Comp. Biochem. Physiol. Vol. 113B, No. 3, pp. 619–623, 1996

ELSEVIER

ISSN 0305-0491/96/$15.00
SSDI 0305-0491(95)02034-I

Milk Composition in the North American Opossum (*Didelphis virginiana*)

Brian Green, William J. Krause and Keith Newgrain*

DIVISION OF WILDLIFE AND ECOLOGY, CSIRO, P.O. BOX 84, LYNEHAM, ACT 2602, AUSTRALIA; AND *DEPARTMENT OF ANATOMY AND NEUROBIOLOGY, SCHOOL OF MEDICINE, UNIVERSITY OF MISSOURI-COLUMBIA, MO 65212, U.S.A.

ABSTRACT. The composition of milk samples collected from captive opossums (*Didelphis virginiana*) was determined at various intervals during lactation. The milk solids increased from 9% at week one to a maximum of 34% at 11 weeks post-partum. There were changes in the relative proportions of protein, lipid and carbohydrate at different stages of lactation. Lipid represented the greatest fraction of the solids except for a period at mid-lactation when there was a peak in protein concentration. The concentrations of sodium, potassium and magnesium were relatively constant, 41 ± 4, 35 ± 11 and 9.2 ± 1.6 mmol respectively, although calcium increased from 13 ± 5 mmol at week one to a peak of 112 ± 35 mmol at 9 weeks. COMP BIOCHEM PHYSIOL 113B, 619–623, 1996.

KEY WORDS. *Didelphis virginiana*, milk composition, marsupial, opossum

INTRODUCTION

The study of Lemon and Barker (1967) on the milk of red kangaroos was the first to show that there are substantial changes in the major milk components relative to the stage of lactation in a marsupial. Most subsequent studies have focussed on the Macropodoidae although compositional data throughout the lactational cycle are available for one phalangerid (*Trichosurus*), one petaurid (*Pseudocheirus*), one peramelid (*Isoodon*) and one dasyurid (*Dasyurus*); reviewed by Munks *et al.*, (1991). Of the 78 American marsupials belonging to the Caenolestoidea, Didelphidea and Microbiotheriidae (Harder, 1992), only the milk of one species, *Monodelphis domestica*, has been analysed in any detail (Crisp *et al.*, 1989, Green *et al.*, 1991).

The female opossum (*Didelphis virginiana*) is a polytocous marsupial which produces more young than are able to attach to the 13 teats. Adult females attain a body mass of 3 kg and lactation lasts for about 14 weeks (Harder, 1992). Weaning commences at 12 weeks when the young weigh 165 g (Cutts *et al.*, 1978). Bergman and Housley (1968) reported on the composition of milk samples from *D. virginiana*, but did not clearly establish that compositional changes were associated with the age of sucklings. The present study was undertaken to determine if milk composition in *D. virginiana* follows the general pattern shown by other marsupials.

MATERIALS AND METHODS

Fifteen wild adult female opossums were trapped in central Missouri (U.S.A.) from February through June using Havaheart traps (Tomahawk Trap Co.) baited with peanut butter and fruit. Following capture the opossums were maintained in large open air enclosures which contained individual animal boxes filled with straw. This facility is located at the Charles Green Wildlife research area maintained by the Missouri Department of Conservation. The captive animals had free access to food (Purina dog chow) and water during the period of study. The diet was supplemented with carrion (primarily cotton-tail rabbit) and fruit. The opossums were captured under Wildlife Collector's permit number 2982 and all animals appeared healthy and free of obvious disease. Ages of the pouch young of wild caught females were estimated from measurements of the snout-rump length (Cutts *et al.*, 1978).

To obtain animals with young of known age, some females had their litters permanently removed, and were then placed in breeding pens with continuous access to males. Opossums usually return to estrus within 7 to 14 days after removal of their young (Krause and Cutts, 1984). The pouches of these females were examined each morning to precisely determine the dates of birth of young. Prior to milking the young were separated from their mothers for 6 h to allow the accumulation of milk in the mammary glands. The nipples and glands were wiped with gauze that had been moistened with alcohol. Females were not anesthetized but restrained by hand and milk collection was assisted by manual squeezing of all of the lactating teats until milk could no longer be expressed from the glands.

In animals at early stages of lactation, an intra-vascular

Correspondence to: Dr. B. Green, CSIRO Division of Wildlife and Ecology, PO Box 84, Lyneham, A.C.T. 2606, Australia Tel. 61 6 2421738; Fax 61 6 2413343.

