Relationship between fatty acid compositions and taurine concentration in breast milk from Chinese rural mothers

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Long chain ω3 and ω6 fatty acids and taurine have been suggested to have structural and/or functional roles in the brain. In this study the levels of fatty acids and taurine in breast milk and their correlations were investigated in 46 lactating women from an area 100 km north-east of Beijing, China. The subjects consisted of two groups: group 1 was 22–47 days postpartum and group 2 was 75–106 days postpartum. Fatty acids and taurine in breast milk were measured by gas-liquid chromatography and high-performance liquid chromatography, respectively. The relative compositions of long chain ω6 and ω3 fatty acids decreased significantly (20:3 ω6, 20:4 ω6, 22:4 ω6, 22:5 ω6 and 22:6 ω3; all $P < 0.01$) in the course of lactation, while long chain saturated (20:0, 22:0) and monounsaturated (20:1, 22:1) fatty acids tended to increase. The ratio of ω6/ω3 fatty acids in breast milk fat appeared to be higher (12.3 ± 4.3 for group 1, and 16.5 ± 7.4 for group 2) than the desired range of 4–10. Breast milk taurine concentrations from Chinese rural mothers were 186 ± 48 nmol/mL and 157 ± 65 nmol/mL for groups 1 and 2, respectively. A significantly inverse relationship was observed between breast milk taurine concentration and the ratio of arachidonic acid ω6 to docosahexaenoic acid ω3 ($r = -0.323$, $P = 0.028$). Further studies on the physiological function(s) of taurine as an antioxidant and as a neurotransmitter are worth pursuing in relation to the balance of long chain ω6 and ω3 fatty acids, especially as regards its role in retina and brain development.

Key words: fatty acid composition, taurine concentration, breast milk, Chinese mothers, rural.

Introduction

Fat constitutes about 50% of the total energy in milk and provides the essential fatty acids, fat soluble vitamins and cholesterol needed for functional and structural development of the brain, retina and many other tissues.[1] Although the amount of fat in human milk is relatively constant, the fatty acid composition can vary considerably depending on many factors including the mother's diet.[2–6] Long chain ω3 fatty acid, docosahexaenoic acid (DHA, 22:6), and ω6 fatty acid, arachidonic acid (AA, 20:4) are known to accumulate rapidly in phospholipid of the human brain cell membrane during the last trimester of gestation and 18 months postnatally.[7–9] The limited accretion of DHA to brain lipids is known to be related to alterations in visual response and learning behaviour in growing rats.[10–12] Also the retina, which is considered an integral part of the central nervous system, contains a fatty acid composition high in DHA and AA.[13] Wiegand *et al.* suggested that retinal degenerative changes induced by oxidants or constant light exposure in rats are accompanied by lipid peroxidation, especially a selective loss of DHA from outer segment membranes.[14] It is speculated that the physiological functions of taurine in the brain and retina may be closely associated with its antioxidant role in a polyunsaturated fatty acid rich environment.

Taurine is the second-most abundant free amino acid in human milk, accounting for 13% of the total free amino acid pool.[15] In contrast, taurine concentration is very low in cow's milk, and is absent from soy-based formulas. The diverse functions of taurine in the brain, retina, heart, reproductive system, and in growth and development have received considerable attention in recent years.[16] The adverse effects of maternal taurine deficiency on reproduction and fetal development involving morphological abnormalities in brain and visual cortex development have been well documented in a series of studies conducted on cats.[17–20] It has been repeatedly proposed that taurine, which has a structure similar to gamma-aminobutyric acid (GABA), a known potent inhibitory neurotransmitter, has a major role in the central nervous system as an inhibitor of nerve impulses.[21,22] More recent findings that taurine protects biomembranes by lipid peroxidation induced by light exposure and oxidants may have physiological implications to many important tissues.[23,24]

In the present study, the fatty acid compositions and the levels of taurine were determined in breast milk samples from Chinese rural mothers, and correlations between them were analysed.

Methods

Subjects and sample collection

Breast milk samples were obtained from 46 healthy lactating

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mothers (26.7 ± 4.5 years old) of full-term infants residing in 23 different villages located 100 km north-east of Beijing, China. The subjects consisted of two groups: group 1 was 22–47 days postpartum and group 2 was 75–106 days postpartum. The average body weight and height of the subjects were 58.2 ± 5.7 kg and 159.5 ± 4.6 cm for group 1, and 59.0 ± 5.9 kg and 159.6 ± 3.7 cm for group 2, respectively, at the time of milk sampling.

