Crude Protein Content and Amino Acid Composition in Taiwanese Human Milk

Tzee-Chung WU, Chin-Chin CHUANG, Beng-Huat LAU, Betau HWANG, Makihiro SUGAWARA[1] and Tadashi IDOTA[1]

Division of Pediatric Gastroenterology and Nutrition, Children's Medical Center, Veterans General Hospital-Taipei and National Yang-Ming University, Taipei, Taiwan, R.O.C.
[1]*Nutritional Science Laboratory, Snow Brand Milk Products Co., Ltd., Kawagoe 350–1165, Japan*

(Received October 25, 1999)

Summary Breast milk provides the essential nutrients for infants in readily available form. The content of nitrogen in human milk is of great importance because it relates to the growth of infants in the early stage, and the composition of nitrogenated compounds varies according to the lactational stage. Three-hundred-and-three human milk specimens were obtained from 240 healthy mothers living in two different districts in Taiwan, and 264 specimens were used for the analysis. The crude protein content, total and free amino acid compositions as well as urea content were evaluated using pooled milk samples according to different lactational stages and geographical location. The crude protein content decreased sharply from colostrum (2.51 g/100 mL) to mature milk (1.25 g/100 mL). Total amino acids account for 80–85% of the crude protein throughout the whole lactation period. Crude protein also contained 30 to 35 mg/100 mL urea and 41 to 48 mg/100 mL free amino acids as non-protein nitrogen components. The ratio of essential to non-essential amino acids remained constant throughout the lactation period in spite of a decline in amino acid content. The amino acid composition per 1 g of nitrogen varied during the lactation period. The differences of these lactational changing patterns of individual amino acids were probably reflected by variation of the protein composition during lactation. The sum of free amino acid content ranged from 43 to 50 mg/100 mL in Taipei and 40 to 45 mg/100 mL in Kaohsiung. Although the variations of free amino acids during the lactation period differed among amino acids, glutamic acid predominated in mature milk while phosphoethanolamine was predominant in colostrum.
Key Words crude protein content, amino acid, Taiwanese human milk, lactational change

Breast milk is the perfect food for infants because it contains the necessary nutrients in readily bioavailable forms while simultaneously providing many anti-infective components. Most researchers agree that breast feeding exclusively will provide adequate amounts of most nutrients for infants for at least 4 mo after birth. A comprehensive evaluation of human milk composition is extremely significant not only for infant nutritional studies, but also to form a basis for developmental research regarding infant formula. Among macronutrients and micronutrients, proteins are the most important determinants and there is a close relationship between the growth rate of mammals and the protein content of milk (*1*). In Taiwan, however, protein content and amino acid composition of human milk have not yet been clarified. To get information on these nutrients in Taiwanese, we collected human milk specimens from different districts and analyzed them.

MATERIALS AND METHODS

Three-hundred-and-three human milk specimens were obtained from 240 mothers between the ages of 19 and 41 and who were living in northern Taiwan (Taipei) or southern Taiwan (Kaohsiung) from March to December, 1995. Sampling criteria were as follows: 1) the milk specimens were collected between 10:00 and 18:00 to avoid circadian changes, 2) the delivery and birth weight of infant was normal, 3) the baby was healthy and growing well, and 4) mother was healthy and had a balanced diet. We selected 264 milk specimens because 39 did not meet all of the criteria described above. We prepared 32 pooled milk samples according to lactational stage and district where the mother lived.

We explained the aim and the method of the study to the mothers, and all of the subjects that agreed to donate their milk filled out a questionnaire about the criteria described above.

Experimental method.

(1) Crude protein: The Micro-Kjeldahl method was used. Crude protein was calculated as total nitrogen multiplied by 6.38.

