The latest survey for the composition of human milk obtained from Japanese mothers

Part 3. Composition of amino acids and related nitrogenous compounds

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Summary

In order to obtain the human milk composition of modern Japanese, 2,727 human milk specimens were obtained from 2,434 mothers living in 46 different districts in Japan. Out of these specimens, 2,279 specimens which met certain predetermined categories including the health conditions of mother and child and growth status of child were selected and divided into two seasonal groups, summer and winter, for an evaluation of their changes in total amino acid composition and free amino acid composition as well as urea contents according to different lactational stages and geographical differences. While the total amino acid composition showed variations according to changes in lactational stages which resembled those of crude protein contents, the variations according to changes in lactational stages of amino acid composition per 1g nitrogen varied among respective amino acid types. Urea content remained stable at 34.23 to 43.82mg/100ml throught the entire lactation period, and urea nitrogen consisted 5.5 to 11.7% of total nitrogen. Although the variations of free amino acids according to changes in lactational stages differed according to type, glutamic acid was most dominant throughout the entire lactational period, followed by taurine for the lactational stage of 31 to 60 days and glutamine for after 61 to 120 days postpartum. Total free amino acid content ranged from 36.81 to 55.2mg/100ml, and although it showed a minor variation due to changes in lactational stages, its percentage among total amino acids increased as lactational stages progressed.

Introduction

A scientific analysis of human breast milk, the ideal source of nutrition for newborns and infants, and a comprehensive evaluation of human milk composition are extremely significant not only for infant nutritional studies, but also to form a basis for developmental research regarding infant breast milk formula(hereinafter referred to as "infant formula")as a valid substitute for human breast milk.

The eating habits of Japanese in recent years have changed drastically, such as increases in the opportunities of eating out and consuming prepared food, and the intake volume of each nutrient has changed accordingly[1)2)].These changes are considered to have major influences on the contents of human breast milk[3)~5)]. Therefore, in order to elucidate the latest composition of Japanese human milk, the authors have conducted an examination of 2,279 specimens obtained from subjects living throughout Japan. As a result, the authors have reported a recent increase in crude protein contents in Japanese human milk.

While this is considered to effect the amino acid composition in human milk as well, almost no reports have been made on the recent status of the amino acid composition of Japanese human milk[8)9)]. Further, the only surveys conducted on a anationwide basis regarding human milk have been by Saito et al.[10] and Yonekubo et al.[11].

On the other hand, human milk contains 20 to 30% of nonprotein nitrogenous elements. These elements have also been reported to be effected by the dietary patterns of the mother[12][13]. The elements include urea, glutamic acid, taurine, phosphoethanolamine and other free amino acids, and their nutritional and physiological significances are suggested to be nitrogen and energy cources, and contribute towards the digestion and absorption of bifidobacteria flora and fat, as well as maintain the functions of the optical and central nervous systems[13)~15].

Unfortunately, there has been only a few reports regarding the free amino acids in Japanese human milk[11][16][17], and none of them are recent studies.

Therefore, an examination was conducted in order to elucidate the variations in the total amino acid composition, free amino acid composition and urea contents in recent Japanese human milk due to differences in lactational stage, district and season. The results were as follows.

Subjects／Method

Subjects:

2,727 human milk specimens were obtained from 2, 434 mothers between the ages of 17 and 41 and living in various districts of Japan. Out of these specimens, 2, 279 which met the various predetermined conditions including the health status of the mother and child, growth status of child, time of day of milking and milking method were selected as subjects[6].

Samples:

As stated in the previous report[6], mixed milk samples were fabricated for the respective lactational stages, districts and seasons to be analyzed.

Method of analysis:

(1)Total amino acid composition:

1ml of human milk was collected in a test tube, and 9ml of 6N hydrochloric acid containing 0.05% β-mercaptoethanol was added. After de-airing and nitrogen substitution, the test tube was sealed for hydrolysis at 110℃ for 2 hours. The solution was then filtered and depressurized／dehydrated to remove tha hydrochloric acid. 0.02N hydrochloric acid was added to the remains. After solution, 50ml samples were measured.

For cystine, 1ml of human milk was collected in an egg plantshaped flask, and added newly prepared 50ml of performic acid (30% hydrogen peroxide:90% formic acid=1:9)was added, and left untouched for 16 hours at 0℃. Then, 20ml of distilled water was added, and while ice-cooling, 4ml of 47% hydrobromic acid was added, then depressurized／dehydrated afterwards. 6N hydrochloric acid containing 0.05% β-mercaptoethanol was used to measure 20ml of the remains. Then, 10ml of the solution was collected in a test tube for de-airing and hydrolyses, and was filtered and depressurized／dehydrated afterwards. 0. 02N hydrochloric acid was added to the remains, and 25ml samples mere measured.

