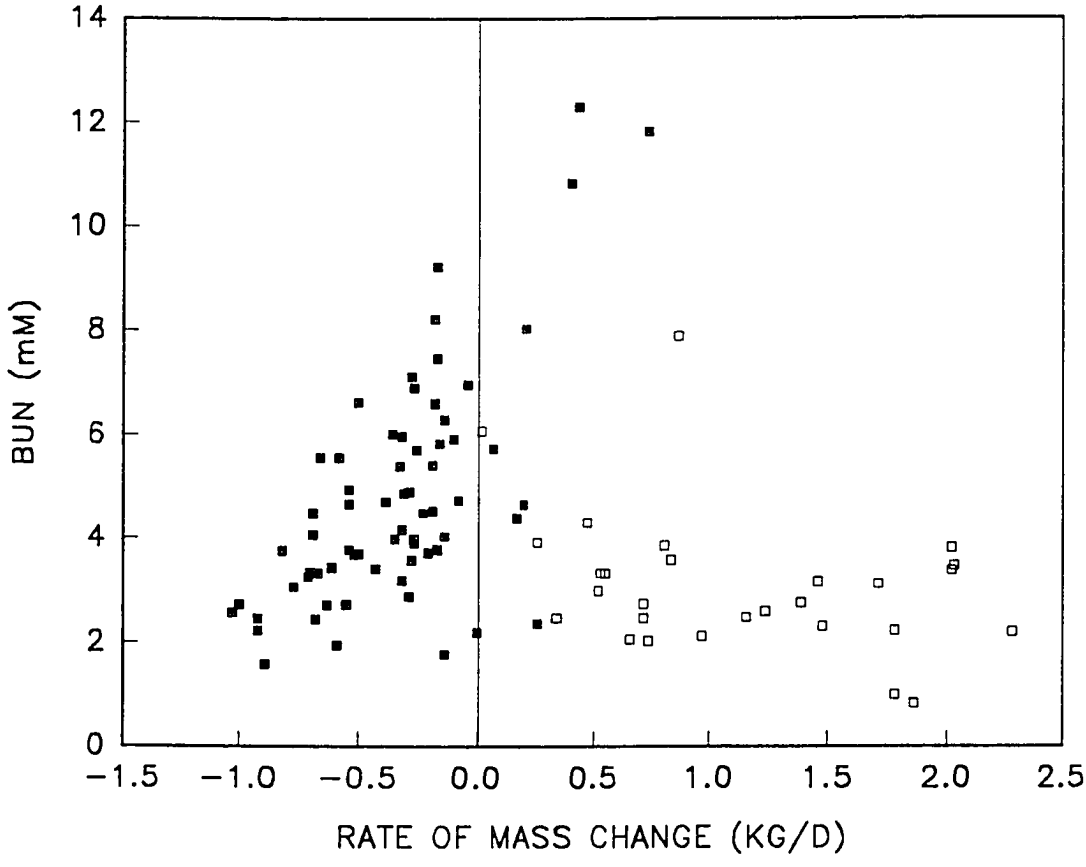

Figure 4.8. Plasma concentrations of blood urea nitrogen (BUN) versus the rate of mass change seen during the preceding 2 week interval. Open squares represent data collected during the week of maximum suckling mass and solid squares represent data collected during the post-weaning period.

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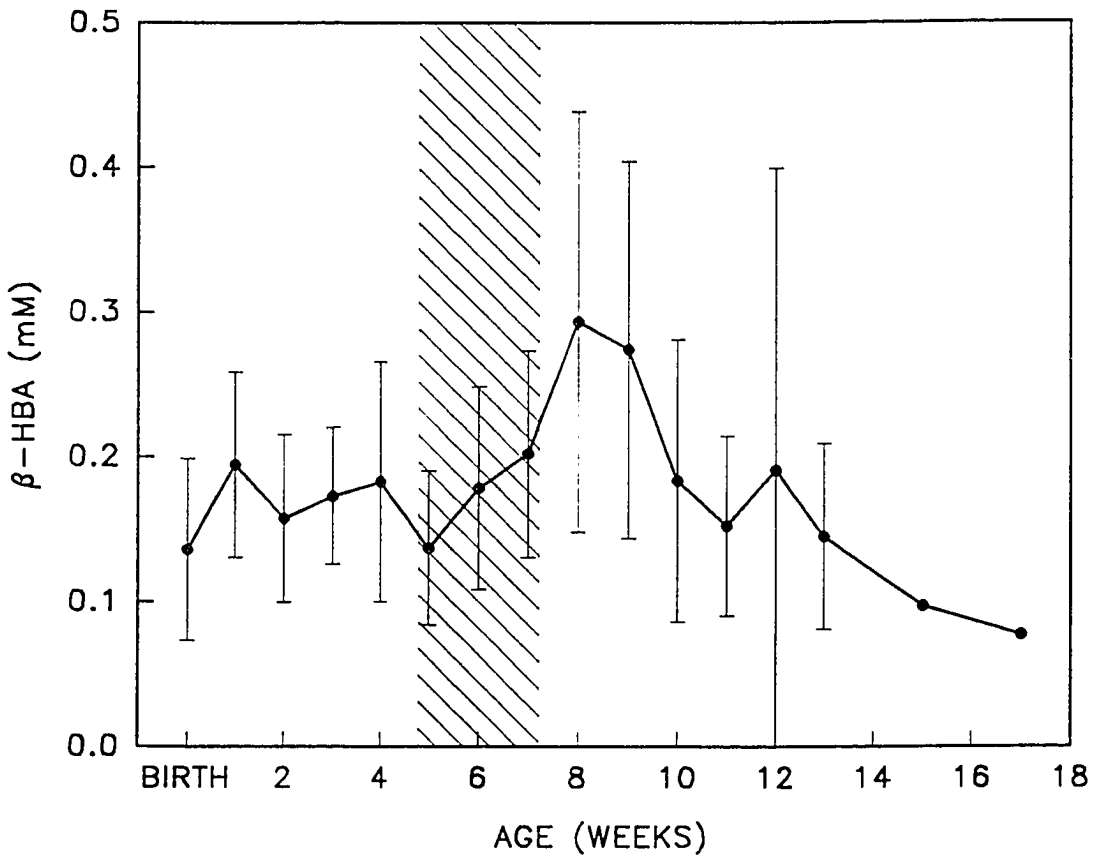

Figure 4.9. Changes in plasma β-hydroxybutyrate (β-HBA) concentration seen during the suckling, weaning and post-weaning periods in Weddell seal pups. The shaded area represents the estimated weaning period.

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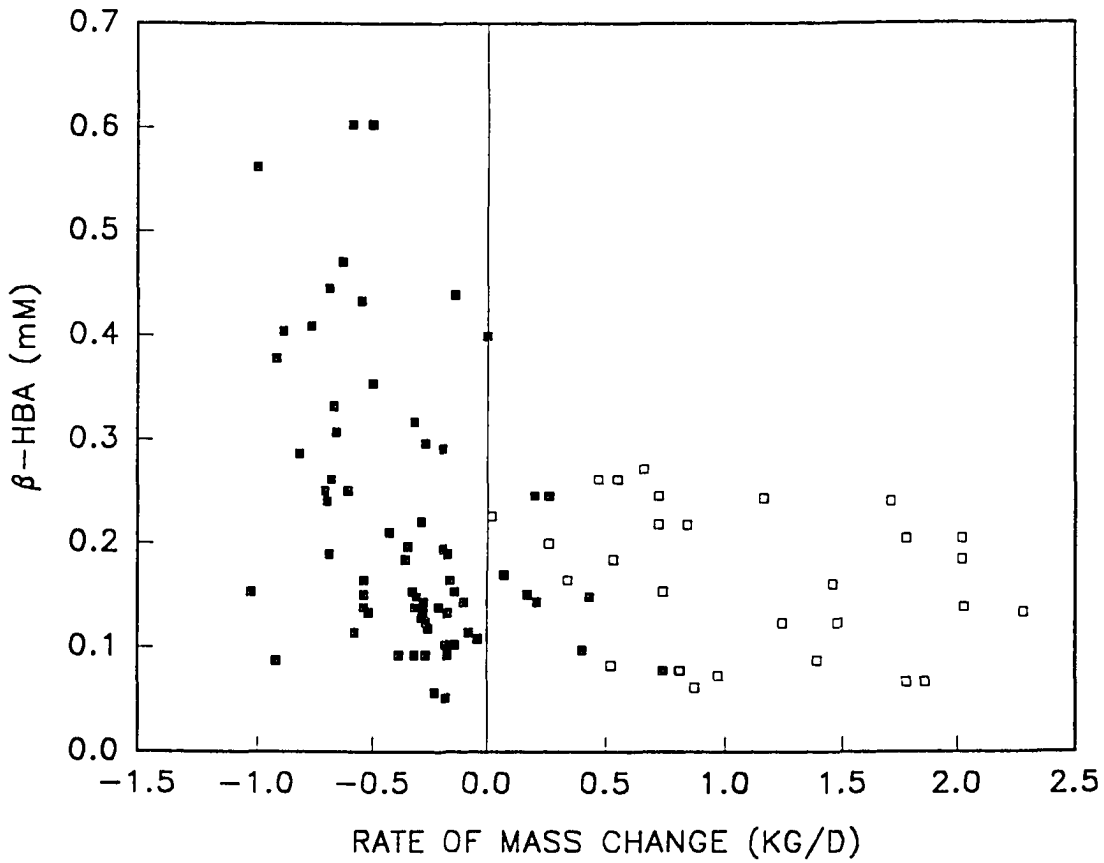

Figure 4.10. Plasma β-hydroxybutyrate (β-HBA) concentration versus the rate of mass change seen during the preceding 2 week interval. Open squares represent data collected during the week of maximum suckling mass and solid squares represent data collected during the post-weaning period.

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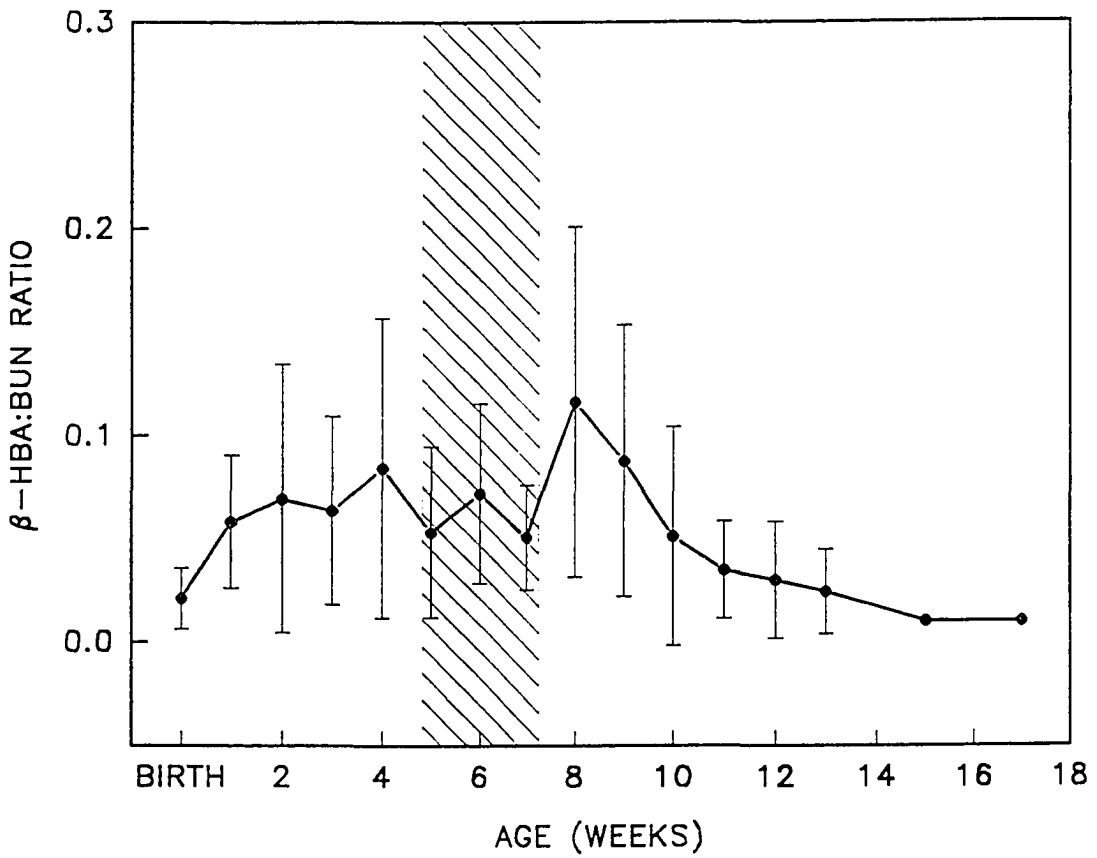

Figure 4.11. Changes in the ratio of β-hydroxybutyrate to blood urea nitrogen (β-HBA:BUN) concentrations seen during the suckling, weaning and post-weaning periods in Weddell seal pups. The shaded area represents the estimated weaning period.

