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Milk secretion in marine mammals in relation to foraging: can milk fatty acids predict diet?

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Synopsis

Patterns of milk composition and delivery differ greatly among mammals. However, despite these species-specific differences, lactation is very expensive in all species and thus diet and nutrient reserves of individual females must play a critical role in lactation performance. Pinnipeds, as well as mysticete whales, exhibit extreme adaptations to the constraints imposed by the separation of maternal feeding from milk transfer. This paper will consider three main questions: (1) how are milk secretion processes adapted to the temporal separation of foraging and milk secretion; (2) how are changes in diet or nutrient reserves likely to affect milk composition or yield; and (3) can specific milk constituents be used to indicate foraging behaviour or diet of individual animals? Substantial quantities of nutrients and metabolites are required by the mammary gland for the secretion of milk constituents. Nutrient partitioning and milk secretion are physiological processes which are both highly regulated and biochemically constrained. The general principles of these processes appear to be shared among all mammals, including marine mammals. It is concluded that neither the levels nor the types of most milk constituents are likely to be affected by maternal diet in marine mammals. However, milk yield may be reduced during a low plane of nutrition. Unlike other constituents, such as protein or carbohydrate, dietary fatty acids essentially remain intact through the digestion process (in carnivorous mammals) and many of these are secreted in milk or deposited in adipose tissue with no or minimal modification. Recent studies on species such as the California sea lion, Antarctic fur seal, hooded seal and harbour seal suggest a strong potential for determining prey items and diet of marine mammals through fatty acid signatures in the milk, particularly given the complex array of fatty acids which exist in marine organisms.

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Introduction

Milk is the complex lacteal secretion of the mammary gland which is responsible for the provision of nutrients and energy to the growing neonate. Among mammals, there are large differences in the composition of milk, as well as in milk output, frequency of nursing and duration of lactation. However, despite species-specific differences in milk secretion patterns, lactation represents the greatest energetic cost of reproduction in female mammals (Blaxter 1962; Millar 1977; Oftedal 1985), requiring large amounts of nutrient transfer and elevated maintenance costs. Thus maternal diet and nutrition must play a critical role in the ability of individual mothers to meet the nutrient requirements of milk secretion.

Most mammals are able to mobilize body reserves, especially fat, to partially compensate for energy deficits during lactation (Young 1976; Bauman & Elliot 1983). This ability has been critical in the evolution of lactation strategies of many species, but in particular to those which must cope with long-term spatial and temporal separation of maternal feeding and milk secretion. Many marine mammals (e.g. pinnipeds and mysticete whales) have evolved unusual patterns in both milk composition and output which reflect this separation of foraging and lactation. Thus, understanding the influence of maternal diet and nutrient reserves on milk secretion in individuals is important to interpretations of reproductive patterns, foraging ecology and life history strategies in these species.

The interaction between maternal feeding and lactation in marine mammals may be considered by posing three questions: (1) how are milk secretion processes adapted to the constraints imposed by the temporal separation of foraging and milk secretion; (2) how are changes in diet or nutrient reserves likely to affect milk composition or yield; and (3) can specific milk constituents indicate foraging behaviour or diet of individual animals? Each of these questions involves understanding the relationship between the supply of nutrients and metabolic substrates to the secretion of milk products at the level of the mammary gland. Although direct data are often lacking for marine mammals, in some cases observations from other species provide reasonable clues; pinnipeds are emphasized in this paper, since most quantitative work has been done on this marine mammal group. Finally, because milk fat represents the primary and most interesting constituent of marine mammal milks and because lipid metabolism plays such a large role in the lactation process in these species, most attention is given here to lipids and their secretion in milk.

The overall objective of this paper is twofold: the first aim is to provide an overview of the physiological and biochemical processes involved in secretion of the major organic constituents of milk, which is necessary to interpret data on milk composition and production. On the basis of understanding these processes, the second aim is to explore the use of milk fatty acids as a means of determining marine mammal diets.

Foraging and lactation in marine mammals: adaptation vs. physiological response

In pinnipeds, two general lactation strategies have evolved to cope with the constraints imposed on the mother which must feed at sea but suckle her pup on land (Bonner 1984; Oftedal, Boness & Tedman 1987). Otariids lactate during an initial perinatal fast of about 5–9 days, then begin alternating regular foraging trips with suckling periods of 1–2 days. Thus milk is initially synthesized from substrates derived from maternal tissues and subsequently from nutrients acquired while foraging during lactation. Lactation generally lasts one year or more in these species. By contrast, phocids, in particular large phocids, are able to store greater energy reserves as blubber prior to parturition, and fast during a brief intense lactation period of 4–50 days. Hence all substrates for milk synthesis are derived from maternal tissues. Smaller phocid species, such as the harbour seal (*Phoca vitulina*), may not be able to store sufficient energy reserves to support lactation, and may initiate foraging during the nursing period (Bowen, Oftedal & Boness 1992).

In mysticete whales, their larger body size (3000–150 000 kg; Macdonald 1984: 214–235) permits far greater energy reserves than phocids possess. Most mysticetes undergo seasonal migrations and give birth and begin the first months of the 6–10 month lactation period in tropical areas of low food availability (Harrison 1969; Rice & Wolman 1971; Gaskin 1982). Hence during the first half of lactation milk must be synthesized primarily from stored nutrients.

Thus, pinnipeds and mysticetes have evolved lactation patterns which may reflect such factors as the need to conserve maternal water and to reduce lactation length during fasting. For instance, milks of these species tend to be concentrated and especially high in fat (30–60%; Gaskin 1982; Oftedal, Boness & Tedman 1987), and are often associated with large daily milk energy outputs. But to evaluate the effects of diet, nutrition, and foraging strategies on milk composition or production, it is important to distinguish between evolutionary adaptations and physiological responses on an ecological time-scale which might affect an individual animal during lactation. For example, milk fat content is correlated with average duration of maternal foraging trips when compared across various species of otariids (Trillmich & Lechner 1986). However, this does not mean that if an individual female otariid changes her foraging trip duration she will be able to 'adjust' the composition of her milk.

Diet, physiology, or even body size have been suggested as factors which might affect the proximate milk composition of an individual (e.g., Kretzmann *et al.* 1991). However, given the physiological mechanisms responsible for milk secretion, it is unlikely that such factors would affect milk composition. For instance, in no mammal does the proximate milk composition reflect the composition of its diet. Although the patterns observed in the proximate composition of milk across species may have evolved in response to nutritional and environmental constraints, they are clearly not a function of the diet itself. Rabbits (order Lagomorpha) and black bears (*Ursus americanus*) produce relatively high-fat milks (15% and 28% fat, respectively; Cowie 1969; Iverson & Oftedal 1992), even though they feed primarily on succulent and leafy vegetation. Pinnipeds uniformly produce high-fat milks regardless of whether species typically feed on high-fat fish or low-fat invertebrates or whether they fast entirely. Within species, the fat concentration of milk is equally unlikely to be directed by diet. The proximate composition of milk within a species is also unlikely to be influenced by body size. For example, although there exist hundreds of breeds of the domestic dog (*Canis lupus*), which vary tremendously in body size (e.g. dachshunds to Saint Bernards), the proximate composition of dog milks exhibits little variability and no correlation to body size (Russe 1961; Oftedal 1984a).

Finally, the factors which might influence the composition of milk are different than those which influence the yield of milk. Therefore, attempts to link ecological factors to the proximate milk composition of individuals should first have a physiological basis. It is for this reason that we must understand milk secretion and nutrient partitioning to the mammary gland as physiological processes, which are both highly regulated and biochemically constrained. Certain aspects of these processes may be highly influenced by diet and nutrition, but most patterns are genetically and evolutionarily determined among differing taxa.

Milk secretion

The concept of nutrient partitioning

Substantial quantities of nutrients and metabolites are required by the mammary gland for the synthesis and secretion of milk. In fact, the demand for nutrients by the mammary gland in all mammals is so great that the general body metabolism of the mother must be altered and organized in such a way that the appropriate nutrients are efficiently partitioned to the mammary glands for milk synthesis and secretion. Thus, milk secretion takes place at the expense of other biological processes (Patton & Jensen 1976; Bauman & Currie 1980; Williamson 1980; Bauman & Elliot 1983).

A number of physical and metabolic changes in tissues occur during lactation to support milk secretion. The hormonal regulation of this process can be viewed as a combination of several types of controls, namely, homeostasis (maintaining physiological equilibrium of the whole body) and homeorhesis ('orchestrated changes for the priorities of a physiological state') (Bauman & Currie 1980). The primary objectives of homeorhesis are to increase the availability of fatty acids, amino acids, glucose and metabolites to the mammary gland. This often begins with increased food intake and digestion, accompanied by hypertrophy of the digestive tract and increased absorptive capacity (Cripps & Williams 1975; Millar 1979), and continues with changes in tissue metabolism, mobilization of body reserves, increased mammary blood flow and a priority of nutrient use by the mammary gland (Hanwell & Peaker 1977; Mepham 1983). A number of hormones may be involved in homeorhetic control, which are not yet fully understood, but these probably include prolactin, progesterone and growth hormone (Bauman & Elliot 1983).

The nutrient requirements of the mammary gland are essentially species-specific or characteristic of a phylogenetic group. Within species, changes in nutrient requirements often occur with stage of lactation (i.e. with changes in milk composition or yield). These changes are hormonally and biochemically directed (Cowie, Forsyth & Hart 1980; Jenness 1986). The regulation of nutrient utilization during lactation in all species allows milk secretion by the mammary gland to be robust to many fluctuations in dietary intake or composition. Thus, during periods of feeding, nutrients from dietary intake are partitioned first to the mammary glands, but during fasting or when nutrient intake is insufficient to meet demands, body nutrient stores are mobilized for milk secretion. In other words, homeorhetic regulatory mechanisms alter body metabolism to make available what the mammary gland requires (Bauman & Currie 1980; Williamson 1980). Although there are numerous examples of homeorhesis in nutrient partitioning, some of the best examples occur in female phocids, mysticete whales and winter-dormant bears, which fast throughout all or a large portion of lactation and produce milk derived entirely from the mobilization of body stores.

