Note

**Long-Term Consumption of Whey Hydrolysate Formula
by Lactating Women Reduces the Transfer of
 β -Lactoglobulin into Human Milk**

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Summary Food antigens transferred into breast milk sometimes cause an allergic reaction in exclusively breast-fed infants. This study will show whether the intake of a whey hydrolysate formula for lactating women (MOM HA) can reduce the appearance of food antigens in breast milk. Lactating women in the MOM group ($n=12$) consumed MOM HA as a substitute for cow's milk and those in the COW group ($n=13$) consumed cow's milk for more than 4 months. After the ingestion of 200 mL of MOM HA and cow's milk by the women in the MOM and COW groups, respectively, the first breast milk samples were obtained and β -lactoglobulin was measured using enzyme-linked immunosorbent assay. The number of subjects with detectable β -lactoglobulin (>0.1 ng/mL) in the MOM group was two (17%), which was significantly less than that in the COW group (11 subjects, 85%, $p<0.01$). The level of β -lactoglobulin was also lower in the MOM group than the COW group ($p<0.01$). Subsequently, the women in the MOM group consumed cow's milk and those in the COW group consumed MOM HA for one week; then a second sampling was performed. β -Lactoglobulin was detected in three (25%) and 8 subjects (62%) in the MOM and COW groups, respectively. The level of β -lactoglobulin was still lower in the MOM group ($p<0.05$). The consumption of whey hydrolysate formula by lactating women over a considerable time reduces the transfer of β -lactoglobulin into their breast milk, and the low level can be maintained even after inadvertent ingestion of cow's milk.

Key Words β -lactoglobulin, breast milk, food antigen, cow's milk, whey hydrolysate formula

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Food antigens, β -lactoglobulin and ovalbumin, which are derived from cow's milk and eggs consumed by lactating women frequently appear in their breast milk (1–3). These antigens in breast milk include intact proteins (1, 3) and sometimes cause allergic reactions in exclusively breast-fed infants (4–6). The avoidance of some allergenic foods including cow's milk and eggs by mothers during lactation is effective in preventing allergy development in risk infants (7, 8), which lowers the incidence of allergic sensitization to food in infants via breast milk (9). Therefore, reducing the food antigen levels in breast milk may help to prevent allergy development in infants.

A hypoallergenic formula for pregnant and lactating women using whey hydrolysate (MOM HA) was newly developed (10–13). In a previous study, we showed that the levels of anti-cow's milk IgE antibody can be reduced in breast-fed infants, when their mothers consumed MOM HA as a substitute for cow's milk (13). This suggests that the hypoallergenic formula has a potential to reduce the frequency of allergic sensitization in breast-fed infants. In this study, we examined whether or not the consumption of the hydrolysate formula by lactating women, as a substitute for cow's milk, reduced the level of β -lactoglobulin in their breast milk.

Experimental

Subjects. Forty healthy pregnant Japanese women without cow's milk intolerance were recruited and allocated to the MOM group and COW group. The women in the MOM group consumed at least 200 mL/d of the hypoallergenic formula MOM HA (Nestlé Japan, Kobe, Japan) as a substitute for cow's milk from late pregnancy to the times of sampling, and they were asked not to consume cow's milk during this period. Those in the COW group consumed at least 200 mL/d of cow's milk during the corresponding period. All women kept a diary of their daily consumption of MOM HA, cow's milk, and eggs from late pregnancy. Four to 10 mo after giving birth, the women who had consumed MOM HA or cow's milk as scheduled and could provide sufficient breast milk were asked to participate in our breast milk sampling. This resulted in 12 lactating women in the MOM group and 13 in the COW group agreeing to participate in the experiment; their ages ranged from 22 to 36 y (median, 32 y). None of them was suffering from any diseases including cold and gastrointestinal defects, nor taking any drugs during the two-week sampling period described below.

