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Changes in composition and energy content of harp seal milk during lactation

by

D.M. Lavigne and R.E.A. Stewart
Department of Zoology, University of Guelph,
Guelph, Ontario, Canada N1G 2W1

and

F. Fletcher
Central Milk Testing Laboratory
Ontario Ministry of Agriculture and Food
240 York Rd., Guelph, Ontario, Canada N1G 2W1

Milk samples were obtained from stomachs of 26 harp seal pups, *Phoca groenlandica*, at various stages during lactation. In addition, morphometric data were obtained for these pups and for 20 of their respective mothers. Fat, and hence, energy content of milk increased during lactation; water content declined, whereas protein and ash remained constant. Fat, water, and energy content were significantly correlated with pup age, a measure of the stage of lactation of the female, and with female condition which also declined during the nursing period. Trends observed in milk composition in the present study were similar to those reported for several other phocid seals but differed significantly from an earlier examination of harp seal milk. The high fat content of harp seal milk transfers large amounts of energy to the pup during a short lactation period, facilitating rapid neonatal growth. In addition, the "replacement" of water with fat in milk as lactation proceeds, conserves water and this may be important in lactating seals with limited sources of fresh water.

INTRODUCTION

Milk of marine mammals (Pinnipedia and Cetacea) contains high concentrations of fat and only small amounts of lactose (Jenness 1974, Ben Shaul 1962, Lavigne *et al. in press*). In both the Weddell seal, *Leptonychotes weddelli* (see Stull *et al.* 1967, Kooyman and Drabek 1968) and northern elephant seal, *Mirounga angustirostris* (see Riedman and

Ortiz 1979), fat content increases as water content decreases during the lactation period. Similar changes in milk composition have been observed in the southern elephant seal, M. leonina, except that there was an abrupt decrease in fat content with a concomitant increase in water content just prior to weaning (Bryden 1968). In contrast, fat and water content reportedly remain constant in the harp seal Phoca groenlandica during the first 10 days of nursing, with fat content dropping as water content increased at 16 days post partum (Van Horn and Baker 1971). In addition to stage of lactation, other factors such as diet, physiological state (which may vary with female age), and genetic constitution may also effect milk composition (Glass et al. 1967).

The objective of the present study was to re-examine proximate composition, and thus energy content, of harp seal milk during lactation in relation to the age of the female and her physiological condition. Such information is required to refine further the energy budget of Northwest Atlantic harp seals (Lavigne et al. in press, Lavigne and Stewart 1979, Stewart and Lavigne 1980, Innes et al. 1981) and to evaluate aspects of maternal investment in this species (Stewart in prep.).

MATERIALS AND METHODS

Obtaining sufficient quantities of harp seal milk for analysis is a recurrent problem. As an alternative to hormone injections (Cook and Baker 1969, Van Horn and Baker 1971), milk was obtained from the stomach of pups, most of which had been observed nursing immediately prior to being collected in an annual scientific sample of mother-pup pairs.

Fresh milk samples, free of obvious contamination, were obtained from 26 of 36 pups between 1 March and 14 March 1980 in the Gulf of St. Lawrence, west of the Magdalen Islands. Age of pups (measure of the stage of lactation of its mother) was estimated using criteria described by Stewart and Lavigne (1980). Morphometrics including standard length, axillary girth, blubber thickness, sculp (blubber with skin attached) weight, and core (lean body) weight were also obtained for each pup sampled and for 30 of their respective mothers (American Society of

Mammalogists 1967, Innes et al. 1981). Age of adult females was estimated from counts of dentinal annuli in canine teeth (Scheffer 1950, Laws 1953, Fisher and Mackenzie 1954, Stewart and Lavigne 1979).

Milk samples were frozen in the field and maintained at -20C until analyses were conducted in May and August 1980. Prior to analysis, milk samples were thawed and homogenized. Subsequent determinations of proximate composition were expressed as per cent of whole milk on a weight-weight basis (g/100 g whole milk).