For tryptophan, 2ml of human milk was collected in a test tube and added 3.0g barium hydroxide, 7ml distilled water, and 2 to 3 drops of β-thioglycol was added, mixed and sealed for hydrolysis at 110℃ for 24 hours. Then, distilled water was added to make 100ml. Carbon dioxide was removed by ventilating with barium carbonate, and the result was used as the sample. For measurement, Hitachi 835-50 high-speed amino acid analyser was used. Analysis of cystine and tryptophan was by the highseparation analysis method, while other amino acids were analyzed by the specific analysis method.

(2)Free amino acid composition:

5ml of 6% sulfosalicylic acid was added to 10ml of human milk. After mixing thoroughly, the solution was centrifuged at 3,000 r. p. m. for 15 minutes. The supernatant liquid was filtered to be used as the sample. For measurement, Hitachi 835-50 high-speed amino acid analyser was used. Analysis by the bioanalysis method.

Results

1.Total amino acid composition

1.1 Time course variation

The results of analyses per 100ml summer and winter samples are indicated in Chart. 1. Also, the time course variations of total amino acid and crude protein con-

Chart.1 Total amino acid composition according to respective lactational stages(for respective seasons)

(mg/100mℓ)

lactational stage	3 to 5 days postpartum		6 to 10 days postpartum		11 to 15 days postpartum		16 to 30 days postpartum		31 to 60 days postpartum		61 to 120 days postpartum		121 to 240 days postpartum		241 to 482 days postpartum	
	winter	summer	winter	summer	winter	summer	winter	summer	winter	summer	winter	summer	winter	summer	winter	summer
Isoleucine	84.8	86.5	83.3	81.9	74.5	76.4	68.5	68.6	63.3	63.2	53.7	55.0	50.4	50.5	48.8	50.3
Leucine	176.0	182.4	166.7	164.9	144.6	146.8	131.2	130.2	121.0	119.5	102.9	106.2	97.1	99.0	97.2	99.1
Lysine	119.6	124.6	114.1	113.2	99.7	102.2	89.7	89.7	82.2	82.1	69.2	72.2	64.5	67.2	64.6	67.1
Methionine	24.7	25.2	23.3	24.8	21.1	21.2	18.7	18.7	17.0	17.3	13.8	15.4	12.9	14.1	12.8	14.0
Cystine	41.6	44.2	38.3	37.2	31.1	31.4	35.9	28.0	34.1	25.3	33.8	22.1	19.7	21.3	21.0	21.9
Phenylalanine	73.2	77.7	68.2	67.6	57.8	58.5	51.5	50.8	46.9	46.2	39.4	41.2	37.6	38.8	38.3	38.4
Tyrosine	79.0	78.4	73.1	69.2	62.7	60.0	55.2	53.0	50.4	48.1	42.0	43.0	40.4	39.8	41.4	39.2
Threonine	93.6	99.0	83.2	82.1	71.2	72.2	62.5	63.9	57.3	57.4	49.6	51.4	47.0	48.6	48.7	49.1
Tryptophan	30.8	31.5	28.7	33.0	25.3	23.4	23.0	18.4	19.5	19.6	19.3	16.3	16.9	15.5	20.3	14.1
Valine	104.4	88.5	97.0	74.5	81.1	62.9	71.0	55.9	63.4	50.5	53.1	44.2	56.3	42.7	57.7	43.8
Arginine	81.9	86.6	72.7	71.3	57.3	57.1	48.8	47.6	42.6	42.3	37.5	38.1	36.1	37.0	38.5	38.8
Histidine	45.1	47.2	42.8	42.3	36.8	37.7	33.3	32.9	31.2	30.7	26.6	27.7	25.1	25.9	24.3	25.9
Alanine	78.8	82.6	71.9	70.8	59.0	60.0	53.2	51.4	47.8	46.4	41.4	42.2	39.4	39.8	41.7	40.4
Aspartic acid	177.1	190.3	166.6	167.0	139.1	142.8	124.1	128.2	111.7	115.0	95.3	97.3	88.7	93.7	90.6	92.8
Glutamic acid	284.6	290.7	272.8	270.1	239.9	241.2	217.8	213.7	207.5	202.8	183.7	189.4	176.0	173.9	171.4	174.7
Glycine	50.1	55.0	44.3	45.2	36.8	37.6	32.0	32.4	28.6	28.5	24.9	25.7	23.5	24.5	25.1	24.9
Proline	148.6	147.1	137.1	139.2	124.5	123.3	109.9	111.7	105.4	103.0	88.3	92.5	82.3	85.6	83.7	84.1
Serine	94.8	104.4	85.8	86.4	70.8	74.0	62.9	63.2	56.7	56.0	47.4	48.9	44.1	46.0	46.7	47.2
total amino acid content	1788.7	1841.9	1669.9	1640.7	1433.3	1428.7	1289.2	1258.3	1186.6	1153.9	1021.9	1028.8	958.0	963.9	972.8	965.8
crude protein content[6] (g/100mℓ)	2.04	2.21	1.94	1.93	1.68	1.63	1.53	1.46	1.36	1.33	1.17	1.18	1.09	1.13	1.13	1.11

