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Short communication

Free amino acids in milks of human subjects, other primates and non-primates

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Preterm and term transitional milks of human subjects and mature milks of human subjects, non-human primates and non-primates were analysed for free amino acids (AA) using precolumn phenylisothiocyanate derivatization and liquid chromatography. Differences in free AA between three types of human milk were small. Milks of pinnipeds (seals and sea lions) contained the highest levels of total free AA (8634–20 862 μmol/l), while the milks of cows and sheep had the lowest levels of total free AA (1061–1357 μmol/l). The milks of human subjects, chimpanzees (*Pan troglodytes*), gorillas (*Gorilla gorilla*), elephants (*Elephas maximus*), horses and pigs had intermediate levels of total free AA (3069–7381 μmol/l). Glutamic acid was the most abundant free AA in milks of human subjects (1339–2157 μmol/l), non-human primates (423–2528 μmol/l), elephants (1332 μmol/l), horses (1119 μmol/l), and cows (349 μmol/l). Taurine was the most abundant free AA in milks of pinnipeds (5776–13 643 μmol/l), pigs (1238 μmol/l), goats (1150 μmol/l) and sheep (341 μmol/l). Taurine was the second most abundant free AA in milks of human subjects and non-human primates, while histidine was the second most abundant free AA in milks of pinnipeds. Milks of each species had a distinctive free AA pattern which may reflect the relative importance of the free AA during early postnatal development.

Amino acids: Milk

The total (including protein-bound) amino acid (AA) concentrations in the milks of human subjects, non-human primates and pinnipeds have been determined (Davis *et al.* 1994*a*,*b*, 1995). Despite the wide variation in total AA concentration among the species, there was a general similarity in the AA patterns of the milks. Human milk is known to contain a pool of free AA, which may have a beneficial role during early postnatal development (Pamblanco *et al.* 1989). Information on free AA in transitional human milks and milks of other species is limited. Only one comparative study on free AA in mature milks of man and some other mammals has been reported (Rassin *et al.* 1978). The transitional milks of human subjects and of pinnipeds were not studied by these workers. Thus, in the present investigation free AA were studied, using accurate and standardized methods, in preterm and term transitional milks of human subjects and mature milks of human subjects and other species including pinnipeds.

Methods

The preterm and term transitional human milk samples (5–10 d postpartum) originated from mothers of preterm (gestations of 25–32 weeks) or term (gestations of >36 weeks) infants admitted to the Toronto Hospital for Sick Children (Sarwar *et al.* 1996). The details of the collections of the mature milks of human subjects, non-human primates (baboon (*Papio cynocephalus anubis*), chimpanzee (*Pan troglodytes*), gorilla (*Gorilla gorilla*) and rhesus monkey (*Macaca mulatta*)), non-primates (cow, goat, sheep, pig, horse and elephant (*Elephas maximus*)) and pinnipeds (Northern elephant seal (*Mirounga angustiros-*

Abbreviations: AA, amino acids.

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Table 1. Free amino acids (AA) (μmol/l) in milks of human subjects, non-human primates, non-primates and pinnipeds

Milk	1st abundant AA*	2nd abundant AA*	3rd abundant AA*	Total AA
Human, preterm	1412	388	342	3397
Human, term	1339	318	316	3069
Human, mature	2157	331	294	2157
Chimpanzee, mature	2528	349	297	4313
Gorilla, mature	1787	453	336	3879
Baboon, mature	526	260	247	1728
Rhesus monkey, mature	423	407	343	1727
Cow, mature	349	148	110	1061
Pig, mature	1238	1204	826	7381
Horse, mature	1119	947	388	3913
Elephant, mature	1332	459	341	3477
Elephant seal, mature	6508	2532	1671	16393
Australian sea lion, mature	8480	2950	268	12196
Antarctic fur seal, mature	13643	5692	362	20862
California sea lion, mature	11901	1907	323	14748

* 1st abundant AA: Glu in human subjects, non-human primates, cows, horses and elephants, Tau in pinnipeds and pigs; 2nd abundant AA: Tau in human subjects and cows, His in baboons, rhesus monkeys and pinnipeds, Ala in chimpanzees and gorillas, Gln in pigs and horses, Gly in elephants; 3rd abundant AA: Ala in human subjects, Tau in baboons and rhesus monkeys, Gln in chimpanzees, Asp in gorillas, Gly in pigs, His in cows and elephants, Pro in elephant seals, Arg in Australian sea lions, Antarctic fur seals and California sea lions.

tris), Antarctic fur seal (*Arctocephalus gazella*) and California sea lion (*Zalophus californianus*)) have been reported previously (Davis *et al.* 1994*a,b*). These samples were pooled before AA analysis in the present study. Milk fat was removed by centrifugation at 3000 ***g*** for 15 min at 4°. The skimmed milk (0·2 ml) was acidified with an equal volume of 0·1 M-HCl, and deproteinized by filtration. Free AA in the deproteinized milk samples were determined by liquid chromatography of precolumn phenylisothiocyanate derivatives (Sarwar & Botting, 1990). Since there was no true replication for each species, the usual ANOVA analyses were not appropriate. Therefore, instead of looking for differences among the species, we looked for similarities by clustering analysis (Massart & Kaufman, 1983).

Results and discussion

The milks of pinnipeds contained the highest levels of total free AA (12 196–20 862 μmol/l); while the milks of cows (1061 μmol/l), sheep (1357 μmol/l) and goats (1999 μmol/l) had the lowest levels of total free AA. The milks of human subjects, chimpanzees, gorillas, elephants, horses and pigs had intermediate levels of total free AA (3069–7381 μmol/l; Table 1). Glutamic acid was the first most abundant free AA in the milks of human subjects, non-human primates, cows, horses and elephants, while taurine was the first most abundant free AA in the milks of pinnipeds, pigs (Table 1), goats (1150 μmol/l) and sheep (341 μmol/l). The three most abundant free AA (Table 1) constituted about 63–70, 60–74, 52–76 and 65–94 % of the total free AA pools in the milks of human subjects, non-human primates, non-primates, and pinnipeds respectively. The differences between the free AA compositions of the transitional preterm and term human milks were small. The non-essential : essential free AA ratio in the mature human milk (6·4) was, however, higher than those in the transitional human milks (3·4–4·0).

The milks of pinnipeds contained the highest proportion of taurine (40–81 % of total free AA) compared with the milks of human subjects (8–11 %), non-human primates (2–20 %), and all non-primates except goats and sheep (1–17 %). Among the non-primates, the milks of goats and sheep contained the highest proportion of taurine (25–57 % of the total free AA). Taurine has an important role in bile salt synthesis (Kendler, 1989). Thus the extremely high proportion of taurine in the milks of pinnipeds may be related to their high fat content (up to 60 %) (Oftedal *et al.* 1987).

The cluster analysis (to determine similarities) indicated that there were three clusters. The first cluster included the milks of human subjects, gorillas, chimpanzees, baboons, horses, rhesus monkeys, cows, sheep, goats, elephants and pigs. The second cluster included the milks of elephant seals and Australian sea lions (*Neophoca cinerea*). The third cluster included the milks of Antarctic fur seals and California sea lions. The statistical analyses indicated that 93·6 % of the variation in the data was due to the first principal component, and that taurine and histidine were the most important variables in the clustering. Despite similarities of the three clusters, milk of each species had a characteristic free AA composition.

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