Amino acid composition of the milk of some mammalian species changes with stage of lactation

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To determine whether the amino acid composition of milk changes during lactation, we compared the amino acid pattern (concentration of each individual amino acid relative to the total amino acid concentration) of colostrum with that of mature milk in six mammalian species. In the human, horse, pig and cow, the pattern of amino acids changed between colostrum and mature milk: glutamate, proline, methionine, isoleucine and lysine increased; cystine, glycine, serine, threonine and alanine decreased. In these four species, the total amino acid concentration also decreased 75% between colostrum and mature milk. In the baboon (*Papio cynocephalus anubis* and *Papio cynocephalus anubis/Papio cynocephalus cynocephalus*) and rhesus monkey (*Macaca mulatta*), however, there was little change in the pattern of amino acids between colostrum and mature milk, and total amino acid concentration decreased only about 25% between colostrum and mature milk. Mature milk rather than colostrum was the most similar among the three primates in both amino acid pattern and total amino acid concentration. We conclude, in those species in which total amino acid concentrations decline substantially between colostrum and mature milk, amino acid patterns also change. The presence of a change in amino acid pattern and total amino acid concentration during lactation appears to be unrelated to phylogenetic order.

Milk composition: Neonatal nutrition: Lactation

Milk is the sole source of nutrients for the neonatal young of most mammalian species. There is wide variation among species in the gross composition of the milk which is secreted including that of protein (Jenness & Sloan, 1970). Mature human milk is known to have the lowest concentration of milk protein of all species. In the human, milk that is produced during the initiation of lactation, colostrum, has been reported to have a higher protein concentration than the mature milk produced during the later stage of copious lactation (Jenness, 1979; Hibberd *et al.* 1982; Neville *et al.* 1991). However, the limited evidence available suggests that the decline in milk protein concentration during lactation seen in the human is not consistent across all species (Lonnerdal *et al.* 1984; Oftedal, 1984; Roberts *et al.* 1985; Fiorotto *et al.* 1991).

In addition to the changes in total protein concentration, the composition of milk proteins has been shown to differ between colostrum and mature milk in some species as indicated by the change in whey:casein ratios (Buss, 1978; Kulski & Hartmann, 1981; Davies *et al.* 1983; Kunz & Lonnerdal, 1983; Jenness, 1986; Sanchez-Pozo *et al.* 1986; Klobasa *et al.* 1987). Given the differences in the amino acid compositions of whey proteins and casein (Kon & Cowie, 1961; Renner, 1983; Heine *et al.* 1991), differences in the amino acid patterns (defined as the concentration of each individual amino acid relative to the total amino acid concentration) between colostrum and mature milk are to be anticipated.

However, knowledge is limited of the amino acid pattern of mature milk, and especially that of colostrum, secreted by different species. We recently described the amino acid pattern of the mature milks of fourteen different species and concluded that there was, in general, commonality in the amino acid pattern of mature milk among species, particularly among the mature milk secreted by species within the same phylogenetic order (Davis *et al.* 1994). The current study compares the amino acid patterns of colostrum and intermediate milk of selected mammalian species with our previous values for the amino acid pattern of the mature milk secreted by those species.