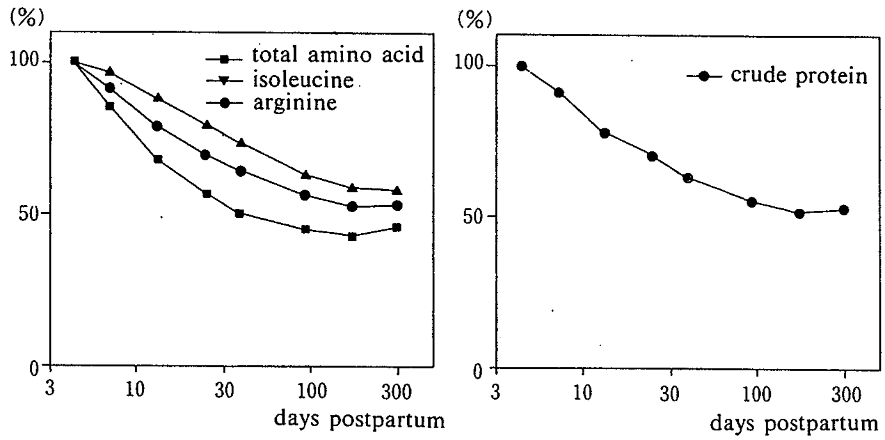

Figure. I The time course variation of total amino acid and crude protein contents per 100ml human milk
Shown in relative values assuming that the contents for 3 to 5 days postpartum are 100%.

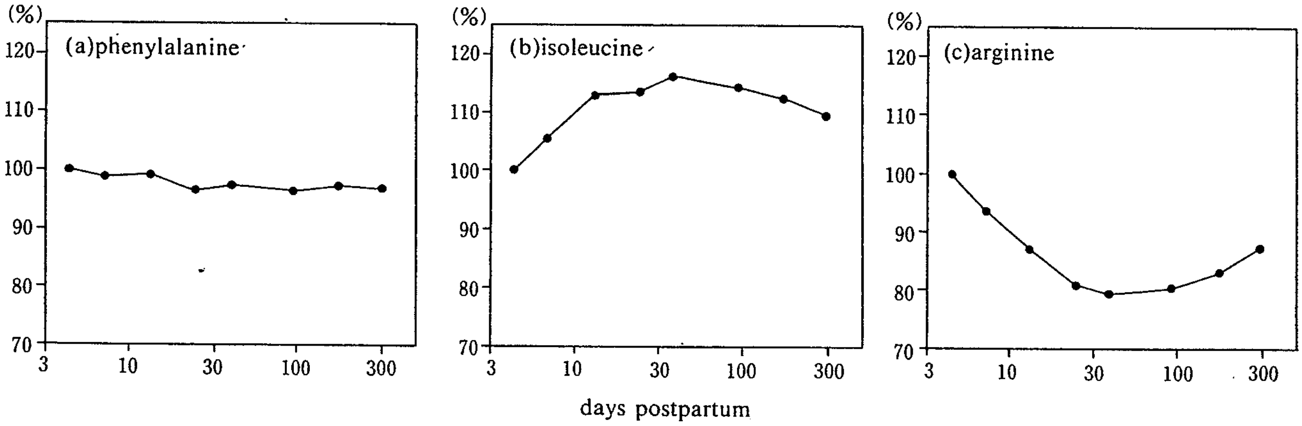

Figure. 2 The time course variation of total amino acid and per 1g nitrogen
Shown in relative values assuming that the contents for 3 to 5 days postpartum are 100%.

tents, as well as the contents of ispleucine and arginine where results differed the most from amino acid and crude protein, are indicated in Figure. 1, The average figures of summer and winter samples, as relative values based on the contents for 3 to 5 days postpartum were used.

Each amino acid as well as total amino acid contents decreased as the lactational stages progressed, and approximated the time course variation of crude protein content.

As for differences among seasons, tyrosine and valine showed tendencies to be high in winter, whereas methionine and aspartic acid were high in summer.

The time course variations of respective amino acids per 1g nitrogen showed completely different results compared to the time course variations per 100ml sample.

The time course variations of phenylalanine, isoleucine and arginine, which were most representative, are indicated in Figure.2, The average figures of summer and winter samples, as relative values based on the contents for 3 to 5 days postpartum were used.