Milk samples were collected from each subject. All mothers breast-fed their babies. Five hand-expressed milk samples (5–10 mL) were collected at five time intervals (8:00–10:00, 10:00–12:00, 12:00–14:00, 14:00–16:00, and 16:00–18:00 h) in the middle of breastfeeding. After the five expressions were completed, 2 mL of each sample was mixed to obtain the 24-h period milk pools, and stored at – 20°C.[25] The research project was reviewed for its scientific values and ethics, and approved by the academic committee of Beijing Children's Hospital.

Analysis of fatty acids in human milk

Pooled breast milk samples were directly methylated by the methods of Lepage and Roy.[26–28] A total of 100 µL of breast milk samples were dissolved in 2 mL of methanol–benzene 4:1(v/v) solution. While stirring, 200 µL of acetyl chloride was slowly added over a period of 1 min. Tubes were tightly closed with Teflon-lined caps and subjected to methanolysis at 100°C for 1 h. After the tubes had been cooled in water, 5 mL of 6% K_2CO_3 solution was slowly added to stop the reaction and to neutralize the mixture. The mixtures were then centrifuged, and an aliquot of the upper benzene phase was injected into the gas-liquid chromatography (GLC) system. Each fatty acid composition of the milk was determined by gas-liquid chromatography (Hewlett-Packard 5890A, USA) with a bonded fused-silica capillary column (Omegawax 250, Supelco, USA; 30 m × 0.25 mm inner diameter). Oven temperature was maintained at 210°C, while the temperature of the injection and detection ports remained at 250°C. Helium was used as a carrier gas at a column flow rate of 1 mL/min with a split ratio of 10:1. Each peak of fatty acid methyl ester was identified by comparing it with the peak of a standard fatty acid methyl ester (Nu-chek-Prep. Inc., USA).

Analysis of taurine in human milk

Pooled milk samples were deproteinized by adding acetonitrile and centrifuging at 17400 g for 30 min at 4°C. The deproteinized milk supernatants were then stored at – 80°C until analysis. Aliquots of the supernatant fluids were analysed for taurine by high performance liquid chromatograph (HPLC, Waters Associates, Milford, MA, USA) as described by Chang *et al.* with a linear 206 Photodiode Array Detector (UV-VIS).[29] Samples were precolumn derivatized with dabsyl chloride, and injected into the reversed phase column (Waters symmetry C18 column, 3.9 × 150 nm, with guard). The taurine peak was obtained at around 8.3 min with acetonitrile:20 mmol/L sodium acetate buffer, pH 6.5 (0.05% triethylamine plus 4% dimethylformamide added; 27:73) as an eluent running at a flow rate of 1.0 mL/min. Taurine recoveries from standard solutions (8–180 nmol/mL taurine concentration) and the milk sample with a known amount of taurine added were found to be from 94.5 to 99.5%.

Statistical analysis

All values appearing in the tables and figures are expressed as a mean ± SEM of 22 (group 1), or of 24 (group 2) samples. The significance of the differences in the values obtained during the two lactating periods was tested using Student's *t*-test at $P < 0.05$. The correlations between the fatty acid series and taurine concentrations in the breast milk were tested using Pearson correlation test at $P < 0.05$.

Results and discussion

Fatty acid compositions in human milk

The composition of fatty acids expressed as a percentage of total fatty acid in the breast milk is shown in Table 1. Medium chain fatty acids (12:0, 14:0, 16:0) constituted a large portion of the total fatty acid of the breast milk, and compositions of all medium chain fatty acids tended to decrease from group 1 to group 2. On the other hand, compositions of long chain saturated fatty acids (20:0 and 22:0) significantly increased and the long chain monounsaturated fatty acids (20:1 and 22:1) tended to increase. The peak of