(2) Amino acid composition: One milliliter of human milk was collected in a test tube, and 9 mL of

Table 1. Amino acid composition and crude protein content for respective lactational stages (mg/100 mL).[1]

Lactational stage (days postpartum)	0–11		22–45		46–65		66–297	
District	T[2]	K[3]	T	K	T	K	T	K
Number of specimens	37	26	49	27	26	10	51	18
Aspartic acid	202	235	123	130	111	103	105	99
Threonine	112	136	63	68	58	54	56	53
Serine	118	150	64	71	59	55	57	54
Glutamic acid	309	362	216	220	208	190	198	188
Proline	154	180	107	106	103	89	97	88
Glycine	65	81	33	38	29	28	29	28
Alanine	92	114	52	60	47	45	47	45
Cystine	51	58	27	31	23	25	23	23
Valine	120	139	73	75	65	60	59	61
Methionine	33	37	21	22	21	18	19	17
Isoleucine	89	89	66	63	53	54	48	53
Leucine	196	232	128	134	124	108	118	108
Tyrosine	85	104	52	58	49	46	45	45
Phenylalanine	87	104	53	58	44	45	42	45
Lysine	137	161	92	96	109	76	102	75
Histidine	53	63	35	37	33	29	31	31
Tryptophan	45	51	24	26	21	20	21	19
Arginine	102	122	52	60	45	43	45	44
Total amino acid	2,016 (80.3)[4]	2,419 (89.5)	1,266 (84.8)	1,348 (84.2)	1,198 (95.8)	1,084 (90.3)	1,140 (87.6)	1,075 (89.6)
Crude protein (g/100 mL)	2.51	2.70	1.49	1.60	1.25	1.20	1.30	1.20
Essential amino acid/ non-essential amino acid	0.74	0.72	0.76	0.75	0.78	0.74	0.77	0.75

[1] Values represent means of the categories (lactational stages and districts). The analyses were done for pooled milk samples described in MATERIALS AND METHODS.
[2] T=Taipei, [3] K=Kaohsiung.
[4] Percentage of total amino acid content among crude protein.

6 M hydrochloric acid containing 0.05% 2-mercaptoethanol was added. After de-aeration and nitrogen substitution, the test tube was sealed for hydrolysis at 110°C for 22 h. The solution was then filtered and evaporated under reduced pressure to remove hydrochloric acid. The sample was prepared by dissolving the residue in 0.02 M hydrochloric acid (final volume 50 mL), and then used for amino acid analysis. A high-performance amino acid analyzer (a Hitachi 835-50 model, Tokyo, Japan) was used. For cystine analysis, 1 mL of human milk was mixed with 50 mL of newly prepared performic acid and allowed to stand for 16 h at 0°C. Next, 20 mL of distilled water was added, and while ice-cooling, 4 mL of 47% hydrobromic acid was added. The solution was evaporated under reduced pressure. The residue was dissolved with 6 M hydrochloric acid containing 0.05% 2-mercaptoethanol (final volume 20 mL). Then, 10 mL of the solution was deaerated, hydrolyzed, and filtered before evaporation under reduced pressure. The residue was dissolved with 0.02 M hydrochloric acid (final volume 25 mL). For tryptophan analysis, 2 mL of human milk was mixed with 3.0 g barium hydroxide, 7 mL distilled water, and 2 to3 drops of 2-mercaptoethanol. The tube was then sealed for hydrolysis at 110°C for 24 h. After letting sit for a while, distilled water was added to a final volume of 100 mL. Carbon dioxide was injected to the solution to remove barium carbonate, and then amino acid analysis was conducted.

(3) Free amino acid composition and urea content: Five milliliters of 6% sulfosalicylic acid was added to 10 mL of a human milk sample. After thoroughly mixing, the solution was centrifuged at 3,000 rpm for 15 min. The supernatant was then filtered and used for the analysis of free amino acids and urea.

RESULTS

Crude protein

Crude protein content in human milk is shown in Table 1. Colostrum had the highest crude protein content (2.51 g/100 mL). Then the content of crude protein decreased sharply, and reached a constant level at postpartum 2 mo (1.20 g/100 mL).