MATERIALS AND METHODS

Milk collection

Milk was obtained from three primate species: human, baboon (*Papio cynocephalus anubis* and *Papio cynocephalus anubis/Papio cynocephalus cynocephalus*), and rhesus monkey (*Macaca mulatta*), as well as from three non-primate species: horse, cow, and pig. Human colostrum samples (*n* 8) were obtained 2 d after parturition. Human milk samples that were obtained from different individuals between 4 and 16 d after parturition were designated intermediate milk (*n* 3) and those obtained between 4 and 16 weeks after parturition (*n* 6) were designated mature milk. Human milk samples obtained during the three stages of lactation were obtained from different individuals. Baboon samples were obtained 1 d after parturition (colostrum, *n* 4), 30–31 d after parturition (intermediate milk, *n* 5), and 60–63 d after parturition (mature milk, *n* 5). Of the baboon milk samples collected, four were obtained from the same individual animals at each of the three lactation stages. Rhesus monkey samples were collected 1–2 d after parturition (colostrum, *n* 5), 30–31 d after parturition (intermediate milk, *n* 4), and approximately 60 d after parturition (mature milk, *n* 5). Of the rhesus milk samples collected, three were obtained from the same individual animals 30 and 60 d after parturition. The samples designated as intermediate milk in the baboon and rhesus monkey were obtained somewhat later in lactation than that generally designated for intermediate or 'transitional' milk in the human. We have labelled them intermediate milk because they represent milk collected at a time point that was intermediate to an early and a later time in lactation. Horse milk samples were obtained from the same individual horses at 1 (colostrum, *n* 6), 7 (intermediate milk, *n* 6), and 30 (mature milk, *n* 6) d after parturition. Bovine milk samples were obtained from different individual animals at 1 d after parturition (colostrum, *n* 4) and more than 2 months after parturition (mature milk, *n* 4). Pig milk samples were collected at 1 d (colostrum, *n* 3) and 21 d (mature milk, *n* 3) after parturition; milk samples from several sows were pooled.

When possible, nipples of the animals were cleaned before milking. Baboons and rhesus monkeys were anaesthetized before collection and were administered oxytocin to promote let-down. The offspring had suckled just before obtaining the milk samples from some but not all animals. Complete evacuation of the glands was not possible for all samples. Milk samples were frozen, transported to the laboratory on dry ice, and stored at −20° until analysis.

Milk analysis

Milk samples were brought to 37° and mixed well. Duplicate portions (approximately 0·20 ml) were weighed and an equal volume of water was added. Samples were centrifuged at 3000 ***g*** for 15 min, frozen for 10 min at −70° and the upper fat layer was skimmed from the lower frozen aqueous layer.

The skimmed milk was hydrolysed in 4 ml 6 M-HCl under a blanket of N_2 at 110° for 24 h. The hydrolysates were dried under vacuum (Speedvac, Savant Instruments, Farmingdale, NJ, USA), 1 ml water was added and evaporated twice, and 1·0 ml 4·0 mM-methionine sulphone was added as an internal standard. The hydrolysates were filtered through a 0·2 μm filter and the amino acid compositions were determined.

Amino acid chromatography

Amino acids in the milk protein hydrolysates were pre-column derivatized with phenylisothiocyanate and separated on a PICO.TAG reverse phase column (Waters, Milford, MA, USA). Derivatized amino acids were detected on-line spectrophotometrically and quantitated by comparing the area under the sample peak against that of an amino acid standard solution (Pierce H standard; Sigma Chemicals, St Louis, MO, USA) of known concentration.

Tryptophan was lost during hydrolysis (McKenzie, 1970), and therefore tryptophan values are not reported. Because glutamine was converted to glutamate and asparagine was converted to aspartate during the hydrolysis, the values reported as glutamate include both glutamate and glutamine and those reported as aspartate include both aspartate and asparagine. We have previously reported (Davis *et al.* 1993) that the recovery of amino acids from milk, as determined by the total amino acids (corrected for the water of hydrolysis) in relation to the protein (using the assay of Lowry *et al.* (1951)), was 97 (SD 2)%. The recovery of individual amino acids from human recombinant insulin (> 98% pure, Boehringer Mannheim Corporation, Indianapolis, IN, USA) and bovine serum albumin (> 98% pure; Sigma Chemicals) was between 97 and 104%.

Calculations

Total amino acid concentration (g/l whole milk) is the sum of all individual amino acids analysed. The amino acid pattern of milk is defined as the amount of each individual amino acid in mg divided by the total amino acid content in g. Tryptophan was not included in the total amino acid content.

