Note

The Relationship between Dietary Vitamin A Intake and the Levels of Sialic Acid in the Breast Milk of Lactating Women

Yang QIAO^{1,2}, Jinlu FENG³, Jianping YANG² and Guixiong GU^{1,*}

¹Department of Child Health, Affiliated Children's Hospital of Soochow University, Suzhou 213001, China

²Department of Child Health, Shanxi Maternal and Child Health Hospital, Taiyuan 030013, China

³Department of Anesthesia, Shanxi Tumor Hospital, Taiyuan 030013, China

(Received November 14, 2012)

Summary Sialic acid (SA) is an important nutrient but few studies have examined the link between dietary intake and breast milk sialic acid. The purpose of this observational study was to assess the potential relationship between human breast milk sialic acid levels and dietary nutrition intake 40 d postpartum. The study population included 90 healthy women who were lactating. Human breast milk SA concentrations were measured using fluorescence detector-high performance liquid chromatography (HPLC-FLD) analysis and nutritional intake was estimated by a computerized validated food frequency questionnaire. SA in human breast milk was bound to free oligosaccharides (82.35%), protein (15.27%) and free sialic acid (2.37%). The findings of this study indicate that subjects with higher milk SA levels showed statistically higher levels of vitamin A compared with subjects with lower SA levels (423.48 ± 172.29 vs 602.22 ± 126.46 $\mu\text{g/d}$, $p=0.000$). In addition, there was a certain association (standardized coefficients=0.713; $p=0.000$) between breast milk SA and vitamin A intake in healthy young subjects. This study demonstrated that dietary vitamin A intake has a certain relationship with breast milk SA concentrations. This may be attributed to the influence of vitamin A on sialic acid glycoprotein and sialic acid mucopolysaccharide in the human body or the common food sources for vitamin A and sialic acid. Additional study is required to further investigate this relationship.

Key Words sialic acid, breast milk, vitamin A, diet, nutrition

Sialic acid (SA) is an integral part of the ganglioside structure in synaptogenesis and neural transmission in humans (1, 2) and because it can improve learning and memory (3), it is regarded as an important nutrient in early life (4). For infants, breast milk is the only rich source of sialic acid (5–7). Thus, the SA levels of breast milk are vitally important. Although maternal dietary intake of nutrients such as fatty acid and choline affects breast milk nutrition during lactation, there have been no studies examining the effects of sialic acid on lactation nutrition (8, 9). The purpose of this observational study was to assess the potential relationship between breast milk SA levels and dietary nutrition intake 40 d postpartum.

Methods and Materials

Subjects. The study population included 90 healthy women who were lactating. None of the study participants were reported to have inflammatory-related diseases, tumor diseases or cardiovascular diseases.

None of the participants were taking vitamin or trace elements supplements. Each participant signed a written informed consent of acceptance that was previously approved by the Regional Ethical Review Board at Maternity and Child Care Hospital of Shanxi. This research was conducted in accordance with the Helsinki Declaration (2003) of the World Medical Association (SXFY2010017).

Preparation of human milk. Milk samples were drawn on day 40 of lactation from breast-feeding mothers of term infants (infants born at a gestational age between 37 and 42 completed weeks) when lactation ended each time. The milk sample (10 mL) was collected within 15 min and was stored at -25°C . Sialic acid concentrations in breast milk were determined by fluorescence detector-high performance liquid chromatography (HPLC-FLD). All the experimental instruments and main chemicals were purchased from Waters (Milford, MA) and Sigma-Aldrich (St. Louis, MO), respectively.

Sialic acid assay.

Determination of free sialic acid: The following procedure was used to determine free sialic acid concentrations (10). Breast milk (500 μL) and an equal volume of 10% trichloroacetic acid (TCA) were placed into a centrifugal pipe, mixed using a vortex, iced for 10 min and centrifuged at 3,000 r/min at 4°C for 30 min. The

*To whom correspondence should be addressed.

