Consumption of cow milk and egg by lactating women and the presence of β-lactoglobulin and ovalbumin in breast milk1,2

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ABSTRACT β-Lactoglobulin and ovalbumin in mature human milk in healthy lactating Japanese women (n = 24) were determined by using an enzyme-linked immunosorbent assay. Subjects consumed ± 200 mL cow milk/d for 1 wk before the sampling day and exactly 200 mL cow milk on the morning of the sampling day. β-Lactoglobulin was detected (> 0.1 µg/L) in breast milk in 15 of the 24 subjects (62.5%), with a maximum concentration of 16.5 µg/L. Ovalbumin was detected in only two subjects (8.3%) after the subjects followed their usual diet. β-Lactoglobulin concentrations were low in the subjects whose cow milk consumption during the entire lactating period was low, even though all subjects consumed the same amount of cow milk before sampling. This result suggests that β-lactoglobulin concentrations in breast milk are related to long-term consumption of cow milk. Amounts of food antigens in breast milk may be controlled by modifying the daily maternal diet. Am J Clin Nutr 1997;65:30-5.

KEY WORDS β-Lactoglobulin, ovalbumin, food allergy, antigen, human breast milk, cow milk, egg, enzyme-linked immunosorbent assay, immunoglobulin A

INTRODUCTION

Breast milk is considered the most appropriate and nutritious food for infants (1). Breast milk has important immunologic functions because it contains secretory immunoglobulin A (IgA) for the prevention of infections and exposure to antigens in the intestines of infants (2, 3). However, food antigens administered orally to lactating women have been detected in their breast milk. β-Lactoglobulin (4-7), bovine IgG (8), ovalbumin (4, 9), ovomucoid (4), and gliadin (10) antigens have been detected in breast milk from lactating women who consumed cow milk, eggs, or gluten before sampling. These antigens were also found frequently in breast milk randomly collected from lactating women who followed a normal diet without any compulsory doses of antigens (11-14).

Allergies in infants have been increasing recently (15). The development of a food allergy may be initiated when infants are exposed to and allergic-sensitized by food antigens. Although antigens in breast milk are measured in micrograms per liter, their presence is nevertheless immunologically important (16, 17). In some cases, infants who were entirely breast-fed and who never received cow milk protein developed an allergy to cow milk, which was then alleviated by having the infants' mothers adhere to a diet free of cow milk (18, 19). This finding strongly suggests that a reduction in antigens in breast milk could be significant in preventing the development of an allergy in infants.

Cow milk and eggs are common sources of food protein; however, both contain well-known food antigens. β-Lactoglobulin is a major antigen in cow milk (20, 21) and is known to be relatively resistant to acid and enzymatic degradation (22, 23). β-Lactoglobulin taken orally is thus absorbed in great quantities compared with other milk proteins, as shown in animal studies (24-26). Ovalbumin is a major antigen in egg white and has been noted by many researchers to be a food allergen (27). Thus, the determination of β-lactoglobulin and ovalbumin in breast milk is important in understanding the frequency of exposure to food antigens in infants via breast milk.

Axelsson et al (11) showed that there was no relation between the transfer of β-lactoglobulin and cow milk consumption in lactating Swedish women (11). However, per capita cow milk consumption in Sweden is approximately three times higher than that in Japan (28). There have been few reports describing the relation between food antigens and cow milk consumption, especially in Japanese women, whose consumption of cow milk is low. This study was undertaken to determine the concentrations of antigens (β-lactoglobulin and ovalbumin) in the breast milk of lactating Japanese women and to determine the influence of a long-term maternal daily diet of cow milk and eggs during lactation on the transfer of food antigens into breast milk.

SUBJECTS AND METHODS

Subjects

Forty healthy pregnant Japanese women who did not suffer from cow milk intolerance were recruited. All women kept a...


**β-Lactoglobulin in Human Milk**

Diary of their daily consumption of cow milk and eggs beginning in late pregnancy. Four to 10 mo after giving birth, women who could provide sufficient breast milk and could keep the food diary as scheduled were invited to participate in our breast milk sampling study. Twenty-four lactating women aged 22–37 y (median: 32 y) agreed to participate. None were suffering from any diseases, including respiratory or gastrointestinal illnesses, or had taken any drugs in the previous week; thus, none were excluded from the study. The study was undertaken in accordance with the Helsinki Declaration of 1975 as revised in 1983.