Other than phenylalanine, the amino acids showing only minor time course variations were tyrosine, tryptophan, and aspartic acid. pther than isoleucine, the amino acids which increased as the lactational stages progressed until 31 to 60 days postpartm and then either remained stable or decreased were leucine, lysine, methionine, histidine, glutamic acid and proline. Finally, Other than arginine, the amino acids which decreased as the lactational stages progressed until 31 to 60 days postpartum, then either remained stable or increased were threonine, valine, alanine, glycine and serine.

The time course variations per 1g nitrogen were smaller than those per 100ml of human milk.

Chart. 2 Total amino acid composition for respective districts

(mg/100mℓ)

district	Hokkaido	Tohoku	Kanto/Koshinetsu	Chubu/Tokai	Kinki	Chugoku/shikoku	Kyushu/Okinawa
Isolecuine	63.6	60.4	65.5	62.8	62.7	62.1	65.3
Leucine	121.8	118.3	124.9	118.2	118.6	117.5	124.8
Lysine	83.2	81.9	85.1	80.3	80.6	79.6	85.0
Methionine	17.4	17.1	17.2	15.7	16.4	16.2	17.3
Cystine	26.6	25.4	25.8	24.1	22.3	23.8	26.0
Phenylalanine	47.7	46.6	48.2	45.0	45.2	45.2	48.7
Tyrosine	50.2	49.3	51.1	47.5	47.7	47.9	51.5
Threonine	59.6	58.7	60.5	55.9	57.2	56.3	61.3
Tryptophan	19.5	19.7	20.7	17.5	18.8	18.9	20.8
Valine	60.9	59.0	61.6	57.3	57.2	56.7	61.5
Arginine	45.5	43.1	45.0	41.5	41.4	41.6	46.6
Histidine	31.4	30.7	31.8	30.2	30.3	30.4	31.9
Alanine	48.9	47.6	49.7	45.6	45.4	45.4	49.9
Aspartic acid	116.3	114.7	120.1	107.6	109.3	110.0	120.1
Glutamic acid	209.8	202.7	207.9	200.3	202.7	202.0	211.7
Glycine	30.3	29.6	30.7	28.2	27.8	27.6	31.0
Proline	105.3	103.1	105.2	97.7	104.4	102.4	106.0
Serine	58.0	55.8	58.9	54.3	53.9	54.4	59.3
total amino acid content	1196	1163	1209	1129	1141	1138	1218
crude protein[6] (g/100mℓ)	1.38	1.35	1.39	1.31	1.32	1.32	1.40

(2)Geographical differences

The results of the analyses of summer and winter samples are shown as average figures in chart 2.

Resembling the geographical differences of crude protein contents, the contents of both respective and total amino acids were lower in the chubu/Tokai, Kinki and Chugoku/Shikoku districts.

As for seasonal differences, tyrosine and valine were found in high percentages in winter.

2.Time course variations of free amino acid and urea contents

(1)Time course variations

The results of the analyses of summer and winter samples are shown as average figures in Chart 2.

The time course variations differed among respective elements. Isoleucine, leucine, methionine, phenylalanine, valine and histidine showed only minor or no variations. Glutamine, serine and glycine increased as the lactational stages progressed, while in contrast, phosphoserine, phosphoethanolamine, lysine, arginine and proline decreased as the lactaional stages progressed. Urea, threonine, and aspartic acid decreased until 16 to 30 days postpartum and then increased afterwards. Taurine remained roughly stable, i. e. 5.95mg/100ml for 3 to 5 days postpartum and 5. 97mg/100ml for 6 to 10 days postpartum, before decreasing to 3.63mg/100ml for 241 to 482 days postpartum.

As for seasonal differences, leucine, proline and seline were found to be higher in summer.

The percentages of free amino acid contents among total amino acids were low in all lactational stages, with the exception of glutamic acid and alanine, and were lower than 1% for isoleucine, leucine, phenylalanine, tyrosine and proline.

The figures for glutamic acid and alanine were at a minimum 5.5% and 2.6% respectively for 3 to 5 days postpartum, then increased afterwards, evevntually indicating a maximum 15.3% and 7.8% respectively for 121 to 240 days postpartum.

