

Short Communication

Amino acid composition of lactating mothers' milk and confinement diet in rural North China

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This study was designed to investigate the amino acids composition of lactating mothers' milk and their confinement diet in a town in Northern China, as well as to assess the relation of amino acids content in human milk and diet. Forty lactating mothers age 19 to 35 years participated in the study. They were 4 to 180 days postpartum. A 24-hour dietary recall was done and amino acids content of maternal milk was analyzed. The main findings are as follows: (1) The protein content of human milk is 1.58 g/dL and the ratio of EAA to NEAA is about 1:2. The most abundant amino acids in human milk are GLU (16.0%), PRO (10.2%), LEU (8.67%) and the lowest two are MET (1.76%) and TRP (0.91%). (2) The diet contains enough energy and protein, but lacks vitamins A, B and C, indicating that it is a characteristic confinement diet. Grain and eggs are the main source of protein, and soy and fish were consumed less frequently. (3) Amino acids composition in diet and milk are similar; and the correlation of the amino acids patterns between diet and milk is 0.989, demonstrating that the amino acid composition of diet is the foundation of that in human milk. However, almost no relation is found between the amino acids concentration in maternal diet and milk, suggesting that the amino acids content of the diet does not have a direct relation with that of human milk.

Key Words: amino acids, human milk, confinement diet, Chinese mothers, rural north China

INTRODUCTION

Amino acids in breast milk are important for infants, mainly in the form of proteins. Proteins are the crucial nutrient in infant growth and development, and have other functions, such as facilitating digestion by increasing uptake of other nutrients and enhancing the infant's immune function against pathogenic bacteria, virus and yeasts.¹ The amino acids composition of human milk has been studied in other countries, such as Thailand, Mexico and America,²⁻⁴ however, not in China so far, although there are some investigations on the nutrient content of breast milk.⁵⁻⁷ Since amino acids composition of human milk is important for infants and may differ between countries owing to different race and eating habits, it makes sense to investigate it in China.

In China, especially rural northern areas, there is a special 'confinement diet' for lactating mothers. In the first several months postpartum, lactating mothers will receive special treatment from her family, namely: no heavy work and given the confinement diets. Besides noodles and steamed bread, which are the ordinary diet of the family, confinement diet also consists of food the family considers is best for the body, which is usually pork, chicken soup, eggs and milk, and with little fresh vegetables and fruits. Because this widespread eating pattern is seldom

studied in China, in this study we will investigate the amino acids composition of human milk in rural northern China, in order to evaluate this special diet and analyze the relation of amino acid in human milk and the diet.

MATERIALS AND METHODS

Subjects

Forty healthy Chinese lactating mothers within 7-180 days postpartum from Laishui, a town in Hebei Province, participated in our study. Total 13, 13 and 14 mothers were within 7-30, 31-90 and 90-180 days postpartum, respectively. These women aged 19 to 35 years old and gave birth to healthy, term infants who were breast-fed for the whole 6 months postpartum. Their mean level of education attained was junior middle school, and most of them were farmers. Mothers who had diabetes mellitus

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and used tobacco or immunosuppressive drugs were excluded from the study.

Milk collection

Breast milk (5 ml) was collected by manual expression in plastic tubes in the morning (8 AM to 12 AM) from May to July, 2007, adhering to Neville & Picciano.⁸ The samples were stored frozen in liquid nitrogen, and then in a -20°C refrigerator until analyzed. Analysis was performed within 3 months of collection.

Diet questionnaire

Before milk collection, a structured questionnaire was implemented by trained investigators. It includes a 24-hour dietary recall and frequency of food consumption after parturition. Nutrient intakes from the 24-hour dietary recall and amino acids composition were derived based on the *Chinese Food Composition 2004*.⁹

Amino acids in milk analysis

Amino acids were analyzed by an amino acids analyzer according to Chinese Standard Guobiao/Tui (GB/T) 5009.124-2003. Skimmed milk samples were hydrolyzed in 4*N*-methanesulfonic acid containing 0.2% 3-(2-aminoethyl)-indole at 110 °C for 22 h, after reducing tryptophan with pyridine borane. After cooling at 4 ° C, the pH was adjusted to between 2.0 and 2.5 with NaOH. Norleucine was added as an internal standard. The amino acid composition of the hydrolysate was determined using a 12-cm high-performance sodium column with sodium buffers by an amino acid analyzer. (Beckman 6300 amino acid analyzer, Beckman Instruments, Palo Alto, California). Amino acids include free amino acids and amino acids in proteins. Proteins were analyzed by Chinese Standard GB/T 5009.5-2003.