**Study design**

The subjects, who had consumed as much cow milk as they wanted from late pregnancy to 7 d before sampling, were advised to consume ≥ 200 mL cow milk/d, without heating the milk, for 7 d before the sampling day, and then 200 mL cow milk the morning of the sampling day. Three samples were obtained from each subject: one in the morning, one at noon, and one in the evening 1–3 h, 4–8 h, and 9–15 h, respectively, after they consumed the cow milk in the morning. Twenty milliliters of breast milk was obtained from 11 breasts by using a milk pump before the infants were breastfed. Samples were stored at −20°C before the analyses. Egg consumption was not regulated throughout the entire study period. Consumption of cow milk, cow milk products, and eggs was recorded daily in the food diary, which was kept beginning in late pregnancy to the day of the samplings. The overall condition of the mothers’ health, including any use of drugs, was also recorded daily.

**Antigen analyses**

β-Lactoglobulin and ovalbumin were measured with a sandwich-type enzyme-linked immunosorbent assay (ELISA) by using the streptavidin-biotin amplification system according to Makinen-Kiljunen and Palosuo (7), with a slight modification (29). Anti-β-lactoglobulin serum was obtained from New Zealand white rabbits immunized with bovine β-lactoglobulin with Freund’s adjuvant and purified with salting out and affinity-column chromatography with β-lactoglobulin to obtain anti-β-lactoglobulin–specific polyclonal IgG. Anti-ovalbumin–specific antibody was purchased from Cosmo Bio Ltd (Tokyo). The lower limit of detection of β-lactoglobulin and ovalbumin was 0.1 μg/L. There was no cross-reactivity with casein and ovalbumin in β-lactoglobulin detection, or with casein and β-lactoglobulin in ovalbumin detection, within concentrations of 1.0 g/L, respectively. The CVs of the within-day and between-day analyses were, respectively, 6.2% and 13.6% at 0.35 μg β-lactoglobulin/L and 3.2% and 9.8% at 0.22 μg ovalbumin/L. The interference by specific IgAs in breast milk during the ELISA was negligible.

**Analyses of specific IgA**

Anti-β-lactoglobulin and anti-ovalbumin–specific IgA were measured by using an ELISA without an amplification system. Microtiter plates were coated with β-lactoglobulin (Sigma Chemical Co, St Louis) or ovalbumin (Sigma Chemical Co). Breast milk samples defatted by centrifugation (17 000 × g, 4°C, 30 min) and a SEROCLEAR reagent (Calbiochem Corp, La Jolla, CA) were added on the plate. Anti-human IgA peroxidase-labeled polyclonal immunoglobulin from rabbits (1: 4000 dilution; Dako Japan Ltd, Kyoto, Japan) was used as a second antibody for detection. Anti-β-lactoglobulin and anti-ovalbumin–specific IgA in breast milk were isolated by using affinity-column chromatography with a β-lactoglobulin- or ovalbumin-bound POROS AL column (Japan Millipore Ltd, Tokyo) and quantified by an IgA detection system using an ELISA. These pooled IgAs were used as the standards for anti-β-lactoglobulin– and anti-ovalbumin–specific IgA in breast milk. The lower limit of detection of specific IgA was 35 μg/L.

**History and development of allergy**

A history of allergies in both the lactating women and their husbands was taken when the subjects were pregnant. Questionnaires concerning the development of allergies in the infants were sent to the participating women 12 mo after birth. The infants were examined by a physician to determine whether they had developed an allergy. Atopic dermatitis was diagnosed when skin areas of scaly erythematous and itchy rash primarily involving the face, scalp, area behind the ears, and flexural folds were evident. Asthma was considered when cough and other respiratory symptoms lasted > 1 d in the absence of infection. Allergic rhinitis was defined as the presence of a clear watery discharge from the nose and no infection.

**Statistical analyses**

The results are expressed as means ± SDs. Statistical analyses were performed by using SPSS software (SPSS Japan Inc, Tokyo), and statistical differences were tested by using the Mann-Whitney U test. Correlations between two variables were shown as Pearson’s correlation coefficients.

**RESULTS**

**β-Lactoglobulin in breast milk**

β-Lactoglobulin concentrations in breast milk are shown in Figure 1. β-Lactoglobulin was detected in 39 (54.2%) breast milk samples from 15 (62.5%) lactating women. The maximum value was detected at 16.5 μg/L. The appearance and disappearance of β-lactoglobulin in a single day varied between individuals. Seven (29%) subjects showed detectable β-lactoglobulin in all three samples, from morning to evening.