Also, the percentage of gross free amino acid con-

Chart. 3 Free amino acid composition and urea content for respective lactational stages

(mg/100mℓ)

lactational stage (days postpartum)	3 to 5	6 to 10	11 to 15	16 to 30	31 to 60	61 to 120	121 to 240	241 to 482
Isoleucine	0.38	0.20	0.20	0.19	0.15	0.20	0.13	0.20
Leucine	0.82	0.43	0.43	0.43	0.41	0.46	0.41	0.43
Lysine	1.35	0.69	0.52	0.43	0.41	0.42	0.39	0.30
Methionine	0.28	0.13	0.18	0.12	0.13	0.14	0.17	0.13
Cystine	0.73	0.74	0.59	0.61	0.68	0.82	0.99	0.74
Phenylalanine	0.39	0.30	0.30	0.31	0.31	0.32	0.29	0.25
Tyrosine	0.53	0.31	0.31	0.30	0.29	0.28	0.28	0.24
Threonine	1.26	1.05	0.95	1.03	1.02	1.29	1.46	1.10
Tryptophan	0.50	0.37	0.22	0.21	0.25	0.19	0.25	0.09
Valine	0.89	0.68	0.67	0.66	0.67	0.66	0.64	0.52
Arginine	0.94	0.52	0.37	0.36	0.28	0.27	0.30	0.26
Histidine	0.50	0.51	0.46	0.49	0.42	0.42	0.45	0.33
Alanine	2.06	2.30	2.03	2.13	2.12	2.36	3.07	2.20
	(2.6)	(3.3)	(3.4)	(4.1)	(4.5)	(5.7)	(7.8)	(5.4)[a]
Aspartic acid	1.16	0.86	0.66	0.68	0.78	0.94	1.17	1.12
Glutamic acid	15.92	18.76	17.04	19.68	20.94	24.41	26.79	21.41
	(5.5)	(6.9)	(7.1)	(9.1)	(10.2)	(13.1)	(15.3)	(12.4)[a]
Glycine	0.44	0.52	0.62	0.78	0.81	0.99	1.23	1.03
Proline	1.08	0.41	0.26	0.18	0.26	0.27	0.16	0.27
Serine	0.97	1.02	1.02	1.15	1.27	1.46	1.81	1.31
Asparagine	0.43	0.34	0.14	0.09	0.06	0.14	0.28	0.20
Glutamine	0.31	0.77	1.32	2.46	3.66	6.54	8.27	5.93
Taurine	5.93	5.97	5.20	4.53	4.22	3.97	3.76	3.63
Hydroxyproline	0.12	0.11	0.11	0.16	0.13	0.16	0.09	tr
α-amino-n-butyric acid	0.08	0.11	0.11	0.12	0.11	0.14	0.17	0.12
α-amino adipic acid	0.08	0.04	tr	tr	tr	tr	0.02	tr
Ornithine	0.21	0.12	0.12	0.11	0.11	0.11	0.16	0.11
Citrulline	0.03	0.03	0.08	0.07	0.17	0.31	0.43	0.30
1-methylhistidine	tr	0.03	tr	0.03	0.03	0.04	0.03	0.03
Phosphoserine	1.83	1.45	1.06	0.80	0.77	0.70	0.65	0.46
Phosphoethanolamine	1.93	1.37	1.37	1.58	1.41	1.12	1.26	1.01
Ethanolamine	0.37	0.47	0.56	0.48	0.46	0.40	0.38	0.32
total free amino acid content	41.45	40.53	36.81	40.10	42.21	49.45	55.26	43.95
	(2.2)	(2.4)	(2.5)	(3.1)	(3.6)	(4.7)	(5.6)	(4.5)[a]
Urea	39.01	36.21	34.81	34.23	34.82	36.51	43.82	35.36
	[5.5]	[5.6]	[6.2]	[6.8]	[7.7]	[9.2]	[11.7]	[9.4][b]
Ammonia	0.60	0.57	0.48	0.50	0.49	0.53	0.55	0.33
Total	81.06	77.31	72.10	74.83	77.52	86.49	99.63	79.64

( )[a]:Percentage of free amino acid content among coresponding total amino acids in respective lactational stages

[ ] [b]:Perentrag of urea nitrogen among total nitrogen in respective lactational stages

Chart. 4 Major components in respective lactational stages lactational stage (days postpartum)

order \ 泌乳期(日)	3 to 5	6 to 10	11 to 15	16 to 30	31 to 60	61 to 120	121 to 240	241 to 482	
1	Urea	Urea	Urea	Urea	Urea	Urea	Urea	Urea	a
2	Glu	Glu	Glu	Glu	Glu	Glu	Glu	Glu	a
3	Tau	Tau	Tau	Tau	Tau	Glun	Glun	Glun	a
4	Ala	Ala	Ala	Glun	Glun	Tau	Tau	Tau	b
5	Pea	Pser	Pea	Ala	Ala	Ala	Ala	Ala	b
6	Pser	Pea	Glun	Pea	Pea	Ser	Ser	Ser	c
7	Lys	Thr	Pser	Ser	Ser	Thr	Thr	Asp	c
8	Thr	Ser	Ser	Thr	Thr	Pea	Gly	Thr	c
9	Asp	Asp	Thr	Pser	Gly	Gly	Pea	Gly	c
10	Pro	Cys	Val	Gly	Asp	Asp	Asp	Pea	c

taurine, phosphoethanolamine and phosphoserine are abbreviated as Tau, Pea and Pser respectively.