E-mail: szggx000@163.com

Abbreviations: HPLC-FLD, fluorescence detector-high performance liquid chromatography; SA, sialic acid; TCA, trichloroacetic acid.

supernatant was removed and the remaining pellet was washed with cold 5% TCA (500 μ L). The pellet and TCA were then centrifuged at 3,000 r/min at 4°C for 30 min. The combined supernatant from both washes (approximately 500 μ L) was filtered using a 0.22 μ m filter membrane. The filtered supernatant (90 μ L) was combined with 1,2-diamino-4,5-methylenedioxybenzene (DMB; 10 μ L) and set aside in the dark for 150 min. Chromatographic analysis proceeded after the solution cooled.

Determination of oligosaccharides-bound sialic acid: The total amount of oligosaccharides-bound sialic acid and free sialic acid was determined using the following method (10). The remaining supernatant (approximately 410 μ L) from the combined washes used to determine the amount of free sialic acid was combined with an equal volume of 0.1 mmol trifluoroacetic acid/L and hydrolyzed for 30 min at 80°C. The supernatant was then filtrated using a 0.22 μ m filter membrane. DMB (10 μ L) was added to the supernatant (90 μ L) and the solution placed in the dark for 150 min. Chromatographic analysis proceeded after the solution cooled.

Determination of protein-bound sialic acid: The precipitate from the initial wash to determine free sialic acid concentrations was combined with sulfuric acid (0.05 mol/L; 2 mL) and hydrolyzed for 120 min at 80°C (10). Again, the supernatant was then filtrated using a 0.22 μ m filter membrane. DMB (10 μ L) was added to filtered supernatant (90 μ L), and placed in the dark for 150 min. Chromatographic analysis proceeded after the solution cooled.

Dietary intake assessment. The dietary intake of the study participants was assessed by a 72-h dietary recall questionnaire. Nutrient intake was computed using a dietary evaluation validated for Chinese people (11). All foods and beverages consumed throughout the 72-h study period were recorded (12). Quantities were weighed when possible and information about foods, including brand names, food preparation methods and all ingredients used in food preparation methods were recorded when possible. Food models, measurement aids and food-specific units were used to estimate amounts consumed. Each individual's intake of energy, fat, fiber, protein, vitamins and minerals was determined from their 72-h dietary recall.

Statistical analysis. Results are presented as arithmetic means and standard deviations. The Kolmogorov-Smirnov test was used to determine variable distribution. Study participants were divided into two groups, lower and higher SA levels, based on the median level of breast milk SA on postpartum day 40 (± 7 d). *t*-tests were used to test the differences in nutritional intake between the two groups. Chi-square tests were used to test the differences of frequency distributions in milk, beef, egg, mutton, and pork intakes between the higher vitamin A group and lower vitamin A group. Multiple linear regression analysis was used to analyze the relation of nutritional intake and breast milk SA on postpartum day 40 (± 7 d). All analyses were performed by SPSS for Windows 17.0 (SPSS Inc, Chicago, IL, USA). A *p* value of less than 0.05 was considered significant.

Table 1. Sialic acid concentrations in human milk 40 $\pm$ 7 d postpartum (mg/L, mean $\pm$ SE).

Distribution of sialic acid	40 d after birth (<i>n</i> =90)	Percent (%)
Free	16.96 $\pm$ 4.38	2.37
Oligosaccharide-bound	588.19 $\pm$ 56.66	82.35
Protein-bound	109.10 $\pm$ 23.63	15.27
Total sialic acid	714.25 $\pm$ 64.39	100

Table 2. Total nutrient intakes of the subjects categorized according to the median (719 mg/dL) of breast milk SA concentration.

	Lower milk SA (<i>n</i> =45)	Higher milk SA (<i>n</i> =44)
Protein (g/d)	70.71 $\pm$ 25.03	77.09 $\pm$ 25.94
Carbohydrate (g/d)	199.47 $\pm$ 65.25	224.93 $\pm$ 82.41
Vitamin A (μ g/d)	423.48 $\pm$ 172.29	602.22 $\pm$ 126.46**
Vitamin E (mg/d)	19.84 $\pm$ 12.63	23.82 $\pm$ 17.81
Vitamin C (mg/d)	56.96 $\pm$ 10.23	60.98 $\pm$ 13.31
Zinc (mg/d)	9.44 $\pm$ 3.52	10.42 $\pm$ 3.25
Calcium (mg/d)	490.16 $\pm$ 184.20	563.18 $\pm$ 243.29
Manganese (mg/d)	4.64 $\pm$ 1.60	5.28 $\pm$ 1.38
Iron (mg/d)	17.87 $\pm$ 5.91	17.12 $\pm$ 6.23
Selenium (μ g/d)	36.66 $\pm$ 14.57	36.26 $\pm$ 14.45
Polyunsaturated fatty acid (mg/d)	11.31 $\pm$ 7.14	13.98 $\pm$ 8.79