Statistics

Data are presented as means and SD. Because many of the milk samples collected at the three stages of lactation were collected from different animals, the data were treated as independent (Snedecor & Cochran, 1967). Two-way analysis of variance was used to test overall differences among species and across the lactation time periods (colostrum and mature milk only). When there was evidence of species × time interactions, specific comparisons were made for the effect of stage of lactation within a species and for differences among species at each stage of lactation. Probability levels of less than 0·05 were considered statistically significant; these levels are not presented in the text.

RESULTS

Total amino acid concentration of milk

The concentration of total amino acids (protein-bound plus free) was greater in colostrum than in mature milk in all species studied (Table 1). On average, there was a 75% decrease in total amino acid concentration between colostrum and mature milk in the human, horse, cow and pig, but a much smaller change was observed for the baboon and rhesus monkey: 34 and 21% respectively. The total amino acid concentration of intermediate milk secreted by the human between 4 and 16 d of lactation and by the horse at 7 d of lactation was intermediate between colostrum and mature milk and differed significantly from both. However, intermediate milk from the baboon and rhesus monkey did not differ significantly from the mature milk of their species.

Table 1. *Total amino acid concentration (g/l) of milk from different species during lactation*†

(Mean values with their standard errors)

	Stage of lactation									
	Colostrum			Intermediate milk			Mature milk			
	Mean	SE	*n*	Mean	SE	*n*	Mean	SE	*n*	Change (%)‡
Human	37·7*	15·4	8	17·2*	1·3	3	8·5	0·9	6	−78
Baboon§	17·5*	2·1	4	12·2	1·5	5	11·5	2·5	5	−34
Rhesus‖	14·5*	2·2	5	13·5	3·4	4	11·5	1·2	5	−21
Horse	37·2*	30·7	6	25·2*	5·1	6	16·8	3·5	6	−88
Cow	61·5*	37·6	4	ND			33·6	4·8	4	−79
Pig	99·0*	16·2	3	ND			35·0	3·5	3	−65

ND, not determined.

Mean values were significantly different from those for mature milk: * $P < 0·05$.

† For details of collection times, see p. 846.

‡ Percentage change from colostrum to mature milk.

§ *Papio cynocephalus anubis* and *Papio cynocephalus anubis*/*Papio cynocephalus cynocephalus*.

‖ *Macaca mulatta*.

Milk amino acid patterns

Because the total amino acid composition of milks varies so widely among species and across the period of lactation, the content of each individual amino acid in each milk sample was expressed relative to the total amino acid concentration in the milk sample so that milk amino acid patterns could be compared across species and across lactation. The proportions of glutamate, proline, methionine, isoleucine and lysine relative to the total amino acids were increased about 25% from colostrum to mature milk in the human, horse, cow and pig (Table 2). However, the pattern of these amino acids did not change significantly over the course of lactation in the baboon and rhesus monkey. Of the amino acids that were decreased from colostrum to mature milk in the human, horse, cow and pig, cystine decreased about 50%, glycine decreased about 40% and serine, threonine and alanine decreased about 25% (Table 3). The methionine:cystine ratio increased about 100% in the human and pig and over 200% in the horse and cow between colostrum and mature milk (results not shown). Small but statistically significant declines during lactation were observed in milk from the baboon for glycine, serine and threonine and in the rhesus monkey for serine. Other amino acids either did not change over the course of lactation or the direction of change was not consistent among species (Table 4). For example, arginine was 38% lower in mature milk than colostrum in the human but 29% higher in mature milk than colostrum in the horse.