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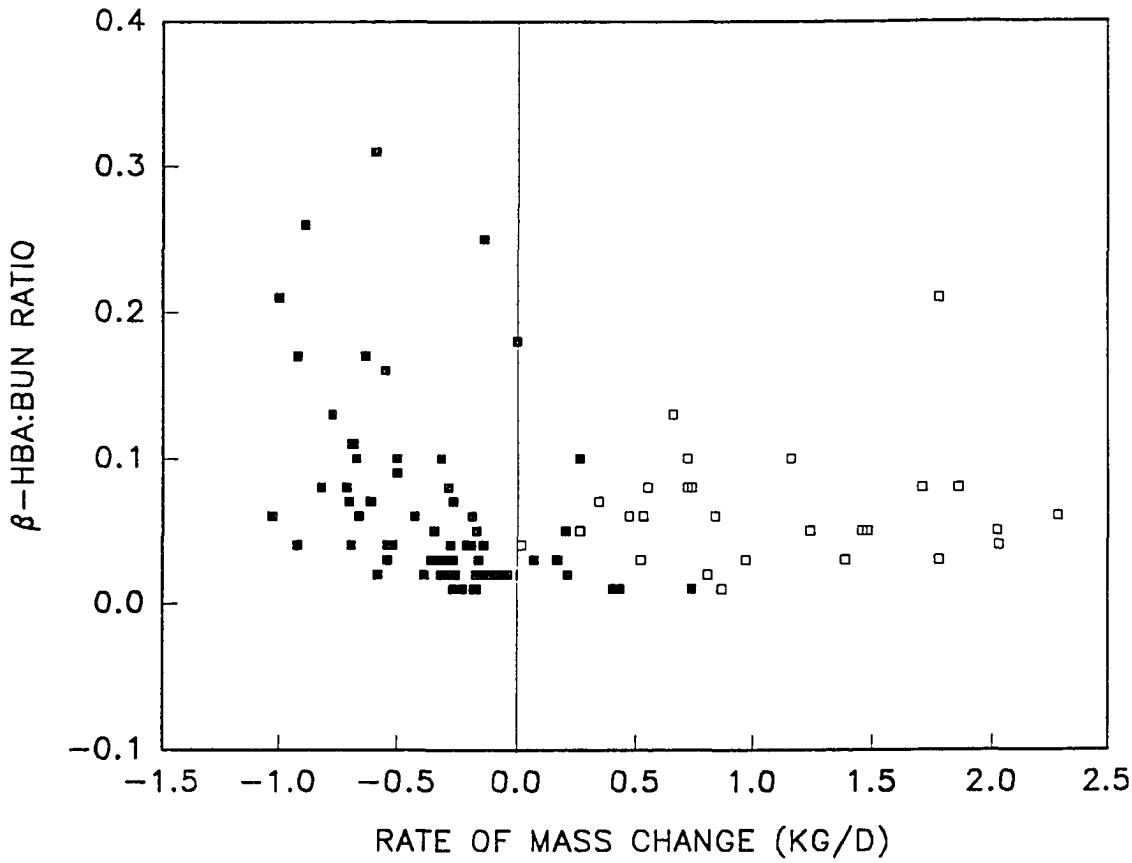

Figure 4.12. The ratio of β-hydroxybutyrate to blood urea nitrogen (β-HBA:BUN) concentrations versus the rate of mass change seen during the preceding 2 week interval. Open squares represent data collected during the week of maximum suckling mass and solid squares represent data collected during the post-weaning period.

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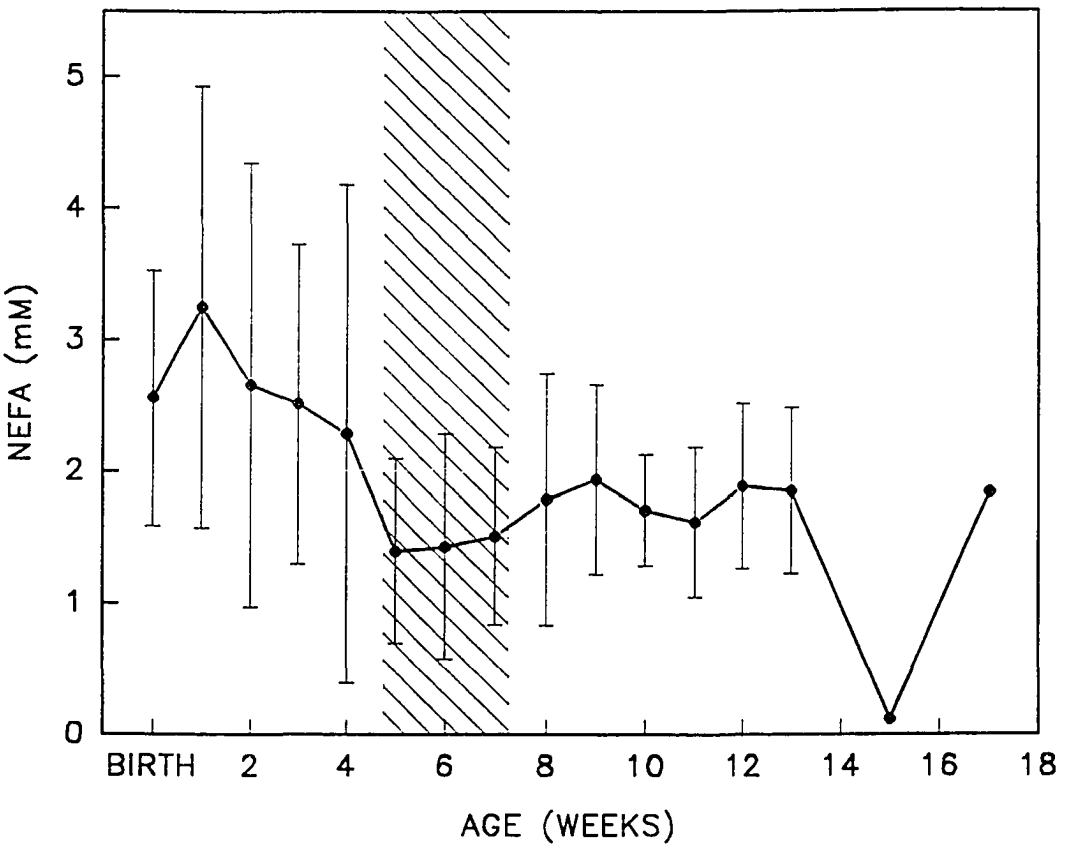

Figure 4.13. Changes in plasma non-esterified fatty acid (NEFA) concentrations seen during the suckling, weaning and post-weaning periods in Weddell seal pups. The shaded area represents the estimated weaning period.

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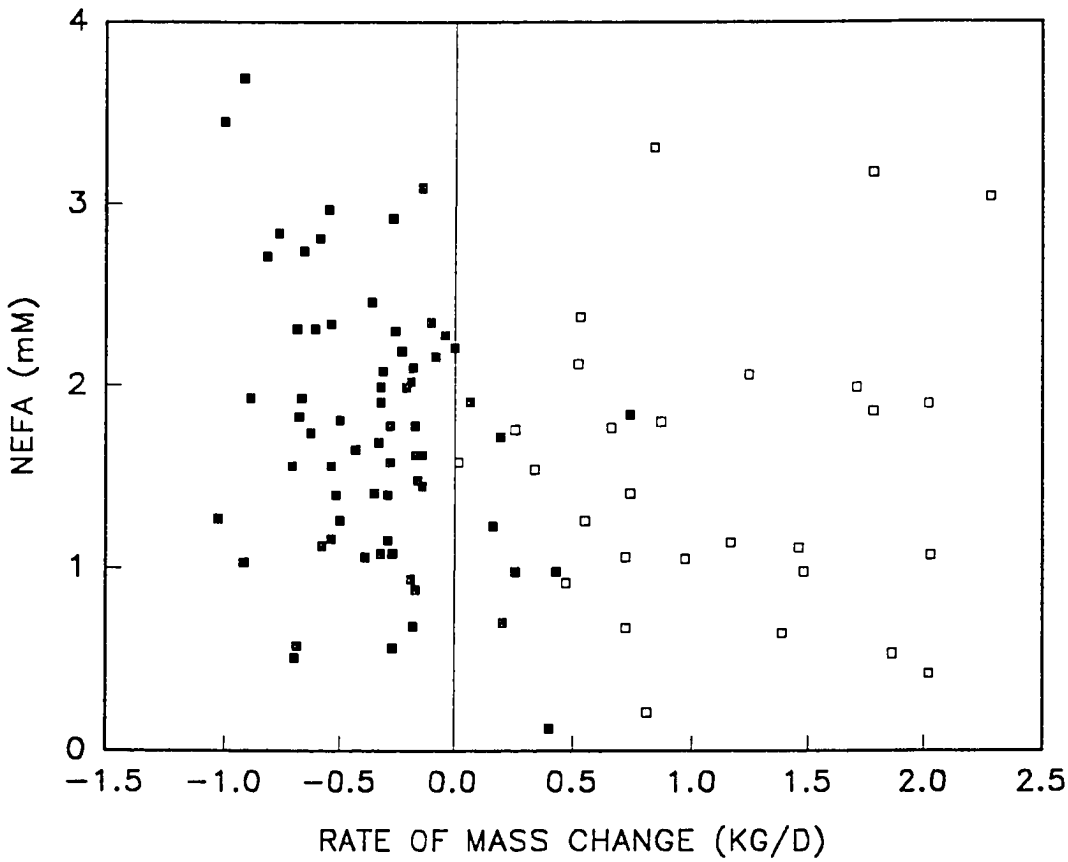

Figure 4.14. Plasma non-esterified fatty acid (NEFA) concentration versus the rate of mass change seen during the preceding 2 week interval. Open squares represent data collected during the week of maximum suckling mass and solid squares represent data collected during the post-weaning period.

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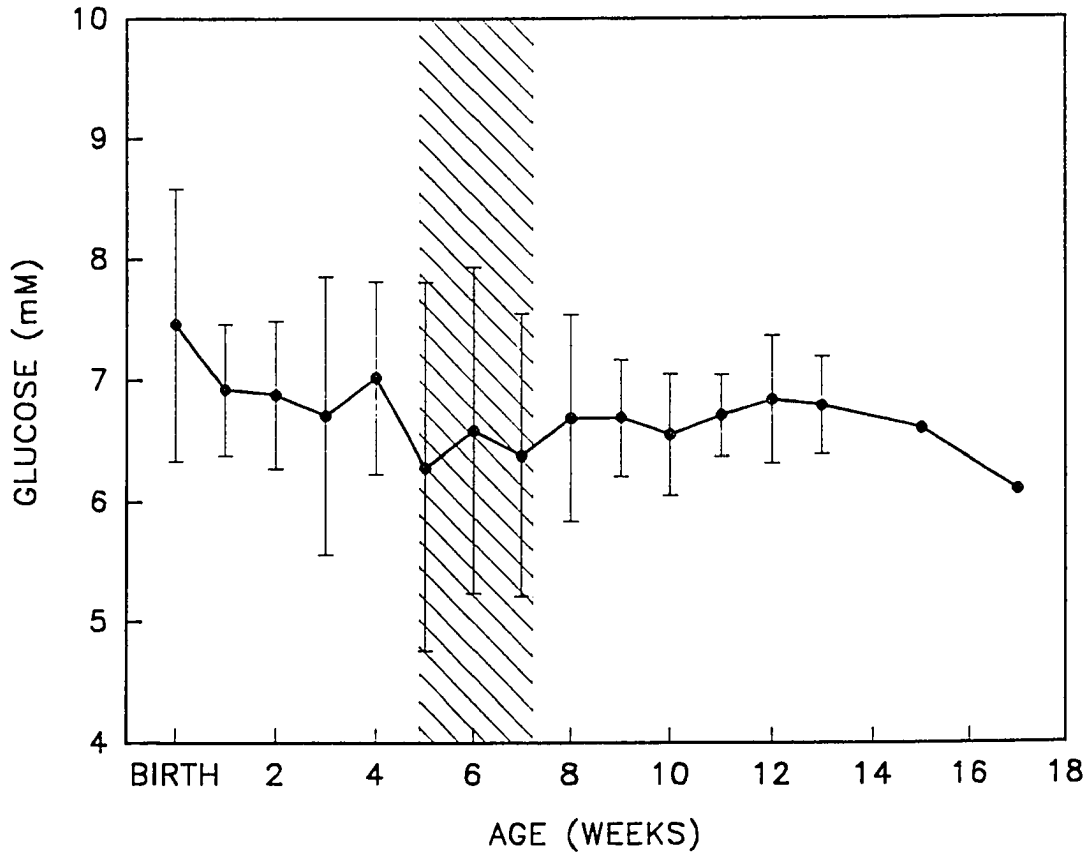

Figure 4.15. Changes in plasma glucose concentration seen during the suckling, weaning and post-weaning periods in Weddell seal pups. The shaded area represents the estimated weaning period.

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Chapter 5. Health status of young Alaskan Steller sea lion pups as indicated by blood chemistry and body condition.