** *p*≤0.01.

Results

Distribution of breast milk SA

There were three major forms in human breast milk SA. Most of the sialic acid in human breast milk was bound to free oligosaccharides (82.35%) and 15.27% of sialic acid in human breast milk was bound to protein. Little of the sialic acid in human breast milk was free sialic acid (2.37%) (Table 1).

Nutrient intakes and breast milk SA

There were no statistically significant differences in macronutrient (carbohydrate, protein, vitamin E, zinc, selenium, iron or polyunsaturated fatty acid) intake between subjects with higher and lower milk SA (Table 2). Subjects with higher breast milk SA presented significantly higher vitamin A intake (*p*<0.01) when compared with individuals with lower milk SA (Table 2).

There was a certain association between breast milk SA and vitamin A intake in healthy young subjects (standardized coefficients=0.713, *p*=0.000) after adjustment for maternal general characteristics (including education and age) as shown in Table 3.

The frequency distributions of Vitamin A intake

Study participants were divided into two groups, lower and higher vitamin A intake, based on the median level of vitamin A intake on postpartum day 40 (± 7 d). Subjects with higher vitamin A intake were 47 cases

Table 3. Multiple linear regression analysis of human milk SA 40 ± 7 d postpartum ($\alpha=0.01$).

Independent variables	Unstandardized B	Unstandardized B	t	p	Standardized coefficients beta
Protein (g/d)	0.069	0.288	0.024	0.811	0.027
Carbohydrate (g/d)	-0.038	0.081	-0.472	0.638	-0.044
Vitamin A ($\mu\text{g/d}$)	0.264	0.035	7.432	0.000	0.713
Vitamin E (mg/d)	-1.006	0.459	-2.189	0.032	-0.241
Vitamin C (mg/d)	-0.143	0.478	-0.299	0.766	-0.027
Zinc (mg/d)	-0.415	1.987	-0.209	0.835	-0.022
Calcium (mg/d)	0.009	0.028	0.311	0.756	0.029
Manganese (mg/d)	-1.659	4.594	-0.361	0.719	-0.039
Iron (mg/d)	-1.735	1.071	-1.621	0.109	-0.163
Selenium ($\mu\text{g/d}$)	0.591	0.437	1.353	0.180	0.132

Table 4. The frequency distributions of vitamin A intake.

Group	Milk	Beef	Pumpkin	Egg	Seafish	Pork	Carrot	Mutton	Corn
Higher group	47 (64.4)	30 (83.3)	21 (70.0)	47 (56.0)	24 (82.8)	43 (81.1)	30 (73.2)	30 (78.9)	28 (87.5)
Lower group	26 (35.6)	6 (16.7)	9 (30.0)	37 (44.0)	5 (17.2)	10 (18.9)	11 (26.8)	8 (21.1)	4 (12.5)

$\chi^2=23.85$, $p=0.002$. $p<0.05$ was considered significant.

Table 5. Vitamin A intake of the subjects categorized according to the median (17 mg/dL) of breast milk free SA concentration.

	Lower SA (n=48)	Higher SA (n=42)
Vitamin A ($\mu\text{g/d}$)	490.42 $\pm$ 171.99	536.11 $\pm$ 175.83
t	1.244	
p	0.217	

Table 7. Vitamin A intake of the subjects categorized according to the median (103 mg/dL) of breast milk SA concentration bound to protein.

	Lower SA (n=44)	Higher SA (n=46)
Vitamin A ($\mu\text{g/d}$)	442.44 $\pm$ 130.30	578.03 $\pm$ 186.26
t	3.99	
p	0.000	

Table 6. Vitamin A intake of the subjects categorized according to the median (598 mg/dL) of breast milk SA concentration bound to oligosaccharides.