Relations between β-lactoglobulin in breast milk and cow milk consumption by the lactating women are shown in Figure 2. The amounts of cow milk consumed during the entire lactating period and at one time 1 wk before sampling were 1527 ± 1099 and 1876 ± 500 mL/wk, respectively; the corresponding concentrations of β-lactoglobulin in cow milk were 4.5 ± 3.3 and 5.6 ± 1.5 g, respectively. β-Lactoglobulin concentrations were low in the subjects whose cow milk consumption during the entire lactating period was low, even though all subjects consumed > 200 mL cow milk for 1 wk before the sampling day and consumed 200 mL cow milk (0.6 g β-lactoglobulin) on the morning of the sampling day. In the subjects whose cow milk consumption during the entire lactating period was < 1000 mL/wk, β-lactoglobulin was detected in only 4 (40%) of the 10 subjects. β-Lactoglobulin was detected in all eight subjects who consumed > 2000 mL cow milk/wk.
during the entire lactating period. Subjects were divided into two groups according to the presence of β-lactoglobulin in their breast milk: a β-lactoglobulin–positive group (n = 15) in which detectable β-lactoglobulin concentrations were > 0.1 μg/L from any sampling, and a β-lactoglobulin–negative group (n = 9) in which β-lactoglobulin was not detectable in any samples (Figure 3). Cow milk consumption during the entire lactating period in the β-lactoglobulin–negative group and the β-lactoglobulin–positive group was 811 ± 465 and 1914 ± 1196 mL/wk, respectively; these amounts of cow milk contain ≈ 2.4 ± 1.4 and 5.7 ± 3.6 g β-lactoglobulin, respectively. The consumption of cow milk by the β-lactoglobulin–negative group was significantly lower than that by the β-lactoglobulin–positive group (P < 0.05). There was no difference in cow milk consumption between the two groups during the week before sampling.

Five subjects (21%) and six of their husbands (25%) had a history of atopic diseases (eg, asthma, atopic dermatitis, and allergic rhinitis). There were no differences in the history of allergy in the subjects or their husbands between the β-lactoglobulin–positive and the β-lactoglobulin–negative groups. The manifestation rate of allergies in the 1-y-old infants of the subjects was 27% in the β-lactoglobulin–positive group and 33% in the β-lactoglobulin–negative group, respectively, and there was no significant difference between the two groups. The concentration of β-lactoglobulin also showed no correlation with infant allergy development. No infants developed cow milk allergy during the study period.

Ovalbumin in breast milk

The ovalbumin concentration in breast milk is shown in Figure 4. Ovalbumin was detected in three samples (4.2%) from two lactating women (8.3%), with a maximum value of 0.7 μg/L. The three samples with detectable ovalbumin also had detectable β-lactoglobulin. Sixteen subjects consumed eggs within 24 h of sampling, and ovalbumin was detected in two (12.5%). One sample showed detectable ovalbumin after consuming a cooked egg > 24 h before sampling. A subject consumed half of a raw egg within 24 h of sampling; however, no ovalbumin was detected in her breast milk. All other subjects consumed cooked eggs.

Egg consumption of lactating women during the entire lactating period and for 1 wk before sampling was 4.2 ± 2.17 and 4.26 ± 1.60 eggs/wk, which contain ≈ 7.4 ± 3.7 and 7.3 ± 2.8 g ovalbumin, respectively. Because of the small number of detectable ovalbumins, we were unable to determine a correlation between egg consumption and ovalbumin in breast milk. The infant of the subject in whom ovalbumin was detectable in two breast milk samples had atopic dermatitis at 1 y of age, but the infant of the other ovalbumin–positive subject had no allergy.

Anti-β-lactoglobulin and anti-ovalbumin–specific IgA in breast milk

Anti-β-lactoglobulin and anti-ovalbumin–specific IgA concentrations in breast milk are shown in Figure 5. Anti-β-lactoglobulin and anti-ovalbumin–specific IgA were detected in all samples. There was no significant correlation between anti-β-lactoglobulin-specific IgA and cow milk consumption during the entire lactating period or 1 wk before sampling, and between anti-ovalbumin–specific IgA and egg consumption during the entire lactating period or 1 wk before sampling. There was no correlation between antigen concentrations and specific IgA concentrations in breast milk. There was also no correlation between these IgA concentrations and allergy development in infants at 1 y of age.
DISCUSSION