Dietary lipid and nutrient partitioning

Of the major physical and metabolic tissue changes which occur in mammals during lactation, those involving lipid metabolism and adipose tissue stores (and thus energy requirements) are probably of greatest importance and magnitude (Bauman & Elliot 1983). In marine mammals this is particularly the case since a considerable component in the reproductive strategy involves alternating between deposition and mobilization of fat reserves for milk synthesis.

Fatty acids are essentially the building blocks of lipids, being the largest constituent of neutral lipid compounds such as triglycerides (glycerol esterified to three fatty acids) and phospholipids. The most common fatty acids in plants and animals are composed of 14–22 carbon atoms in even-numbered straight chains, containing a terminal methyl end and a terminal carboxyl end. The chain may be saturated with no double bonds or unsaturated, containing from one to six double bonds[1]. Organisms are able to biosynthesize, modify chain-length and introduce double bonds to fatty acids, but can only do so subject to biochemical limitations and differences in these processes depending on the phylogenetic group.

In monogastrics, ingested lipid is hydrolysed in the stomach and small intestine to monoglyceride, glycerol and free fatty acids. These products are transported across the mucosal lining of the small intestine during which they are re-esterified into triglycerides and incorporated into circulating lipoproteins (i.e. the chylomicrons) for transport to tissues (Borgstrom 1977; Patton 1981). Thus, with the exception of ruminants, fatty acids essentially remain intact during digestion and those found in chylomicrons reflect the original dietary composition. Circulating very low density lipoproteins (VLDL) carry endogenously derived triglycerides, for instance from the mobilization of adipose tissue. The circulating chylomicrons along with VLDL are the only lipoprotein classes which have been found to contribute triglyceride fatty acids to the mammary gland (Annison 1983; Hamosh & Hamosh 1985).

Lipoprotein lipase (LPL) is the key enzyme involved in channelling circulating triglycerides to various organs. LPL is a tissue-bound enzyme responsible for hydrolysing circulating triglyceride carried in chylomicrons and VLDL, thus facilitating the uptake of fatty acids by most tissues, including adipose tissue and the mammary gland (Hamosh & Hamosh 1985). During fasting or starvation, activity of LPL in adipose tissue decreases dramatically, which stops the uptake of fatty acids, while adipocyte hormone-sensitive lipase becomes active, which catalyses the release of fatty acids from adipose tissue into the circulation (Allen 1976).

The homeorhetic control of LPL activity, studied primarily in humans, rodents and ruminants, is also one of the more striking examples of nutrient partitioning during lactation. Shortly before parturition, LPL activity in adipose tissue decreases sharply, while LPL activity in the mammary gland (previously non-existent) appears (Hamosh *et al.* 1970; Bauman & Elliot 1983; Hamosh & Hamosh 1985). Although numerous factors may be involved in this change, prolactin is believed to be primarily responsible for LPL regulation during lactation (Zinder *et al.* 1974; Bauman & Elliot 1983). LPL activity in adipose tissue remains almost completely depressed

[1] According to IUPAC nomenclature, fatty acids are herein designated as carbon chain length:number of double bonds and n-*x*, if present, denotes the position (*x*) of the last double bond relative to the terminal methyl carbon.

throughout most of lactation because of the continued secretion of prolactin, particularly in response to suckling (Hamosh & Hamosh 1985), while LPL activity in the mammary gland rises sharply after parturition (McBride & Korn 1963; Robinson 1963; Hamosh 1989) and may continue to rise during lactation (Mehta, Jones & Hamosh 1982). Thus most dietary fatty acids as well as those mobilized from body stores are directed to the mammary gland during lactation. In humans, evidence suggests that the increase in milk fat content from colostrum to mature milk is accompanied by an increase in milk LPL levels (Mehta *et al.* 1982; Hamosh 1989). In guinea pigs and rats, LPL activity is correlated with the degree of uptake of lipid by the mammary gland (McBride & Korn 1964; Mendelson & Scow 1972; Scow 1977).

Recent evidence suggests that a similar pattern may occur in phocids. In harbour seal and grey seal (*Halichoerus grypus*) females at parturition, general activity levels of LPL (released into the circulation after heparin administration) are low or non-existent, consistent with depression in adipose tissue as well as perhaps a delay in its rise in the mammary gland. However, during mid to late lactation, LPL levels in both species are up to tenfold higher than levels at parturition and sixfold higher than those in humans (Iverson, Hamosh & Bowen 1991, and unpubl. data). During late lactation, both the concentration of milk fat and the amount secreted are at maximum levels in fasting grey seal mothers (Iverson, Bowen *et al.* 1993). Thus, this dramatic rise in LPL activity is probably due to mammary gland activity, since adipose tissue will only be releasing fatty acids. The pattern of rapidly increasing lipid content of milk during early lactation in some phocids (e.g. Peaker & Goode 1978; Riedman & Ortiz 1979; Iverson, Bowen *et al.* 1993), might be in part explained by rising LPL levels at the onset of lactation, although this has not yet been verified. This hypothesis is supported by evidence that prolactin (which regulates these changes in mammary LPL) is essential for the maintenance of lactation, at least in otariids (Boyd 1991). Given that milk fat content increases over lactation in these phocid species while females fast, the pattern of change is clearly not related to diet.

The milk secretory process and pinnipeds

Despite the extreme variation in milk composition and output among mammals, there appear to be only a few mechanisms responsible for the secretion of all major organic constituents of milk and these mechanisms appear to be identical among all mammals including pinnipeds (Tedman & Bryden 1981; Mather & Keenan 1983; Tedman 1983). The morphology and ultrastructure of the mammary gland, at the level of ducts and alveoli, is also relatively homogeneous among all species studied (Patton & Jensen 1976; Mepham 1983; Oftedal, Boness & Tedman 1987). In the developed mammary gland, milk is synthesized in secretory (epithelial) cells which line the central alveolar cavities (lumina). Milk is then secreted from the cells, each of which produces the complete milk product, into the alveolar lumina which drain into the system of ducts towards the body surface and teat (Jenness 1974; Mepham 1983). Thus, although relatively little work has been done on pinnipeds, it appears that the underlying processes of milk secretion are similar to those of other mammals. The unusual patterns observed in milk delivery in many pinnipeds suggest that these species may have some physiological adaptations uncommon in other mammals. Is there evidence for such adaptations?

The secretion of milk fat globules in all mammalian species occurs by apocrine action (the extrusion of lipid droplets from the cell, taking with them some of the surrounding cell membrane) (Mather & Keenan 1983; Tedman 1983). Since milk fat content of most mammals studied ranges from about 4 to 8%, we might expect differences in the total amount of membrane required and in turnover of membrane components in pinnipeds secreting milk containing 50–60% fat. However, pinnipeds may partially alleviate membrane utilization by secreting larger diameter globules which require less membrane per unit fat secreted (Tedman 1983).

Given the magnitude of daily nutrient output by lactating phocids, we might expect the mammary glands in these species to be unusually large compared to those of other mammals. Mammary glands isolated from a single lactating Weddell seal and a single hooded seal (Oftedal, Boness & Tedman 1987) were larger than predicted by the allometric equation for terrestrial mammals (Hanwell & Peaker 1977). However, data from lactating harbour seals ($n = 13$) indicate that mammary gland weight (1.9 kg; Bowen *et al.* 1992) is reasonably predicted (1.8 kg) from the allometric equation. If mammary glands are not larger than expected in most phocids, we might expect a greater synthetic capacity of secretory cells or a greater efficiency and degree of the homeorhetic regulation of nutrient partitioning to the secretory cells. Phocids may be particularly efficient in the provisioning (from blubber through LPL regulation as discussed above) and transport (through increased mammary blood flow; e.g. Linzell 1974, Hanwell & Peaker 1977) of nutrients, metabolites and energy to the sites of synthesis.

Since the evacuation of milk from the alveolar lumina is of critical importance in sustaining and maximizing the secretory process in other mammals (Mepham 1983), we might expect that the phocid neonate is more proficient at milk removal and is able to digest greater loads of nutrients than are the young of other mammals. Pinniped neonates are extremely efficient at lipid digestion compared to other species (Iverson, Sampugna & Oftedal 1992) but we do not know whether it is the mother or pup that controls milk delivery (e.g. see Iverson, Bowen *et al.* 1993).

In otariids, the secretion mechanisms which enable females to maintain lactation despite long intervals of sometimes many days between suckling are not understood. However, if we assume that a constant rate of milk secretion into alveoli occurs during both suckling and foraging trips, the maximum volume of milk stored in mammary glands during the foraging trip of 2.5–6 days in the California sea lion (*Zalophus californianus*) or northern fur seal (*Callorhinus ursinus*), would be 60 and 170 g/kg$^{0.75}$, respectively (calculated from Costa & Gentry 1986, and Oftedal, Iverson & Boness 1987). Assuming that mammary glands in otariids scale to body mass as in other species, this maximum is similar to the range of the total daily secretion volumes (45–158 g/kg$^{0.75}$; Oftedal 1984b) of a number of terrestrial carnivores and rodents, as well as the rabbit which suckles only once per day. Furthermore, milk secretion rates in otariids are likely to be somewhat reduced during foraging trips in any case owing to end-product inhibition and increased hydrostatic pressure within the mammary gland (e.g., Mepham 1983). However, the extraordinary length (greater than 20 days) of foraging trips in some Juan Fernandez fur seals (*Arctocephalus philippii*) (J. Francis & D. J. Boness pers. comm.) cannot easily be explained by the above argument.

Do diet or nutrient reserves affect milk composition or yield in marine mammals?

Dietary constituents and milk products

The principal constituents of all milks are water, lipids secreted in the form of globules, proteins comprising caseins and whey, carbohydrates and major inorganic salts (Jenness 1974; Johnson 1974; Davies, Holt & Christie 1983; Tedman 1983). The major organic constituents of diets in general are proteins, carbohydrates and lipids. In monogastrics, the primary digestion products of these dietary components are amino acids, simple monosaccharides (e.g. glucose) and fatty acids, respectively.