	Lower SA (n=45)	Higher SA (n=45)
Vitamin A ($\mu\text{g/d}$)	426.78 $\pm$ 173.29	596.70 $\pm$ 129.14
t	5.274	
p	0.000	

Vitamin A intake of the subjects categorized according to the median of breast milk free SA concentration

Study participants were divided into two groups, lower and higher breast milk free SA concentration based on the median level of free SA concentration on postpartum day 40 (± 7 d). Subjects with a higher breast free SA concentration presented significantly higher vitamin A intake ($t=1.244$, $p<0.01$) when compared with individuals with a lower free SA concentration, SA concentration bound to oligosaccharides and SA concentration bound to protein (Table 5).

Vitamin A intake of the subjects categorized according to the median of breast milk SA concentration bound to oligosaccharides

Study participants were divided into two groups, lower and higher breast milk SA concentration bound to oligosaccharides based on the median level of SA concentration bound to oligosaccharides on postpartum day 40 (± 7 d). Subjects with higher breast SA bound to oligosaccharides presented significantly higher vitamin A intake ($t=5.274$, $p<0.01$) when compared with indi-

and lower vitamin A intake were 43 cases. The frequency distributions of vitamin A intake in the two groups are presented in Table 4. The frequency distributions of milk, beef, egg, mutton, and pork intakes in the higher vitamin A group were significantly higher when compared with individuals with lower vitamin A intake ($\chi^2=23.85$, $p=0.002$).

viduals with lower breast SA bound to oligosaccharides (Table 6).

Vitamin A intake of the subjects categorized according to the median of breast milk SA concentration bound to protein

Study participants were divided into two groups, lower and higher breast milk SA concentration bound to protein based on the median level of SA concentration bound to protein on postpartum day 40 (± 7 d). Subjects with a higher breast SA concentration bound to protein presented significantly higher vitamin A intake ($t=3.99$, $p<0.01$) when compared with individuals with a lower breast SA concentration bound to protein (Table 7).

Discussion

The distribution of breast milk sialic acid during lactation was reported previously by Wang et al. (7). Carlson reported that the sialic acid concentration in human milk oligosaccharides was higher than sialic acid in human milk glycoproteins (13). Our results reveal that most sialic acid in human breast milk was bound to free oligosaccharides, whereas little was free sialic acid or bound to protein. The main reason is that as there are higher levels of oligosaccharides in breast milk than in bovine milk (14, 15), it would lead to the higher SA concentration in human milk oligosaccharides.

Although many essential nutrients are secreted into breast milk, including the fatty acid, docosahexaenoic acid (DHA) (16, 17), there have been few reports describing the relationship between maternal diet and sialic acid in breast milk. In this study it was observed that vitamin A intake during maternal lactation at 40 d had a high correlation with sialic acid concentration in breast milk. Wolf et al. (18, 19) indicated that vitamin A exerted its influence on mucopolysaccharide synthesis. Wolf et al. (20–22) also pinpointed the defect in glycoprotein synthesis caused by vitamin A deficiency at the protein-glycosylation step. Whereas sialic acid exists only as free mucosaccharides in very low concentrations in the body fluids of humans and animals, it usually occurs in glycosidic linkages of mucopolysaccharide, glycoproteins, gangliosides and lipopolysaccharides (23, 24). Although from questionnaire survey there was poor evidence, we suppose vitamin A impacted the occurrence of sialic acid by influencing the synthesis of glycoproteins and mucopolysaccharide by affecting the binding of sialic acid. We hope it will serve as a basis for future clinical research and has an important meaning for the research of human milk SA. Additional study is required to further investigate this relationship. Another explanation for the relationship between vitamin A intake and human breast milk sialic acid 40 d postpartum may lie in the native food sources of vitamin A and sialic acid. In the current study we found the volunteers whose milk SA was higher could have greater food sources of vitamin A. The best sources for vitamin A are liver (3,000–15,000 μg retinol/100 g) and fish liver oils. Significant levels of vitamin A are also present in milk, whole eggs and egg products (25). Our data displayed the higher vitamin A intake group consumed more milk, eggs, beef, pork, and mutton. Tangvoranun-