DISCUSSION

Change in milk amino acid pattern during lactation

The results of our previous study (Davis *et al.* 1994) demonstrate that there is commonality in the amino acid pattern of the mature milk of different species, particularly in those species of the same phylogenic order. For example, the amino acid pattern of mature milk from the human is similar to that of other primates, particularly the great apes. In the current study we wished to determine whether there was a change during the course

Table 2. *Increases in milk amino acids (mg/g total amino acids) in some species during lactation*†

(Mean values with their standard errors)

	Stage of lactation									
	Colostrum			Intermediate milk			Mature milk			
	Mean	SE	*n*	Mean	SE	*n*	Mean	SE	*n*	Change (%)‡
Glutamate+glutamine										
Human	144*	11	8	164*	17	3	190	8	6	+32
Baboon§	189	1	4	194	8	5	194	6	5	+2
Rhesus‖	194	4	5	195	3	4	191	5	5	−3
Horse	156*	11	6	212	4	6	217	9	6	+39
Cow	166*	9	4	ND			208	2	4	+25
Pig	167*	13	3	ND			208	5	3	+25
Proline										
Human	73*	8	8	90	6	3	95	5	6	+30
Baboon	103	4	4	106	6	5	107	6	5	+3
Rhesus	106	5	5	110	5	4	111	4	5	+5
Horse	78*	4	6	86	5	6	93	7	6	+19
Cow	83*	3	4	ND			100	4	4	+20
Pig	92*	3	3	ND			117	3	3	+27
Methionine										
Human	13*	3	8	16	1	3	16	1	6	+24
Baboon	21	2	4	22	2	5	21	2	5	0
Rhesus	25	1	5	23	3	4	25	2	5	+1
Horse	16*	1	6	23	1	6	22	1	6	+37
Cow	21*	2	4	ND			26	1	4	+26
Pig	19*	1	3	ND			22	1	3	+12
Isoleucine										
Human	36*	5	8	50	5	3	53	3	6	+47
Baboon	56	2	4	55	1	5	54	1	5	−4
Rhesus	60	2	5	57	2	4	58	2	5	−4
Horse	32*	2	6	41	2	6	39	1	6	+19
Cow	37*	2	4	ND			47	1	4	+29
Pig	36	3	3	ND			40	2	3	+9
Lysine										
Human	59*	10	8	71	5	3	71	6	6	+21
Baboon	67	6	4	64	5	5	69	6	5	+3
Rhesus	73	3	5	72	7	4	74	6	5	+1
Horse	60*	7	6	78	4	6	73	5	6	+23
Cow	74*	2	4	ND			86	2	4	+16
Pig	67*	14	3	ND			79	4	3	+17

ND, not determined.
Mean values were significantly different from those for mature milk: * $P < 0.05$.
† For details of sampling times, see p. 846.
‡ Percentage change from colostrum to mature milk.
§ *Papio cynocephalus anubis* and *Papio cynocephalus anubis/Papio cynocephalus cynocephalus*.
‖ *Macaca mulatta*.

of lactation in the amino acid pattern of milk from different species, particularly the human. Although we previously reported changes in the amino acid pattern of rat milk over the course of lactation (Davis *et al.* 1993), these changes were small and not of the magnitude noted by other investigators in the pig (Elliott *et al.* 1971). Previous studies of the amino acid content of human (Chavalittamrong *et al.* 1981; Britton, 1986; Janas &

Table 3. *Decreases in milk amino acids (mg/g total amino acids) in some species during lactation*†

(Mean values with their standard errors)