Received 6 April 1995; revised 12 July 1995; accepted 16 July 1995.

injection with 0.75 ml oxytocin (Sigma, 10 USP units/ml) was administered via the lateral mammary vein. Milk appeared at the teat tips within a minute of injection. Milk samples were obtained at days 2, 3 and 16 for 3 females; they were collected as two distinct subsamples; one obtained without the use of oxytocin, the other obtained immediately following the intravascular injection of oxytocin. This was done to assess the possible effects of this hormone on milk composition (Oftedal, 1984) in the early stages of milk production when only small volumes of milk can be obtained for analysis.

Milk from animals at early stages of lactation was collected in 370 ul Caraway Micro collecting tubes (Fisher Scientific); milk from animals at later stages was collected directly into 10 ml plastic vials. In the latter instances, three to six ml were collected at each milking. After milking the young were reunited with their mothers, except for young that were less than 60 days old. These young were found to be incapable of reattachment to the teat and were sacrificed. It was these females that were rebred to obtain further litters of known age. In those animals whose young could reattach, milking was done at 7 day intervals until natural weaning occurred.

All milk samples were maintained frozen until analysed.

The methods used to analyse the milk samples were similar to Green *et al.*, (1991) except that four samples were analysed for total nitrogen by a microdiffusion method (Conway, 1962) after Kjeldahl digestion. Total nitrogen was converted to milk protein using a factor of 6.38 (Horwitz, 1980) and all of the results from samples analysed by the protein dye binding method were subsequently corrected by a factor of 1.245.

For statistical purposes data were grouped into consecutive fortnightly intervals. Significance between mean values where $n \geq 3$ was tested by Tukey's HSD test following ANOVA, (Systat, 1990). All mean values in the test are given ± 1 SD.

RESULTS

The milk solids showed a distinct pattern of increasing concentration as lactation progressed; ranging from 9% at 2 days to a peak of around 34% at week 11 post-partum (pp) ($P < 0.001$). There was then a significant decline ($P = 0.043$) in total solids over the subsequent two weeks to around 27% which was then maintained until the completion of lactation at about 15 weeks pp. (Fig. 1a). Two samples of milk associated with cessation of lactation showed high solids values about this time (38% and 47%). The respective mammary glands were greatly reduced in size and attempts to obtain milk one week later were unsuccessful.

Total hexose concentrations reached a maximum of about 7% at 7 weeks. After this there was a continuous and significant decline in carbohydrate levels ($P = 0.002$) until, at the conclusion of lactation, hexose concentrations were about 3% of whole milk (Fig. 1b).

Protein concentrations displayed a similar pattern to total solids (Fig. 1c); peak protein concentrations of 10% were measured at 11 weeks pp, however the difference in mean protein concentrations from week 5 to week 11 pp was not sigificant ($P = 0.086$).