The amino acid pattern means the proportion of essential amino acids in proteins. The amino acid score of the

diet was also calculated.

Amino acid score = mg of amino acid in 1 g of the test protein (confinement diet)/mg of amino acid in 1 g of the reference protein (human body) × 100.¹⁰

Statistics

Data were analyzed using SPSS 15.0 (SPSS Inc., Chicago, IL, USA). Values are expressed as mean ± SD. Pearson correlation was performed to determine the correlation of the amino acids composition in diet and milk. We define *p* value less than 0.05 as significant.

Ethics

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving mothers were approved by the Medical Ethics Research Board of Peking University. Written informed consent was obtained from all mothers.

RESULTS

Amino acids composition of maternal milk

The average protein content was 1.58 g/dL and the average amino acids content was 1.54 g/dL. The average essential amino acids (EAA) content was 0.535 g/dL and the average nonessential amino acids (NEAA) content was 1.01 g/dL. The ratio of EAA to NEAA was about 1:2. The amino acids composition of human milk samples are shown in Table 1. The top five amino acids were GLU (16.0%), PRO (10.2%), LEU (8.67%), ASP (8.39%) and ARG (7.15%) and the lowest two were MET (1.76%) and TRP (0.910%).

24-hour diet analysis for the lactating mothers

Comparing the dietary intakes of lactating mothers with Chinese Dietary Reference Intakes 2000 and defining 80% DRIs as sufficient (Table 2),¹¹ we found that the energy, protein, fat, glucose, Fe, Cu intake were sufficient,

Table 1. Amino acids composition of maternal milk and confinement diet (mean±SD)

	Human milk (n=40)		Confinement diet (n=40)	
	Content (mg/dL)	Proportion %	Content (g)	Proportion %
EAA				
THR	64.6±21.4	4.16±0.26	2.63±1.58	3.56±0.68
TRP	14.3±6.91	0.91±0.29	1.12±0.63	1.55±0.17
VAL	73.8±23.6	4.78±0.49	3.39±2.14	4.55±0.79
MET	27.3±8.76	1.76±0.22	1.48±0.97	1.98±0.41
PHE	66.1±19.7	4.29±0.38	3.39±1.81	4.73±0.34
ILE	72.8±18.0	4.77±0.37	3.21±1.82	4.43±0.35
LEU	133±40.6	8.67±1.09	5.64±3.12	7.77±0.66
LYS	83.5±27.7	5.37±0.45	3.82±2.59	5.01±1.24
NEAA				
ASP	130±42.1	8.39±0.80	5.63±3.32	7.67±1.08
GLU	244±62.2	16.0±1.65	16.8±7.64	24.7±4.93
SER	65.7±25.6	4.19±0.57	3.83±2.22	5.28±0.64
HIS	36.3±16.0	2.32±0.46	1.78±0.98	2.46±0.28
GLY	31.8±12.6	2.03±0.25	2.97±1.51	4.22±0.82
ALA	113±47.5	7.11±1.63	3.47±1.97	4.78±0.55
ARG	112±47.0	7.15±0.22	3.99±2.29	5.47±0.65
TYR	73.9±24.4	4.77±0.81	2.63±1.45	3.64±0.30
CYS	48.5±23.2	3.10±1.14	1.56±0.76	2.24±0.30
PRO	153±33.0	10.2±1.50	4.28±2.63	5.97±1.84

Table 2. Maternal daily nutrient intakes (mean±SD)

Food source	Total (n=40)	0-30 days (n=13)	31-180 days (n=27)	Chinese DRI for lactating mothers
Energy (kcal)	2270±826	2364±971	2219±753	2600
Protein (g)	78.7±39.7	102±47.5	66.3±28.8	85
Fat (g)	87.9±35.2	96.2±42.4	83.4±30.7	25% of total energy
Glucose (g)	299±126	279±152	310±112	65% of total energy
Vitamin A (ug)	667±690	933±635	524±688	1200
Vitamin B1 (mg)	0.99±0.61	1.29±0.73	0.84±0.47	1.8
Vitamin B2 (mg)	1.31±0.86	1.75±1.01	1.07±0.67	1.7
Vitamin C (mg)	60.6±44.4	35.8±23.8	74.0±47.4	130
Ca (mg)	493±405	620±579	424±260	1200
Fe (mg)	22.2±9.55	24.4±11.9	21.0±8.05	25
Zn (mg)	10.9±5.33	13.1±6.79	9.76±4.06	21.5
Cu (mg)	1.86±0.82	1.88±0.76	1.85±0.87	2.0