INTRODUCTION

Alaskan Steller sea lion populations in the Gulf of Alaska and Aleutian Islands have declined by over 70% since the 1960's (Merrick et al. 1987; Loughlin et al. 1992; Sease et al. 1993; Mello 1995). Between 1992 and 1994, a 13% drop was seen in the Aleutian Islands while Gulf of Alaska numbers declined by 10% (Mello 1995). In comparison, populations in Southeast Alaska have remained relatively stable. One hypothesis suggests that recruitment failures could contribute to the pattern of declines (York 1994). However, it is unknown at what age recruitment might be failing. Perhaps a decrease in pup survival due to disease or to inadequate nursing could contribute to a decline in juvenile recruitment.

Body condition, blood chemistry and hematology were examined in 168 Steller sea lion pups to test the hypothesis that pups less than one month of age were nutritionally or physiologically compromised such that they may be unable to survive the nursing period. Of particular interest were physiological factors that would make pups poor divers (thus limit foraging ability) or evidence of metabolic disorders that would decrease their ability to survive to nutritional independence. This approach has been used previously to investigate the health and nutritional condition of marine and terrestrial mammal species (Seal et al. 1975; Geraci et al. 1979; McConnell and Vaughan 1983; Kuiken 1985; Payne and Payne 1987; DelGiudice et al. 1992;

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Castellini et al. 1993).

Body mass, standard length (SL) and axillary girth (AG) are routinely used for body condition indices in mammals. Plasma concentrations of blood urea nitrogen, glucose, non-esterified fatty acids and β-hydroxybutyrate reflect protein, carbohydrate and fat utilization, respectively, and may indicate conditions of fasting or starvation (Castellini and Costa 1990; Nordøy and Blix 1991; Castellini and Rea 1992; Kirby 1992; Nordøy et al. 1992). Additional veterinary clinical profiles of blood chemistry are used to identify metabolic and/or physiological problems. Hydration state of the blood is reflected in the specific gravity and water content of the plasma and whole blood (Castellini et al. 1990) and the oxygen carrying capacity is defined by the hematocrit, hemoglobin concentration and mean corpuscular hemoglobin concentration (MCHC) of the blood.

Together these morphological and physiological variables can provide a profile from which to judge the relative health of individual pups up to one month old. Data were considered for each of the separate areas (Aleutian Islands, Gulf of Alaska and Southeast Alaska) to identify potential correlations with areas of greater population decline. The Aleutian Islands area has exhibited more dramatic population declines than the Gulf of Alaska, prompting these areas being reported individually in Sease et al. (1993) and Mello (1995). In addition, these morphometric and blood values can be compared with historical samples collected in California before the decline (Hubbard 1968), with values for other pinnipeds (Hunter and Madin 1976; Cargill et al. 1979; Bossart and Dierauf 1990; Roletto 1993) and with recent studies of Steller sea lion

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pups in more limited areas (Castellini et al. 1993).

METHODS

Animal collection

One hundred and sixty-eight Steller sea lion pups were captured for study at 10 locations in the Gulf of Alaska, the Aleutian Islands, and Southeast Alaska during June and July of 1991, 1992, 1993 and 1994 (Table 5.1). With the exception of two locations, pups were randomly selected from haul-out areas after the adults were cleared from the beach. Pups ranged in age from newborn (with fresh umbilicus) to approximately four weeks of age (based on the extent of physical development). Exact ages of most pups were not known.

Morphometrics

Beginning in 1992, pups were weighed in a hoop net using an electronic hanging load cell (Ohaus Model I-20W, capacity of 500 kg $\pm$ 0.1 kg). Before this, pups were weighed using a mechanical scale ($\pm$ 1.0 kg). Standard length (SL, straight-line measure from the tip of the nose to tip of tail; not along the curved dorsal surface) and axillary girth (AG, upon exhalation) were measured while the animals were restrained flat with their abdomen on the ground. Mass data reported for the Aleutians and Gulf of Alaska are a subsample of the data reported by Merrick et al. (1995). Morphometric data for Southeast Alaska were provided by E. Brandon, R. Davis and T. Williams (pers. comm.). Morphometric measurements were used to derive two indices of condition. A condition index (CI) was calculated as (AG/SL)·100

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(Pitcher 1986, Ryg et al. 1990). A density index (DI) was calculated using body mass divided by a volume index ((Mass/SL·AG^2)·10^6) following Castellini and Calkins (1993) as modified by Fadely (in press)). Larger values for CI reflect a greater relative girth, while larger values for DI infer a greater mass per volume, consistent with a higher density and therefore leaner body condition. To normalize these ratios, CI and DI data were arcsine transformed for statistical analysis.

Blood collection and analysis

Blood samples (approximately 20 mL) were drawn from the pelvic venous plexus of manually restrained pups with 18 or 20 gauge needles directly into heparinized Vacutainer® blood collection tubes. Samples were held on ice until return to the ship or laboratory less than four hours later. Heparinized whole blood was then rewarmed and gently mixed. Hematocrit was determined in duplicate using a battery operated field microhematocrit centrifuge (Compur M1100, samples spun at 5400 g (11500 rpm) for 3.5 min) or a standard clinical microhematocrit centrifuge (IEC MB Centrifuge, samples spun at 13460 g (11500 rpm) for 3 to 5 min). Hemoglobin concentration was measured spectrophotometrically using methanocyanide (Sigma Chemicals Kit 525-A). The remaining blood samples were centrifuged and plasma removed and frozen. Samples were stored at -20°C while in the field (shipboard up to 3 weeks) then transferred to a -80°C freezer at UAF until analysis. Glucose and lactate concentrations of the plasma were measured using a YSI Model 2300 STAT glucose/L-lactate autoanalyzer. Methods for the determination of water content and SG of the plasma and whole blood, and the concentrations of BUN, NEFA and β-HBA

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were previously described by Castellini et al. (1993). All values are presented as mean ± standard deviation (SD).

Additional 5 mL blood samples from 52 pups were collected into Vacutainer® blood collection tubes with EDTA additive for later analysis of complete blood count (CBC). Blood smears were made on glass slides for differential white blood cell counts. Samples were then refrigerated in a dark container for 2 to 10 days until total red blood cell and white blood cell counts were performed using a Coulter S-Plus IV at Fairbanks Memorial Hospital, Fairbanks, AK. Clinical chemistry profiles were run on a Kodak Ektachem analyzer using frozen plasma from 62 pups. Ten plasma samples analyzed from Southeast Alaska had no accompanying EDTA whole blood for CBC analysis.

Statistical analyses and sample grouping

One-way analysis of variance (ANOVA) was performed to identify significant differences in morphometrics, metabolite concentrations or water content in pups among areas or age groups ($p \leq 0.05$; Statistix®). For recaptures, only data collected on the first capture were included in the statistical analysis. Captures at Chirikof Island in 1993 and at Marmot Island in 1994 were treated separately in the statistical analysis, because pups were not selected randomly from the population as at other locations. On-going studies at these locations necessitated the selection of neonatal pups, thus biasing the sample towards younger (presumably smaller) pups. Previously published results from pups captured at Marmot Island, Gulf of Alaska in 1990 and 1991 (n=18; Castellini et al. 1993) were also included for comparison. At Chirikof

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Island in 1993, 11 pups of known birth dates were sampled as neonates (1-3 days of age), 8 were recaptured at 4-6 days and all 11 were again handled at 21-30 days of age. Paired Student's t-tests were used to detect significant differences between the measured parameters for the recaptured pups ($p \leq 0.05$). These samples provide the only data available on how metabolic chemistry and body condition change with the age of Steller sea lion pups during the first month of life.

In total, 33 pups were sampled between birth and 3 days of age (based either on known date of birth or the presence of a fresh umbilical cord) and are reported separately as neonatal values. A separate statistical analysis was performed among neonates and older pups captured over the entire range. In addition, samples were collected from five emaciated pups (presumably abandoned or orphaned pups). These data were treated separately and reported as orphaned pups. Similarly, two pups found injured on the rookeries were reported separately.

RESULTS

Morphometrics

Random pups

Pups captured in the Aleutian Islands were of larger mass and AG than those sampled in the Gulf of Alaska or Southeast Alaska. There were no significant differences seen in body morphometric measurements for pups captured in the latter two areas (Table 5.2). The Aleutian pups showed a significantly increased CI and decreased DI when compared with Gulf of Alaska pups. Although the CI of Aleutian

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pups was also higher than seen in pups from Southeast Alaska, there was no significant difference in DI values. Throughout the range, male pups were of larger mass, AG and SL than females (Table 5.3). However, there were no differences in CI or DI between sexes. To correct for differences in capture date among rookeries, Pearson correlation tests were utilized to examine correlations between measured variables and the date of sampling ($p \leq 0.05$). All morphometric measurements were significantly correlated with the julian date of sample (p values ranged from <0.0001 to 0.0013). However, there was no significant correlation between date and the area of study or the sex of the pup (p=0.10 and p=0.94 respectively).

Neonates and orphans

The five emaciated orphaned pups were of unknown age, but they were within the same size range of neonatal pups (Table 5.2). Neither CI nor DI could distinguish among neonates, orphans, or older pups. Morphometric data were available for only one of the two injured pups observed during this study. The body mass (14.7 kg) was similar to that seen in the two smallest orphans, and the AG (52 cm) was the smallest measured in this study. However, the SL (97 cm) fell within the ranges observed for all other groups. The calculated DI could not distinguish this injured pup from any other group. However, CI was significantly lower than those seen in neonates and older pups.

In recaptured pups studied at Chirikof Island in 1993, no change in mass was seen during the first week, but by four weeks of age, mass had increased from 21.9 ± 2.4 kg to 32.7 ± 2.8 kg accompanied by significant increases in both AG and SL

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(Table 5.4). Pup conditions at birth and at 4 weeks of age were not significantly different as assessed by either CI or DI.

Plasma chemistry

Random pups

Blood urea nitrogen and NEFA levels were similar throughout the entire geographical range in the randomly selected pups (Table 5.5). Southeast Alaska pups, however, showed elevated β-HBA and depressed glucose levels when compared with animals of the other two regions. No sex differences were seen in any of the plasma metabolites, hematology measures or in the water content or specific gravity of the plasma (Table 5.3).

Neonates and orphans

Circulating levels of BUN and NEFA were elevated in neonates compared to older pups, but there were no significant differences in β-HBA concentrations (Table 5.5). Orphaned pups also had higher plasma concentrations of BUN and NEFA than those of the older group.

β-hydroxybutyrate was the only plasma metabolite shown to change during the first week in pups studied across time at Chirikof Island in 1993 (Table 5.4). Ketone body concentrations increased 0.10 to 0.36 mM over the first 1 to 3 day period. By 3 weeks of age plasma glucose had significantly increased, while circulating levels of all other metabolites measured declined.

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Hematology

Random pups

There were no significant differences in Hct, Hb or MCHC between the Aleutian Island pups and the Gulf of Alaska animals (Table 5.5). Hemoglobin concentrations of Southeast Alaska pups were slightly higher than those seen in Aleutian Island samples. There was no significant difference in the water content of the plasma of randomly selected pups among the areas. The mean plasma water concentration over the whole range was 93.3 ± 0.98%. There was, however, a higher plasma SG measured in pups captured in SE Alaska, compared with pups from the rest of the range. The water content of the whole blood was closely related to the Hct of the blood sample (Figure 5.1) with the exception of three individuals. Pups labelled A, B, and C showed lower whole blood water content than would be expected, given the hematocrit values measured.

Neonates and orphans

Hematocrit, Hb concentration and calculated MCHC were all significantly higher in neonatal pups than in older pups (Table 5.5). Hematocrit, Hb and MCHC values measured in orphaned pups were not significantly different from either group. In recaptured pups, Hct declined by more than 10% over the first month and Hb fell from 17.2 ± 2.5 g/dL at birth to 11.5 ± 1.6 g/dL at four weeks of age (Table 5.4). There was no significant difference in the plasma SG or the plasma water content among these three groups. Orphans and neonates did not depart from the regression line values of Hct and whole blood water concentration (Figure 5.1).

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In the Chirikof pups, plasma water concentration did not change significantly during the first week. However, a significant increase was seen by one month of age. No change was observed in the SG of the plasma with age of the pup.

Clinical chemistry and hematology

Random pups

The clinical chemistry panels for pups captured in Southeast Alaska showed significant differences when compared with pups in the other two areas (Table 5.6). Southeast pups showed lower glucose concentrations than other pups and higher concentrations of bilirubin, total protein, albumin, AST and creatine phosphate. Alkaline phosphatase levels were low compared with Aleutian pups. Plasma concentrations of sodium were significantly different in Gulf of Alaska and Aleutian pups. There were no other differences seen among pups from these two areas.

Similarly, Southeast Alaska pups showed significantly higher red blood cell (RBC) counts, Hb concentration, and MCHC than pups from the other two areas (Table 5.7). Conversely, mean cell volume (MCV) and monocyte counts were lower in Southeast animals than in pups from the Aleutians. There were no other differences seen in the hematology of the blood among pups from the three different areas.

DISCUSSION

Morphometrics

When corrected for sexual dimorphism, Aleutian pups are bigger than those captured in the Gulf of Alaska or in Southeast Alaska. Merrick et al. (1995) suggest

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that this is a geographical trend of decreasing pup mass that extends to the Oregon populations. Since pups in the areas of population decline are larger than those in the non-decline areas, and larger than in the historical measurements, they concluded that there is no widespread decline in early pup condition. It is unlikely that the difference in pup mass in various areas studied is related to pup age. There were no significant differences in hematological parameters or plasma water values, which were shown to change with increasing age of the pup.