The major milk proteins of both casein and whey are synthesized within the mammary gland from individual amino acids present in the secretory cells; these amino acids are in turn derived either from direct uptake (all essential and some non-essential amino acids) or from biosynthesis (non-essential amino acids) within the mammary gland (Mepham 1971; Mather & Keenan 1983). The synthesis and secretion rates of these milk proteins are directed by a specific group of genes which become hormonally expressed during pregnancy (Mather & Keenan 1983; Mercier & Gaye 1983).

The primary milk carbohydrates of most mammals are synthesized within the mammary gland from simple monosaccharides taken up by the gland.

Carbohydrate concentration and composition tend to be specific to species and lactation stage (Messer & Green 1979; Oftedal 1984b), and are regulated by the presence of specific enzymes and proteins in the mammary gland under hormonal influence (Brew, Vanaman & Hill 1968; Brew 1970; Kuhn 1983). In most pinniped milks, carbohydrate is absent or less than 1% by weight throughout lactation (Oftedal, Boness & Tedman 1987). This is probably true also of many cetaceans and hence is of minor importance in these species. Thus, by analogy to other species, neither the nature nor the amount of protein and carbohydrate in the milks of marine mammals will be directed by the composition of diet.

However, the relationship between diet and the nature of milk fat differs from that of protein or carbohydrate. Milk lipid is composed of 98–99% triglyceride in all mammals studied, including otariids and phocids (Iverson, Sampugna *et al.* 1992). However, striking differences occur among species in the composition of the triglyceride fatty acids. Milk fatty acids originate from two sources, namely (1) the direct uptake of circulating fatty acids and (2) *de novo* synthesis within the mammary gland from metabolites such as acetate and NADPH which provide sources of carbon and energy. Thus, the variation in milk fatty acid composition among species must arise from differences in the composition of circulating fatty acids (from diet or mobilization) and from species-specific differences in types and rates of *de novo* synthesis.

The extent to which *de novo* synthesis occurs in the mammary glands of carnivores or pinnipeds is not known. *De novo* synthesis of fatty acids is easily detectable when short- (4:0 and 6:0) or medium-chain (8:0–12:0) fatty acids are present in milk, since the enzymes which produce these fatty acids are found only in the mammary glands of some species (Strong & Dils 1972; Dils, Clark & Knudsen 1977; Dils 1983). The fatty acids usually produced in other tissues (e.g. liver, adipose and some species' mammary tissue) are 16:0 or 18:0. The short- and medium-chain fatty acids are found in relatively specific proportions primarily in the milks of ruminants and some monogastrics (e.g. primates, rodents, rabbits and elephants), but not in carnivores and marine mammals. Thus, milk fatty acids in carnivores are thought to be derived primarily from direct uptake from the circulation. Given the nature of dietary lipid digestion and absorption in monogastric species (see pp. 267–268), milk fatty acid composition will be strongly directed by dietary fatty acid composition.

The relationship of diet and nutrient reserves to the proximate composition and yield of milk in marine mammals

Given that lactation is essential for the survival of mammalian offspring, we might expect high priority to be given to milk secretion and to the constancy of nutrient ratios to which the young are adapted. The effects of diet on proximate milk composition and production have been studied primarily in dairy breeds, other domestic animals, and humans. Although no direct studies have been conducted on marine mammals, given the similarity in the milk secretion processes in all mammals studied to date, observations from non-dairy species may be informative. The protein sequencing genes of the mammary gland require a specific array of amino acids (Bauman & Elliot 1983) and only if important component amino acids are unavailable are rates of synthesis likely to be limited; however, the product is unlikely to be altered. Considerable variations in diet, including a poor-quality diet, do not affect the nature of milk proteins and appear to have only a small effect on milk protein content in humans (Lonnerdal *et al.* 1976; Jenness 1979). During starvation in some species, there may be differences in the precursors used for milk fat synthesis (Annison 1983), but in non-dairy species there are virtually no reliable data which indicate that milk fat concentration will be altered by changes in diet (e.g. National Research Council 1991). Numerous studies on the effects of diet composition, quality, quantity or malnutrition on milk fat in humans have concluded that milk fat concentration is little if at all changed by such variables (World Health Organization 1985: 3–22; Jensen 1989). In species adapted to fasting during lactation, total milk fat must be independent of dietary fat. The fact that fasting phocids produce milk containing a relatively constant protein content, up to 60% fat, and increasing milk energy output during the fasting period (e.g. Iverson, Bowen *et al.* 1993), serves as a good example of the control of nutrient partitioning and the unresponsiveness of proximate milk composition to diet.

Despite regulation mechanisms which 'place a priority' on milk secretion, lactation cannot continue without sufficient metabolic substrates for synthesis. When nutrients or reserves become severely limited, production cannot be sustained. However, in non-dairy species there is little evidence that the proximate composition of milk will become significantly altered, unless disease states or metabolic disorders occur. Instead, milk yield will decrease or stop altogether. This has been demonstrated in studies of both penned and pasture-fed ungulates (reviewed in Oftedal 1985). There is also evidence that this occurred in the California sea lion during the reduced food availability associated with the 1983–84 El Niño (Iverson, Oftedal & Boness 1991). Milk composition was not altered, but milk output was reduced. Thus, on a low plane of nutrition, milk output rather than milk composition appears to be altered. Milk yield might also be influenced by body size if body size is an indicator of total nutrient availability, as in phocids adapted to using only body stores to support lactation. In grey seals, smaller females produce less milk than larger females, but milk composition follows the normal postpartum increase in fat content with lactation (Iverson, Bowen *et al.* 1993).

Analysis and interpretation

To assess the relationship between diet and proximate composition of milk in any species, it is important to account for species-specific changes in composition which occur as a function of the lactation process and its regulation, e.g., with lactation stage (Oftedal 1984b; Oftedal, Boness & Tedman 1987; Jensen 1989). Existing data for most marine mammal species are often characterized by small sample sizes and little or imprecise information on lactation stage (Oftedal, Boness & Tedman 1987). Thus it is important to evaluate sampling schemes.

Given the predominance of lipid over all other components in marine mammal milk, accurate assays for milk fat are critical. Firstly, gastric samples cannot be used to assess fat content of milk, not even on a relative basis. Gastric lipase is present in neonates of both phocids and otariids and may result in up to 56% hydrolysis of milk triglyceride in the stomach (Iverson, Sampugna *et al.* 1992). Hydrolysis is associated with more rapid passage of fat than other components of dry matter and both are a function of time since ingestion (Iverson, Kirk *et al.* 1991; Iverson 1988). Thus estimated fat concentration (on both a fresh and a dry weight basis) will also vary with time since ingestion.

The use of extraction methods appropriate to the condition of the sample is also essential for total isolation and accurate quantitation of lipid. Milks, including those of pinnipeds, possess endogenous lipases which hydrolyse triglyceride to free fatty acids and partial glycerides (Hernell & Olivecrona 1974; Freed *et al.* 1986). The Roese-Gottlieb method for analysis of total milk fat is widely recommended for appropriate samples (Horwitz *et al.* 1975; Jensen *et al.* 1985). However, it may significantly underestimate fat content (by as much as 25%) for samples of milk which have undergone extensive hydrolysis, since this method does not extract free fatty acids (Iverson 1988). This has important implications for the analysis of milks that have been stored in various preservatives, such as sodium azide, which do not inhibit hydrolysis. In these cases, extractions using chloroform/methanol, such as the Folch or Bligh and Dyer procedures (reviewed in Christie 1982), should be used. In all cases, gravimetric methods which isolate the fat are clearly the most accurate methods of analysis (Horwitz *et al.* 1975; Jensen 1989). Volumetric methods, such as the Gerber method or creamotocrit, are designed to measure milk fat of the cow, and if used on other species must be calibrated to the specific gravity of the milk fat of that species under the conditions measured (Jenness & Patton 1959).

Lastly, although gastric samples from pups cannot be used to indicate total milk fat content, they can be used to assess maternal milk fatty acids. The fatty acid composition of milk and gastric samples from five harp seal (*Phoca groenlandica*) and five hooded seal (*Cystophora cristata*) mother-pup pairs was identical, even after extensive hydrolysis (Iverson 1988). Gastric hydrolysis products (except for short- and medium-chain free fatty acids) remain inside the intact milk fat globule until the intestine (Patton *et al.* 1982). Thus, in marine mammals, although the original milk may be both diluted and hydrolysed, any given globule will still contain the original fatty acid composition.

Fatty acids as indicators of diet composition in marine mammals

Fatty acids in the marine food web

Perhaps the most intriguing aspect of the relationship between maternal diet and milk secretion is the question of what the milk lipid composition can tell us about the foraging ecology of a species or individual. Marine mammal diets are high in unusual fatty acids which often occur in specific individual arrangements depending on the prey and geographical location. Thus, differences in the fatty acid composition of marine mammal milks can lead to the identification of individual prey types and perhaps to establishing the species composition of diets (Iverson 1988).

An important consequence of the differing restrictions to fatty acid biosynthesis or modification within animals, plants and bacteria is that individual isomers (fatty acids with the same chain length and number of double bonds, but with different configurations or positions of double bonds) as well as 'families' of fatty acids arise in the food chain which can be attributed to specific origins (Cook 1985). Since many animals deposit these dietary fatty acids in body tissues with no or minimal modification, it is possible to distinguish between fatty acids that could be biosynthesized by the animal and those that could only come from the diet.