Table 3. Food source of daily protein intake

	Total (n=40)
Meat (g)	92.1
Fish (g)	6.80
Eggs (g)	150
Milk (ml)	133
Soy (g)	33.3
Grains (g)	436

while the vitamins A, B₁, B₂ and C, Zn, Ca intakes were deficient. The average energy intake was 2270 kcal, while protein provides 13.9% of total energy, fat provides 34.9% and carbohydrate provides 52.8%. Then we compared the nutrient intake between the first month postpartum group and the other group, and found that the protein ($p=0.026$), vitamin B₁ ($p=0.029$), vitamin B₂ ($p=0.042$) intakes were significantly higher in the first month postpartum group. Vitamin A intake was also higher in the first month postpartum group ($p=0.085$). Vitamin C ($p=0.01$) intake was significantly higher in the other group. There was no significant difference of energy, fat, glucose, Ca, Zn, Fe, Mg intake between the two groups. With regard to food source of protein, generally mothers consumed more grains, milk, eggs and meat, and less fish and soy (Table 3). The amino acids composition of the mothers' protein intake was also calculated (Table 1). The highest five proportion of amino acids were GLU (24.7%), LEU (7.77%), ASP (7.67%), PRO (5.97%), ARG (5.47%) and the lowest two were MET (1.98%) and TRP (1.55%). The ratio of EAA to NEAA was about 1:2.

Relationship of amino acids composition and content between maternal milk and diet

According to table 1, the amino acid pattern of human milk is ILE 5.2, LEU 9.5, LYS 5.9, MET 1.9, PHE 4.7, THR 4.6, VAL 5.3, TRP 1.0; the amino acid pattern of confinement diet is ILE 2.9, LEU 5.0, LYS 3.2, MET 1.3, PHE 3.1, THR 2.3, VAL 2.9, TRP 1.0. The amino acids pattern of human body is ILE 4.0, LEU 7.0, LYS 5.5, MET 3.5, PHE 6.0, THR 4.0, VAL 5.0, TRP 1.0.¹⁰ The correlation of the amino acid pattern of human milk and

confinement diet was as high as 0.989 ($p<0.05$). The correlation of the amino acids pattern of human body and the confinement diet is 0.902 ($p<0.05$). The first limiting amino acid of the confinement diet is MET with an amino acid score of 37 and the second is PHE with a score of 50.

The correlation analysis about the content of each amino acids in diet and human milk shows that there was no significant correlation between human milk and the previous day's diet except for MET ($r=0.340$), THR ($r=0.352$), HIS ($r=0.465$), ALA ($r=0.332$).

DISCUSSION

The protein content of human milk is 1.58 g/dL, which is higher than reports from other countries, such as Sweden, Pakistani and ivory coast.¹²⁻¹⁴ The proportions of amino acids in human milk is similar to the studies of the Food and Agriculture Organization (FAO), on Thai mothers, and Swedish mothers.^{2,4,15} Compared with the FAO report, LYS, LEU, ILE, THR proportions of the mothers' milk were lower and ALA, ARG, CYS were higher.

The energy, protein, fat, carbohydrate, Fe and Cu intakes of the mothers were sufficient while vitamins A, B₁, B₂ and C, Zn, Ca intake were deficient. This is in agreement with the 'confinement diet', considering the fact that the confinement diet contains an abundance of chicken soup, pork, and eggs high in protein but little fruit and vegetables high in vitamins. To further illustrate the character of the confinement diet, we compared the nutrition intake between the first month postpartum group and the other group, because the confinement diet is gradually changed to the normal diet as time extends (although this usually happens after six months postpartum, when solid foods can be introduced to the baby's diet). It can be seen that the first month postpartum group consumed more protein, vitamins A, B₁, and B₂, while the other group consumed more vitamin C, demonstrating that the confinement diet contains more protein and less vitamin C. The food source of proteins for the confinement diet is mainly grains, eggs, milk, meat, with little soy and fish. The mothers eat little fish because Laishui is in the middle of China and far from the sea, and the inhabitants prefer meat and eggs to fish. Surprisingly Laishui lactating mothers ate little soy, for it seems that soy is a daily consumed food in China. In fact, a recent study has demonstrated that soy consumption is still low in China, espe-

cially in rural areas,¹⁶ which accords with our study. However, it is not economic of them to eat so little soy, because soy is inexpensive in China and contains adequate quantities of essential amino acids, especially lysine.^{17,18} The amino acid pattern of the confinement diet has a correlation of 0.902 with that of the human body, indicating that the amino acid composition is similar to human body and this diet is of high nutritional value in terms of amino acids. The limiting amino acids are MET and PHE, and maybe it is because the mothers eat too little soy, which is high in PHE. In all, the confinement diet can provide mothers enough energy and protein, which is of high nutritional value. At the same time, mothers should consume more fruit and vegetables to make up for their insufficient vitamins, as well as consume more soy to improve the amino acid pattern of the confinement.