Results of the CI analysis show that pups sampled in the Aleutian Islands were of greater girth for a given length and had a lower DI, suggesting a higher proportion of body fat. However, the inability of the CI or the DI distinguish the body condition of visibly emaciated, orphaned pups and healthy neonates suggests that these indices are not sensitive enough to accurately detect differences under these conditions. Both condition models assume that changes in AG adequately reflect condition changes over the entire body. Blubber thickness or girth changes occurring at other areas of the body may not be strongly correlated with changes in AG (Beck et al. 1993) and thus would not be reflected in the CI. McLaren and Smith (1985) suggested that morphometric indices alone may not be sensitive enough to indicate changes in condition and may require other physiological indices, such as blood variables, to discriminate differences.

Plasma chemistry

With the exception of pups sampled in Southeast Alaska in 1992, BUN, β-HBA and NEFA levels seen in the randomly sampled pups were similar to those seen

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in other pinniped species which were feeding or that had undergone a short fast (Nordøy and Blix 1991; Kirby 1992; Nordøy et al. 1992). In particular, newly weaned elephant seal pups show ketone body concentrations that range from 0 to about 0.3 mM (Castellini and Costa 1990). Approximately 80% of the older sea lion pups studied fell within this range. This is consistent with a maternal investment strategy where adult Steller sea lion females suckle their pups intermittently between short foraging trips to sea. The pups are typically left fasting on the rookery for 1 to 3 days (Oftedal et al. 1987; Higgins et al. 1988). The variability seen in the plasma metabolite concentrations is attributed to differences between pups recently fed and those awaiting the return of the foraging female. However, blood chemistry should be distinctive for pups that have fasted for longer than the standard at-sea foraging period. The magnitude of these potential differences was evident in the orphaned pups. In two of the orphaned pups, elevated β-HBA and FFA values suggest Phase II fasting (protein sparing phase), while the other two pups showed classic signs of entering Phase III fasting (or terminal starvation). In this state, body fat resources have been depleted such that β-HBA and FFA decline and body protein is used as the primary fuel, resulting in dramatic increases in BUN, up to 17 mM in one pup (Nordøy and Blix 1991; Castellini and Rea 1992; Kirby 1992; Nordøy et al. 1992).

The increase in ketone body concentrations seen in 4-7 day old pups at Chirikof Island in 1993 suggest that these pups may not have suckled since first being sampled. The mothers of these pups had been anaesthetized for another study which may have influenced their attendance behavior or milk production. However, these

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pups were not abandoned, and blood samples collected 3 weeks later suggested no long-term adverse effects.

The elevated β-HBA levels in Southeast Alaska pups suggest that they may have been fasting for longer periods than pups from other areas. This is supported by the observation of longer foraging trips made by females at Forrester Island in 1992 (Brandon and Davis, pers. comm.). Over 50% of the pups captured there had β-HBA concentrations above 0.3 mM. Considering how quickly β-HBA concentrations changed in the recaptured pups, this may reflect an increase of only a day or two in the mothers' at-sea foraging interval.

Slightly higher Hb concentrations and SG seen in Southeast Alaska pups were probably not due to dehydration, since the water content of the plasma was within ranges for other pinnipeds (Castellini et al. 1990). Unfortunately, there were no other hematological values available for these animals.

Hematology

Mean Hct, Hb and MCHC values reported in this study fall within the ranges reported by Roletto (1993) for juvenile California sea lions (Zalophus californianus) and by Bossart and Dierauf (1990) for northern fur seal adults (Callorhinus ursinus). Although Hct and Hb values measured here are slightly higher than those found for other Steller sea lion pups by Castellini et al. (1993) or by Hubbard (1968), MCHC values are within the ranges for those previously reported. Hematocrit varies widely in all pinniped species depending on a number of different factors (Castellini and Castellini 1989, Castellini et al. in press). While Hb is directly proportional to Hct,

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MCHC is a ratio of these two variables and is not altered by instantaneous changes in Hct. Two pups sampled in the Aleutian Islands in 1992 had very low MCHC (24.0 and 24.7 $mg \cdot dL^{-1}$) and Hb (8.6 and 9.1 $mg \cdot dL^{-1}$) values. These pups did not show clinical signs of poor health, and there were other indications from plasma metabolite levels that the pups were feeding well.

Consistent with other neonatal mammals (Spensley et al. 1987) neonatal Steller sea lions had high MCHC, Hct and Hb values. Based on data from pups recaptured at Chirikof Island, these variables did not decrease until after the first week post-partum. Spensley et al. (1987) noted that Hct remained high in horse foals until 2 weeks of age, and a significant decrease was evident by 4 weeks. MCHC, Hb and Hct measured in orphans were intermediate to those in the other two groups. Because of the unknown ages of the emaciated pups, it is difficult to determine if these differences in hematology were due to nutritional factors or to age. However, these differences were not likely due to hydration state since they exhibited a higher plasma water content than seen in either neonates or randomly selected older pups and showed the same relationship between Hct and whole blood water content as seen in other pups in this study.

The regression line calculated for Hct versus whole blood water content in the Steller sea lion pups of this study was compared with data available for northern elephant seal pups (Mirounga angustirostris). Three individual pups showed lower water content of the whole blood than would be expected, given the Hct of the samples collected. Pup A was found injured and immobile on the beach during a pup

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census, possibly with a broken or dislocated pectoral flipper. The remaining two pups (B and C) were neonates sampled at Chirikof Island in 1992, and both showed higher whole blood SG than many of the other pups (1.047 $g \cdot mL^{-1}$). These pups were observed on the rookery 3 weeks later, and blood samples showed no unusual parameters at that time. In particular, plasma SG had decreased to 1.025 $g \cdot mL^{-1}$ (from 1.047 $g \cdot mL^{-1}$) and whole blood water content had increased to 83.3%. All other pups, including the remaining neonates and orphaned pups, fell within the 95% confidence limits of the regression line.

Neonates had a lower plasma water content than older pups, unlike the trend seen in phocids, which have high plasma waters at birth and gradually decrease throughout the suckling period (Castellini et al. 1990). Interestingly, otariid milk shows an opposite trend to that of phocid milk with high fat and low water content at parturition and decreasing fat and increasing water concentrations over the first 10 days (Costa and Gentry 1986). Low water values in the recaptured pups on Chirikof Island persisted over the first week. This is within the time frame of the first half of the perinatal suckling period (3 to 12 days; Higgins et al. 1988).

Clinical chemistry and hematology

Although statistically significant differences in plasma ion levels between the Gulf of Alaska and the Aleutian pups were measured, sodium concentrations for pups in both areas fall within ranges reported for clinically healthy juvenile California sea lions (140 - 154 mM; Roletto 1993). Caution must be used when making comparisons with published values as it is difficult to compare blood chemistry profiles run by

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different analytical laboratories without some indication of the degree of variability between those labs.

Several differences were seen in clinical plasma chemistries among the Southeast Alaskan pups and those captured in other regions of Alaska. Low glucose levels, with high bilirubin, albumin and total protein concentrations may indicate that the 15 pups sampled in Southeast Alaska for clinical chemistry were of a younger developmental stage than those sampled over the rest of the range (Kerr 1989).

Clinical blood chemistry values measured for Alaskan pups were significantly different from levels reported for pups in California in the 1960's (Hubbard 1968). Sodium, potassium, BUN, total bilirubin and total protein levels were much higher in Hubbard's pups, whereas glucose and albumin concentrations were much lower than measured in Alaskan samples. Apart from interlab analytical variability, the age composition of Hubbard's pups was younger than that of the Alaskan pups reported here. Seven of their ten pups were classified as 1 week old. High BUN, and low glucose concentrations were also seen in the neonates of this current study. High bilirubin concentrations were also reported for neonatal common seals (McConnell and Vaughan 1983).

Conclusion

Several of the variables investigated in this study are useful in identifying gross physiological problems in body condition or metabolic chemistry that would impact the ability of a pup to survive the nursing period. Plasma metabolite concentrations of BUN and β-HBA proved to be sensitive indicators of nutritional status, and

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hematology factors such as Hct, Hb and water content of the blood showed typical mammalian relationships. Others, such as CI and DI, did not possess the sensitivity to identify differences in body condition over small ranges of body size. The clinical plasma chemistry profiles showed no indication of general poor health for pups from any of the areas studied. It was also apparent that many blood parameters change significantly during the pups' first month of growth, and these developmental influences must be considered in any interpretation.

Since only pups up to 5 weeks of age could be studied, these findings can only suggest the health status of pups up to that stage of nursing. It cannot be assumed that healthy neonatal pups will be successfully reared by the female to weaning or that they will be able to forage effectively, independently at sea. With the few exceptions noted, the Steller sea lion pups examined in the areas of decline were healthy, robust individuals that exhibited no obvious indications of poor health or nutritional condition.

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Table 5.1. Locations of Steller sea lion captures during the 1991-1994 research cruises and for data cited from Castellini et al. (1993), indicated by an asterisk.

AREA	LOCATION	YEAR	Total n	Neonates n	Orphans n
Gulf of Alaska	MARMOT*	1990	9	1	
	MARMOT*	1991	9		1
	MARMOT	1993	11		3
	MARMOT	1994	10	6	
	ATKINS	1991	9		1
	ATKINS	1993	10		
	SUGARLOAF	1992	10	2	
	CHURNABURA	1992	7		
	CHIRIKOF	1993	22	20	
Aleutian Islands	UGAMAK	1991	9		
	UGAMAK	1993	10		
	AKUN	1991	6		
	AKUTAN	1992	11		
	BOGOSLOF	1993	10		
Southeast Alaska	LOWRIE	1992	13		
	LOWRIE	1994	13	4	

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TABLE 5.2. Body morphology of Steller sea lion pups in Alaska. Data are represented as mean ± (SD), with sample size noted below.

AREA	Mass (kg)	SL (cm)	AG (cm)	CI	DI
ALEUTIAN ISLANDS	32.9 (6.0) 45	107.2 (6.0) 41	78.9 (7.3) 41	74.1 (3.8) 40	48.2 (3.1) 40
GULF OF ALASKA	28.2 (5.5) 57	104.8 (5.7) 57	72.8 (6.1) 56	69.5 (3.9) 56	50.3 (3.9) 56
SOUTHEAST ALASKA	26.7 (4.6) 21	105.2 (8.0) 21	70.8 (4.9) 21	67.5 (4.0) 21	50.3 (2.3) 21
GULF OF ALASKA NEONATES	20.8 (2.5) 33	95.9 (5.4) 32	65.4 (3.9) 32	68.3 (5.0) 32	50.6 (3.9) 32
GULF OF ALASKA ORPHANS	16.7 (2.6) 5	92.2 (6.9) 5	61.3 (3.8) 5	66.8 (6.7) 5	47.9 (2.2) 5

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Table 5.3. Sex differences in blood chemistry and body morphology of Steller sea lion pups (including neonates). Values are represented as mean ± (SD), with sample size noted below. * denotes significant difference between means at $p \leq 0.05$.

Sex	Mass (kg)	SL (cm)	AG (cm)	CI	DI	Glucose (mM)	BUN (mM)	NEFA (mM)	β-HBA (mM)	Hct (%)	Hb (mg/dL)	MCHC (mg/dL)	SG (g/mL)	H_2O (%)
Males	29.7*	105.5*	74.7*	70.4	50.4	8.6	5.3	1.9	0.21	41.2	14.5	35.0	1.014	93.4
	(6.7)	(7.0)	(7.5)	(5.4)	(5.3)	(2.2)	(1.9)	(1.3)	(0.14)	(6.5)	(2.8)	(3.2)	(0.005)	(0.9)
	89	84	83	94	94	88	88	87	88	66	73	66	84	81
Females	25.0*	101.1*	69.8*	69.4	49.5	9.1	5.9	1.5	0.22	42.1	15.3	36.4	1.015	93.1
	(5.4)	(6.9)	(6.7)	(4.1)	(4.0)	(3.0)	(2.3)	(0.8)	(0.12)	(6.9)	(3.1)	(3.0)	(0.006)	(1.0)
	73	73	73	86	86	72	72	72	72	54	62	54	72	71

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Table 5.4. Blood chemistry and body morphology for known age pups recaptured at Chirikof Island, June/July 1993. Values are presented as mean ± (SD).

AGE (wk)	n	Mass (kg)	SL (cm)	AG (cm)	Hct (%)	Hb (g/dL)	Glucose (mM)	Lactate (mM)	BUN (mM)	NEFA (mM)	β-HBA (mM)	SG (g/mL)	H_2O (%)
Neonate	11	21.9 (2.4)	95.4 (6.6)	67.0 (4.5)	48.3 (4.6)	17.2 (2.6)	5.9 (1.9)	5.0 (1.8)	8.4 (2.1)	3.2 (1.2)	0.20 (0.11)	1.013 (0.003)	92.9 (0.3)
1	8	21.2 (2.1)			47.8 (3.4)	17.6 (2.4)	6.7 (0.3)	3.9 (0.4)	6.9 (1.3)	3.0 (1.6)	0.48 (0.18)	1.011 (0.003)	92.7 (0.4)
4	11	32.7 (2.8)	107 (4.4)	78.8 (3.9)	32.8 (2.9)	11.45 (1.6)	10.9 (1.8)	4.2 (1.4)	5.8 (1.5)	1.0 (0.6)	0.15 (0.05)	1.010 (0.003)	93.3 (0.3)

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TABLE 5.5. Blood chemistry and hematology of Steller sea lion pups in Alaska. Data is represented as mean ± (SD), with sample size noted below. ND denotes no data available.