a:Components with contents of 5mg/dl or more

b:Components with contents of 2mg/dl or more

c:Components with contents of 1mg/dl or more

Chart. 5 Free amino acid composition and urea content for respective districts (mg/100mℓ)

district	Hokkaido	Tohoku	Kanto/ Koshinetsu	Chubu/ Tokai	Kinki	Chugoku/ Shikoku	Kyushu/ Okinawa
Isoleucine	0.20	0.18	0.23	0.19	0.18	0.19	0.21
Leucine	0.44	0.40	0.49	0.42	0.42	0.42	0.46
Lysine	0.42	0.39	0.61	0.43	0.37	0.42	0.54
Methionine	0.13	0.12	0.19	0.13	0.13	0.13	0.14
Cystine	0.71	0.74	0.71	0.77	0.71	0.73	0.72
Phenylalanine	0.25	0.29	0.33	0.31	0.30	0.31	0.31
Tyrosine	0.27	0.29	0.38	0.30	0.24	0.24	0.32
Threonine	1.10	1.01	1.04	1.11	1.06	1.05	1.04
Tryptophan	0.31	0.25	0.33	0.27	0.24	0.24	0.33
Valine	0.69	0.62	0.76	0.68	0.65	0.65	0.69
Arginine	0.33	0.32	0.43	0.28	0.30	0.31	0.37
Histidine	0.46	0.43	0.47	0.52	0.49	0.51	0.45
Alanine	2.31	2.23	2.33	2.37	2.32	2.21	2.29
Aspartic acid	0.76	0.72	0.74	0.79	0.79	0.73	0.78
Glutamic acid	20.76	20.31	21.27	22.77	22.98	22.22	21.17
Glycine	0.82	0.84	0.87	0.84	0.82	0.80	0.83
Proline	0.25	0.13	0.30	0.22	0.23	0.19	0.12
Serine	1.30	1.24	1.11	1.37	1.37	1.29	1.22
Asparagine	0.32	0.31	tr	tr	tr	tr	tr
Glutamine	3.92	4.14	3.36	3.76	4.16	3.46	2.90
Taurine	4.36	3.92	4.10	4.19	4.41	4.06	3.91
Hydroxyproline	tr	tr	0.11	tr	0.11	0.16	0.09
α-amino-n-butyric acid	0.11	0.11	0.11	0.11	0.12	0.12	0.11
α-amino adipic acid	tr	0.02	tr	tr	tr	tr	tr
Ornithine	0.12	0.11	0.11	0.12	0.11	0.11	0.12
Citrulline	0.19	0.14	0.18	0.18	0.19	0.14	0.19
1-methylhistidine	tr	0.03	0.03	0.03	0.03	0.03	0.03
Phosphoserine	0.79	0.76	0.89	0.90	0.75	0.73	0.80
Phosphoethanolamine	1.52	1.60	1.56	1.58	1.54	1.50	1.52
Ethanolamine	0.46	0.45	0.47	0.46	0.46	0.44	0.45
total free amino acid content	43.30	42.10	43.51	45.10	45.48	43.39	42.11
Urea	31.78	35.67	35.20	35.52	36.65	35.27	34.61
Ammonia	0.46	0.46	0.57	0.49	0.48	0.50	0.54
total	75.54	78.23	79.28	81.11	82.61	79.16	77.26

tents among total amino acid contents for respective lactational stages was at a minimum 2.2% for 3 to 5 days postpartum, and increased afterwards to eventually indicating a maximum 5.6% for 121 to 240 days postpartum.

The main elements in respective lactational stages are shown in Chart.4.

Most dominant among all elements in alllactational stages was urea followed by glutamic acid. Glutamic acid was followed by taurine until 31 to 60 days postpartum, then by glutamine after 61 to 120 days postpartum. Among other elements, alanine contents was higher than 2mg/100ml.

The percentage of urea nitrogen content among total nitrogen content in respective lactational stages ranged from a minimum 5.5% for 3 to 5 days postpartum a maximum 11.7% for 121 to 240 days postpartum.

(2)Geographical differences

The results of the analyses of summer and winter samples are shown as average figures in Chart 5. No significant relations were found to exist regarding geographical and seasonal differences.

Examination

For any mammal in the newborn stage, the milk secreted from their mother is the sole nutritional source for infants, and it has been indicated that there is a close relationship between the growth rate of mammals and the protein content of milk. It is known that mammals with fast growth rates have high protein contents in their milk, while humans and other mammals with slow growth rates have low protein contents[14]. This also suggests that breast milk is ideal as the nutritiona source of infants.