Table 1. Composition of fatty acids in breast milk from Chinese rural mothers

Fatty Acids	Group 1 [a]	Group 2 [b]	*t*-value
	g/100 g of total fatty acids		
Saturates			
10:0	1.83 ± 0.08	1.59 ± 0.05	–1.89
12:0	6.44 ± 0.40	5.48 ± 0.21	–1.51
14:0	5.36 ± 0.36	4.81 ± 0.21	–0.96
16:0	19.6 ± 0.43	18.8 ± 0.43	–0.94
18:0	5.55 ± 0.14	5.50 ± 0.23	–0.13
20:0	0.22 ± 0.01	0.29 ± 0.02	2.35*
22:0	0.13 ± 0.01	0.21 ± 0.02	2.72**
24:0	0.12 ± 0.01	0.13 ± 0.01	0.94
Total (SFA)	39.2 ± 0.88	36.7 ± 0.55	–1.79
Monounsaturates			
16:1	2.34 ± 0.13	2.20 ± 0.14	–0.57
18:1 ω9 (*cis* & *trans*)	31.7 ± 0.53	34.1 ± 0.52	2.39*
18:1 ω7	0.06 ± 0.00	0.05 ± 0.00	–2.47*
20:1	0.55 ± 0.02	0.65 ± 0.05	1.49
22:1	0.16 ± 0.03	0.28 ± 0.07	1.16
24:1	0.11 ± 0.01	0.10 ± 0.01	–0.24
Total (MUFA)	34.8 ± 0.59	37.2 ± 0.58	2.26*
Polyunsaturates			
18:3 ω3	1.28 ± 0.10	1.18 ± 0.09	–0.56
20:3 ω3	0.08 ± 0.00	0.07 ± 0.00	–0.54
20:5 ω3	0.13 ± 0.00	0.13 ± 0.01	0.29
22:5 ω3	0.17 ± 0.01	0.14 ± 0.01	–1.64
22:6 ω3	0.41 ± 0.03	0.22 ± 0.02	–3.82**
Σ ω3	1.99 ± 0.11	1.65 ± 0.10	–1.61
18:2 ω6	20.4 ± 0.68	22.2 ± 0.69	1.35
18:3 ω6	0.17 ± 0.00	0.14 ± 0.01	–1.82
20:3 ω6	0.59 ± 0.02	0.46 ± 0.02	–3.39**
20:4 ω6	0.67 ± 0.02	0.54 ± 0.02	–3.88**
22:4 ω6	0.15 ± 0.00	0.12 ± 0.00	–3.96**
22:5 ω6	0.09 ± 0.00	0.05 ± 0.00	–6.98**
Σ ω6	22.1 ± 0.68	23.5 ± 0.69	1.07
Total (PUFA)	24.2 ± 0.16	25.6 ± 0.74	0.72
Others	2.18 ± 0.20	2.05 ± 0.24	–0.42
P/M/S	0.64/0.91/1.00	0.71/1.02/1.00	

Values are mean ± SEM. * $P < 0.05$, ** $P < 0.01$.
[a] 22–47 days postpartum ($n = 22$), [b] 75–106 days postpartum ($n = 24$).

18:1 ω9 was *cis* and *trans* form, the majority portion of which was oleic acid, and was the predominant monounsaturated fatty acid (91–97% of total monounsaturates).

α-Linolenic acid (18:3 ω3) was the major ω3 polyunsaturated fatty acid (4.6–5.3% of total polyunsaturates), while linoleic acid (18:2 ω6) was the major ω6 polyunsaturated fatty acid (84–87% of total polyunsaturates). Most of the long chain ω3 and ω6 polyunsaturated fatty acids (20:3 ω6, 20:4 ω6, 22:4 ω6, 22:5 ω6 and 22:6 ω3) decreased significantly ($P < 0.01$) in the course of lactation. According to Makrides *et al*., ω6 series fatty acids, 18:3, 20:3, 20:4 and 22:5 were reduced with time; from the 6th week to the 30th week of lactation.[30] The percentage of linoleic acid (18:2 ω6), the precursor of long chain ω6 fatty acids, was very much higher in the milk from Chinese rural mothers than in the milk from those of other countries.[30–33] This phenomenon appears to reflect a high intake of vegetable foods by Chinese rural mothers. Of the 48.8 g of total fats and oils consumed by the subjects, 72.9% came from vegetable sources (data not shown). The major cooking oil used most frequently was found to be soybean oil which provided ω3 series fatty acid (18:3) in the study area. Rapeseed oil, which used to be the major oil used in the study area, has been changed to soybean and peanut oil during last 6–7 years. The percentage of breast milk DHA of Chinese rural mothers was not lower than that of other countries. A relatively high level of 18:3 ω3 provided by soybean oil must have contributed to the level of DHA in breast milk. Chulei *et al.* studied milk composition in women from five different regions of China and observed that the level of α-linolenic acid in the milk from mothers in rural and pastoral regions, who consume a high amount of soybean oil and negligible amounts of fish, was much higher than the values from mothers in other regions of China.[34] It was known that the intake of fats and oils varied according to the economic status of the subjects. It has also been noted that the fatty acid patterns of human milk are known to be affected by the length of lactation, the nutritional status of the lactating mothers, the number of deliveries, and by geography.[34–36]