Total amino acid composition

The amino acid composition of human milk is given in Table 1. Total amino acid content tended to decrease as lactation was prolonged, but account for more than 80% of the crude protein content over the period of lac-

Table 2. Amino acid composition for respective lactational stages (mg/g nitrogen).[1]

Lactational stage (days postpartum)	0–11		22–45		46–65		66–297	
District	T[2]	K[3]	T	K	T	K	T	K
Aspartic acid	521	549	524	530	565	535	515	537
Threonine	289	318	270	278	293	279	274	284
Serine	303	349	271	289	301	285	277	293
Glutamic acid	799	846	924	901	1,058	989	979	1,017
Proline	398	421	456	434	520	464	478	476
Glycine	166	189	142	154	148	146	142	151
Alanine	238	265	223	242	240	232	228	241
Cystine	131	136	117	126	114	128	114	125
Valine	308	324	312	307	331	313	293	330
Methionine	84	87	91	91	105	94	94	94
Isoleucine	231	207	281	256	267	282	238	289
Leucine	505	543	547	547	631	564	578	587
Tyrosine	218	244	221	236	246	238	222	245
Phenylalanine	223	244	225	234	221	233	204	243
Lysine	354	376	395	391	553	405	502	406
Histidine	136	147	150	149	167	151	153	166
Tryptophan	114	118	102	105	104	105	102	101
Arginine	262	285	221	241	226	222	220	235
Sum	5,205	5,643	5,409	5,502	6,084	5,661	5,609	5,815

[1] Values represent means of the categories (lactational stages and districts). The analyses were done for pooled milk samples described in MATERIALS AND METHODS.
[2] T=Taipei, [3] K=Kaohsiung.

tation. Changes in individual amino acids during lactation are shown in Table 2. These values (mg/g nitrogen) varied little compared to those given in Table 1 (mg/100 mL milk). Isoleucine, leucine, lysine, histidine, glutamic acid and proline tended to increase. Phenylalanine, tyrosine, aspartic acid, alanine, methionine, valine and threonine showed little variation throughout lactation. While arginine, glycine, serine, cystine, tryptophan, alanine and threonine rather decreased in early in lactation and reached a stable state.

Lactational changes of free amino acid and urea contents

The contents of free amino acids and urea in human milk collected in Taipei and Kaohsiung are shown in Table 3. Their lactational changes differed with components, for example, taurine, proline, isoleucine, leucine, tyrosine, tryptophan, lysine and arginine decreased at later lactational stages, while glutamine, glutamic acid, glycine and alanine increased although cystine and histidine remained constant. The percentage of free amino acids increased from 2.4% on postpartum 0–11 d to 4.2% on postpartum 66–297 d in Taipei and from 1.9 to 3.9% in Kaohsiung as well. The percentage of urea nitrogen increased from 4.2 to 7.6% in Taipei and from 3.8 to 7.4% in Kaohsiung. The major components of human milk at each lactational stage are rearranged as shown in Table 4.

DISCUSSION

Balanced nutrition is essential for the sound development of infants. In this respect, breast milk serves as an ideal source of nutrition, and infant formula, a substitute for breast milk, should be made to resemble human milk more closely. Therefore, characterization of the human milk composition is of great important to further improve infant formula as well as gain an exact understanding of infant nutrition.

Among essential nutrients, protein is the most important determinant. Human milk provides all of the amino acids necessary for infant growth, so we analyzed the crude protein and amino acid compositions of Taiwanese human milk.

The aim of this study was to determine the average amino acid composition of Taiwanese human milk. To analyze individual milk requires a large amount of each specimen. Unfortunately, all of the milk specimens we collected was in an amount insufficient to be analyzed. Therefore we prepared pooled milk samples and analyzed them.

As shown in Table 1, there were no differences of crude protein content and amino acid composition in Taiwanese human milk. This observation was similar, in principle, to previous observations (2–4). It is reported that there are wide variations in the protein content of human milk between individuals (2). In some infants, much protein is required for optimal growth. In the case of infant formula, care should be taken so as not to cause protein deficiency in any infant. Incidentally human milk contains such physiologically important proteins as immunoglobulins and lactoferrin (5). High protein intake may lead to metabolic acidosis

Table 3. Free amino acid composition and urea content (mg/100 mL).[1]