CATEGORY	Glucose (mM)	BUN (mM)	NEFA (mM)	β-HBA (mM)	Hct (%)	Hb (mg/dL)	MCHC (mg/dL)	SG (g/mL)	H_2O (%)
ALEUTIAN ISLANDS	10.0 (1.9) 45	4.9 (1.7) 45	1.4 (0.8) 45	0.18 (0.10) 45	38.0 (3.9) 40	13.1 (1.6) 42	34.5 (2.2) 40	1.013 (0.004) 42	93.3 (0.5) 40
GULF OF ALASKA	10.0 (2.2) 56	5.2 (2.0) 56	1.4 (1.0) 56	0.19 (0.10) 56	39.7 (6.0) 46	13.9 (2.0) 47	35.2 (2.5) 46	1.014 (0.005) 54	93.2 (1.3) 52
SOUTHEAST ALASKA	7.5 (1.8) 21	4.1 (1.7) 21	1.6 (1.1) 21	0.31 (0.19) 21	ND	15.0 (1.5) 12	ND	1.019 (0.007) 21	93.6 (0.8) 21
NEONATES	6.2 (1.7) 33	7.9 (2.3) 33	2.9 (1.1) 33	0.25 (0.15) 33	48.9 (5.3) 29	18.5 (3.3) 29	37.6 (4.4) 29	1.013 (0.004) 33	93.1 (0.7) 33
ORPHANS	9.5 (1.6) 4	11.7 (3.9) 4	3.9 (3.0) 4	0.51 (0.30) 4	43.6 (5.0) 4	16.0 (2.3) 4	36.7 (2.6) 4	1.014 (0.002) 4	94.2 (1.4) 4

Table 5.6. Clinical chemistry for Steller sea lion pups captured in the Aleutian Islands (n=15), the Gulf of Alaska (n=32) and Southeast Alaska (n=15). Values are represented as mean (± SD).

Plasma Constituent	Aleutian Islands	Gulf of Alaska	Southeast Alaska
Sodium; mmol/L	141 (7)	144 (2)	147 (4)
Potassium; mmol/L	4.3 (0.5)	4.3 (0.4)	4.3 (0.6)
Chloride; mmol/L	107 (4)	107 (2)	107 (3)
Calcium, mmol/L	2.7 (0.1)	2.7 (0.1)	2.5 (0.6)
Phosphorus; mmol/L	2.5 (0.3)	2.3 (0.2)	2.4 (0.5)
Glucose, mmol/L	9.5 (1.8)	10.6 (1.7)	5.9 (3.8)
BUN; mmol/L	2.5 (1.3)	2.4 (0.9)	1.7 (0.5)
Creatinine; µmol/L	37 (12)	44 (5)	42 (8)
Cholesterol; mmol/L	4.66 (0.89)	4.64 (0.88)	5.17 (0.63)
Bilirubin, total; µmol/L	4.9 (4.8)	3.3 (1.4)	7.7 (5.6)
Bilirubin, direct; µmol/L	2.3 (5.0)	3.2 (1.3)	7.9 (5.2)
Total Protein; g/L	57.3 (4.6)	57.3 (3.4)	62.1 (5.0)
Albumin; g/L	32 (3)	34 (3)	37 (4)
Albumin:Globulin	1.3 (0.3)	1.4 (0.2)	1.5 (0.1)
Alkaline Phosphatase; iu/L	144 (32)	129 (32)	104 (50)
ALT (formerly SGPT); iu/L	31 (18)	42 (21)	49 (12)
AST (formerly SGOT);iu/L	24 (26)	26 (38)	59 (54)
Creatine Phosphokinase; iu/L	279 (185)	285 (178)	1041 (1193)

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Table 5.7. Hematology data for Steller sea lion pups captured in the Aleutian Islands (n=15), the Gulf of Alaska (n=32) and Southeast Alaska (n=5). Values are represented as mean (± SD).

Blood Parameter	Aleutian Islands	Gulf of Alaska	Southeast Alaska
WBC Count (x 10^9/L)	14.5 (3.3)	15.7 (3.7)	13.7 (4.7)
RBC Count (x 10^{12}/L)	3.67 (0.35)	3.52 (0.48)	4.51 (0.52)
Hemoglobin (mg/dL)	12.8 (1.1)	12.6 (2.0)	15.4 (1.0)
Hematocrit (L/L)	40.5 (4.9)	40.0 (5.4)	44.7 (3.4)
Mean Corpuscular Vol. (fL)	109.9 (6.8)	113.7 (4.4)	99.5 (6.6)
Mean Corpuscular Hb (pg)	34.9 (2.1)	35.7 (2.0)	34.3 (2.5)
Mean Corpuscular Hb Concentration (mg/dL)	31.9 (2.6)	31.5 (1.5)	34.4 (0.4)
Platelets	345.9 (99.6)	342.3 (104.0)	191.8 (128.6)
Differential WBC Counts (x 10^9 / L)			
Neutrophils	8.94 (2.53)	10.35 (3.35)	7.78 (3.23)
Eosinophils	0.16 (0.13)	0.12 (0.19)	0
Lymphocytes	4.77 (1.91)	4.81 (2.12)	5.94 (2.63)
Monocytes	0.68 (0.49)	0.41 (0.36)	0.06 (0.09)

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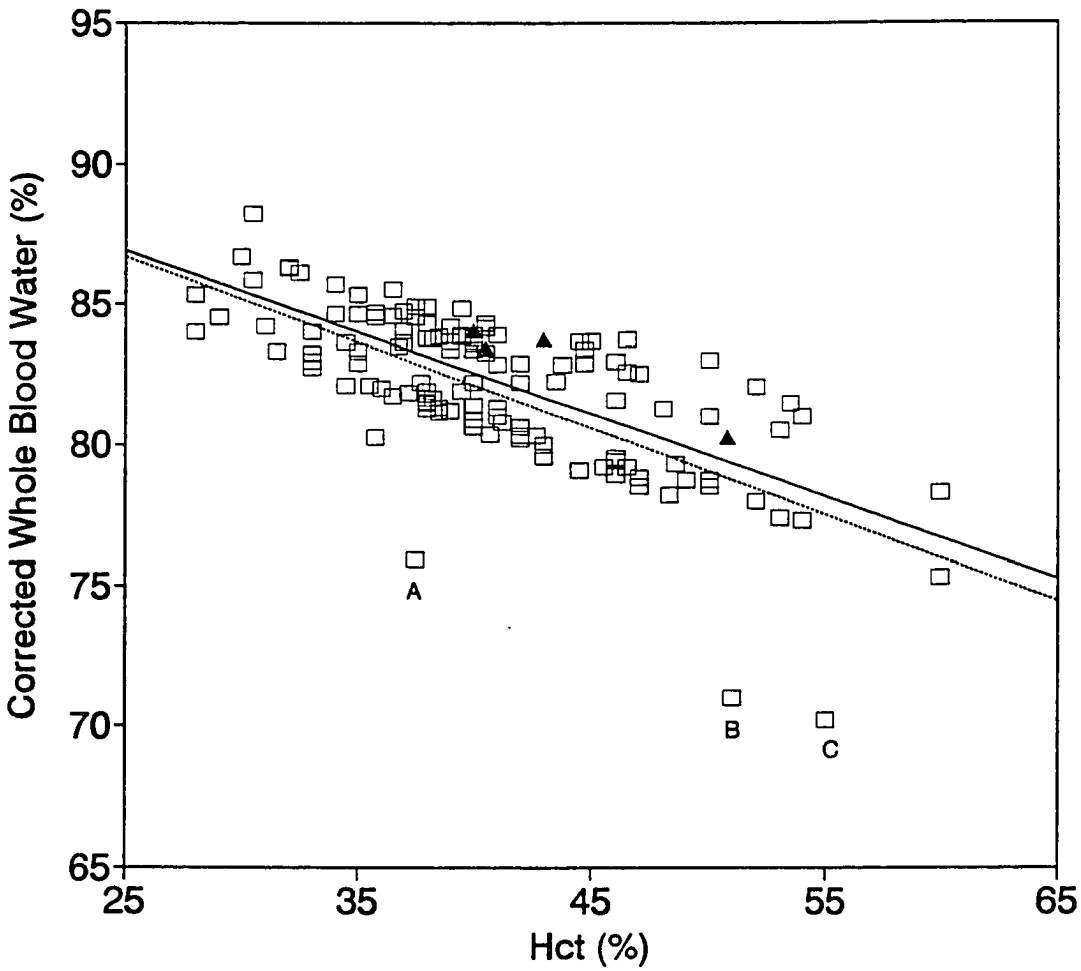

Figure 5.1. Relationship between corrected whole blood water content (%) and hematocrit (%) for Steller sea lion pups including randomly selected pups, neonates and orphans (represented by filled triangles). The three pups positioned outside the 95% confidence limits were labelled A, B and C. The regression line for this relationship was calculated as Whole blood water = -0.29 (Hct) + 94.1 (r^2 = 0.48, n=128) and is represented by the solid line. The dashed line represents the regression equation for this relationship in northern elephant seal pups (Whole blood water = -0.305 (Hct) + 94.3 ; r^2 = 0.80, n = 36), adapted from Castellini et al. (1989) and Castellini and Castellini (1990).

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Chapter 6. Prolonged fasting in young phocids; current knowledge and future research directions.

During the past two decades, considerable information has been gathered on the behavior, ecology and physiology of fasting in pinnipeds. A period of post-weaning fast, ranging in length from 2 to 12 weeks has been documented in at least 8 species of phocid seals (Table 6.1). With the exception of two species in which pups spend a great deal of time in the water (Phoca vitulina and Leptonychotes weddellii), the duration of the post-weaning fast tends to increase with increasing mean body mass at weaning (Figure 6.1). This is attributed primarily to increased body fuel reserves available in larger species. Under natural conditions, phocid pups lose from 0.3 to 0.7 $kg \cdot d^{-1}$ and decrease mass by 13 to 40% during the post-weaning fast. This represents from 0.4 to 1.1% of original weaning mass lost per day. The mean daily percent mass loss decreases linearly with increasing mean weaning mass seen among species (Figure 6.2). This lower rate of body mass loss was not closely related to percent fat composition at weaning in these species but can be attributed to a lower mass specific metabolic rate seen in larger species (Table 6.1). The combination of lower mass specific metabolic rates, lower relative rates of mass loss and larger initial weaning mass contribute to the ability of elephant seal pups to fast for up to twice as long as other species.

Although there was no significant relationship between body composition at weaning and the rate of post-weaning mass loss, those species with relatively low

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body fat contents (harbor and Weddell seals each have less than 40% body fat) also show the shortest post-weaning fast durations. It could be that these pups have begun to develop important diving skills earlier than species that do not enter the water until after weaning. In addition, Weddell seal pups are older when weaned (typically 5 to 7 weeks of age) than many other species (4 days to 4 weeks). Thus if the main purpose of the post-weaning fast is to keep pups in a relatively predator free environment until diving behavior and physiology has reached a minimum stage of development for efficient foraging, then harbor and Weddell seal pups may not require a prolonged fast. It is also possible that low body fat content may influence hormonal regulation of fasting metabolism as seen in LWM northern elephant seals, such that pups with lower body fat are unable to spare protein efficiently for maintenance of a long fast.

The predictable pattern of changes in body mass and plasma metabolites seen during prolonged fasting in pinnipeds indicate that phocid seal pups are metabolically adapted to fast for weeks to months during the post-weaning period. As the duration of the post-weaning fast varies from species to species, so does the ability to sustain a protein sparing metabolism. Each species in which metabolite levels have been studied to date (harp, grey and northern elephant seals) has proven the ability to minimize protein loss by progressively increasing circulating NEFA and β-HBA levels (thus decreasing the need for glucose production from amino acid precursors), but northern elephant seals can maintain this fasting-adapted metabolism for much longer periods than grey or harp seals. This is, in part, attributed to the larger body size in northern

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elephant seal pups. Weaned elephant seal pups are 2 to 3 times the body mass of harp and grey seal pups, and typically show fasting durations 2 to 3 times longer than the other two species. Elephant seal pups are capable of these longer duration fasts even while maintaining higher activity levels (ie. diving and social interactions) than pups fasted in captivity. Figure 6.3a. demonstrates that after 2 weeks of fasting, larger weaning mass pups show lower circulating BUN concentrations. After 4 weeks of fasting this contrast becomes even more apparent, as captive harp seals show entrance into Phase III fasting with elevated BUN levels up to 57 mM (Figure 6.3b). There was little relationship between plasma β-HBA levels seen at 2 weeks and the weaning mass of pups (Figure 6.4a), but after 4 weeks of fasting, β-HBA concentrations seen in smaller body mass pups increases considerably (Figure 6.4b). These data illustrate that changes in plasma metabolite levels do not progress at the same rate in all species adapted to fasting. If all species were similar, elephant seal pups would be expected to show similar concentrations of plasma metabolites as those seen in harp and grey seals during week 4 of the post-weaning fast and then maintain these metabolite levels for the remainder of the 9 to 12 week fast. Instead, northern elephant seal pups show lower circulating levels of both BUN and β-HBA during early fasting (2 to 4 weeks) than those seen in grey and harp seal pups, implying that they may be able to accomplish a higher degree of protein sparing (relatively early in the fast) than the species with shorter duration fasts.