Three grams of homogenized milk were placed in a steam bath for 30 minutes, then transferred to an oven at 102 C for 24 hours to determine total solids, and by subtraction, the water content of the milk. Fat content was determined using the Mojonnier extraction method (= Roese-Gottlieb method used by Cook and Baker 1969, Van Horn and Baker 1971, Association of Official Analytical Chemists 1970). Protein was estimated using a semi-micro (0.5 ml samples) Kjeldahl nitrogen determination (A.O.A.C. 1970). Nitrogen recovery (g) was multiplied by 6.83 to obtain an estimate of total protein (Cook and Baker 1969, Van Horn and Baker 1971). To determine ash content two milk samples of known mass were placed in oven-dried crucibles and allowed to evaporate at temperatures increasing from ~22^{o}C to 600^{o}C over six hours. Samples were then maintained at 600^{o}C for an additional 3 h, and cooled in dessicators, prior to reweighing.

A polarimetric determination for lactose used routinely for bovine milk (Biggs and Szijarto 1963) failed to produce repeatable results. Consequently, lactose was indirectly estimated by:

$$\%\ \text{lactose} = \%\ \text{total solids} - \%\ \text{fat} - \%\ \text{protein} - \%\ \text{ash} \qquad [1]$$

Caloric density (or calorific value, kcal g^{-1} whole milk) was estimated indirectly from proximate composition using caloric equivalents for fat, protein, and carbohydrate of 9.40, 5.65, and 4.15 kcal g^{-1} dry weight (Pike and Brown 1975), and directly using an adiabatic bomb calorimeter (Lavigne and Stewart 1979).

Blubber thickness (mm) and axillary girth (cm), measures of the amount of stored energy or fatness, were standardized for variations in body size (i.e. blubber thickness ÷ length, girth ÷ length) to provide "condition indices" (McLaren 1958, Usher and Church 1969, Innes et al.

1981) for comparisons of mothers and pups during the lactation period.

Simple correlations were examined between female age, physiological condition, stage of lactation (estimated pup age), milk composition and energy content. Relationships between various parameters were examined using least squares regression analyses (Draper and Smith 1966).

RESULTS

Only those milk samples (n=26) which were of sufficient volume and appeared uncontaminated with blood or gastric secretions were analysed. Volume of milk in 19 samples recorded ranged from 90 to 650 ml ($\bar{x} \pm SD$ = 298.2 $\pm$ 133.6 ml).

Changes in per cent water and fat, and caloric density occurred throughout the nursing period. Water content of milk obtained from stomachs of newborn seals less than one day of age was about 68% (n=5), declining to near 50% (n=4) in pups aged about 9 days (Fig. 1). In contrast, fat increased from about 23% in newborn pups to more than 40% in 9-day old animals. The correlation between per cent water and per cent fat was highly significant (Fig. 2).

Fat content of milk was significantly correlated with the stage of lactation (pup age) ($r = 0.76$, $p = 0.00001$, $n = 26$), and female condition ($r = -0.63$, $p = 0.0069$, $n = 17$) but not with the age of the female ($r = 0.31$, $p = 0.22$, $n = 17$). There were also significant correlations between caloric density and stage of lactation, per cent water, and per cent fat (Fig. 1).

Protein content remained constant throughout the nursing period (Fig. 1; $\bar{x} \pm SD = 6.56 \pm 0.75$ g/100 g whole milk) as did the indirect estimates of lactose levels (2.26 $\pm$ 0.83 g/100 g whole milk) and ash content (0.56 $\pm$ 0.09 g/100 g whole milk). There was no correlation between the age and condition of lactating females ($r = 0.06$, $p = 0.83$, $n = 17$).

Female condition was negatively correlated with pup age (stage of lactation) (blubber thickness $\div$ length, $r = -0.74$, $p < 0.00001$, $n = 29$; girth $\div$ length, $r = -0.69$, $p = 0.00004$, $n = 29$) indicative of a decline in her stored energy throughout lactation (Fig. 3). The correlation between pup condition and pup age was also highly significant (blubber thickness $\div$ length, $r = 0.90$, $p < 0.00001$, $n = 30$) reflecting a concomitant increase in blubber stores as lactation progressed (Fig. 3).

Indirect estimates of caloric density from proximate composition were highly correlated with the direct and independent estimates determined using bomb calorimetry ($r = 0.97$, $p < 0.00001$, $n = 26$) (Fig. 4).