Lactational stage (days postpartum)	0–11		22–45		46–65		66–297	
District	T[2]	K[3]	T	K	T	K	T	K
Phosphoserine	8.51	8.54	2.65	3.16	2.32	2.16	5.83	2.22
Taurine	6.26	5.99	4.48	3.81	3.53	4.02	3.59	4.34
Phosphoethanolamine	9.48	9.55	9.19	8.45	7.61	7.90	5.55	6.76
Aspartic acid	0.65	0.64	0.39	0.42	0.51	0.46	0.72	0.67
Threonine	1.05	0.89	0.75	0.67	0.85	0.84	0.90	0.76
Serine	0.70	0.55	0.71	0.61	0.83	0.66	0.90	0.70
Asparagine	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Glutamic acid	8.28	6.63	15.17	12.87	16.33	15.55	18.15	15.99
Glutamine	0.33	0.32	1.81	1.70	3.46	2.76	4.03	3.07
Proline	1.75	1.60	0.33	0.40	0.30	0.26	0.41	0.31
Glycine	0.45	0.36	0.70	0.63	0.63	0.68	0.78	0.78
Alanine	1.30	1.02	1.72	1.55	1.74	1.71	1.97	1.81
Citrulline	0.15	0.09	0.18	0.18	0.28	0.24	0.37	0.31
Valine	1.14	0.90	0.57	0.54	0.53	0.51	0.55	0.52
Cystine	0.67	0.63	0.69	0.61	0.64	0.66	0.72	0.70
Methionine	0.72	0.50	0.33	0.32	0.38	0.31	0.29	0.28
Isoleucine	0.75	0.60	0.35	0.35	0.31	0.30	0.30	0.36
Leucine	1.61	1.13	0.53	0.53	0.53	0.46	0.51	0.49
Tyrosine	0.93	0.75	0.39	0.43	0.37	0.28	0.34	0.33
Phenylalanine	0.65	0.55	0.43	0.45	0.40	0.38	0.44	0.42
Ethanolamine	0.32	0.26	0.35	0.32	0.26	0.25	0.24	0.22
Tryptophan	0.50	0.19	0.00	0.00	0.00	0.00	0.00	0.00
Ornithine	0.31	0.35	0.15	0.16	0.14	0.14	0.18	0.15
Lysine	1.81	1.35	0.46	0.44	0.28	0.30	0.40	0.29
Histidine	0.34	0.31	0.34	0.31	0.32	0.34	0.29	0.28
Arginine	1.28	1.03	0.32	0.32	0.21	0.23	0.31	0.21
Sum of free amino acid	50.4 (2.4)[4]	45.16 (1.9)[4]	43.43 (3.4)	39.65 (2.9)	43.16 (3.6)	41.72 (3.8)	48.22 (4.2)	42.40 (3.9)
Urea	35.84 [4.2][5]	34.77 [3.8]	31.98 [6.3]	33.87 [6.3]	33.08 [7.8]	29.06 [7.2]	30.45 [7.6]	30.10 [7.4]
Ammonia	0.50	0.47	0.49	0.44	0.43	0.39	0.47	0.45

[1] Values represent means of the categories (lactational stages and districts). The analyses were done for pooled milk samples described in MATERIALS AND METHODS.
[2] T=Taipei. [3] K=Kaohsiung.
[4] Percentage of total nitrogen. [5] The ratio of urea nitogen to total nitrogen (%).

and hyperaminoacidemia that in turn stimulate insulin secretion. To design an infant formula, these non-nutritional protein components should also be considered so as not to cause a metabolic burden for the infant.

Although the protein content in whole milk gradually decreases with the lapse of lactation, the essential/non-essential rate of amino acids remains unchanged both in Taipei and in Kaohsiung. The variation of amino acid content per gram of nitrogen in human milk was only little compared to that per 100 mL. Harzer et al. (*6*) reported changes of individual amino acids (mg/g nitrogen) from 2 to 36 d postpartum. They showed that isoleucine, leucine, lysine, histidine, glutamic acid and proline had a tendency to increase during lactation, while phenylalanine tyrosine, aspartic acid, methionine, and tryptophan remained stable, and arginine, glycine, serine, cystine, threonine, alanine and valine had a tendency to decrease. Our results were similar to the results of Harzer et al. except for valine and tryptophan. The differences in changing patterns of individual amino acids were probably reflected in the variation of the protein components in human milk during the lactation period (*6*).