Studies on LWM and HWM northern elephant seal pups suggest that

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differences in the magnitude and timing of plasma metabolite changes are not simply differences among species, but they are more closely related to differences in initial body reserves. Among fasting elephant seal pups, size at weaning plays a strong role in determining the duration of the post-weaning fast. Northern elephant seal pups weaned at less than 75 kg body mass fast for an average of only 4 weeks, compared with the 9 to 12 week durations seen in larger pups. During the shortened fast, LWM pups show evidence of higher NEFA and β-HBA concentrations than seen during the same period in AWM and HWM pups (Figures 6.4a & 6.4b), and BUN levels are slightly (although not significantly) higher in small pups. This suggests that pups may need to reach a threshold mass by weaning that enables them to conserve energy and lean body mass efficiently during fasting. In contrast, the levels of circulating metabolites in LWM pups do not reach those seen in harp and grey seal pups at 4 weeks fasting, even though these 3 groups fast for similar lengths of time. The additional 20 kg of body size seen in LWM pups may still give these pups some advantage in their ability to maintain a protein sparing metabolism (Figures 6.3a & 6.3b).

Studies of LWM elephant seal pups also provide some insight into how body fat content at weaning may influence the ability of pups to sustain a fasting-adapted metabolism. Due to a shorter suckling period, LWM pups attain a lower relative fat content (about 36% of the total body mass) than larger pups of that species (48 to 50% body fat). In fact, most fasting-adapted phocid species reach 40 to 50% body fat

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at weaning. Low weaning mass pups of this study did not fast for as long as the average weaned elephant seal pups, and they also showed higher circulating lipids and ketone body levels. Low weaning mass pups also did not decrease BUN concentrations to the extent seen in larger pups late in the fast. These differences in metabolite levels may be due to higher rates of lipolysis in LWM pups due to depressed insulin levels seen in leaner animals (Kirby and Ortiz 1994). Higher lipid metabolism due to low body fat (and its effect on hormone regulation) may also explain why LWM pups do not fast for substantially longer periods than harp or grey seal pups, as would be expected from their larger weaning mass. LWM elephant seal pups typically weigh at least 20 kg more than harp or grey seal pups at weaning but all 3 species fast for 3 to 4 weeks post-weaning.

Unlike results from captive fasting studies on harp and grey seals, free-ranging elephant seal pups showed no evidence of entrance into Phase III fasting at the end of the post-weaning fast. This implies that under natural conditions, northern elephant seal pups do not reach their physiological limit of fasting. Thus, weaned pups leave the beach with energy reserves of up to 50% body fat. Studies on harbor seals have shown that independently feeding pups take several weeks to improve foraging skills such that prey intake can allow maintenance of body mass (Muelbert and Bowen 1993). Remaining energy resources in weaned elephant seal pups may be necessary to compensate for a similar period of inefficient foraging when they begin to feed independently.

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Since northern elephant seal pups were able to maintain Phase II fasting until they departed the beach, there were no precipitous changes seen in any of the blood chemistry levels studied that might be considered a signal or trigger to cue pups to initiate feeding. The possibility exists that rapid changes in BUN concentration (indicative of Phase III) were missed due to the weekly sampling regime and because all pups were not sampled immediately prior to departure. However, the one captive elephant seal pup that showed plasma chemistry changes near the end of the fast, showed a progressive increase in BUN concentration over several days (Figure 6.5). Also, it seems unlikely that these indications would have been missed in all of the LWM pups that were presumably forced to leave the beach before the customary duration of fasting due to lower energy reserves.

Future research directions

Smaller weaning mass pups lost mass at a higher relative rate while fasting than did larger pups (Figure 6.2). It is these small species (grey and harp seals in particular) that also show higher metabolite concentrations during the post-weaning fast, implying lesser ability to spare protein. It would be of particular interest to examine plasma metabolite levels during the post-weaning fast in harbor seals and ringed seals. These species are small (20 to 25 kg at weaning) and harbor seals show a high relative rate of mass loss post-weaning. Ringed seal pups also show an elevated metabolic rate which would result in a high relative rate of mass loss. From body mass relationships alone, one might expect these species to be poorly adapted for

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protein sparing, but the ability to fast for 2 to 5 weeks in pups that also spend considerable time in the water warrants further attention.

While we have greatly improved our understanding of how body mass and metabolite levels change during prolonged fasting in phocid pups, there is much to learn about the physiological mechanisms that regulate fasting and the transition to refeeding. It has been suggested that the transition to Phase III fasting occurs in other species when lipid stores are depleted. This is an unlikely occurrence in seal pups, since body stores are utilized equally from fat and lean body mass compartments during the post-weaning fast, allowing pups to maintain a constant percent body fat content throughout the fast. This is also true for LWM northern elephant seal pups. Considerable fat reserves remained in the captive elephant seal pup that showed early signs of entrance into Phase III. However, depletion of specific fatty acids in the adipose stores may be occurring, even though adequate total fat content (i.e., for thermoregulation) is maintained. Data available for fasting emperor penguins suggest that certain chain length fatty acids are selectively mobilized during the fast, which results in a change of the fatty acid composition of adipose stores by the end of the fasting period (Groscolas 1990). Preliminary data from a collaborative project with René Groscolas at CNRS in Strasbourg, France suggest that the fatty acid composition of the plasma also changes in Weddell seal pups during the post-weaning period (Rea, Groscolas and Castellini, unpublished data). This implies that fasting pups may also selectively mobilize some specific fatty acids from adipose stores. Changes in the

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circulating levels of certain fatty acids should be given more attention, since depletion of particular fatty acids could provide a cue or trigger to initiate feeding at the end of the post-weaning fast. Recent work on the influence of the obesity gene product protein on appetite control and metabolism in mice (Pelleymounter et al. 1995) also poses some interesting questions about the possibilities of biochemical mechanisms involved in the cue to end fasting and the initiation of independent feeding in seal pups.

To date the majority of the research attention has focused on phocid pups during the post-weaning fast due to their ease of handling and the simplicity of their energetic demands. Although similar patterns of metabolism have been shown in many phocid species studied to date, one would not expect to see the same magnitude or exact timing of metabolite shifts due to fasting when comparing phocid and otariid pups. Steller sea lions do not typically experience long-term fasting as pups, but instead are subjected to repetitive short fasts (a few days in duration) while lactating females forage at sea. It would be expected from the relationship seen between metabolite levels and body size in phocids that smaller, leaner Steller sea lion pups would show faster shifts in metabolite concentrations than seen in northern elephant seal pups. The phocid model of fasting metabolism may not accurately reflect metabolic changes that occur in otariid pups when faced with repeated short (order of days) interruptions in suckling or possibly when faced with longer periods of food limitation as juveniles or adults. The extent of our information on how metabolite

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levels change in Steller sea lion pups during food deprivation is based on sampling of abandoned pups and repeated sampling of recaptured individuals. What is needed is a controlled study of young otariids that can document plasma metabolite concentrations when food intake is closely regulated. These conditions are most easily met in a captive setting. It is only with the results of such a study that we can begin to understand whether ecological differences in the maternal investment patterns between phocids and otariids is reflected in the physiology of how these groups tolerate fasting.

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Table 6.1. Duration of the post-weaning fast in 8 species of phocid seals and rates of metabolism and mass loss during this period. Asterisks denote studies conducted on captive pups, thus durations of fast may not reflect natural fasting durations.

Species	Weaning Mass (kg)	Fat content (%)	Mass loss ($kg \cdot d^{-1}$)	Mass loss ($\% \cdot d^{-1}$)	Duration of fast (weeks)	Cumulative mass loss (%)	Metabolic rate (ml $O^2 \cdot min^{-1} \cdot kg^{-1}$)
HWM northern elephant seal[a]	149.6 ± 9.2	50.1[b,c]	0.65	0.40	9-12	24.6 ± 3.5	4.9 ± 0.4[c]
AWM northern elephant seal[d]	102.9 ± 15.1	48.9[b,c]	0.55	0.55	9 - 12	37.4	5.6 ± 0.9[c]
LWM northern elephant seal[a]	66.1 ± 4.0	35.6[b]	0.54	0.80	4	25.0 ± 4.3	6.6 ± 0.7[c]
Weddell seal[e]	105.8 ± 16.1	37[f]	0.66	0.60	2 - 3	8.5 to 13	-
Grey seal[g]	40.7 ± 1.8	43	0.45	1.10	2	16.2 ± 1.3	4.8 ± 0.3[h] 3.0 - 4.2[i]
Grey seal[j,*]	41.7 ± 2.3	43[g]	0.36	0.87	7-8	40	4.8 ± 0.3[j] 3.0 - 4.2[q]
Harp seal[j,*]	33.8 ± 0.9	42.3[k]	0.36	1.06	4	31	6.0 - 9.0[j]
Harbor seal[l]	24.9 ± 0.45	35[m]	0.28	1.10	2	18	-
Hooded seal[n]	43.7 ± 1.9	44.7[o]	0.40	0.92	4	29	-
Ringed seal	22.1[p]	46.3[q]	-	-	5	-	20[r]
Southern elephant seal[s]	125.0 ± 8.6	-	0.97	0.78	6-7	32 ± 0.7	-

a) This study, Chapter 3.
b) Rea and Costa 1992
c) Rea 1990
d) This study, Chapter 2.
e) This study, Chapter 4.
f) Tedman and Green 1987
g) Reilly 1991
h) Nordøy et al. 1990
i) Worthy and Lavigne 1987
j) Nordøy et al. 1993
k) Worthy and Lavigne 1983
l) Muelbert and Bowen 1993
m) Bowen et al. 1992
n) Bowen et al. 1987
o) Oftedal et al. 1993
p) Hammill et al. 1991
q) Lydersen et al. 1992
r) Smith et al. 1991
s) Arnbom et al. 1993

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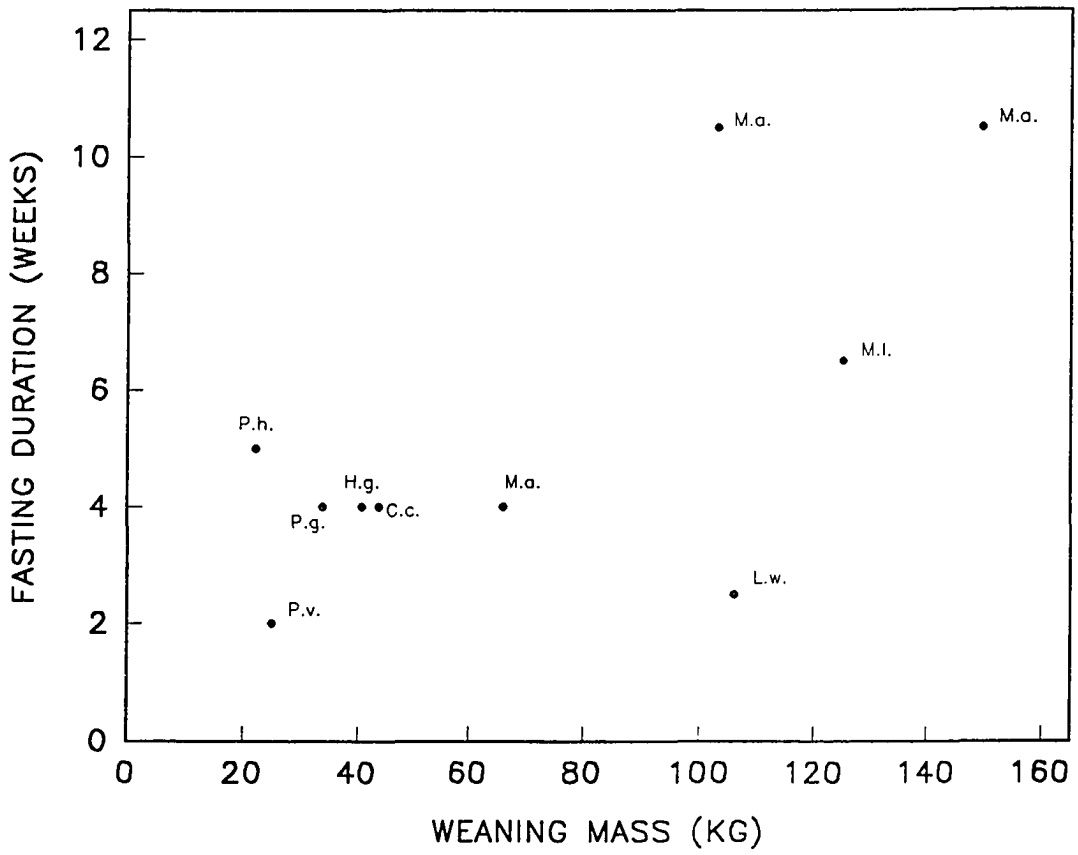

Figure 6.1. Duration of the post-weaning fast (weeks) in relation to the weaning mass (kg) of 8 species of phocid seals. Symbols represent the scientific name of each species: C.c. - hooded seal; H.g. - grey seal; L.w. - Weddell seal; M.a. - northern elephant seal; M.l. - southern elephant seal; P.g. - harp seal; P.h. - ringed seal; P.v. - harbor seal.