DISCUSSION

Female harp seals give birth to a single pup weighing 10.8 ± 0.65 kg ($\bar{x}$ ± SD) (Lavigne and Stewart 1979, Stewart and Lavigne 1980) and lacking a subcutaneous layer of fat characteristic of older seals. Nursing begins shortly after birth, and lasts for about nine days, during which time the pup grows at a rate of about 2.5 kg per day (Stewart and Lavigne 1980). Weight loss in the lactating female is primarily from the blubber layer, and not from the lean body mass (Innes *et al*. 1978).

As the growth rate of neonatal harp seals appears to remain constant during the lactation period (Stewart and Lavigne 1980), the increase in caloric density of milk (Fig. 1) suggests that the amount of milk ingested also changes during the nursing period, or alternatively, that changes in digestive efficiency occur as the animal grows. There are no data on the latter, but field observations (Stewart and Lavigne 1980) suggest that frequency of nursing may decline during the nursing period. It is thus consistent to suggest that the total daily caloric intake could remain constant during nursing.

Our results differ from the reported changes in milk composition in harp seals during lactation (Van Horn and Baker 1971). However, in the earlier study, a single female and her pup were maintained in captivity and milk samples were obtained only after injection of oxytocin. Minimal fat and protein levels were observed on day 16 *post partum* (Van Horn and Baker 1971). In view of the fact that nursing in non-captive harp seals appears to last on the average, about nine days (Stewart and Lavigne 1980), these data may be somewhat anomalous in that the duration of lactation may have been artificially extended, either because the female was unable to wean her pup naturally, or because she received injections of oxytocin prior to donating milk for analysis. Furthermore, the female did not eat during the first 10 days of nursing but was subsequently fed frozen herring during the last six days of the experiment. In view of the very different conditions under which the previous determination was conducted, further speculation about differences in our results is unwarranted.

Other studies of milk composition in the harp seal were based on single samples from two animals which had been nursing for unknown periods of time (Sivertsen 1941) and from three females whose pups were reportedly less than one week of age (Van Horn and Baker 1971, in reference to Cook and Baker 1969). Their results correspond to values obtained from animals in the present study which had been nursing for six to nine days.

Lactose levels determined indirectly in the present study were somewhat higher than those determined previously (Sivertsen 1941, Cook and Baker 1969, Van Horn and Baker 1971). Further direct estimates are required to characterize lactose levels in harp seals milk.

Changes in milk composition observed in the harp seal in the present study are similar to those reported previously in the Weddell seal (Kooyman and Drabek 1968) and northern elephant seal (Riedman and Ortiz 1979). In harp seals stage of lactation appears to be the most significant variable influencing fat and water content of milk. In a multiple regression analysis of fat content as a function of pup age, female age, and condition, pup age (stage of lactation) explained 62% of the variation in the data ($n = 17$) whereas the contributions of the other two independent variables were not significant ($p > 0.05$). Similarly stage of lactation explained 51.1% of the variation in water content and neither female age nor condition made a significant contribution to the regression. Lactating females of all three species apparently fast (Kooyman and Drabek 1968, Sergeant 1973, Reidman and Oritz 1979). "Replacement" of water with fat during lactation has been observed in a number of mammalian species but is most pronounced when water is in limited supply (Kooyman and Drabek 1968, Riedman and Ortiz 1979). The increasing fat and decreasing water content of seal milk (e.g. Fig. 2) may thus be important in maintaining water balance in lactating (and fasting) seals as suggested by previous authors (Kooyman and Drabek 1968, Riedman and Ortiz 1979). The high water content of seal milk at the onset of nursing would supply the newborn pup with an adequate supply of free water at a time when it lacks a well-developed blubber layer, and when it is presumably dependent on the catabolism of carbohydrate and protein, and in the case of harp seals at least, brown fat (Blix et al. 1975, 1979, Grav et al. 1974, Grav and Blix 1976) to meet various metabolic demands. As growth, initially the deposition of blubber, followed by increases in the lean body mass (Stewart and Lavigne 1980), and development continue, a transition to lipid catabolism might be expected as nursing proceeds (Dawes 1968). The progressive decline in water content of harp seal milk thus occurs at a time when the pup should be less dependent on free water in milk (Riedman and Oritz 1979) being able to meet its needs using metabolic water derived from lipid metabolism.