Marine lipids are well-known for their characteristically high levels of long-chain and polyunsaturated fatty acids (PUFA), which originate from various unicellular phytoplankton and seaweed (Ackman 1980). However, just as noteworthy are the occurrences in some marine lipids of unusual or novel fatty acids (e.g. those that are odd- or branched-chained or non-methylene interrupted, or that contain functional groups such as cyclopropane rings) which may be attributed to a single phylogenetic group or even to a single species from a specific ecological community. For instance, jellyfish were indicated in the diet of ocean sunfish, *Mola mola* (Hooper, Paradis & Ackman 1973) on the basis of the presence of a single unusual fatty acid, *trans*-6-hexadecenoic acid, originally discovered in the giant leatherback turtle, *Dermochelys coriacea coriacea* (Ackman, Hooper & Sipos 1972). Rare, individual isomers are also found among various species. For instance 16:2n-4 and 16:4n-1 are metabolically inert in animals and arise from certain algae (Ackman & McLachlan 1977). Thus, their presence in the depot fats of some fish (e.g. sturgeon, *Acipenser oxyrhynchus*) can be indicative of feeding habits (Ackman, Eaton & Linke 1975). The unique monounsaturate 22:1n-11 originates, together with very large concentrations of 20:1n-9, from the fatty alcohols (wax esters) of some copepods (Pascal & Ackman 1976; Ackman, Sebedio & Kovacs 1980), and appears as a marker in omnivorous fish such as the Atlantic cod, *Gadus morhua*, and herring, *Clupea harengus harengus* (Ackman 1980). Specific odd-chain fatty acids with unique double-bond positions have been found to vary with season and geographical location in the mullet, *Mugil cephalus* (Deng *et al.* 1976) and in the smelt, *Osmerus mordax* (Paradis & Ackman 1976), apparently as a result of consuming specific amphipods. Thus, previous evidence indicates that individual fatty acids or their positional isomers in many marine animals must or most likely arise from dietary intake of specific phyla, classes or even species. The presence of intact wax esters and glycerol ethers (fatty acid-containing lipids) can also be used in tracing trophic relations (Lewis 1967; Ackman 1980).

Given the nature of lipid digestion in carnivores where dietary fatty acids remain largely intact (see pp. 267–268), the concept of fatty acids as trophodynamic tracers readily extends to marine mammals. For example, some of the first examples of such tracers included the presence of the unusual methyl-branched fatty acids in lipids of the ocean sunfish and blubber of the sperm whale, *Physeter macrocephalus* (Pascal & Ackman 1975), and high levels of the long-chain fatty acids 20:1 and 22:1 distinguishing oils of the North Atlantic from those of the Antarctic finwhale, *Balaenoptera physalus* (Ackman & Eaton 1966).

Milk fatty acids as indicators of prey type and diet in marine mammals: evidence

There are many difficulties inherent in determining the diet of pinnipeds posed by collecting data from free-ranging animals at sea (Harwood & Croxall 1988). Information on the diets of whales is even less accessible. The use of fatty acid composition in milk, in combination with information from more traditional methods (i.e., recovered hard parts from stomach contents and faeces), may provide a better understanding of marine mammal diets both nearshore and offshore. Over the past few decades, growing sophistication in methodology and equipment (e.g. gas liquid chromatography—GLC—and mass spectroscopy) has enabled the increasingly complex and rapid analysis of lipid classes and their fatty acids. Although the 'basic' composition of any marine oil may be summarized by about 14 fatty acids, 50 to 70 fatty acids and isomers can routinely be identified and quantified in most marine lipids (Ackman, Ratnayake & Olsson 1988; Iverson 1988). This allows extremely detailed examination of any sample.

In the following discussion, all isomers have been identified and quantified; however, to simplify presentation only a summary of fatty acids is shown in most figures.

The relationship between foraging and milk fatty acid composition can be considered in two ways. The first is the use of fatty acids as tracers, i.e. the presence of a single unusual component which can be traced to a specific prey species, as described above. The second is to consider the array of fatty acids present; i.e. to match the pattern (components and relative levels of components) of all fatty acids in prey to the pattern in milk. This signature method may potentially be more useful because, in addition to identification of prey types, we may be able to evaluate the relative contribution of various prey to the overall diet.

The use of fatty acid signatures is possible because the array of fatty acids can differ substantially among various prey species. To use these differences to infer diet from milk, fatty acids must be generally grouped into three categories: (1) those components which could be readily biosynthesized by the animal or mammary gland (primarily 16:0, 16:1, 18:0 and 18:1); (2) those components that could be biosynthesized but at the levels found are probably mostly of dietary origin (e.g. 14:0, 20:1, some 22:1); and (3) those components which could originate only from the diet (e.g. 22:1n-11, all long chain PUFA with n-3 and n-6 double bond positions, and unusual fatty acids). One long-chain PUFA, 22:5n-3, cannot be used to evaluate diet because it is believed to be an intermediate of 20:5n-3 and 22:6n-3 (Ackman, Ratnayake *et al.* 1988). However, since it is a relatively minor component (usually $< 1\%$ in prey and 1–4% in marine mammal lipids: Iverson 1988) it does not interfere with prey identification. Generally, categories 2 and 3 represent 'indicator' fatty acids (dappled areas, Fig. 1) which contain information about the types of food eaten. The potential differences among prey in the indicator fatty acids 20:1, 22:1, 20:5n-3 and 22:6n-3 are clearly illustrated in Fig. 1. Thus, the extent to which marine mammals can biosynthesize fatty acids should be taken into account when evaluating fatty acid patterns as indicators of diet.

Fatty acids in marine mammal blubber can also be used to infer diet. However, blubber and milk may tell us different things about the diet. The fatty acids in the blubber of a pinniped or cetacean represent an integration of the dietary history of the individual. In an animal which undergoes seasonal periods of fasting and extensive depletion of fat stores (e.g. during breeding or moulting), followed by intensive blubber deposition (e.g. prior to the subsequent breeding season), blubber lipids may reflect dietary intake over the period of several months. Thus, milk lipids secreted while the female is fasting (e.g. in a large phocid) may directly reflect blubber lipids and thus dietary history. However, in species which feed during lactation (e.g. an otariid), milk lipids will reflect the most recent dietary intake, since

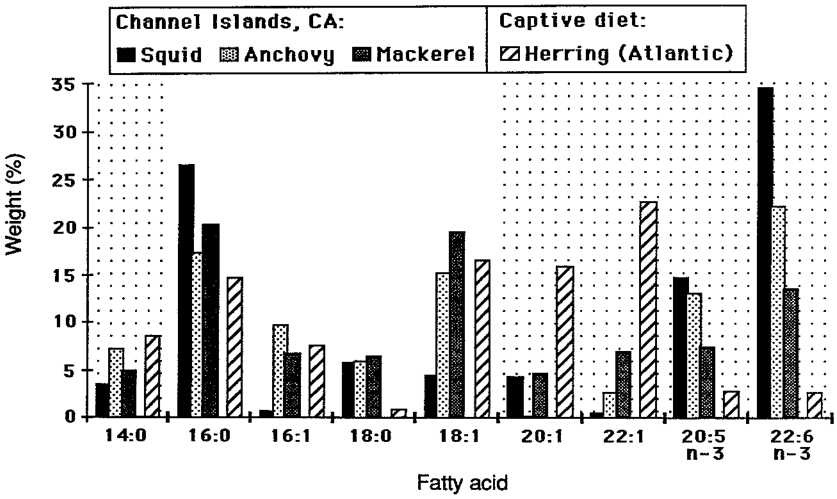

Fig. 1. Fatty acid composition of selected prey items of the California sea lion. Dappled areas represent typical 'indicator' fatty acids (see text). References for prey fatty acid values: Ackman (1980); Ackman (1982); U.S.D.A. (1987).

dietary fatty acids will be directed to the mammary gland by LPL. If dietary intake is insufficient to meet demands of milk lipid secretion, fatty acids may reflect a combination of both diet and blubber mobilization.

Does the fatty acid composition of marine mammal blubber reflect that of diet? Unfortunately little experimental work has been done to date. Grahl-Nielsen & Mjaavatten (1991) studied incorporation of dietary fatty acids into the blubber of adult grey and harbour seals. However, in their study a period of fasting did not precede the diet trial and animals appear to have been kept at maintenance rations during the trial, so that no new dietary fatty acids would have been deposited. Thus, their study was poorly designed to evaluate the use of fatty acid signatures and their conclusion that fatty acids could not be used in this matter must be doubted. Although there are few data available for adults, lipid deposition processes are likely to be similar to those in the young for which there are data. Hooded seal pups are born with a blubber layer which represents fatty acid biosynthesis by the foetus or transfer across the placental membrane, but includes none of the typical indicator (dietary) fatty acids of mothers' milk (Fig. 2; Iverson, Oftedal, Bowen *et al.* in prep.). However, at weaning, the fatty acid composition of pup blubber mirrors the fatty acids which have been transferred over the four-day lactation period (Fig. 2). Thus, initial deposition from diet is unmodified in pups, and this may be true also for adults. Ackman, Epstein & Eaton (1971) suggest that some modification of fatty acids may occur after deposition; however, modification of fatty acids in

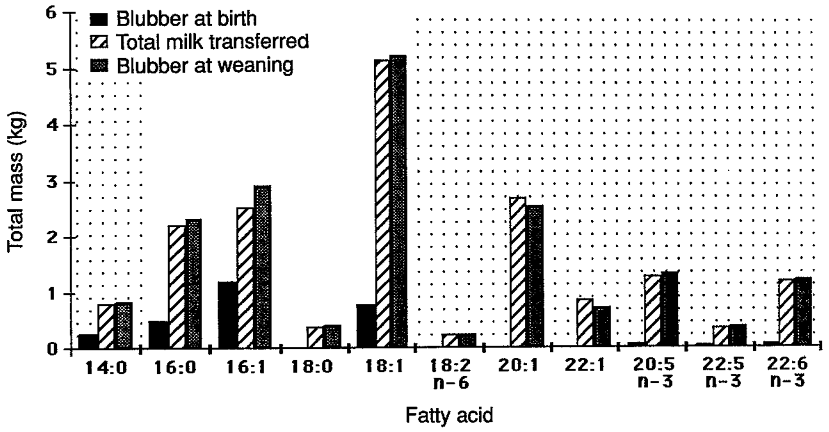

Fig. 2. Total deposition of dietary fatty acids over lactation in suckling hooded seal pups in comparison to the fatty acid content of milk delivered. Dappled areas represent typical 'indicator' fatty acids (see text). From Iverson, Oftedal, Bowen *et al.* (in prep.).

adipose tissue is unlikely to occur at significant rates during lactation (e.g. Bauman & Elliot 1983; Turner, Anderson & Blintz 1989).