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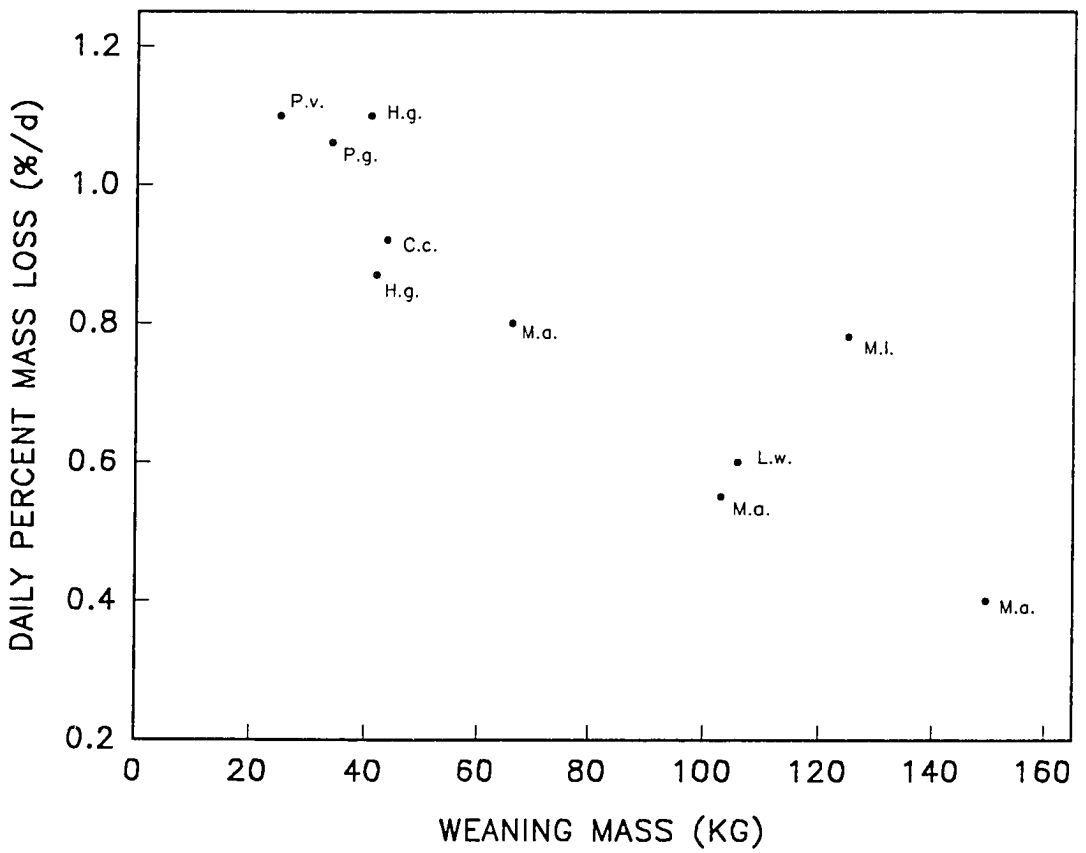

Figure 6.2. Mean daily percent mass loss ($\%\cdot d^{-1}$) in relation to weaning mass (kg) in 7 species of phocid seals. Symbols represent the scientific name of each species: C.c. - hooded seal; H.g. - grey seal; L.w. - Weddell seal; M.a. - northern elephant seal; M.l. - southern elephant seal; P.g. - harp seal; P.v. - harbor seal.

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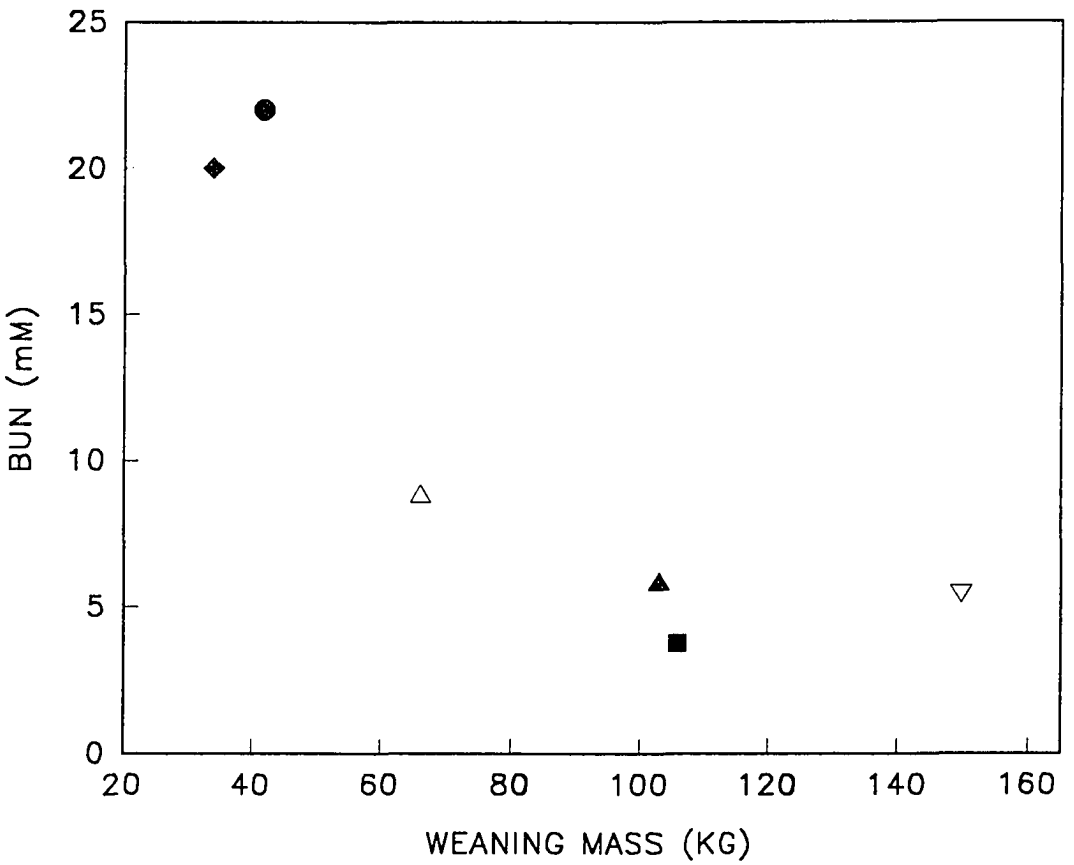

Figure 6.3a. Plasma blood urea nitrogen (BUN) concentration (mM) in relation to weaning mass (kg) in 4 phocid species after 2 weeks of fasting. Symbols represent: ▽ HWM northern elephant seal; ▲ AWM northern elephant seal; △ LWM northern elephant seal; ■ Weddell seal; ● grey seal; ◆ harp seal.

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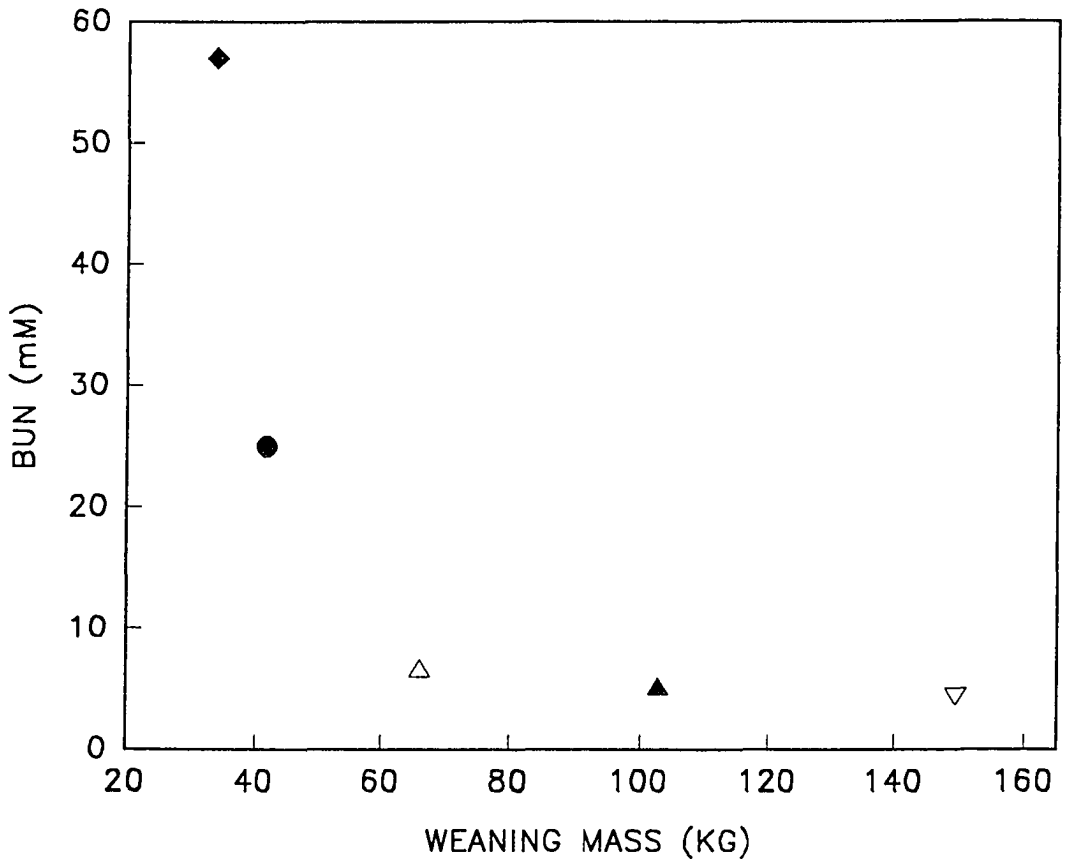

Figure 6.3b. Plasma blood urea nitrogen (BUN) concentration (mM) in relation to weaning mass (kg) in 3 phocid species after 4 weeks of fasting. Symbols represent: ▽ HWM northern elephant seal; ▲ AWM northern elephant seal; △ LWM northern elephant seal; ● grey seal; ◆ harp seal.

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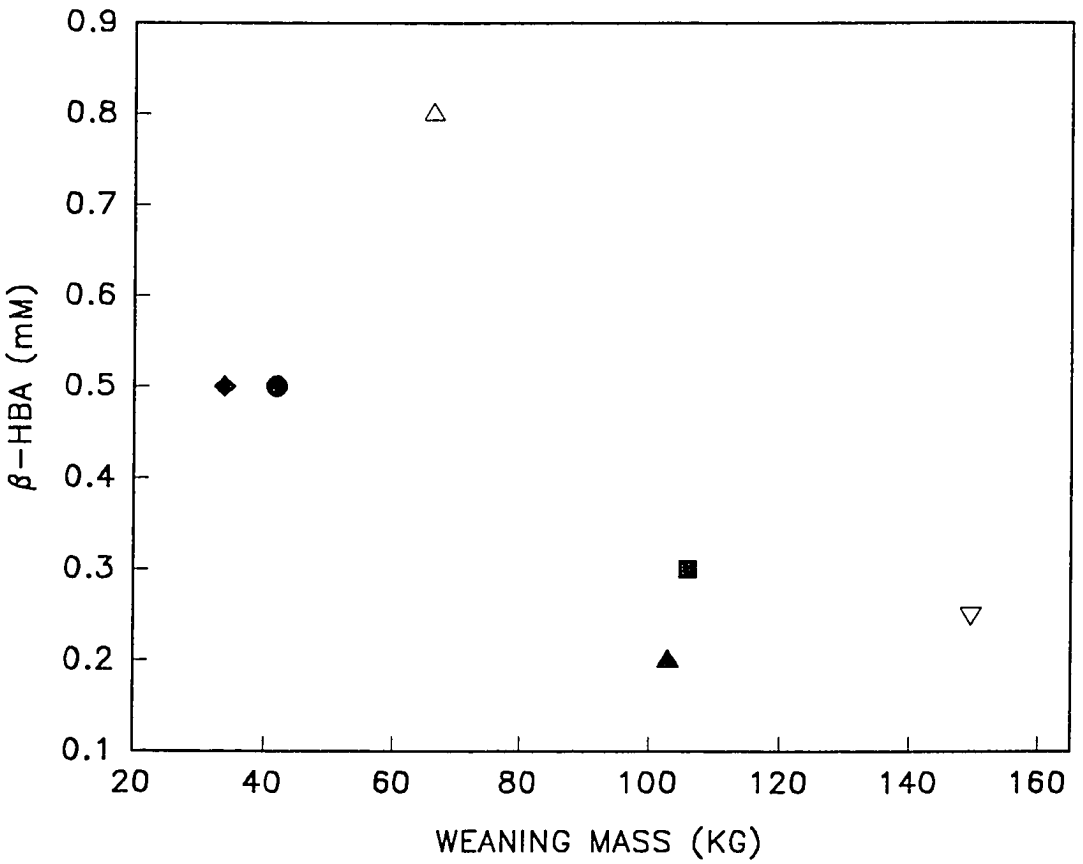

Figure 6.4a. Plasma β-hydroxybutyrate (β-HBA) concentration (mM) in relation to weaning mass (kg) in 4 phocid species after 2 weeks of fasting. Symbols represent: ▽ HWM northern elephant seal; ▲ AWM northern elephant seal; △ LWM northern elephant seal; ■ Weddell seal; ● grey seal; ◆ harp seal.