The concentration of milk fats were stable at about 8% up

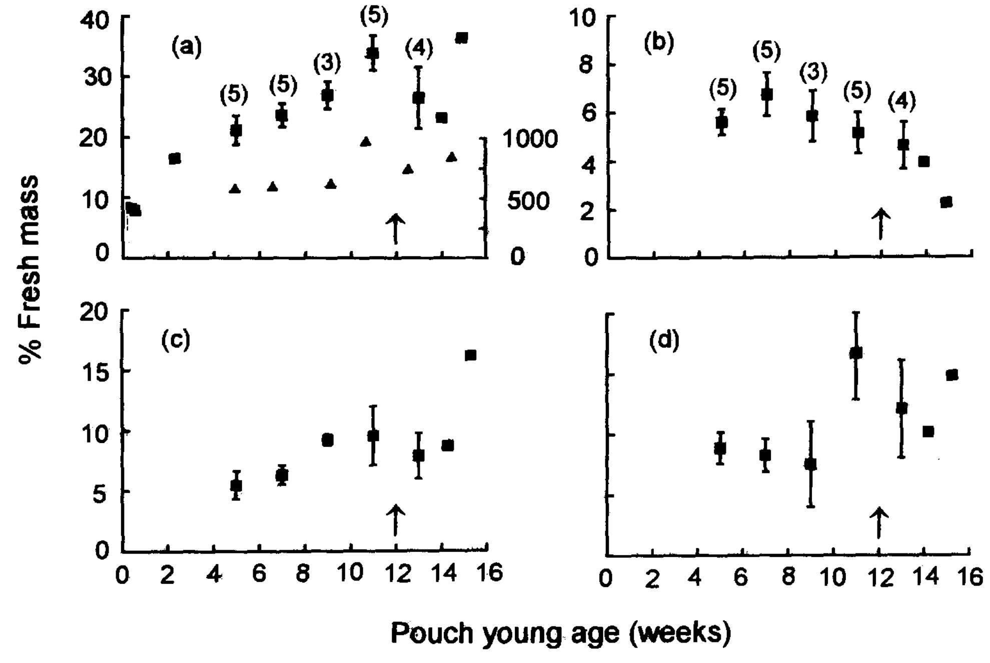

FIG. 1. Changes in (a) solids ■, % of fresh mass and energy ▲, kJ 100 ml^{-1}, (b) carbohydrate, (c) protein, (d) lipid in *D. virginiana* milk. Arrow indicates onset of weaning. Number of samples in parentheses, error bar ± 1SD.

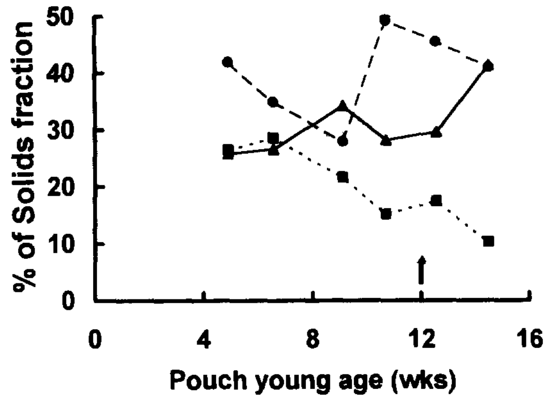

FIG. 2. Changes in carbohydrate (■), protein (▲) and lipid (●) as a percentage of the solids fraction in *D. virginiana* milk. Arrow indicates onset of weaning.

to week 9, after which there was a significant increase (P = 0.002) to a peak of about 17% at 11 weeks. Subsequently there was a decline (P = 0.036) to about 11% at 13 weeks (Fig. 1d).

In comparative terms, lipid was the major component of the milk solids throughout lactation apart from an interval of about two weeks when protein reached peak concentrations of 34% of the solids fraction at about 9 weeks (Fig. 2).

The estimated energy content of milk increased to a maximum of 966 kJ 100 ml^{-1} at around 11 weeks pp, reflecting the increasing contribution made by lipid to the solids fraction (Fig. 1a).

There were no obvious and consistent changes in the concentrations of sodium, potassium or magnesium throughout the course of lactation (Fig. 3a,b). The mean concentrations of these elements were respectively; 41 ± 4 mmol Na, 35 ± 11 mmol K and 9.2 ± 1.6 mmol Mg (n = 27). However, the concentrations of calcium increased from 13 to about 100 mmol during the first 7 weeks of lactation, remaining at this level until around 10 weeks pp (Fig 3a).

There were no obvious or consistent differences between early stage milk samples collected before and after the administration of oxytocin with respect to electrolytes and solids. The values for carbohydrate, protein and lipid for these early samples were beyond the sensitivity of the analyses and it is thought likely that some degradation of milk components had occurred prior to completion of analyses, consequently, the lipid, protein and hexose data for these samples have not been included in the analysis of data.

DISCUSSION

A previous report on milk composition in this species (Bergman and Housley, 1968) provided a range of values for milk components; 9.4 to 31.3% for total solids, 6.7 to 11.8% for protein, 4.9 to 17.9% lipid and 0.7 to 3.2% for "carbohydrate." However, the method used by these authors to estimate carbohydrate is suspect since milk hexose in marsupials is mostly in the form of oligosaccharides that are not measured as reducing sugars (Messer and Green, 1979). Therefore, it is likely that the values for milk carbohydrates reported by Bergman and Housley (1968) are underestimated. These authors also showed that at the end of lactation the milk contained oligosaccharides, but no lactose. The other values for milk components in *D. virginiana* reported by these authors are within the range of concentrations described in the present study. It appears that Bergman and Housley (1968) did not clearly discern the sequential nature of changes in milk composition, although they attributed the time of milk collection as partially responsible for the broad range of values they obtained for milk components.

The sequential pattern of changes recorded in the composition of milk from different species of marsupials is generally similar, i.e., increasing solids with a reversal of the contributions of carbohydrate and lipid as lactation proceeds. Protein concentration remains at a relatively constant fraction of the solids component. While most studies have focussed on the Macropodoidae a similar pattern is also observed in a Peramelid, *Isoodon macrourus* (Merchant and Libke, 1988), a Dasyurid, *Dasyurus viverrinus* (Green *et al.*, 1987) and in a Phalangerid *Trichosurus vulpecula*, (Cowan, 1989). The milk of the folivorous Petaurid (*Pseudocheirus peregrinus*) is different due to lipid remaining at lower levels than carbohydrate throughout lactation (Munks *et al.*, 1991).