The ratios between different species of ω6 and ω3 polyunsaturated fatty acids in the breast milk are shown in Table 2. The ratio of total ω6 to total ω3 fatty acids in human milk has been reported to range from 4 to 10,[37] but the results of this study on Chinese rural mothers were higher (12.3 in group 1 and 16.5 in group 2) than these values. Rapeseed containing a considerable amount of ω3 fatty acid (18:3) with a lower linoleic acid (18:2 ω6) content is a good choice for Chinese people because rapeseed oil is one of the major traditional oils used for everyday cooking, and in some parts of China soil and climate conditions are well suited for rapeseed cultivation. Because dietary fatty acids can influence the fatty acid patterns of breast milk, Chinese women involved in the present study are recommended to consume more rapeseed oil, legumes and fish in their diet.

Table 2. Ratios among ω6 and ω3 polyunsaturated fatty acids in breast milk from Chinese rural mothers

	Group 1[a]	Group 2[b]	*t* -value
22:5 ω6/22:4 ω6	0.58 ± 0.02	0.39 ± 0.01	–5.59**
22:4 ω6/20:4 ω6	0.22 ± 0.00	0.23 ± 0.06	1.03
22:6 ω3/22:5 ω3	2.54 ± 0.12	1.66 ± 0.10	–4.15**
22:5 ω3/20:5 ω3	1.38 ± 0.12	1.18 ± 0.12	–0.86
DHA/AA	0.62 ± 0.05	0.40 ± 0.02	–2.66*
Σ ω6/Σ ω3	12.3 ± 0.63	16.5 ± 1.09	2.42*

Values are mean ± SEM. *$P < 0.05$, **$P < 0.01$.
[a]22–47 days postpartum ($n = 22$), [b]75–106 days postpartum ($n = 24$).

The ratio of 22:5 ω6 fatty acid to 22:4 ω6 fatty acid denoting desaturation of long chain ω6 fatty acid, and the ratio of 22:6 ω3 fatty acid to 22:5 ω3 fatty acid representing desaturation of long chain ω3 fatty acid in the milk decreased significantly ($P < 0.01$) as the length of the lactating period increased (Table 2). This may be due to the decreased activities of Δ4-desaturase.

Taurine concentrations in human milk

Taurine concentrations in the breast milk from Chinese rural mothers were 186 ± 48 nmol/mL (23.3 ± 6.0 μg/mL) during period 1 (22–47 days postpartum), and 158 ± 65 nmol/mL (19.7 ± 8.1 μg/mL) during period 2 (75–106 days postpartum) (Table 3). In the present study, milk taurine concentrations had a tendency to decrease as lactation progressed from period 1 to 2, but the difference was not statistically significant. Breast milk taurine concentrations in Chinese rural mothers have not been reported elsewhere, and the direct comparison of our data with other data from China is impossible. However, reports from other countries suggest that human milk taurine concentrations decrease gradually in the course of lactation,[37–39] and the values obtained from the present study on Chinese rural mothers are much lower than the previous reports from other countries during the equivalent lactation periods.

Longitudinal studies on Korean mothers reported that taurine concentrations in breast milk decreased from 420–430 nmol/mL (3–5 days postpartum) to 300 nmol/mL (30 days postpartum), and finally to 250 nmol/mL (150 days postpartum) in the course of lactation.[25,40] Human milk samples from European countries also had much higher taurine levels than the values obtained from Chinese rural mothers in the present study. Taurine concentrations in milk samples from Ethiopian and Swedish mothers with infants in the age range of 2–5 months were 761 ± 143 and 667 ± 70 nmol/mL, respectively.[41] Spanish mothers had much lower milk taurine concentrations (228 ± 59 nmol/mL) compared with mothers from other European countries at a similar length of lactation period.[41–43]

Taurine biosynthesis from methionine and cysteine is known to be extremely limited in humans due to the absence of enzyme activities involved in the biosynthetic pathways.[15,16] Therefore, the taurine concentration in human milk appears to reflect the amount of taurine consumed by the subject. This has been partially proven by studies conducted on

Table 3. Concentrations of taurine in breast milk from Chinese rural mothers

	Group 1[a]	Group 2[b]	*t* -value
Taurine (nmol/mL)	186 ± 7.1	158 ± 9.6	–1.57

Values are Mean ± SEM. [a]22–47 days postpartum ($n = 22$), [b]75–106 days postpartum ($n = 24$).