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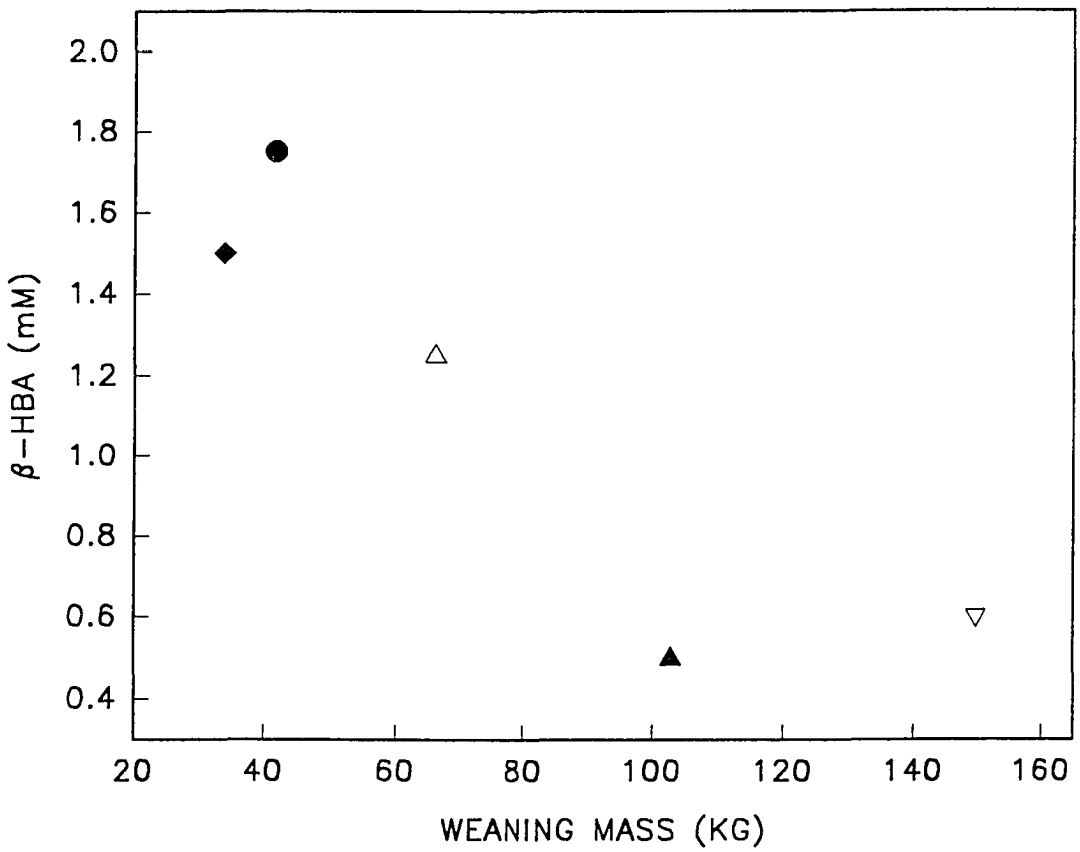

Figure 6.4b. Plasma β-hydroxybutyrate (β-HBA) concentration (mM) in relation to weaning mass (kg) in 3 phocid species after 4 weeks of fasting. Symbols represent: ▽ HWM northern elephant seal; ▲ AWM northern elephant seal; △ LWM northern elephant seal; ● grey seal; ◆ harp seal.

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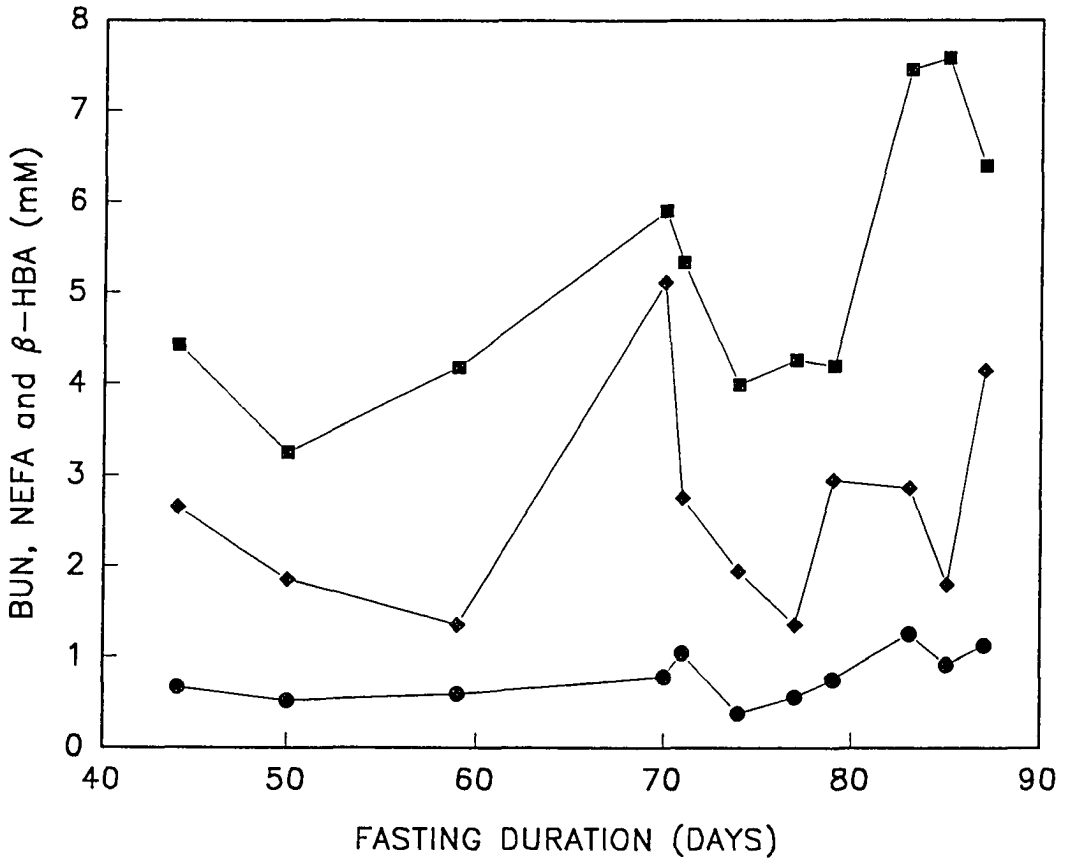

Figure 6.5. Changes in plasma blood urea nitrogen (■ BUN), non-esterified fatty acid (◆ NEFA) and β-hydroxybutyrate (● β-HBA) concentrations (mM) during the post-weaning fast in a northern elephant seal pup. This pup was held at Long Marine Lab during the last 2 weeks of fasting (up to 87 days post-weaning).

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Chapter 7. The biochemistry of prolonged fasting and applications to assessing physiological state; Conclusion.

Over the past two decades a variety of studies have contributed to our current knowledge of how pinnipeds are adapted to prolonged fasting. Decreases in metabolic rate have been documented during the post-weaning fast in several species along with decreases in the contribution of protein catabolism to total energy expenditure (as evidence of protein sparing metabolism). Changes in the concentrations of key plasma metabolites have been described for two species of captive pinnipeds, grey and harp seals, relatively small species that fast for 3 to 6 weeks. Independent studies have shown that levels of these individual metabolites also change during the post-weaning fast in northern elephant seal pups. Our understanding remains relatively incomplete, however, with regards to how these key plasma metabolites change in relative concentration during fasting in free-ranging pinnipeds which are maintaining natural activity patterns. Also, very little attention has been given to how body size influences the duration of fast and how body reserves are utilized during prolonged fasting.

The first objective of this project was to determine how plasma metabolite concentrations changed in relation to duration of the post-weaning fast in large body mass phocids, in particular the northern elephant seal. Particular attention was given to whether free-ranging pups showed similar patterns of change in metabolite levels during fasting as those seen in captive species previously studied, and if the absolute

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metabolite concentrations in elephant seal pups were distinctive to a species that can maintain the post-weaning fast up to 12 weeks. In addition, by contrasting the patterns of metabolite concentrations seen in LWM and HWM elephant seal pups, I intended to illustrate the influence of body size on the ability of pups to maintain a prolonged fast.

The next objective of this work was to develop a model of how metabolite concentrations changed under known conditions of fasting which could be used to assess the nutritional condition of other species for which we were unable to determine feeding behavior. The first application of this model was to determine occurrence of weaning in Weddell seal pups and to determine how soon pups of this species begin to feed independently. Thus we were interested in knowing if Weddell seal pups showed similar patterns of plasma metabolite changes as fasting elephant seal pups, indicating that they, too, undergo a post-weaning fast. Secondly, I intended to use the knowledge of how metabolite levels change in elephant seal pups during fasting to assess the nutritional status of Steller sea lion pups captured in different regions of Alaska. Dramatic declines in the populations of Steller sea lions in Alaska prompted concern over the health and nutritional status of juvenile sea lions. It was the objective of this study to determine if young Steller sea lion pups showed changes in metabolite concentrations that indicate prolonged fasting in elephant seals or any other indication from blood or body condition parameters that would suggest that pups were not healthy.

Northern elephant seal pups exhibited similar patterns of plasma metabolite

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changes during the post-weaning fast as were seen in the smaller harp and grey seal pups during fasting. However, this study illustrated that body size (and possibly body fat content) influenced the length of fast that pups are able to sustain and the degree of protein sparing attained. Average northern elephant seal pups are able to fast for 2 to 3 times as long as the smaller harp and grey seal pups while losing the same relative amount (about 30%) of body mass during the fast. Studies on LWM and HWM elephant seal pups show that body mass can also dictate the duration of the post-weaning fast within species. Low weaning mass pups did not have the body reserves required to sustain the customary 9 to 12 week post-weaning fast seen in elephant seals and left the beach at an average of 4 weeks. These low weaning weight pups also did not indicate as great an ability to spare protein as larger pups.

From data collected on northern elephant seal pups during the post-weaning fast, a model of how body mass and metabolite concentrations change in large phocids with duration of fasting was developed. Comparison of plasma metabolite concentrations and mass changes with those seen in fasting northern elephant seal pups should provide reasonable indicators of fasting metabolism since these species are very close in weaning size. A period of post-weaning fast is common among phocid species, even in harbor seals which also spend considerable amounts of time in the water during the suckling period (Muelbert and Bowen 1993). Thus the occurrence of a post-weaning fast was not unexpected for Weddell seals pups, but it was impossible to accurately judge from previous body mass loss data alone when pups made the transition to independent foraging. When compared with changes in mass and plasma

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metabolite levels seen in Weddell seal pups, it was clearly apparent that after weaning Weddell seal pups typically fast for 2 to 3 weeks before foraging independently on live prey. From body mass changes and metabolite data it can also be deduced that pups experience an initial period of inefficient foraging such that body mass can not be maintained.

Using the knowledge of how metabolite concentrations change during fasting in the elephant seal model and the magnitude of changes observed in emaciated Steller sea lion pups, it was determined that randomly captured Steller sea lion pups showed no signs of inadequate suckling or other indications of poor health. Higher ketone body concentrations seen in pups captured in Southeast Alaska suggested that pups in this area may experience longer periods separated from their mothers. This conclusion is supported by behavioral data collected in that region which shows that females from Southeast Alaska spend longer periods at sea foraging followed by longer periods on land nursing their pups. This longer fast would account for higher β-HBA levels but does not necessarily suggest that pups in Southeast Alaska receive less nourishment since overall attendance (% time with pup) is the same as in other areas (ie. Gulf of Alaska).

This study provides a comprehensive description of how body mass and plasma metabolite concentrations change during the post-weaning fast in large body mass, free-ranging pinnipeds. Data from this study supports the conclusion that elephant seal pups are able to attain a higher degree of protein sparing than smaller species that fast for only 3 to 6 weeks. And, in contrast to captive studies, there is no evidence that

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free-ranging elephant seal pups enter into Phase III fasting before departing the beach to feed at sea. This work also provides insight into the role of body size (and possibly body composition) in regulating the length of the post-weaning fast and in particular the ability of pups to maintain a protein sparing metabolism. In this dissertation I have shown the usefulness of applying models of known fasting metabolite levels to assess the nutritional status of species for which we have no data on current feeding activity. In Weddell seal pups a combination of body mass changes and plasma metabolite levels were effectively used to document a 2 to 3 week post-weaning fast. These same criteria were used to evaluate the nutritional status of Steller sea lion pups. Steller sea lion pups in Alaska showed no indication that they were physiologically compromised such that they might not survive the nursing period. By the study of plasma metabolite concentrations, we were able to distinguish animals that were experiencing slightly longer fasting durations, even differing by as little as a few days.

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