	Stage of lactation									
	Colostrum			Intermediate milk			Mature milk			
	Mean	SE	n	Mean	SE	n	Mean	SE	n	Change (%)‡
Cystine										
Human	40*	9	8	25	3	3	20	3	6	−50
Baboon§	13	2	4	11	2	5	10	2	5	−20
Rhesus‖	12	2	5	12	2	4	11	2	5	−8
Horse	31*	4	6	14	1	6	12	3	6	−63
Cow	24*	2	4	ND			9	1	4	−63
Pig	26*	1	3	ND			16	1	3	−41
Glycine										
Human	38*	5	8	25	3	3	22	2	6	−42
Baboon	16*	1	4	15	1	5	14	1	5	−12
Rhesus	15	2	1	14	1	4	14	2	5	−6
Horse	36*	2	6	17	1	6	16	1	6	−55
Cow	31*	3	4	ND			18	1	4	−43
Pig	35*	1	3	ND			32	1	3	−6
Serine										
Human	82*	5	8	79*	6	3	61	4	6	−25
Baboon	60*	3	4	53	2	5	53	1	5	−11
Rhesus	52*	1	5	50	1	4	48	3	5	−8
Horse	77*	4	6	50	5	6	52	9	6	−33
Cow	80*	6	4	ND			56	1	4	−29
Pig	61*	6	3	ND			51	3	3	−17
Threonine										
Human	55*	8	8	45	4	3	44	1	6	−21
Baboon	43*	1	4	40	1	5	39	1	5	−8
Rhesus	41	2	5	40	1	4	39	3	5	−5
Horse	65*	2	6	36	2	6	39	2	6	−40
Cow	61*	3	4	ND			42	1	4	−32
Pig	52*	3	3	ND			37	1	3	−28
Alanine										
Human	50*	4	8	41	2	3	40	2	6	−19
Baboon	37	1	4	38	2	5	38	2	5	+2
Rhesus	42	1	5	38	2	4	40	2	5	−5
Horse	50*	3	6	36	3	6	38	2	6	−24
Cow	39*	1	4	ND			32	1	4	−19
Pig	44*	2	3	ND			36	2	3	−17

ND, not determined.
Mean values were significantly different from those of mature milk: * $P < 0.05$.
† For details of sampling times, see p. 846.
‡ Percentage change from colostrum to mature milk.
§ *Papio cynocephalus anubis* and *Papio cynocephalus anubis/Papio cynocephalus cynocephalus*.
‖ *Macaca mulatta*.

Picciano, 1986) and baboon (Buss, 1978) milk did not report the composition for milk secreted during the first 2 d of lactation, i.e. for colostrum, which we hypothesized may differ from that obtained during later periods of lactation.

The results of the present study suggest that the amino acid pattern of milk does change during the course of lactation, but the extent varies among species. Moreover, the presence

Table 4. *Amino acids in milk (mg/g total amino acids) that change little or with inconsistent direction of change among species during lactation*†

(Mean values with their standard errors)

	Colostrum			Intermediate milk			Mature milk			
	Mean	SE	n	Mean	SE	n	Mean	SE	n	Change (%)‡
Aspartate										
Human	100*	9	8	86	13	3	86	9	6	−14
Baboon§	82	2	4	81	2	5	80	4	5	−2
Rhesus‖	76	7	5	76	1	4	73	9	5	−4
Horse	89	2	6	94	5	6	94	5	6	+6
Cow	81*	1	4	ND			70	5	4	−13
Pig	81	6	3	ND			78	5	3	−4
Phenylalanine										
Human	43*	4	8	42*	2	3	37	1	6	−14
Baboon	42	2	4	42	2	5	43	2	5	+1
Rhesus	41*	2	5	44	2	4	45	2	5	+8
Horse	39*	2	6	45	2	6	43	3	6	+10
Cow	42*	1	4	ND			50	1	4	+19
Pig	45	6	3	ND			43	2	3	−3
Arginine										
Human	55*	2	8	42	7	3	36	3	6	−38
Baboon	32	5	4	55	2	5	56	2	5	+6
Rhesus	50	1	5	47	1	4	47	5	5	−7
Horse	46*	2	6	57	2	6	60	2	6	+29
Cow	41*	2	4	ND			34	1	4	−18
Pig	47*	2	3	ND			44	1	3	−8
Leucine										
Human	91*	5	8	104	5	3	104	1	6	+14
Baboon	103	3	4	104	1	5	105	3	5	+2
Rhesus	107	2	2	108	2	4	112	2	5	+4
Horse	83*	2	6	94	3	6	93	2	6	+13
Cow	86*	2	4	ND			99	1	4	+14
Pig	96	7	3	ND			89	4	3	−7
Histidine										
Human	23	1	8	25	2	3	23	2	6	+2
Baboon	22	2	4	22	1	5	21	2	5	−3
Rhesus	22	1	5	21	2	4	20	2	5	−9
Horse	27*	1	6	23*	1	6	21	1	6	−22
Cow	22	1	4	ND			24	1	4	+9
Pig	25	3	3	ND			24	1	3	−4
Tyrosine										
Human	44	2	8	44	3	3	46	2	6	+3
Baboon	41	1	4	41	1	5	40	1	5	−1
Rhesus	42	1	5	41	1	4	42	1	5	−1
Horse	50*	2	6	46	2	6	44	3	6	−13
Cow	50*	1	4	ND			47	1	4	−6
Pig	47*	2	3	ND			39	1	3	−17
Valine										
Human	54	3	8	51	2	3	51	2	6	−5
Baboon	52	1	4	56	1	5	55	3	5	+6
Rhesus	53	1	5	51	2	4	52	2	5	−2
Horse	64*	2	6	47	1	6	47	2	6	−26
Cow	61*	3	4	ND			52	1	4	−14
Pig	59*	3	3	ND			46	1	3	−22