To evaluate the incorporation of blubber fatty acids into milk during fasting, milk and blubber must be taken from the same individuals. The hooded seal produces a milk which is 61% fat and secretes about 5 kg milk lipid per day entirely from body reserves (Oftedal, Boness & Bowen 1988). Evidence from this species indicates that the fatty acids in milk are derived mostly from blubber stores (Fig. 3), although minor differences occur in

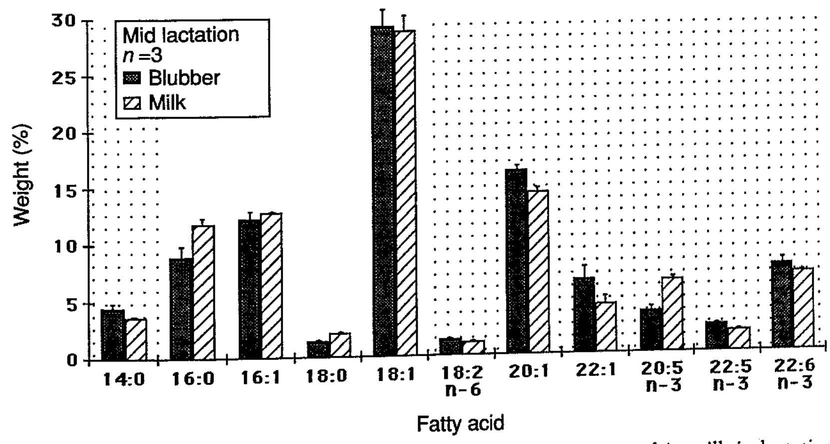

Fig. 3. Fatty acids of maternal blubber in comparison to those secreted in milk in lactating hooded seals at 2 days postpartum. Dappled areas represent typical 'indicator' fatty acids (see text). From Iverson, Oftedal, Bowen *et al.* (in prep.).

some components at different lactation stages (Iverson, Oftedal, Bowen *et al.* in prep.). Lipid transfer from diet to deposition and subsequently to milk is extraordinarily efficient and probably little modification occurs during the process. Thus even if there is some *de novo* synthesis of 16:0–18:1 and some differences or selectivity in mobilized fatty acids, the array of fatty acids secreted in milk while fasting is still highly similar to the array in maternal blubber throughout lactation (Fig. 3). These limited results suggest that both milk and blubber may be used to infer information about dietary history. To the extent that blubber can be used, then the diets of males or juveniles can also be investigated.

If the pattern of milk fatty acids resembles that of blubber when the female is fasting, does the pattern change when feeding resumes? During the perinatal fast in otariids, milk fatty acid secretion must be derived from the mobilization of body stores as described above for a phocid. During this fast, milk lipid presumably reflects blubber and thus dietary history prior to parturition. However, after the onset of foraging trips, lipids from dietary intake will be channelled to the mammary gland by LPL, and thus milk lipid should reflect recent intake. If the diet prior to arrival on the rookery is different from the diet consumed during short foraging trips, the pattern in milk fatty acids should reflect this change, as illustrated by the following examples.

During the breeding season at South Georgia, Antarctic fur seals (*Arctocephalus gazella*) feed almost exclusively on krill (Croxall & Pilcher 1984); however, prior to parturition the fur seal diet is unknown. The fatty acid composition of milk samples obtained from lactating females during the perinatal fast is substantially different than that of milk obtained at the return from foraging trips (Fig. 4a). Although actual prey items have not been analysed, krill (*Euphausia superba*) from the Southern Ocean, which feed upon a specific diatom, have a characteristic fatty acid composition (Ackman & Eaton 1966) which is remarkably similar to the pattern of milk fatty acids during foraging trips (Fig. 4a).

Similarly, milk fatty acid composition of California sea lions also changes dramatically between the perinatal period and intervals between foraging trips (Fig. 4b). In 1983, sea lions in the Channel Islands consumed a mixture of mackerel, *Trachurus symmetricus* (22% mass, 5.3% fat), squid, *Loligo opalescens* (45% mass, 1.0% fat), anchovy, *Engraulis mordax* (14% mass, 2.4% fat) and whiting, *Merluccius productus* (19% mass, 1.2% fat) (Costa, Antonelis & DeLong 1991). Fatty acid contribution of each prey species to the diet can be estimated by using the reported mass contribution corrected for fat content and literature values for the fatty acid compositions of these species (e.g. Fig. 1). Even with this rough estimate of dietary intake, milk fatty acids secreted by females during foraging intervals in 1983 reflect levels of indicator fatty acids of prey species (e.g. 14:0, 20:1–22:6; Fig. 1,

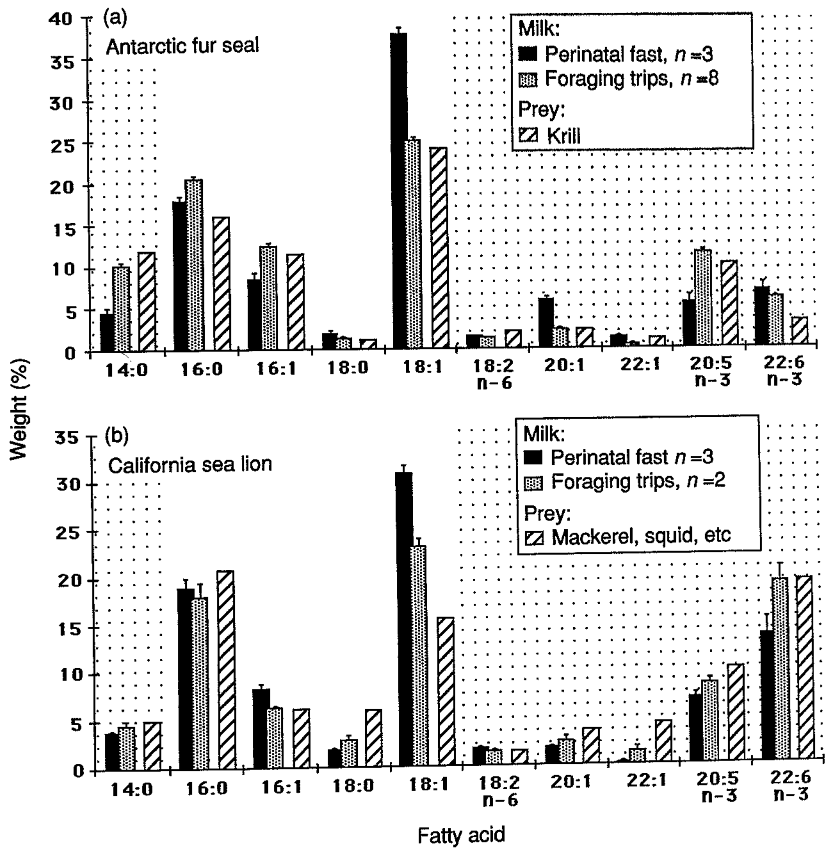

Fig. 4. Changes in milk fatty acid composition from the perinatal fast to feeding in the Antarctic fur seal (a) and the California sea lion (b). The diet during foraging trips in the fur seal (a) was assumed to be krill and in the sea lion (b) to be a mixture of mackerel, squid, anchovy and whiting (see text and Fig. 1). Dappled areas represent typical 'indicator' fatty acids (see text). Most data from Iverson (1988).

Fig. 4b). Additionally, although category 1 fatty acids may originate in part from *de novo* synthesis, in both species of otariid, changes in the levels of 16:1, 18:0 and 18:1 during feeding reflect the differences in the respective prey eaten (Fig. 4a, b).

The use of fatty acid signatures extends beyond marine ecosystems to other species with unique dietary lipid compositions. A lactation pattern comparable to that of otariids is found in the black bear, which secretes milk both during a period of fasting in winter dormancy and during spring and summer foraging. The indicator fatty acid isomers of primary

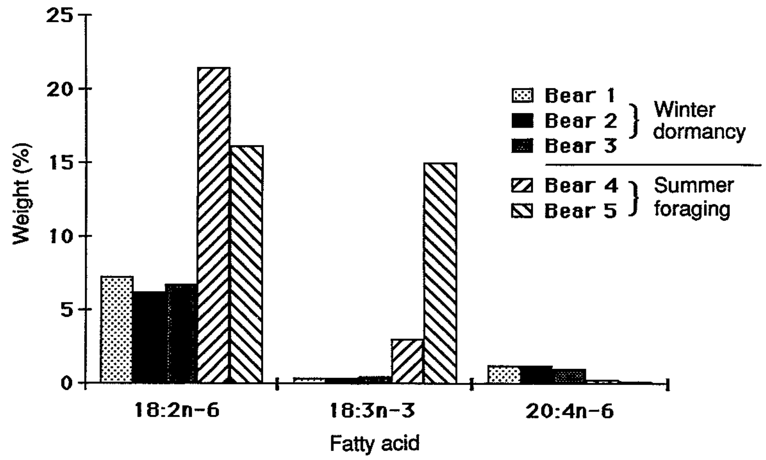

Fig. 5. Important milk fatty acids of dietary origin as indicators of individual foraging habits in the black bear: comparison of milk secreted during and after the fast of winter dormancy. Adapted from data of Iverson & Oftedal (1992).

importance in this case are 18:2n-6 and 18:3n-3, which in marine mammals and their prey usually account for less than 2% and 1% of total fatty acids, respectively. During the period of winter dormancy, levels of 18:2n-6, 18:3n-3 and 20:4n-6 in black bear milk (n = 9) exhibit extremely little variation (Iverson & Oftedal 1992). However, during summer foraging, levels of 18:2n-6 and 18:3n-3 in milks of individual females are variable and extraordinarily high (15–22%), while 20:4n-6 disappears (Fig. 5). Unfortunately, information about the specific dietary items of these individual females is unavailable. However, these patterns clearly reflect individual differences in foraging habits and diets that are high in fruits and leafy vegetation, lipids of which may contain 5–50% of either 18:2n-6 or 18:3n-3, depending on the item, and 0% of 20:4n-6 (Kamel & Kakuda 1992; Peng 1992). Such high levels of 18:2n-6 and especially 18:3n-3 are found only in the milks of other species which feed solely on vegetation, e.g. the koala, *Phascolarctos cinereus*, and the horse, *Equus caballus* (Parodi 1982), red and mantled howler monkeys, *Alouatta* sp. (S. J. Iverson & O. T. Oftedal unpubl. data), and human vegetarians (Jensen 1989).