The amino acid composition of mature human milk in Taiwanese was similar to that of mature human milk in other countries (*6, 7*). This implies that the amino acid composition of mature human milk is not significantly different among countries. However, all of the human milk proteins do not undergo digestion and absorption. Thus, it is necessary to study the amino acid composition of the nutritionally available protein in vivo. Well-designed studies from nutritional, socioeco-

Table 4. Major components of free amino acid at respective lactational stages.

Order	Lactational stage (days postpartum) 0–11	22–45	46–65	66–297	
1	Urea	Urea	Urea	Urea	
2	Pea	Glu	Glu	Glu	a
3	Pser	Pea	Pea	Pea	
4	Glu	Tau	Tau	Pser	
5	Tau	Pser	Gln	Gln	b
6	Pro	Gln	Pser	Tau	
7	Lys	Ala	Ala	Ala	
8	Leu	Thr	Thr	Thr	c
9	Arg	Ser	Ser	Ser	
10	Ala	Gly	Gly	Gly	

Major components of urea and free amino acids are listed in the order of the contents at each lactational stage.
a; contents of 5 mg/100 mL or more, b; contents of 2 mg/100 mL or more, c: contents of 1 mg/100 mL or more.
Abbreviations: Glu=glutamic acid, Pro=proline, Lys=lysine, Leu=leucine, Arg=arginine, Ala=alanine, Thr=threonine, Ser=serine, Gly=glycine, Tau=Taurine, Pea=phosphatidylethanolamine, Pser=Phosphatidylserine.

nomic and environmental aspects will be needed in order to obtain a full understanding of the biological utilization of human milk.

In human milk, the non-protein nitrogen (NPN) fraction comprises about one-quarter of the total nitrogen, compared with about several to 5% in bovine milk (*8*). The NPN fraction of human milk consists of urea, free amino acids, peptides, nucleotides and polyamines. Among the NPN fraction, urea predominates, ranging from 11 to 20 mg/100 mL milk as nitrogen (*7, 9–11*). In our study, urea ranged from 29.06 to 35.84 mg/100 mL (13.56 to 16.72 mg/100 mL as nitrogen). The rate of urea nitrogen to total nitrogen increased with the lapse of lactation and no differences were observed between Taipei and Kaohsiung (Table 3). A similar tendency was also observed for Japanese human milk (*7*). Although some studies have suggested a nutritional role for urea in human milk (*12–14*), further studies are required to establish its nutritional and/or physiological significance.

There were no differences in free amino acid composition between Taipei and Kaohsiung (Table 3). However, the contents of phosphoethanolamine and phosphoserine were especially high in Taiwanese human milk in comparison with other reports (*7, 9*). The relative amounts of both phosphoethanolamine and phosphoserine are much higher in milk than in serum (*15*). It is worth noting that the total amount of phosphoamino acids in human milk remains constant, as shown in Table 3. Phosphoethanolamine has been recognized as a constituent of the growth modulator fraction in human milk (*9*). However, it is not clear whether this phospho-amino acid is synthesized in the mammary gland, or why Taiwanese human milk contains it in abundance.

As shown in Table 4, glutamic acid ranked second in the amino acid on postpartum 22 d later, although it was predominant over the period of lactation in Japanese (*7*) and American series (*9*). In practice, the glutamic acid content of Taiwanese colostrum was much lower than that of Japanese (*7*). Taurine was another dominant free amino acid component which was found at the highest concentration in colostrum and on the decrease late in lactation (Table 3). The change of taurine in Taiwanese human milk during lactation was quite similar to that in Japanese human milk (*7*). Harzer et al. have suggested that urea, glutamic acid, taurine and phosphoethanolamine are beneficial for the growth of infants through bile acid conjugation, zinc bioavailability, energy or nitrogen supply for the intestine, and bifidus colonization (*9*). The fact that the concentrations of those substances increase or remain constant during lactation despite the dilution of milk protein suggest their importance in the growth of infants. In our study, the amount of free amino acids ranged from 39.65 to 50.40 mg/100 mL through the lactation period, and its percentage of total amino acids increased from 2.4 to 4.2% in Taipei and from 1.9 to 3.9% in Kaohsiung. This result was similar to those of other studies (*7, 15, 16*). It also seems likely that free amino acids maintain the osmotic pressure of human milk (*11*) and preferentially contribute to the initial change in plasma free amino acid concentration of the infant because of their early intake (*16*). The nutritional and physiological significance of these free amino acids remains to be investigated further.