Further physiological and biochemical observations are required to elucidate the adaptive significance of changes in composition and energy content of marine mammal milk. Coincident with the present study, we obtained tissue samples (muscle and blubber) from 28 females and their pups. These were freeze-dried (Lavigne and Stewart 1979) and analysed for water content. Water content in muscle and blubber of pups followed the decline in water content of the milk consumed, and at weaning, the amount of water in pup tissues was similar to that of their mothers (Fig. 5). In contrast, water content in blubber of lactating females remained constant and there was some indication of a slight increase in the amount of water in muscle as lactation progressed (Fig. 5). Some fat in muscle tissues (George et al. 1971, George and Ronald 1975) and possibly protein may be utilized during lactation and replaced with water. In addition, female harp seals ovulate and moult shortly after weaning their pups and the influence of estrogens on water balance (Novales et al. 1973) should not be overlooked.

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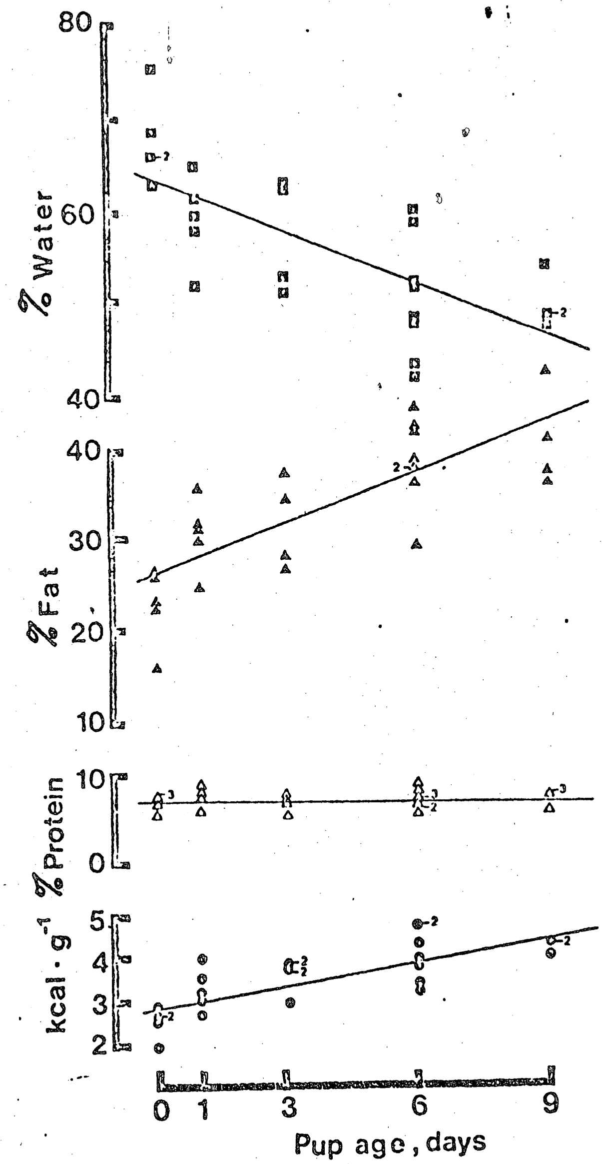

Fig. 1. Changes in proximate composition (g/100 g whole milk and calorid density (kcal g^{-1}, determined by bomb calorimetry) of harp seal milk during lactation. The regression equations are:

% water = 63.48 - 1.87 pup age, R^2 = 51.1%

% fat = 26.33 + 1.81 pup age, R^2 = 57.5%

% protein = 6.88 ± 0.92, ($\bar{x}$ ± SD)