If milk fatty acids resemble those of recent diet, do they change with a switch in diet? Although there are no controlled studies, a comparison of captive animals with their wild counterparts suggests that fatty acid signatures do indeed reflect changes in diet. The California sea lion in the Channel Islands consumes a mixed diet of mackerel, squid, anchovy and whiting during foraging trips (Costa *et al.* 1991; e.g. Fig. 4b). The fatty acid composition of milk obtained from a captive California sea lion fed

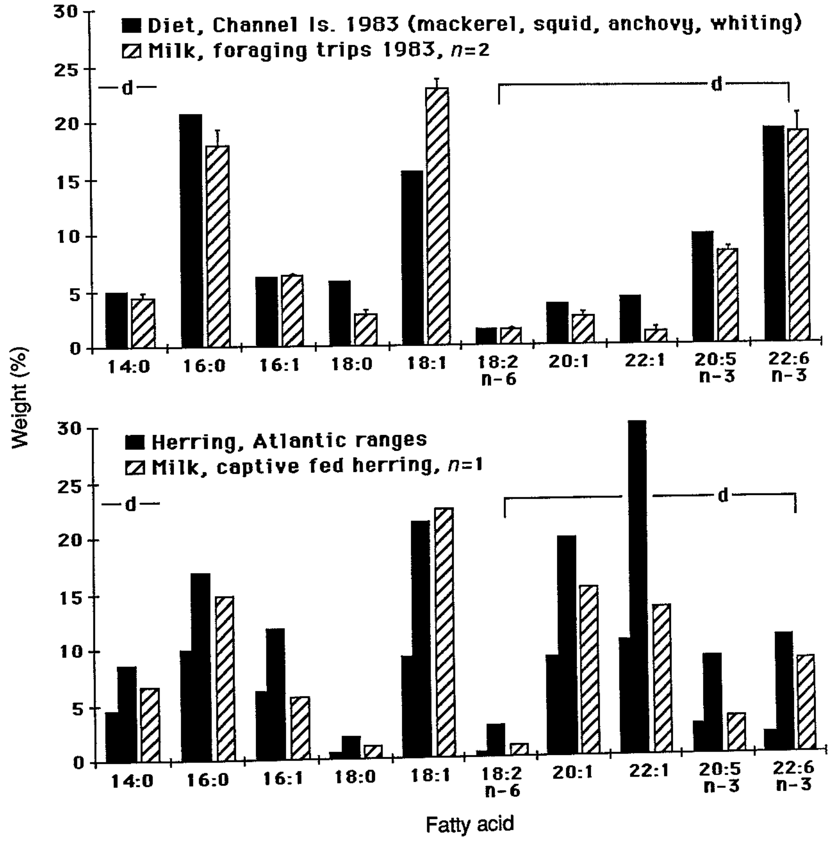

Fig. 6. Milk fatty acid composition of wild (Channel Islands, CA) and captive California sea lions in relation to estimated diets (see text and Fig. 1). Areas marked with '-d-' represent important dietary (or 'indicator') fatty acids (see text). Most data from Iverson (1988).

exclusively on Atlantic herring was substantially different from that in the wild population and the pattern in both milks reflected that estimated for their prey (Fig. 6). Herring are notable for their high levels of isomers of fatty acids 20:1 and 22:1 (Fig. 1), and levels of these components found in the captive sea lion milk have not been encountered in milks from wild sea lions (Iverson 1988). Because the fatty acid composition of lots of herring may vary substantially with factors such as geographical origin (Ackman 1980), a range of values from the literature is shown (Fig. 6). Despite the lack of a controlled study, it is apparent that the changes in diet drive the changes in milk fatty acid patterns.

Additional evidence comes from data on wild and captive harbour seals (S. J. Iverson, W. D. Bowen & D. J. Boness unpubl.). During mid to late

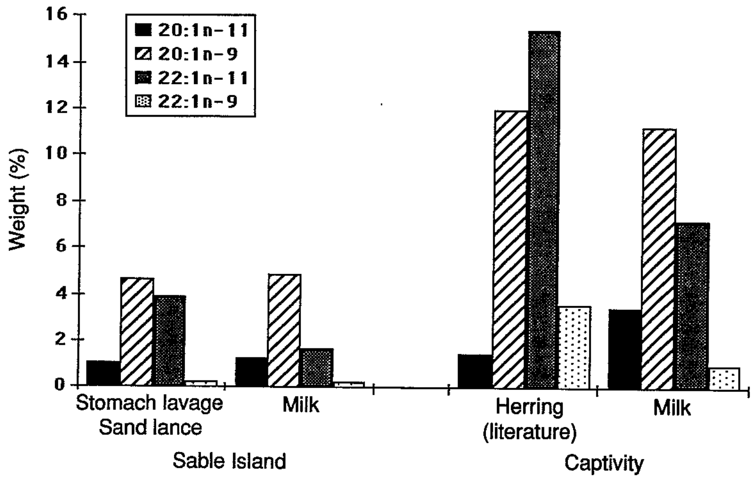

Fig. 7. Long-chain fatty acid isomers of 20:1 and 22:1 in milk from a wild (Sable Island, Nova Scotia) and a captive harbour seal in comparison to wild and captive diets. Wild diet (sand lance) was assessed from fatty acid analysis of stomach contents; captive diet (herring) was derived from literature. From S. J. Iverson, W. D. Bowen & D. J. Boness (unpubl.)

lactation, harbour seals on Sable Island, Nova Scotia, begin regular foraging trips (Bowen *et al.* 1992). Stomach lavage data reveal that the primary prey item at this time is sand lance (*Ammodytes dubius*). Levels of indicator isomers of 20:1 and 22:1 in milk from a harbour seal female that had begun foraging trips are comparable to levels found in lavage samples analysed (Fig. 7). However, the fatty acid pattern of milk from a captive harbour seal, fed solely on herring, reflects the large increases primarily in levels of 20:1n-9 and 22:1n-11 found in herring. Again, the composition of actual herring consumed is unavailable and must be derived from literature values, which probably accounts for discrepancies in some components (Fig. 7). Similarly high levels in these fatty acids are observed in oils from finwhales feeding on the krill *Meganyctiphanes norvegica* (high in 20:1 and 22:1) in Nova Scotia waters as compared with oils from finwhales feeding on the krill *E. superba* (low in 20:1 and 22:1) in the Southern Ocean (Ackman & Eaton 1966).

In conclusion, the potential for use of fatty acid patterns in milks of marine mammals to identify and perhaps quantify prey species appears to be great. During ongoing feeding, fatty acids are partitioned to the mammary gland and thus resemble dietary intake. Unfortunately, information on trophic relationships involving marine mammals through fatty acid signatures is primarily based upon literature values for expected prey items and therefore precludes reliable conclusions. Furthermore, much of the descriptive data from previous marine mammal and prey studies are difficult or impossible to interpret owing to incomplete reports, apparent misidentification of fatty acid peaks and/or failure to separate components with similar retention times during GLC analysis (reviewed in Iverson 1988). Most reports are also based on the analysis of a single lot of fish, seal or whale oil comprising many individuals and often reports list only the analysis of 'edible parts' for human consumers. Thus, detailed and systematic studies of marine mammals and their prey must be conducted in order to exploit fully the potential of using fatty acids to determine trophic interactions.

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References

Ackman, R. G. (1980). Fish lipids, part 1. In *Advances in fish science and technology*: 86–103. (Ed. Connell, J. J.). Fishing News Books Ltd, Surrey, UK.

Ackman, R. G. (1982). Fatty acid composition of fish oils. In *Nutritional evaluation of long-chain fatty acids in fish oil*: 25–88. (Eds Barlow, S. M. & Stansby, M. E.). Academic Press, London & New York.

Ackman, R. G. & Eaton, C. A. (1966). Lipids of the fin whale (*Balaenoptera physalus*) from North Atlantic waters. III. Occurrence of eicosenoic and docosenoic fatty acids in the zooplankton *Meganyctiphanes norvegica* (M. Sars) and their effect on whale oil composition. *Can. J. Biochem.* **44**: 1561–1566.

Ackman, R. G., Eaton, C. A. & Linke, B. A. (1975). Differentiation of freshwater characteristics of fatty acids in marine specimens of the Atlantic sturgeon, *Acipenser oxyrhynchus*. *Fish. Bull. US. Fish Wildl. Serv.* 73: 838–845.

Ackman, R. G., Epstein, S. & Eaton, C. A. (1971). Differences in the fatty acid compositions of blubber fats from northwestern Atlantic finwhales (*Balaenoptera* *physalus*) and harp seals (*Pagophilus groenlandica*). *Comp. Biochem. Physiol.* **40B**: 683–697.

Ackman, R. G., Hooper, S. N. & Sipos, J. C. (1972). Distribution of *trans*-6-hexadecenoic and other fatty acids in tissues and organs of the Atlantic leatherback turtle, *Dermochelys coriacea coriacea* L. *Int. J. Biochem.* **3**: 171–179.

Ackman, R. G. & McLachlan, J. (1977). Fatty acids in some Nova Scotian marine seaweeds: a survey for octadecapentaenoic and other biochemically novel fatty acids. *Proc. Nova Scotian Inst. Sci.* **28**: 47–64.

Ackman, R. G., Ratnayake, W. M. N. & Olsson, B. (1988). The 'basic' fatty acid composition of Atlantic fish oils: potential similarities useful for enrichment of polyunsaturated fatty acids by urea complexation. *J. Am. Oil Chem. Soc.* **65**: 136–138.

Ackman, R. G., Sebedio, J.-L. & Kovacs, M. I. P. (1980). Role of eicosenoic and docosenoic fatty acids in freshwater and marine lipids. *Mar. Chem.* **9**: 157–164.