Table 4. Correlations between fatty acids and taurine concentrations in breast milk from Chinese rural mothers

	Fatty acid (%) or ratios of fatty acids	Correlation coefficients (r) with taurine concentrations
Group 1[a]	18:0	0.46*
Group 1[a]	18:3 ω3	–0.48*
Group 1[a]	Σ ω3	–0.44*
Group 2[b]	18:3 ω3	0.53**
Group 2[b]	Σ ω3	0.56***
Group 2[b]	Σ ω3/Σ ω6	0.49*
Total (pooled)	AA	0.29*
Total (pooled)	AA/DHA	–0.32*

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.
[a]22–47 days postpartum ($n = 22$), [b] 75–106 days postpartum ($n = 24$).

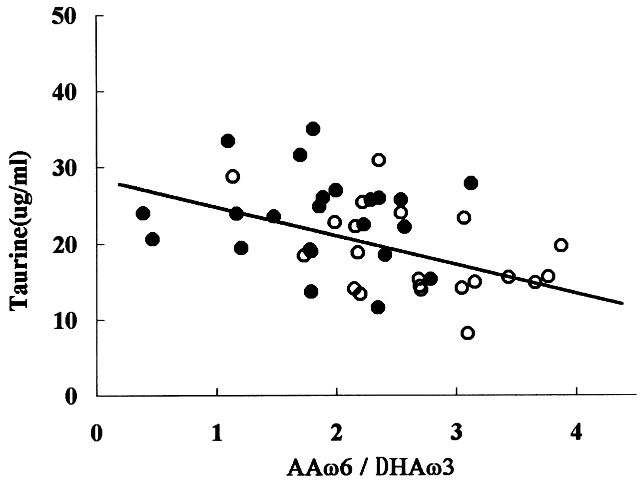

Figure 1. Correlations between taurine concentration and arachidonic acid (ω6)/docosahexaenoic acid (ω3) ratio in the breast milk from Chinese rural mothers. Correlation coefficient (r) = –0.32 at $P < 0.05$.

Korean mothers at days 60–150 of lactation,[25,39] which showed that milk taurine levels are much lower in lacto-ovo vegetarians (262–153 nmol/mL) than their non-vegetarian counterparts (306–248 nmol/mL). Taurine is present in meat and fish in high quantities, while it is virtually absent from the diet of strict vegetarians.[44] Low taurine concentrations in the breast milk of Chinese rural mothers in our study presumably reflect the low dietary intake of animal-derived foods by the subjects. The large individual variations in milk taurine concentrations found in the present study also appear to be due to large variations in the taurine consumption level, which was likely influenced by the diverse economic status of the subjects.

A conditional essentiality of taurine has been proposed for human infants who have a limited capacity for endogenous taurine synthesis as well as a high demand of taurine for rapid growth.[15] Concern has been focused on infants fed commercial formulas that contain little or no taurine, and substantial evidence has suggested that dietary provision of taurine in infancy is important for optimal nutriture. Therefore, since 1984, most cow milk and soy protein-based formulas have been supplemented with taurine in order to reach a level similar to that in human milk; that is, 35 µmol/dL.[45] Children nourished totally by parenteral nutrition[46,47] or on strict vegetarian diets[48,49] also have a high chance of taurine depletion.

Our data showing suboptimal levels of taurine in the breast milk from Chinese rural mothers suggests that more attention should be paid to the taurine status of lactating mothers. Taurine consumption levels and plasma taurine concentrations in mothers and infants fed breast milk remain to be evaluated in future studies.

Correlation between levels of fatty acids and taurine in human milk

It is interesting that the concentrations of taurine and composition of ω-linlolenic acid (18:3 ω3) was negatively correlated in group 1, but positively correlated in group 2. The concentration of taurine was positively correlated with ω3 fatty acids ($P < 0.05$), and also with the ratio of total ω3 to total ω6 fatty acids ($P < 0.05$) in the breast milk of 75–106 day postpartum (group 2) (Table 4). When the data were pooled from groups 1 and 2, taurine concentrations were found to be negatively correlated with the ratios of AA ω6 to DHA ω3 (r = –0.32, $P < 0.05$) even though a positive correlation ($P < 0.05$) was found between taurine concentration and the percentage of AA ω6. These correlations may suggest a close interaction among taurine, ω3 and ω6 fatty acids during growth and development.