caloric density = 2.99 + 0.169 pup age, R^2 = 54.0%

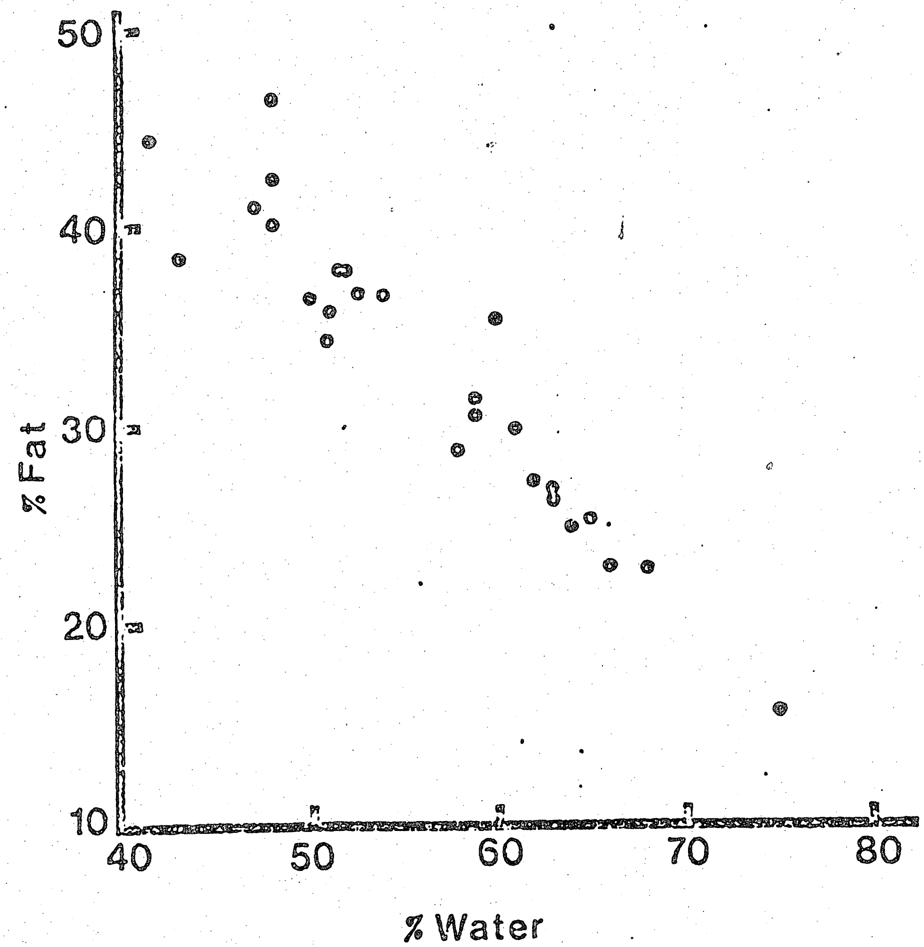

Fig. 2. Changes in fat content of harp seal milk in relation to changes in water content. r = -0.938, $p < 0.00001$, n = 26). The predictive equation for % fat as a function of % water is:

$$\% \text{ fat} = 81.83 - 0.86 \% \text{ water}, R^2 = 88.0\%$$

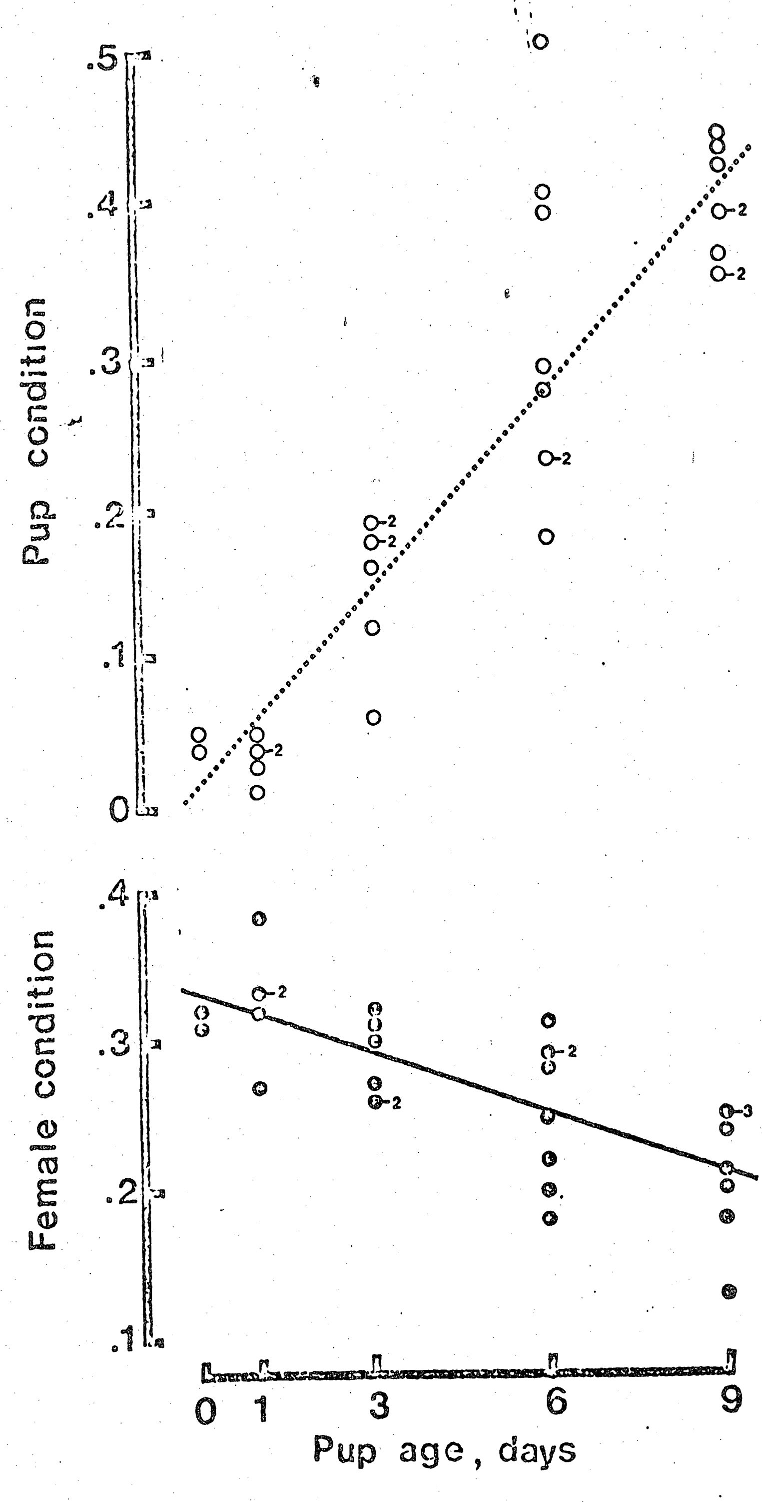

Fig. 3. Changes in condition (blubber thickness (mm) ÷ length (M)) of female harp seals and their pups during lactation. The regression equations are:

female condition = 0.329 - 0.0128 pup age, R^2 = 54.6%

pup condition = 0.0225 + 0.0432 pup age, R^2 = 81.7%

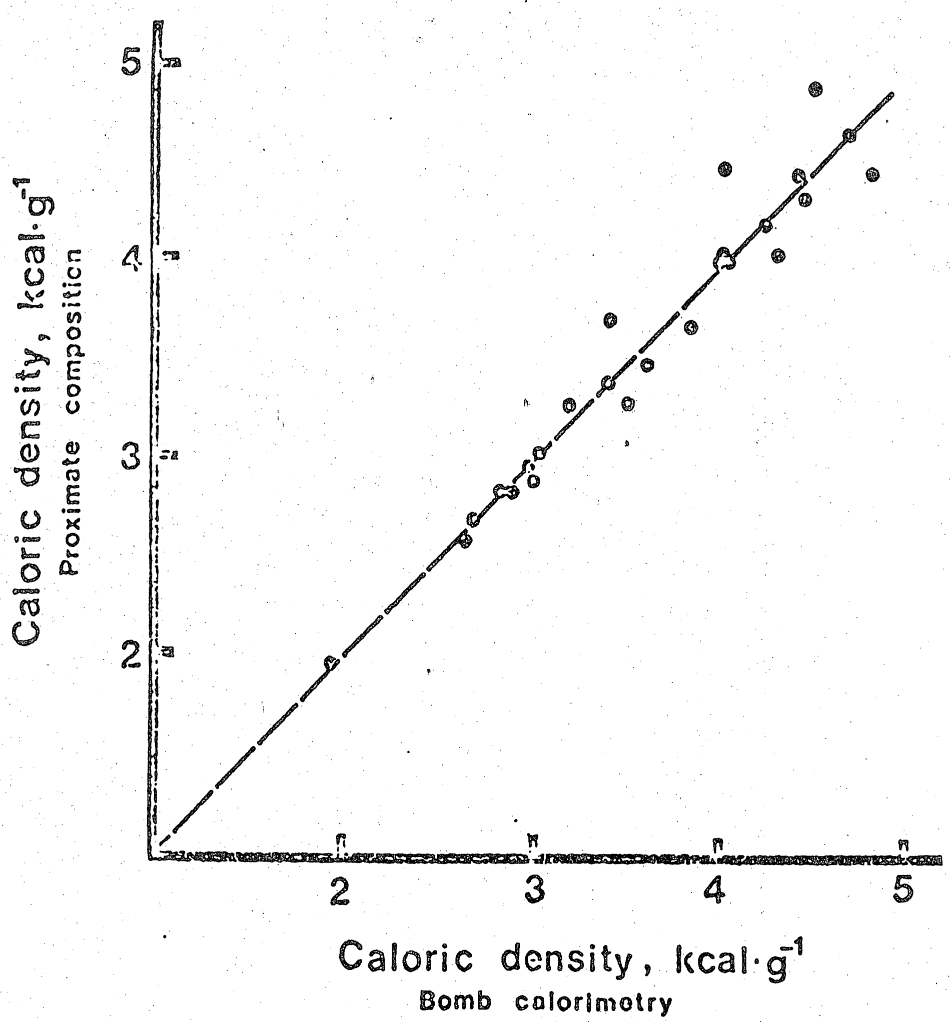

Fig. 4. The correlation between caloric density of harp seal milk (kcal g^{-1}) estimated indirectly from proximate composition, and caloric density estimated by bomb calorimetry.

$r = 0.973$, $p < 0.00001$, $n = 25$.

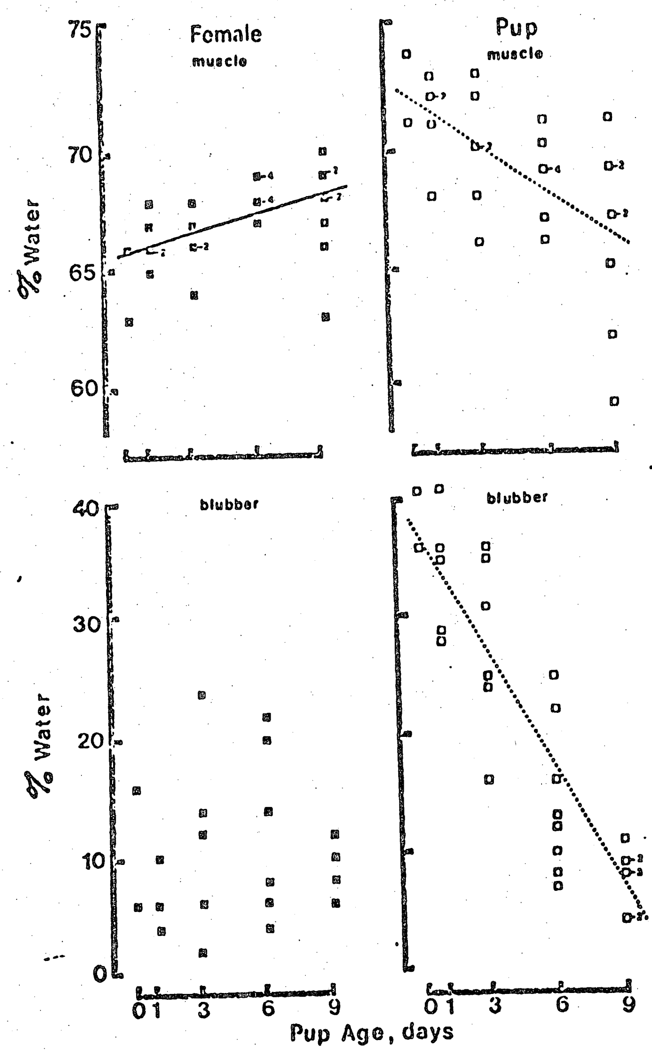

Fig. 5. Changes in water content (%) in muscle and blubber of female harp seals and their pups during lactation. For female tissues water content in blubber was not significantly correlated with pup age (r=0.0891, p=0.652, n=28); whereas the correlation of water content in muscle was significantly correlated with pup age (r=0.447, p=0.0171, n=28). The regression equation is:

Muscle water content = 65.85 + 0.252 pup age, R^2 = 20.0%

For pup tissues water content was negatively correlated with pup age in both muscle (r=-0.655, p=0.00015, n=28) and blubber (r=0.898, p < 0.00015, n=28). The regression equations are:

Muscle water content = 72.34 - 0.66 pup age, R^2 = 42.5%

Fat, water content = 37.34 - 3.45 pup age, R^2 = 80.6%