Allen, W. V. (1976). Biochemical aspects of lipid storage and utilization in animals. *Am. Zool.* **16**: 631–647.

Annison, E. F. (1983). Metabolite utilization by the ruminant mammary gland. In *Biochemistry of lactation*: 399–435. (Ed. Mepham, T. B.). Elsevier Science Publishers, Amsterdam.

Bauman, D. E. & Currie, W. B. (1980). Partitioning of nutrients during pregnancy and lactation: a review of mechanisms involving homeostasis and homeorhesis. *J. Dairy Sci.* **63**: 1514–1529.

Bauman, D. E. & Elliot, J. M. (1983). Control of nutrient partitioning in lactating ruminants. In *Biochemistry of lactation*: 437–468. (Ed. Mepham, T. B.). Elsevier Science Publishers, Amsterdam.

Blaxter, K. L. (1962). *The energy metabolism of ruminants.* Hutchinson, London.

Bonner, W. N. (1984). Lactation strategies in pinnipeds: problems for a marine mammalian group. *Symp. zool Soc. Lond.* No. **51**: 253–272.

Borgstrom, B. (1977). Digestion and absorption of lipids. *Int. Rev. Physiol.* **12**: 305–323.

Bowen, W. D., Oftedal, O. T. & Boness, D. J. (1992). Mass and energy transfer during lactation in a small phocid, the harbor seal (*Phoca vitulina*). *Physiol. Zool.* **65**: 844–866.

Boyd, I. L. (1991). Changes in plasma progesterone and prolactin concentrations during the annual cycle and the role of prolactin in the maintenance of lactation and luteal development in the Antarctic fur seal (*Arctocephalus gazella*). *J. Reprod. Fert.* **91**: 637–647.

Brew, K. (1970). Lactose synthetase: evolutionary origins, structure and control. *Essays Biochem.* **6**: 93–118.

Brew, K., Vanaman, T. C. & Hill, R. L. (1968). The role of α-lactalbumin and the A protein in lactose synthetase: a unique mechanism for the control of a biological reaction. *Proc. natn. Acad. Sci. U.S.A.* **59**: 491–497.

Christie, W. W. (1982). *Lipid analysis.* Pergamon Press, New York.

Cook, H. W. (1985). Fatty acid desaturation and chain elongation in eucaryotes. In *Biochemistry of lipids and membranes*: 181–211. (Eds Vance, D. E. & Vance, J. E.). The Benjamin/Cummings Publishing Co., Inc., Menlo Park, CA.

Costa, D. P., Antonelis, G. A. & DeLong, R. L. (1991) The effects of El Niño on the foraging energetics of the California sea lion. In *Pinnipeds and El Niño: responses to environmental stress*: 156–165. (Eds Trillmich, F. & Ono, K. A.). Springer-Verlag, Berlin, Heidelberg.

Costa, D. P. & Gentry, R. L. (1986). Free-ranging energetics of northern fur seals. In *Fur seals: maternal strategies on land and at sea*: 79–101. (Eds Gentry, R. L. & Kooyman, G. L.). Princeton University Press, Princeton.

Cowie, A. T. (1969). Variations in the yield and composition of the milk during lactation in the rabbit and the galactopoietic effect of prolactin. *J. Endocr.* 44: 437–450.

Cowie, A. T., Forsyth, I. A. & Hart, I. C. (1980). *Hormonal control of lactation.* Springer-Verlag, Berlin, Heidelberg.

Cripps, A. W. & Williams, V. J. (1975). The effect of pregnancy and lactation on food intake, gastrointestinal anatomy and the absorptive capacity of the small intestine in the albino rat. *Br. J. Nutr.* 33: 17–32.

Croxall, J. P. & Pilcher, M. N. (1984). Characteristics of krill *Euphausia superba* eaten by Antarctic fur seals *Arctocephalus gazella* at South Georgia. *Bull. Br. Antarct. Surv.* No. 63: 117–125.

Davies, D. T., Holt, C. & Christie, W. W. (1983). The composition of milk. In *Biochemistry of lactation*: 71–117. (Ed. Mepham, T. B.). Elsevier Science Publishers, Amsterdam.

Deng, J.-C., Orthoefer, F. T., Dennison, R. A. & Watson, M. (1976). Lipids and fatty acids in the mullet (*Mugil cephalus*): seasonal and locational variations. *J. Food Sci.* 41: 1479–1483.

Dils, R. R. (1983). Milk fat synthesis. In *Biochemistry of lactation*: 141–157. (Ed. Mepham, T. B.). Elsevier Science Publishers, Amsterdam.

Dils, R. R., Clark, S. & Knudsen, J. (1977). Comparative aspects of milk fat synthesis. *Symp. zool. Soc. Lond.* No. 41: 43–55.

Freed, L. M., York, C. M., Hamosh, M., Sturman, J. T., Oftedal, O. T. & Hamosh, P. (1986). Bile salt stimulated lipase; the enzyme is present in non-primate milk. In *Human lactation* 2. *Maternal-environmental factors*: 595–602. (Eds Hamosh, M. & Goldman, A. S.). Plenum Press, New York.

Gaskin, D. E. (1982). *The ecology of whales and dolphins.* Heinemann Educational Books, London.

Grahl-Nielsen, O. & Mjaavatten O. (1991). Dietary influence on fatty acid composition of blubber fat of seals as determined by biopsy: a multivariate approach. *Mar. Biol., Berlin* 110: 59–64.

Hamosh, M. (1989). Enzymes in human milk: their role in nutrient digestion, gastrointestinal function, and nutrient delivery to the newborn infant. In *Textbook of gastroenterology and nutrition in infancy*: 121–134. (Ed. Lebenthal, E.). Raven Press, New York.

Hamosh, M., Clary, T. R., Chernick, S. S. & Scow, R. O. (1970). Lipoprotein lipase activity of adipose and mammary tissue and plasma triglyceride in pregnant and lactating rats. *Biochim. Biophys. Acta* 210: 473–482.

Hamosh, M. & Hamosh, P. (1985). Lipoproteins and lipoprotein lipase. In *Handbook of physiology—the respiratory system* 1: 387–418. (Eds Fishman, A. P. & Fisher, A. B.). The American Physiological Society, Washington D.C.

Hanwell, A. & Peaker, M. (1977). Physiological effects of lactation on the mother. *Symp. zool. Soc. Lond.* No. 41: 297–312.

Harrison, R. J. (1969). Reproduction and reproductive organs. In *The biology of marine mammals*: 253–348. (Ed. Andersen, H. T.). Academic Press, New York & London.

Harwood, J. & Croxall, J. P. (1988). The assessment of competition between seals and commercial fisheries in the North Sea and the Antarctic. *Mar. Mammal Sci.* **4**: 13–33.

Hernell, O. & Olivecrona, T. (1974). Human milk lipases. I. Bile salt stimulated lipase. *J. Lipid Res.* **15**: 367–372.

Hooper, S. N., Paradis, M. & Ackman, R. G. (1973). Distribution of *trans*-6-hexadecenoic acid, 7-methyl-7-hexadecenoic acid and common fatty acids in lipids of the ocean sunfish *Mola mola*. *Lipids* **8**: 509–516.

Horwitz, W., Senzel, A., Reynolds, H. & Park, D. L. (Eds) (1975). *Official methods of analysis*. Association of Official Analytical Chemists, Washington D.C.

Iverson, S. J. (1988). *Composition, intake and gastric digestion of milk lipids in pinnipeds*. PhD thesis: Univ. of Maryland, College Park.

Iverson, S. J., Bowen, W. D., Boness, D. J. & Oftedal, O. T. (1993). The effect of maternal size and milk energy output, and its effect on pup growth in grey seals (*Halichoerus grypus*). *Physiol. Zool.* **66**: 61–88.

Iverson, S. J., Hamosh, M. & Bowen, W. D. (1991). Lipid clearing enzymes during lactation in species adapted to high milk fat output and intake. *Abstr. pediatr. Res.* **29**: 44A.

Iverson, S. J., Kirk, C. L., Hamosh, M. & Newsome, J. (1991). Milk lipid digestion in the neonatal dog: the combined actions of gastric and bile salt stimulated lipases. *Biochim. biophys. Acta* **1083**: 109–119.

Iverson, S. J. & Oftedal, O. T. (1992). Fatty acid composition of black bear (*Ursus americanus*) milk during and after the period of winter dormancy. *Lipids* **27**: 940–943.

Iverson, S. J., Oftedal, O. T. & Boness, D. J. (1991). The effect of El Niño on pup development in the California sea lion (*Zalophus californianus*). II. Milk intake. In *Pinnipeds and El Niño: responses to environmental stress*: 180–184. (Eds Trillmich, F. & Ono, K. A.). Springer-Verlag, Berlin, Heidelberg.

Iverson, S. J., Oftedal, O. T., Bowen, W. D., Boness, D. J. & Sampugna, J. (In preparation). *The transfer of lipids from mother to pup in the hooded seal* (Cystophora cristata): *comparison of maternal depot fatty acids to milk fatty acids and subsequent incorporation in the pup*.

Iverson, S. J., Sampugna, J. & Oftedal, O. T. (1992). Positional specificity of gastric hydrolysis of long-chain n-3 polyunsaturated fatty acids of seal milk triglycerides. *Lipids* **27**: 870–878.

Jenness, R. (1974). Biosynthesis and composition of milk. *J. invest. Derm.* **63**: 109–118.

Jenness, R. (1979). The composition of human milk. *Semin. Perinatol.* **3**: 225–239.

Jenness, R. (1986). Lactational performance of various mammalian species. *J. Dairy Sci.* **69**: 869–885.

Jenness, R. & Patton, S. (1959). *Principles of dairy chemistry*. John Wiley & Sons, New York.

Jensen, R. G. (1989). Lipids in human milk—composition and fat-soluble vitamins. In *Textbook of gastroenterology and nutrition in infancy*: 157–208. (Ed. Lebenthal, E.). Raven Press, New York.