ND, not determined.

Mean values were significantly different from those of mature milk: * $P < 0.05$.

† For details of sampling times, see p. 846.

‡ Percentage change from colostrum to mature milk.

§ *Papio cynocephalus anubis* and *Papio cynocephalus anubis/Papio cynocephalus cynocephalus*.

‖ *Macaca mulatta*.

of a large change in amino acid pattern during lactation appeared to be unrelated to phylogenetic order. The change in amino acid pattern during lactation was similar in the human, horse, cow and pig but there was little change in amino acid pattern during lactation in species of the same phylogenetic order as the human, i.e. the baboon and rhesus monkey. Differences among the three primates in amino acid pattern were substantial for colostrum, whereas mature milk was generally similar in amino acid pattern among the primates. Marked differences were also observed among the three primates for the total amino acid concentration of colostrum, but not for the total amino acid concentration of mature milk. Thus, while the total amino acid concentration of mature milk was lowest in the human, the total amino acid concentration of colostrum in the human was over two-fold higher than that of the baboon and rhesus monkey.

Intermediate milk in the human and horse was, in general, more similar to mature milk than to colostrum, in both total amino acid concentration and amino acid pattern. Milks labelled as intermediate milk for the baboon and rhesus monkey were obtained at 30 d of lactation, and therefore are not truly representative of intermediate or 'transitional' milk. However, we have presented the composition of 30-d milk separately from the 60-d milk in these species because of the paucity of information in the literature on the amino acid pattern of the milk of primates during any stage of lactation.

Relationship between total concentration and pattern of milk amino acids

The change in amino acid pattern during the course of lactation appeared to be related to the change in total amino acid concentration during the course of lactation. Thus, in those species (the human, horse, cow and pig) whose total amino acid concentrations differed markedly between colostrum and mature milk, the amino acid pattern also differed between colostrum and mature milk. This is consistent with our previous finding in the rat, in which the changes in both amino acid pattern and total amino acid concentration over the course of lactation were small, although statistically significant (Davis *et al.* 1993).

Because most milk amino acids are derived from milk proteins, the change in milk amino acid pattern during the course of lactation can probably be ascribed to a change during the course of lactation in the relative distribution of milk proteins containing different amino acid patterns. Indeed, the relative proportion of the caseins to the whey proteins, particularly immunoglobulins and α-lactalbumin, increases during the course of lactation in the milk of the human, sow, cow and baboon (Buss, 1978; Kulski & Hartmann, 1981; Davies *et al.* 1983; Kunz & Lonnerdal, 1983; Jenness, 1986; Sanchez-Pozo *et al.* 1986; Klobasa *et al.* 1987). Comparison of the amino acid patterns of different whey proteins with the caseins suggests that whey proteins are generally lower in glutamate, proline and methionine and higher in cystine, glycine and threonine than the caseins (Kon & Cowie, 1961; Renner, 1983; Heine *et al.* 1991). These differences in amino acid patterns between the whey proteins and the caseins are consistent with our observed differences in amino acid patterns between colostrum and mature milk.

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