Protein is an intrumental component of muscles, hones, skin, and hair which form the body of an infant, as well as enzymes, antibodies and hormones which are responsible for various life phenomena. Therefore, a protein intake both qualitatively and quantitatively appropriate is necessary for the infant stage where growth takes place at a remarkably high rate.

while the nutritional value of protein is determined by the types and quantity of its component amino acids, breast milk fulfills all essential amino acids of infants[18].

On the other hand, the dietary patterns of Japanese in recent years have changed drastically, for example increases in dining-out opportunities and the use of prepared foods. This is considered to have an effect on the composition of human milk. The authors have previously reported that the crude protein content in Japanese human milk has increased by approximately 14% in the last 30 years[6].

While this change in the crude protein content of human milk is considered to influence the amino acid composition of human milk, there have been no recent surveys on the amino acid composition of Japanese human milk[8][9]. Also, other than those by Saito et al.[10] and Yonekubo et al.[11], there have been no nationwide reports on the amino acid composition of human milk.

Respective amino acid contents per 100ml human milk are known to decrease, in the same manner as crude protein contents, as lactational stages progress[10][11][19]. The same tendencies were found in the results obtained by the authors.

However, the authors' results regarding isoleucine, leucine, tryptophan, valine and proline contents were higher than the human milk figures in the Revised Food Amino Acid Composition in Japan[9], and lower in all lactational stages than the results of Yonekubo et al[11].

Also, regarding primary milk for the 3 to 5 days postpartum period and matured milk from 241 to 482 days postpartum, the results of the authors for all lactational stages were lower than those by Yonekubo et al. Although this difference is assumed to be influenced by the dietary pattern and age of the mother, days postpartum and the volume of sample collection[10], its source still remains uncertain.

The time course variations of amino acid contents per 1g nitrogen in human milk is significantly smaller than those per 100ml of human milk, and it is known that the patterns of variations differ among respective types of amino acid.

Harzer et al.[19] have reported after analyses of human milk amino acid compositions from 2 to 36 days postpartum that glutamic acid, proline, isoleucine, leucine, histidine and lysine increased while threonine, serine, glycine, alanine, cystine, valine and arginine decreased as the lactational stages progressed. and that asparagine, methionine, tyrosine, phenylalanine and tryptophan remained stable at certain levels.

The results of the authors resembled those of Harzer et at., with the exception of methionine and cystine.

However, Harzer et al. have not reported the time course variations after 36 days postpartum. From the results of the authors, the amino acids which increase as lactation progresses until 31 to 60 days postpartum remain stable or slightly decrease afterwards, while those which decrease as lactation progresses until 31 to 60 days postpartum either remain stable or slightly increase afterwards. However, the variations after 31 to 60 days postpartum seemed minor compared to those prior to this lactational stage.

Regarding such differences of the time course variations of amino acid contents per 1g nitrogen among various types of amino acid, Harzer et al.[19] have reported that these differences are caused by the variations of respective proteinic elements composing protein in human milk according to changes in lactational stages. In other words, the contents of casein, a component of human milk protein, as well as secreted IgA') (immunoglobulin A), a serum protein, and α-lactoalbumin and lactoferrin vary among respective lactational stages, which means that the various amino acid compositions of these proteins very as well.

The FAO／WHO／UNU Joint Special Commission and the Commission of the European Communities both incorporate the essential amino acid composition of human matured milk as standards for the essential amino acid requirements for infants as well as evaluations of protein in infant foods[18)20)]. The results of the authors approximated these figures, as well as the human milk figures of The Revised Food Amino Acid Composition in Japan[9)] and those of Saito et al.[10)].

On the other hand, while the essential amino acid contents per 1g nitrogen in human milk vary for matured milk as well, the variations within the matured milk stage are minor. Therefore, it was considered that the FAO／WHO／UNU Joint Special Commission figures can also be applied to the essential amino acid patterns of Japanese infants.

Other than protein, nitrogenous contens of human milk contains 20 to 30% of. non-protein nitrogenous elememts[13)].

Regarding the significance of free amino acids in human milk, Nishikawa et al.[16)] have reported that since they exist in far smaller volumes than total amino acids, they are less significant as nutritional sources. On the other hand, Pamblanco et al.[21)] and Yonekubo et al.[11)] have argued that they are valuable, due to the immaturity of the digestive functions of newborns. Harzer et al.[15)] have suggested that taurine, glutamic acid, phosphoethanolamine and urea are significant in their contributions in the containment of bile acid and absorption of zinc, supplying energy and nitrogen to the small intestines, contributing to bifidobacteria flora, and their effects as components of growth adjustment.