takul et al. reported that sialic acid was present in red meats, milk and eggs (26) and Varki (27) also indicated that red meat and milk were sialic acid dietary sources. Wang reviewed sialic acid levels in beef, lamb, pork and chicken (4). Although high levels of sialic acid are usually found in animal sources, sialic acid can also be found in plant foods. Cooked corn, surprisingly, not only contained relatively high sialic levels (4), but also contained high levels of beta carotene, which is an effective source of vitamin A (28, 29). Interestingly, our data also showed higher corn intake was consistent with higher vitamin A intake. This research indicates that there is some commonality in foods that are rich in sialic acid and vitamin A. Based on our clinical data, we can assume when mothers who were lactating consumed food enriched with vitamin A such as red meat, eggs, milk, and corn, they were also consuming foods which contained relatively high levels of sialic acid. This would have resulted in high levels of sialic acid secreted into breast milk.

In conclusion, this study demonstrated that dietary vitamin A intake has a certain relationship with maternal milk SA concentrations. This relationship may be attributed to the influence of vitamin A on the sialic acid glycoprotein and sialic acid mucopolysaccharide or the common food sources for vitamin A and sialic acid. Additional study is required to further investigate this relationship.

Acknowledgments

We would like to thank the mothers and all volunteers who participated in this study. We would also like to mention the efforts of physician Yanjun Geng in providing assistance in the nutritional analysis and Professor Hongmei Yu for data analysis. We would like to extend our thanks to Miss Dandan Bai, who provided assistance and support along with the team of dedicated research nurses and ancillary staff.

This project was supported by the scientific research innovation projects of college graduate students in Jiangsu Province, China (CXLX11_0085).

Author contribution

Yang Qiao carried out the clinical study, data acquisition and manuscript preparation. Guixiong Gu participated in the design of the study and manuscript review. Jinlu Feng performed the statistical analysis. Jianping Yang carried out data acquisition. All the authors read and approved the final manuscript. There were no conflicts of interest.

Conflict of interest

The authors have declared that no conflict of interest exists.

REFERENCES

- 1) Bruses JL, Rutishauser U. 2001. Roles, regulation, and mechanism of polysialic acid function during neural development. *Biochimie* **83**: 635–643.
- 2) Wang B, Brand-Miller J. 2003. The role and potential