Jensen, R. G., Clark, R. M., Bitman, J., Wood, D. L. & Clandinin, M. T. (1985). Methods for the sampling and analysis of human milk lipids. In *Human lactation: milk components and methodologies*: 97–112. (Eds Jensen, R. G. & Neville, M. C.). Plenum Press, New York.

Johnson, A. H. (1974). The composition of milk. In *Fundamentals of dairy chemistry*: 1–57. (Eds Webb, B. H., Johnson, A. H. & Alford, J. A.). AVI Publishing, Westport, Conn.

Kamel, B. S. & Kakuda, Y. (1992). Fatty acids in fruits and fruit products. In *Fatty acids in foods and their health implications*: 263–295. (Ed. Chow, C. K.). Marcel Dekker, Inc., New York.

Kretzmann, M. B., Costa, D. P., Higgins, L. V. & Needham, D. J. (1991). Milk composition of Australian sea lions, *Neophoca cinerea*: variability in lipid content. *Can. J. Zool.* **69**: 2556–2561.

Kuhn, N. J. (1983). The biosynthesis of lactose. In *Biochemistry of lactation*: 159–176. (Ed. Mepham, T. B.). Elsevier Science Publishers, Amsterdam.

Lewis, R. W. (1967). Fatty acid composition of some marine animals from various depths. *J. Fish. Res. Bd Can.* **24**: 1101–1115.

Linzell, J. L. (1974). Mammary blood flow and methods of identifying and measuring precursors of milk. In *Lactation* **1**: 143–225. (Eds Larson, B. L. & Smith, V. R.). Academic Press, New York & London.

Lonnerdal, B., Forsum, E., Gebre-Medhin, M. & Hambraeus, L. (1976). Breast milk composition in Ethiopian and Swedish mothers. II. Lactose, nitrogen and protein contents. *Am. J. clin. Nutr.* **29**: 1134–1141.

Macdonald, D. (Ed.) (1984). *The encyclopedia of mammals* **1**. George Allen & Unwin, London; Facts on File Publications, New York.

Mather, I. H. & Keenan, T. W. (1983). Function of endomembranes and the cell surface in the secretion of organic milk constituents. In *Biochemistry of lactation*: 231–283. (Ed. Mepham, T. B.). Elsevier Science Publishers, Amsterdam.

McBride, O. W. & Korn, E. D. (1963). The lipoprotein lipase of mammary gland and the correlation of its activity to lactation. *J. Lipid Res.* **4**: 17–20.

McBride, O. W. & Korn, E. D. (1964). Uptake of free fatty acids and chylomicron glycerides by guinea pig mammary gland in pregnancy and lactation. *J. Lipid Res.* **5**: 453–458.

Mehta, N. R., Jones, J. B. & Hamosh, M. (1982). Lipases in preterm human milk: ontogeny and physiologic significance. *J. pediatr. Gastroenterol Nutr.* **1**: 317–326.

Mendelson, C. R. & Scow, R. O. (1972). Uptake of chylomicron-triglyceride by perfused mammary tissue of lactating rats. *Am. J. Physiol.* **223**: 1418–1423.

Mepham, T. B. (1971). Amino acid utilization by the lactating mammary gland. In *Lactation*: 297–315. (Ed. Falconer, I. R.). Butterworths, London.

Mepham, T. B. (1983). Physiological aspects of lactation. In *Biochemistry of lactation*: 3–28. (Ed. Mepham, T. B.). Elsevier Science Publishers, Amsterdam.

Mercier, J.-C. & Gaye, P. (1983). Milk protein synthesis. In *Biochemistry of lactation*: 177–227. (Ed. Mepham, T. B.). Elsevier Science Publishers, Amsterdam.

Messer, M. & Green, B. (1979). Milk carbohydrates of marsupials. II. Quantitative and qualitative changes in milk carbohydrates during lactation in the tammar wallaby (*Macropus eugenii*). *Aust. J. biol. Sci.* **32**: 519–531.

Millar, J. S. (1977). Adaptive features of mammalian reproduction. *Evolution, Lawrence, Kans.* **31**: 370–386.

Millar, J. S. (1979). Energetics of lactation in *Peromyscus maniculatus*. *Can. J. Zool.* **57**: 1015–1019.

National Research Council (1991). *Nutrition during lactation*. Institute of Medicine, Subcommittee on Nutrition during Lactation. National Academy Press, Washington D.C.

Oftedal, O. T. (1984a). Lactation in the dog: milk composition and intake by puppies. *J. Nutr.* **114**: 803–812.

Oftedal, O. T. (1984b). Milk composition, milk yield and energy output at peak lactation: a comparative review. *Symp. zool. Soc. Lond* No. **51**: 33–85.

Oftedal, O. T. (1985). Pregnancy and lactation. In *Bioenergetics of wild herbivores*: 215–238. (Eds Hudson, R. J. & White, R. G.). CRC Press, Boca Raton, Florida.

Oftedal, O. T., Boness, D. J. & Bowen, W. D. (1988). The composition of hooded seal (*Cystophora cristata*) milk: an adaptation to postnatal fattening. *Can. J. Zool.* **66**: 318–322.

Oftedal, O. T., Boness, D. J. & Tedman, R. A. (1987). The behavior, physiology, and anatomy of lactation in the Pinnipedia. *Curr. Mammal.* **1**: 175–245.

Oftedal, O. T., Iverson, S. J. & Boness, D. J. (1987). Milk and energy intakes of suckling California sea lion *Zalophus californianus* pups in relation to sex, growth, and predicted maintenance requirements. *Physiol. Zool.* **60**: 560–575.

Paradis, M. & Ackman, R. G. (1976). Localization of a source of marine odd chain-length fatty acids. I. The amphipod *Pontoporeia femorata* (Kröyer). *Lipids* **11**: 863–870.

Parodi, P. W. (1982). Positional distribution of fatty acids in triglycerides from milk of several species of mammals. *Lipids* **17**: 437–442.

Pascal, J. C. & Ackman, R. G. (1975). Occurrence of 7-methyl-7-hexadecenoic acid, the corresponding alcohol, 7-methyl-6-hexadecenoic acid, and 5-methyl-4-tetradecenoic acid in sperm whale oils. *Lipids* **10**: 478–482.

Pascal, J. C. & Ackman, R. G. (1976). Long chain monoethylenic alcohol and acid isomers in lipids of copepods and capelin. *Chem. Phys. Lipids* **16**: 219–223.

Patton, J. S. (1981). Gastrointestinal lipid digestion. In *Physiology of the gastro-intestinal tract*: 1123–1146. (Ed. Johnson, L. R.). Raven Press, New York.

Patton, S. & Jensen, R. G. (1976). *Biomedical aspects of lactation with special reference to lipid metabolism and membrane functions of the mammary gland*. Pergamon Press, Oxford.

Patton, J. S., Rigler, M. W., Liao, T. H., Hamosh, P. & Hamosh, M. (1982). Hydrolysis of triacylglycerol emulsions by lingual lipase; a microscopic study. *Biochim. biophys. Acta* **712**: 400–407.

Peaker, M. & Goode, J. A. (1978). The milk of the fur-seal, *Arctocephalus tropicalis gazella**; in particular the composition of the aqueous phase. *J. Zool.,* *Lond.* **185**: 469–476. [*Species incorrectly identified; species is actually the southern elephant seal, *Mirounga leonina*.]

Peng, A. C. (1992). Fatty acids in vegetables and vegetable products. In *Fatty acids in foods and their health implications*: 185–236. (Ed. Chow, C. K.). Marcel Dekker, Inc., New York.

Rice, D. W. & Wolman, A. A. (1971). The life history and ecology of the grey whale (*Eschrichtius robustus*). *Spec. Publ. Am. Soc. Mammal.* No. 3: 1–142.

Riedman, M. & Ortiz, C. L. (1979). Changes in milk composition during lactation in the northern elephant seal. *Physiol. Zool.* **52**: 240–249.

Robinson, D. S. (1963). Changes in the lipolytic activity of the guinea pig mammary gland at parturition. *J. Lipid Res.* **4**: 21–23.

Russe, I. (1961). Die Laktation der Hunden. *Zentbl. VetMed.* **8**: 252–281.

Scow, R. O. (1977). Metabolism of chylomicrons in perfused adipose and mammary tissue of the rat. *Fed. Proc.* **36**: 182–185.

Strong, C. R. & Dils, R. R. (1972). The fatty acid synthetase complex of lactating guinea-pig mammary gland. *Int. J. Biochem.* **3**: 369–377.

Tedman, R. A. (1983). Ultrastructural morphology of the mammary gland with observations on the size distribution of fat droplets in milk of the Weddell seal *Leptonychotes weddelli* (Pinnipedia). *J. Zool., Lond.* **200**: 131–141.

Tedman, R. A. & Bryden, M. M. (1981). The mammary gland of the Weddell seal, *Leptonychotes weddelli* (Pinnipedia). 1. Gross and microscopic anatomy. *Anat. Rec.* **199**: 519–529.

Trillmich, F. & Lechner, E. (1986). Milk of the Galapagos fur seal and sea lion, with a comparison of the milk of eared seals (Otariidae). *J. Zool., Lond.* (*A*) **209**: 271–277.

Turner, J. J., Anderson, B. D. & Blintz, G. L. (1989). Whole-body lipids and fatty acid synthetase activity in Richardson's ground squirrels, *Spermophilus richardsonii*. *Physiol. Zool.* **62**: 1383–1397.

U.S.D.A. (1987). The composition of foods: finfish and shellfish products. *Agric. Handb. U.S. Dep. Agric.* No. 8–15: 19–190.

Williamson, D. H. (1980). Integration of metabolism in tissues of the lactating rat. *FEBS Lett.* **117**: K93–K105.

World Health Organization (1985). *The quantity and quality of breast milk.* World Health Organization, Geneva.

Young, R. A. (1976). Fat, energy and mammalian survival. *Am. Zool.* **16**: 699–710.

Zinder, O., Hamosh, M., Clary Fleck, T. R. & Scow, R. O. (1974). Effect of prolactin on lipoprotein lipase in mammary gland and adipose tissue in rats. *Am. J. Physiol.* **226**: 774–748.