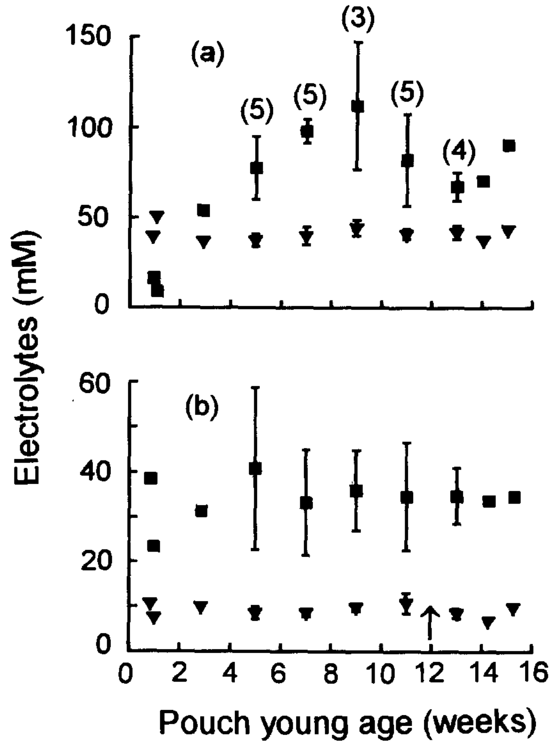

FIG. 3. Changes in (a) sodium (▼), calcium (■) and (b) potassium (■) and magnesium (▼) in *D. virginiana* milk. Arrow indicates onset of weaning. Number of samples in parentheses, error bar ± 1SD.

The lipid levels (expressed as a proportion of the solids fraction) of both *D. virginiana* and *M. domestica* are relatively high in the early stages of lactation but conform with the general pattern in late lactation when high lipid and low carbohdyrate levels prevail.

Peak carbohydrate concentrations in *D. virginiana* are quite low (7%) and remain relatively high at the latter part of lactation. This is similar to the pattern observed in the carnivorous Dasyurid *D. viverrinus* (Messer *et al.*, 1987) and in the omnivorous Peramelid *I. macrourus* (Merchant and Libke, 1988) but differs from the herbivorous Macropodid pattern (Merchant, 1989) where peak concentrations of greater than 10% are followed by a rapid decline to less than 1% of whole milk. The high carbohydrate level (13%) and subsequent rapid decline is also seen in the milk of *M. domestica.* The decline in carbohydrate levels in the milk of *D. viverrinus* coincides with the young first detaching from the teat (Messer *et al.*, 1987). Similarly the young of *D. virginiana* release the teat between 50 and 67 days (Cutts *et al.*, 1978) which also coincides with the decline in carbohydrate. However, the young of *M. domestica* release the teat at 2 weeks of age (VandeBerg, 1990), 5 weeks prior to the decline in carbohydrate. Thus, the stimulus for the decline in milk carbohydrate remains unclear.

The absence of any obvious change in the sodium content of the milk of *D. virginiana* is similar to that found with *M. domestica* milk (Green *et al.*, 1991) but is atypical to the general pattern shown by Australian marsupials where there is a significant decline in sodium in the period between birth and pouch emergence followed by an increase in sodium concentration up to weaning. There is a general pattern of change between potassium and sodium levels in marsupial milk with potassium values exceeding sodium in mid-lactation (Green and Merchant, 1988). However, in *D. virginiana* milk there were no consistent changes in potassium concentration during lactation, with potassium concentrations being slightly lower than sodium. The milk of *M. domestica* shows a different pattern still; potassium concentrations are much higher than sodium in the early stages but lower in late lactation (Green *et al.*, 1991).

There are few published data available for the amounts of calcium and magnesium in marsupial milk, however the values are within the range of those observed for other marsupials (Green and Merchant, 1988). It is difficult to interpret relative changes in milk constituents and the physiological/developmental changes of the pouch young in the absence of estimates of milk consumption rates.

The general pattern of changes in milk consumption in *D. virginiana* is consistent with other marsupials in that solids increase until late lactation and the increase in lipid and energy levels occurs as there is a rapid increase in the growth of the young. However, the mechanisms and stimuli for the large qualitative and quantitative changes in marsupial milk have yet to be fully elucidated (Nicholas *et al.*, 1995). Some insights could be gained from observing the physical and physiological developmental responses of marsupial young following the experimental transfer of young to mammary glands at different stages of milk production, or hand rearing on experimental milk formulae.