It has been reported that the urea content in human milk is approximately 11 to 20mg／100ml as nitrogen[13)22)23)]. The results of the authors were similar, ranging from 34.23 to 43.82mg／100ml(15.96 to 20.43mg／100ml as nitrogen).

Also, while it has been reported that urea nitrogen in human milk consists of 7 to 10% of total nitrogen[24)], Hein et al.[25)] and Donovan et al.[26)] have indicated that the urea contained in human milk is used by infants. The results of the authors showed that the contents were a minimum of 5.5% for 3 to 5 days postpartum and a maximum of 11.7% for 121 to 240 days postpartum, suggesting that the significances as a source of nitrogenare not to be ignored.

As for the variation patterns of free amino acid contents in human milk according to changes in lactational stages, three basic patterns have been reported, i. e. increase, decrease and roughly stable. However, the types of free amino acids which display the respective patterns differ among reports[11)17)].

In the results of the authors, there were substances which showed different patterns from the above, Threonine, aspartic acid and urea decreased until 16 to 30 days postopartum, then increased afterwards.

Also, taurine, known to have an unique time course variation, has been reported to remain stable in one case[15)] while other reports show that it increases until 4 to 5 days postpartum, then decreases[11)17)27)]. The results of the authors indicated that taurine remained stable until 6 to 10 days postpartum, then decreased afterwards. The contents were similar to those of Nishikawa et al.[17] and Zaima et al.[27].

It has been reported that the most dominant free amino acid in human milk is glutamic acid[11)17)28)29]. However, no certain relations have been determined between other major free amino acids and the respective lactational stages[17]. Nishikawa et al.[17] have reported that, other than glutamic acid, the major free amino acids were taurine, proline and alanine primary milk for the period of 2 to 4 days postpartum; taurine, alanine and phosphoethanolamine from transitionary milk from 6 to 10 days postpartum; taurine, glutamine and alanine from matured milk for the period of 24 to 35 days postpartum; and serine, alanine, threonine from milk from 97 to 117 days postpartum[16]. On the other hand, Yonekubo et al.[11] have reported that taurine and alanine were major free amino acids in human milk from 21 days to 3 months postpartum.

From the results of this survey, the authors have recognized a certain relation between major free amino acids and the respective lactational stages, and that the orders of taurine, alanine and phosphoethanolamine dropped as the lactational stages progressed, while glutamine and serine became major components.

It has been reported that free amino acid contained in human milk is only contained by 3 to 6mg/100ml as nitrogen, very insignificant among the total acid content, and has a minor variation according to changes in lactational stages[13)19)29]. Nishikawa et al.[16] have reported that the average content was 30.8mg/100ml from 97 to 117 days postpartum, and composed approximately 3.3% of total amino acids, while Svanberg et al.[30] have reported that the contents in human milk from 2 to 5 months postpartum was approximately 5%. On the other hand, Pamblanco et al.[21] have reported that the free amino acid content in matured milk of 4 weeks postpartum was twice of that in primary milk of 1 week postpartum.

The results of the authors resembled those of Nishikawa et al. and Svanbery et al. Free amino acid contents for all lactational stages ranged from 36.81 to 55.26/100ml(1.28 to 2.49mg/100ml as nitrogen)and roughly remained stable, and its percentage among total amino acids increased by approximately 2.5 times from 2.2% for 3 to 5 days postpartum to 5.6% from 121 to 240 days postpartum.

Clark et al.[23] have assumed that the significandce of the free amino acid contents remaining roughly stable for the entire lactational period is in order to maintain the osmotic pressure of human milk at a certain level. However, there still remains many questions regarding the nutritional and physiological significances of these free amino acids which must be answered in further research.

Conclusion

Breast milk the amino acid composition of breast milk is the ideal nutritional source for infants.

However, while significant improvements based on human milk composition have been made on artificial milk for infants in Japan, there have been only a few reports on the amino acid composition of Japanese milk. Since the recent Japanese human milk contents were comsidered likely to be changed, the above survey was conducted to elucidate the amino acid composition of recent Japanese human milk. The facts that the components of human milk differ greatly according to changes in lactational stages and there are various characteristics of the mother and child were particularly taken into consideration.

Total amino acid composition per 1g nitrogen resembled the figures of the FAO/WHO/UNU Joint Special Commission and The Commission of the European Communities, and it was concluded that these figures can also be adapted as essential amino acid patterns of Japanese infants.

Also,urea nitrogen composed of 5.5% to 11.7% of the total nitrogen, and their significance as a nutritional source is not to be ignored. Further, although it has been assumed that free amino acids have untritional significances on gestationally immatured infants, and also contribute to the maintainance of the osmotic pressure of human milk, they are far from being elucidated, and further research is necessary.

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