- of sialic acid in human nutrition. *Eur J Clin Nutr* **57**: 1351–1369.
- 3) Wang B, Yu B, Karim M, Hu H, Sun Y, McGreevy P, Petocz P, Held S, Brand-Miller J. 2007. Dietary sialic acid supplementation improves learning and memory in piglets. *Am J Clin Nutr* **85**: 561–569.
 - 4) Wang B. 2009. Sialic acid is an essential nutrient for brain development and cognition. *Annu Rev Nutr* **29**: 177–222.
 - 5) Nakano T, Sugawara M, Kawakami H. 2001. Sialic acid in human milk: composition and functions. *Acta Paediatr Taiwanica (Taiwan er ke yi xue hui za zhi)* **42**: 11–17.
 - 6) Pan XL, Izumi T. 2000. Variation of the ganglioside compositions of human milk, cow's milk and infant formulas. *Early Hum Dev* **57**: 25–31.
 - 7) Wang B, Brand-Miller J, McVeagh P, Petocz P. 2001. Concentration and distribution of sialic acid in human milk and infant formulas. *Am J Clin Nutr* **74**: 510–515.
 - 8) Fischer LM, da Costa KA, Galanko J, Sha W, Stephenson B, Vick J, Zeisel SH. 2010. Choline intake and genetic polymorphisms influence choline metabolite concentrations in human breast milk and plasma. *Am J Clin Nutr* **92**: 336–346.
 - 9) Peng Y, Zhou T, Wang Q, Liu P, Zhang T, Zetterstrom R, Strandvik B. 2009. Fatty acid composition of diet, cord blood and breast milk in Chinese mothers with different dietary habits. *Prostaglandins Leukot Essent Fatty Acids* **81**: 325–330.
 - 10) Hai-jiao C. 2011. Determination of total sialic acid content in human milk by HPLC-FLD. *Food Sci* **32**: 308–311.
 - 11) Chinese Nutrition Society. 2006. Chinese Dietary Reference Intakes. China Light Industry Publishing House, Beijing.
 - 12) Thompson JM, Wall C, Becroft DM, Robinson E, Wild CJ, Mitchell EA. 2010. Maternal dietary patterns in pregnancy and the association with small-for-gestational-age infants. *Br J Nutr* **103**: 1665–1673.
 - 13) Carlson SE. 1985. N-acetylneuraminic acid concentrations in human milk oligosaccharides and glycoproteins during lactation. *Am J Clin Nutr* **41**: 720–726.
 - 14) Bode L. 2006. Recent advances on structure, metabolism, and function of human milk oligosaccharides. *J Nutr* **136**: 2127–2130.
 - 15) Bode L. 2009. Human milk oligosaccharides: prebiotics and beyond. *Nutr Rev* **67** (Suppl 2): S183–S191.
 - 16) Innis SM, Kuhnlein HV. 1988. Long-chain n-3 fatty acids in breast milk of Inuit women consuming traditional foods. *Early Hum Dev* **18**: 185–189.
 - 17) Vuori E, Kiuru K, Makinen SM, Vayrynen P, Kara R, Kuitunen P. 1982. Maternal diet and fatty acid pattern of breast milk. *Acta Paediatr Scand* **71**: 959–963.
 - 18) Wolf G, Johnson BC. 1960. Vitamin A and mucopolysaccharide biosynthesis. *Vitam Horm* **18**: 439–455.
 - 19) Wolf G, Varandani PT, Johnson BC. 1961. Vitamin A and mucopolysaccharide synthesizing enzymes. *Biochim Biophys Acta* **46**: 59–67.
 - 20) Chan VT, Wolf G. 1987. The role of vitamin A in the glycosylation reactions of glycoprotein synthesis in an 'in vitro' system. *Biochem J* **247**: 53–62.
 - 21) Kiorpes TC, Molica SJ, Wolf G. 1976. A plasma glycoprotein depressed in vitamin A deficiency in the rat: alpha 1-macroglobulin. *J Nutr* **106**: 1659–1667.
 - 22) Wolf G, Kiorpes TC, Masushige S, Schreiber JB, Smith MJ, Anderson RS. 1979. Recent evidence for the participation of vitamin A in glycoprotein synthesis. *Fed Proc* **38**: 2540–2543.
 - 23) Schauer R, Kamerling J. 1997. Vol 29, Pt B: Chemistry, biochemistry and biology of sialic acids. In: New Comprehensive Biochemistry (Montreuil J, Vliegenthart J, Schachter H, eds), p 243–402.
 - 24) Varki A. 1992. Diversity in the sialic acids. *Glycobiology* **2**: 25–40.
 - 25) Olson JA. 1987. Recommended dietary intakes (RDI) of vitamin A in humans. *Am J Clin Nutr* **45**: 704–716.
 - 26) Tangvoranuntakul P, Gagneux P, Diaz S, Bardor M, Varki N, Varki A, Muchmore E. 2003. Human uptake and incorporation of an immunogenic nonhuman dietary sialic acid. *Proc Natl Acad Sci USA* **100**: 12045–12050.
 - 27) Varki A. 2009. Multiple changes in sialic acid biology during human evolution. *Glycoconj J* **26**: 231–245.
 - 28) Muzhingi T, Gadaga TH, Siwela AH, Grusak MA, Russell RM, Tang G. 2011. Yellow maize with high beta-carotene is an effective source of vitamin A in healthy Zimbabwean men. *Am J Clin Nutr* **94**: 510–519.
 - 29) Tang G, Gu X, Hu S, Xu Q, Qin J, Dolnikowski GG, Fjeld CR, Gao X, Russell RM, Yin S. 1999. Green and yellow vegetables can maintain body stores of vitamin A in Chinese children. *Am J Clin Nutr* **70**: 1069–1076.