We gratefully acknowledge the assistance of Pamela K. Thorne and Sammy L. Eber in gathering the opossum milk samples and the helpful comments from P. Bird, L. Hinds, and J. Merchant.

References

Bergman, H. C.; Housley, C. Chemical analyses of American opossum (Didelphys virginiana) milk. Comp. Biochem. Physiol. 25: 213–218;1968.

Conway, E. J. Microdiffusion analysis and volumetric error. Crosby Lockwood and Son, London, 1962.

Cowan, P. E. Change in milk composition during lactation in the common brushtail possum, Trichosurus vulpecula (Marsupialia: Phalangeridae). Reprod. Fertil. Dev. 1:325–335;1989.

Crisp, E. A.; Messer, M; VandeBerg, J. L. Changes in milk carbohydrates during lactation in a Didelphid marsupial, Monodelphis domestica. Physiol. Zool. 62:1117–1125;1989.

Cutts, J. H.; Krause, W. J.; Leeson, C. R. General observations on the growth and development of the young pouch opossum, Didelphis virginiana. Biol. Neonate 33:264–272;1978.

Green, B.; Merchant, J. C. The composition of marsupial milk. In The Developing Marsupial. Models for Biomedical Research (Edited by Tyndale-Biscoe, C. H.; Janssens, P. A.) 1988: pp. 41–54. Springer-Verlag, Berlin.

Green, B.; Merchant, J. C.; Newgrain, K. Milk composition in the eastern quoll, Dasyurus viverrinus (Marsupialia: Dasyuridae). Aust. J. Biol. Sci. 40:379–387;1987.

Green, B.; VandeBerg, J. L.; Newgrain, K. Milk composition in an American marsupial (Monodelphis domestica). Comp. Biochem. Physiol. 99B:663–665;1991.

Harder, J. D. Reproductive biology of South American marsupials. In Reproductive Biology of South American Vertebrates (Edited by Hamlett, W. C.) 1992: pp. 211–228. Springer-Verlag, New York.

Horwitz, W. E. Official Methods of Analysis of the Association of Official Analytical Chemists. ACOA, Washington, 1980.

Krause, W. J.; Cutts, J. H. Scanning electron microscopic observations on the 9-day opossum (Didelphis virginiana) embryo. Acta Anat. 120:93–97;1984.

Lemon, M.; Barker, S. Changes in milk composition of the red kangaroo, Megaleia rufa (Desmarest), during lactation. Aust. J. Exp. Biol. Med. Sci. 45:213–219;1967.

Merchant, J. C. Lactation in macropodoid marsupials. In Kangaroos, Wallabies and Rat-kangaroos (Edited by Grigg, G.; Jarman P.; Hume I.), 1989: pp. 355–366. Surrey Beatty & Sons Pty. Ltd., N.S.W. Australia.

Merchant, J. C.; Libke, J. A. Milk composition in the northern brown bandicoot, Isoodon macrourus (Peramelidae, Marsupialia). Aust. J. Biol. Sci. 41:495–505;1988.

Messer, M.; Green, B. 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;1979.

Messer, M.; FitzGerald, P. A.; Merchant J. C.; Green, B. Changes in milk carbohydrates during lactation in the eastern quoll. Dasyurus viverrinus (Marsupialia). Comp. Biochem. Physiol. 88B: 1083–1086;1987.

Munks, S. A.; Green, B.; Newgrain, K.; Messer, M. Milk composition in the common ringtail possum, Pseudocheirus peregrinus (Petauridae: Marsupialia). Aust. J. Zool. 39:403–416;1991.

Nicholas, K. R.; Wilde, C. J.; Bird, P. H.; Hendry, K. A. K.; Tregenza, K.; Warner, B. Asynchronous concurrent secretion of milk proteins in the tammar wallaby (Macropus eugenii) In Intercellular Signalling in the Mammary Gland (Edited by Wilde, C. J.; Knight, C.; Peaker, M.) 1995: pp. 153–170. Plenum Press, London.

Oftedal, O.T. Milk composition, milk yield and energy output at peak lactation: A comparative Review. Symp. Zool. Soc. London 51:33–85;1984.

VandeBerg, J. L. The gray short-tailed opossum (Monodelphis domestica) as a model didelphid species for genetic research. Aust. J. Zool. 37:325–347;1990.

Wilkinson, L. Systat: The System for Statistics. Systat, Inc., Evanston, Illinois, 1990.