Comparing the amino acids composition of the confinement diet and human milk, it was found that the most abundant are all GLU, LEU, ASP, PRO and ARG, and MET and TRP are less abundant. The ratios of EAA to NEAA are both about 1:2. The correlation of the two amino acids patterns is 0.989. All these demonstrate that the amino acids composition of food is similar to that of diet and amino acids composition of food is the base for that of human milk. Our result agrees with one cross sectional study on Mexican mothers consuming a low-fat, high carbohydrate diet and American mothers eating a higher-fat, lower-carbohydrate diet, which found that the amino acids pattern of the mothers' milk are not the same.¹⁹

To investigate whether there is any correlation of the amino acids content in human milk and the special confinement diet, we conducted a correlation analysis on each amino acid content in the diet and human milk, and found that most of the amino acids in human milk don't have a correlation with the diet except MET, THR, HIS and ALA. This suggests the amino acids content of diet does not directly relate to human milk. Studies on the relationship of protein content of human milk and diet could not conclude: some studies show that protein concentration maybe affected by diet;^{20,21} some studies show that the protein content is not when mothers were malnourished,²²⁻²⁴ while other studies indicated that the protein content may reduce;^{25,26} one study found that for well nourished mothers, the protein content may decrease when the mothers consumed a diet low in protein.²⁷ In recent studies, researchers prefer to consider more factors, such as body composition, milk volume, lactation time, into consideration when analyzing this relationship,^{28,29} but there is still no certain answer.

For our result, mechanism was proposed: dietary amino acids are digested and absorbed by the gut, and then go to human milk through blood. During this process, first, the amino acids content of blood may differ from that of diet. One study has proved that there is no association between the concentration of amino acids supplied by the diet and the plasma.³ Second, milk proteins are mostly synthesized *de novo* and the mammary gland has a barrier to control movement of proteins, peptides, and amino acids into the gland,^{30,31} so the amino acids content of blood may also differ from that of human milk. All

these factors can affect the relationship between amino acids content in diet and that of human milk. However, the composition of amino acids in human milk calculated is formed on the foundation of daily diet and it is relatively stable, so the correlation of the amino acids pattern of the diet and human milk can be as high as 0.989.

There is another important reason for the non-significant correlation of the amino acids content in human milk and the diet. When the nutrient of the mother's diet goes into breast milk is a question that needs further research, so the 24-hour dietary recall maybe is too short to truly reflect the relation of human milk and the diet. Because our study showed that the amino acid composition of human milk was similar to that of the diet and the amino acids concentration is not, lactating mothers need to pay attention to the nutrition balance of the diet to satisfy their daily demand, and to avoid consuming too much fat and protein to reduce the risk of becoming obese.

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DM and LW performed the data analyses, and drafted the paper. XLW QHS and YMZ designed the study. AZ and XHZ aided with the amino acids measurement. YMZ was responsible for all of the amino acids measurement and contributed to drafting the manuscript.

AUTHOR DISCLOSURES

The authors state that there are no financial or other contractual agreements that might cause conflicts of interest.

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中国北方地区乳母饮食与母乳中氨基酸组成的相关性

本研究描述中国北方乡镇母乳中氨基酸的组成和含量，乳母的饮食特点，并对二者的相关性进行分析。40名产后4-180天的乳母参加了此研究，她们的年龄为19-35岁。我们对乳母进行了24小时膳食回顾调查，并测量了母乳中蛋白质和氨基酸的含量。主要结果如下：(1)母乳中平均蛋白质含量为1.58 g/dL，奶中必需氨基酸(EAA)和非必需氨基酸(NEAA)之比为1:2。奶中含量最高的氨基酸为GLU(16.04%)，PRO(10.18%)，LEU(8.67%)，含量最低的为MET(1.76%)和TRP(0.91%)。(2)乳母的膳食中，能量和蛋白质的摄入量充足，维生素A、维生素B及维生素C的摄入量不足，这与北方地区农村“坐月子”的饮食习惯相符。蛋白质的食物来源主要是谷类和蛋类，大豆和鱼的摄入量较少。(3)饮食和母乳的氨基酸组成相似，两者的氨基酸模式相关性高达0.989，说明饮食中的氨基酸组成是母乳中的基础。但是，母乳中大部分的氨基酸含量和饮食摄入的量没有显著相关性。

关键词：氨基酸、母乳、产后饮食、中国产妇、中国北方农村