REFERENCES

1) Davis TA, Nguyen HV, Garcia-Bravo R, Fiorotto ML, Jackson EM, Reeds PJ. 1994. Amino acid composition of the milk of some mammalian species changes with stage of lactation. *Br J Nutr* **72**: 845–853.
2) Idota T, Sakurai T, Ishiyama Y, Murakami Y, Kubota J, Ii N, Sakamoto T, Doki R, Shimoda K, Asai Y. 1991. The latest survey for the composition of human milk obtained from Japanese mothers Part 1. The contents of gross components and minerals. *Jpn J Pediatr Gastoenterol Nutr* **5**: 145–158 (in Japanese).
3) Harzer G, Bindels JG. 1994. Main compositional criteria of human milk and their implications in early infancy. *In*: New aspects of nutrition in pregnancy, infancy and prematurity. p 83–94. Elsevier Scientific Publishers, Amsterdam.
4) Raiha NCR, Heinonen K, Rassin D. 1976. Milk protein quantity and quality in low-birthweight infants: I. Metabolic responses and effects on growth. *Pediatrics* **57**: 659–674.
5) Raiha NCR, Minoli I, Moro G. 1986. Milk protein intake in the term infant. I. Metabolic responses and effects on growth. *Acta Paediatr Scand* **75**: 881–886
6) Harzer G, Haug M, Bidels JG. 1986. Biochemistry of human milk in early lactation. Z *Ernahrungswiss* **25**: 7–90.
7) Idota T, Sakurai T, Sugawara M, Ishiyama Y, Murakami Y, Maeda T, Yano M, Shimoda K, Asai Y. 1991. The latest survey for the composition of human milk obtained from Japanese mothers Part III. Composition of amino acids and related nitrogenous compounds. *Jpn J Pediatr Gastoenterol Nutr* **5**: 209–219 (in Japanese).

8) Atkinson SA, Schnurr CM, Donovan SM. 1989. The nonprotein nitrogen components in human milk: Biochemistry and potential functional role. *In*: Protein and Non-Protein Nitrogen in Human Milk (Atkinson SA, Lonnerdal B, eds), p 117. CRC Press, Boca Raton, FL.
9) Harzer G, Franzke V, Bindels JG. 1984. Human milk nonprotein nitrogen components: changing patterns of free amino acids and urea in the course of early lactation. *Am J Clin Nutr* **40**: 303–309.
10) Ross SA, Clark RM. 1985. Nitrogen distribution in human milk from 2 to 16 weeks postpartum. *J Dairy Sci* **68**: 778–781.
11) Clark RM, Ross SA, Hill DW, Ferris AM. 1987. Within-day variation of taurine and other nitrogen substances in human milk. *J Dairy Sci* **70**: 776–780.
12) Heine W, Tiess M, Wutzke KD. 1986. 15N tracer investigations of the physiological availability of urea nitrogen in mother's milk. *Acta Paediatr Scand* **75**: 439–443.
13) Fomon SJ. 1991. Requirements and recommended dietary intakes of protein during infancy. *Pediatr Res* **39**: 391–395.
14) Jackson AA. 1994. Urea as a nutrient: bioavailability and role in nitrogen economy. *Arch Dis Child* **70**: 3–4.
15) Armstrong MD, Yates KN. 1963. Free amino acids in milk. *Proc Soc Exp Biol Med* **113**: 660–683.
16) Schanler RJ, Garza C. 1987. Plasma amino acid differences in very low birth weight infants fed either human milk or whey-dominant cow milk formula. *Pediatr Res* **21**: 301–305.