There is evidence that β-lactoglobulin in cow milk is transferred into breast milk (4–7, 11–13). The frequency of the detection of β-lactoglobulin has been reported to range from 33% to 95% in the studies described above. In the present study, β-lactoglobulin was found in 15 of the 24 lactating women (63%). The transfer of the β-lactoglobulin antigen into breast milk occurred at almost the same frequency in lactating Japanese women as in Europeans. We also showed that β-lactoglobulin was detected in all breast milk samples from morning to evening in seven lactating women (29%). Some authors have shown that food antigens remain in breast milk for > 24 h after the consumption of certain foods (9, 12, 30). Several breast-fed infants were thus thought to be exposed to food antigens for a long time after their mother’s consumption of cow milk. Ovalbumin was detected in only two subjects (8.3%). Ogura et al (14) showed that ovalbumin was detected in 17% of the breast milk from lactating Japanese women who followed their usual diet. Native ovalbumin from raw eggs consumed by lactating women seems to be transferred into breast milk as frequently as is β-lactoglobulin (4, 31). The low frequency of detectable ovalbumin in breast milk compared with β-lactoglobulin in this study could have been due to the subjects that consumed cooked eggs in their usual diet, in which ovalbumin was denatured and digested in the intestine more efficiently (32).

We found that the transfer of β-lactoglobulin into breast milk was influenced by the maternal consumption of cow milk. Low β-lactoglobulin concentrations in breast milk were observed in the subjects whose cow milk consumption was low during the entire lactating period, and high β-lactoglobulin concentrations were found in the subjects whose cow milk consumption was high during the entire lactating period.
high during the same period, despite the fact that all subjects consumed a certain amount of cow milk just before sampling. The lactating women in the Swedish study, which reported that there was no correlation between the amount of β-lactoglobulin transfer and cow milk consumption, ingested 1400–10 000 mL cow milk/wk (11), which was much higher than the amount consumed by our subjects. We were also unable to observe any correlation in our subjects who consumed >1400 mL cow milk/wk. We found that β-lactoglobulin concentrations were especially low in the breast milk from subjects who consumed <1000 mL cow milk containing 3 g β-lactoglobulin/wk during the entire lactating period. Such low consumption of cow milk is not rare in Japanese lactating women; 36% of 429 women surveyed consumed <1000 mL cow milk/wk in our in-house survey (data not shown).

None of the subjects, including those with a high β-lactoglobulin concentration in breast milk, suffered from any diseases (eg, diarrhea); therefore, the incorporation of β-lactoglobulin from intestine to blood and to breast milk was not caused by illness. Appearance of β-lactoglobulin in breast milk was not influenced by the consumption of cow milk for several days just before sampling but was influenced after several months of consumption, implying that the long-term consumption of cow milk influenced some responses to food antigens in individuals. The effect of long-term consumption of cow milk on β-lactoglobulin transfer into breast milk may have occurred as a result of changes in the immune response to β-lactoglobulin in the subjects. Specific IgG in serum is important for the systematic clearance of antigens from the circulation (33). Dannaeus et al (34) reported that the concentration of antigens was significantly less in atopic children with a high serum concentration of specific IgG. Secretory IgA in the intestine is also known to play a significant role as a barrier in preventing antigen transfer into the circulation (35). If specific IgG, secretory IgA, or both are retarded in the subjects whose consumption of food antigens is greater for an extended period, the exclusion and clearance of the antigen could occur less efficiently, which may result in the transfer of more antigen into breast milk. Oral tolerance to dietary protein antigens is a well-known reaction, which occurs when subjects are continuously administered antigens orally, and in most cases specific immunoglobulins are suppressed (36). In this study the concentrations of specific IgA in breast milk were not related to the diet, a finding that agrees with the findings of Fålh-Magnusson (37). However, the amount of secretory IgA in the intestine does not always correlate with the amount of IgA in other organs (38). This hypothesis—that the changes in the concentrations of systematic and local immunoglobulins by the long-term consumption of food antigens modifies the level of antigen transfer into breast milk—should be confirmed by further studies.

In this study we showed that the transfer of food antigens, especially β-lactoglobulin, into breast milk occurred frequently in lactating Japanese women. An antigen in breast milk does
not always cause an allergic reaction in infants; however, the antigen may cause allergic reactions in some breast-fed infants (16, 17). We found that there was a positive correlation between long-term consumption of cow milk and the β-lactoglobulin concentration in breast milk, which suggests that the low-level, long-term consumption of food antigens could contribute to a reduction in the transfer of food antigens into breast milk. The concentrations of food antigens in breast milk may be controllable by modifying long-term food consumption.

REFERENCES