In conclusion, the finding that taurine, one of the most abundant free amino acids in animal tissues, has a significant correlation with the ratio of AA ω6 and DHA ω3, the two most important fatty acids for the development of brain, will shed light on pursuing interactive roles including antioxidant and neurotransmitter of taurine and long chain ω6 and ω3 fatty acids in tissues, especially in the retina and brain, during the period of rapid growth and development.

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요 약 문

ω3와 ω6계의 장사슬 지방산과 타우린은 뇌조직에서 구조적 또는 기능적 역할을 담당하고 있음이 제안된 바 있다. 본 연구에서는 중국 북경에서 동북쪽으로 100 km 밖에 위치한 지역에 거주하는 수유부 46명을 대상으로 모유내 지방산과 타우린 농도를 측정하고 이들간의 상관관계를 연구하였다. 연구대상자는 분만후 22-47일이 경과한 수유부 (I군)과 75-106일이 경과한 수유부 (II군)로 구성되었으며, 모유내 지방산과 타우린의 농도는 각각 GLC와 HPLC로 분석하였다. ω6 및 ω3계 장사슬 다불포화지방산 (20:3ω6, 20:4ω6, 22:4ω6, 22:5ω6와 22:6ω3)의 조성은 수유기간이 경과함에 따라 유의하게 ($p<0.01$) 감소한 반면, 장사슬 포화지방산(20:0, 22:0)과 장사슬 단일불포화지방산(20:1, 22:1)의 조성은 수유기간에 의해 증가하는 경향을 보였다. 중국 수유부의 모유내 ω6/ω3 지방산의 비율은 이상적인 비율인 4~10 보다 높게 나타났다 (I군, 12.3±4.3; II군, 16.5±7.4). 모유내 타우린의 농도는 I군의 경우 186±48 nmole/ml, 그리고 II군의 경우 157±65 nmole/ml 로 나타나 수유기간에 따라 감소하는 경향을 보였다. 중국인 수유부의 모유내 타우린 농도는 아라키도닉산 (ω6)/ 도코사헥사에노익산 (DHA, ω3)의 비율과 유의적인 음의 상관관계 ($r=-0.323$, $p=0.028$)를 보인 점이 특이하다. 망막과 두뇌발달 측면에서, 항산화제 및 신경 전달물질로서의 타우린의 생리적인 기능은 ω3 및 ω6계 장사슬 지방산의 균형과 밀접한 연관성이 있을 것으로 생각되며, 앞으로 이에 관한 연구가 계속되어져야 하겠다.

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摘要

中国农村母乳脂肪酸组成和牛磺酸浓度及其相互关系

有人提出长链ω3与ω6脂肪酸和牛磺酸在大脑起着结构和/或功能的作用。本研究，在中国北京东北100公里的农村选择 46 名乳母作为实验对象，研究了她们乳汁中脂肪酸和牛磺酸水平及其相互关系。 实验对象分成两组；Ⅰ组为产后22-47天；Ⅱ组为产后75-106天。母乳中的脂肪酸和牛磺酸分别用气相色谱和高效液相色谱测定。

在哺乳过程中，长链多不饱和 ω6 和ω3 脂肪酸的相对组成明显下降 (20:3ω6, 20:4ω6, 22:4ω6, 22:5ω6和 22:6ω3, p<0.001)，长链饱和(20:0, 22:0)及单不饱和(20:1, 22:1)脂肪酸则有增加趋势。母乳中ω6/ω3脂肪酸比值(Ⅰ组为12.3±4.3, Ⅱ组为16.5±7.4)高于预期的(4-10)范围，母乳牛磺酸浓度Ⅰ组为186±48nmol/ml, Ⅱ组为157±65nmol/ml，母乳牛磺酸浓度和花生四烯酸与DHA比值(AAω6/DHAω3)呈明显负相关(r=-0.323, p=0.028)。

做为抗氧化剂及神经传导剂的牛磺酸， 它的生理功能与长链ω6和ω3脂肪酸间平衡的关系，特别是对视网膜及脑发育的作用， 值得进一步研究。

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