Study design. The subjects of the MOM group ingested 200 mL of MOM HA and those of the COW group ingested 200 mL of cow's milk on the morning of the first sampling day. On the same day, three samples were obtained from each subject, 1 to 3 h, 4 to 8 h, and 9 to 15 h after the ingestion of MOM HA or cow's milk in the morning. Twenty milliliters of breast milk was obtained using a breast pump from both breasts before nursing. Samples were stored at -20°C before analyses. After the first sampling day, the diets in each group were switched for the second sampling. The subjects of the MOM group consumed at least 200 mL/d of cow's milk and those of the COW group consumed the same amount of MOM

HA for seven d. During this week, cow's milk was not consumed by any subject in the COW group. On the morning of the second sampling day, each subject of the MOM and COW groups ingested 200 mL of cow's milk or MOM HA, respectively, and breast milk samples were obtained in the same manner as the first sampling. Egg consumption was not regulated during the entire study period. The consumption of cow's milk, cow-milk products and eggs was recorded daily in a food diary kept from late pregnancy to the day of the second set of samplings. The overall condition of each mother's health, including any use of drugs, was also recorded daily.

Antigen analyses. β -Lactoglobulin and ovalbumin were measured by a sandwich-type ELISA using the streptavidin-biotin amplification system as described in a previous report (14). The lower limit of detection is 0.1 ng/mL for each β -lactoglobulin and ovalbumin. There was no cross-reactivity with casein and ovalbumin in β -lactoglobulin detection nor with casein and β -lactoglobulin in ovalbumin detection within respective concentrations of 1.0 mg/mL.

Statistical analyses. The maximum amounts of β -lactoglobulin and of ovalbumin among the three samples obtained on each sampling day are expressed as the amount per subject. Statistical analyses were performed using SPSS (SPSS Japan, Tokyo), and statistical differences were tested using the chi-square test and Mann-Whitney test.

Results and discussion

From birth to one week before the first sampling, the weekly consumption of MOM HA and cow's milk (mean $\pm$ SD) was $1,642 \pm 658$ mL (MOM group) and $1,943 \pm 916$ mL (COW group), respectively. The subjects of the MOM group consumed $1,647 \pm 397$ mL of MOM HA for one week just before the first sampling and $1,511 \pm 624$ mL of cow's milk for one week just before the second sampling. The subjects of the COW group consumed $1,871 \pm 407$ mL of cow's milk for a week just before the first sampling and $1,519 \pm 637$ mL of MOM HA for a week just before the second sampling. There were no differences in the amounts of MOM HA and cow's milk consumed between the MOM and COW groups at any period.

The levels of β -lactoglobulin in the breast milk are shown in Fig. 1. The antigen was detected (>0.1 ng/mL) in two subjects (17%) of the MOM group and in 11 subjects (85%) of the COW group at the first sampling. The number of subjects who showed detectable β -lactoglobulin was significantly less in the MOM group than in the COW group ($p < 0.01$). The level of β -lactoglobulin was also lower in the MOM group than in the COW group ($p < 0.01$). At the second sampling, three subjects (25%) of the MOM group and eight subjects (62%) of the COW group showed β -lactoglobulin detectable. The frequency of subjects with detectable β -lactoglobulin of the MOM group was not significantly lower than that of the COW group, but the levels of β -lactoglobulin were still lower in the MOM group than in the COW group ($p < 0.05$), even though the subjects in the MOM group had ingested cow's milk prior to the second sampling. These results suggest that

Fig. 1. Level of β -lactoglobulin in breast milk. At the first sampling, breast milk samples in the MOM group were collected after MOM HA ingestion, and those in the COW group were collected after cow's milk ingestion. At the second sampling, the samples were collected after cow's milk ingestion by the MOM group, and after MOM HA ingestion by the COW group.

long-term consumption of MOM HA can lower the level of β -lactoglobulin in breast milk, and that the low level is maintained after following short-term consumption of cow's milk. In our previous study, we found that the level of β -lactoglobulin was also low in women who consumed cow's milk at a low level during the entire period of lactation, even when they ingested cow's milk prior to sampling (14). A long-term consumption of β -lactoglobulin by lactating women may influence the level of the antigen transferred into their breast milk.

Hydrolyzed peptides in MOM HA are hypoallergenic but can be slightly detected as antigens by the ELISA method. We detected β -lactoglobulin in the breast milk from women who had ingested MOM HA. In an attempt to fractionate a few breast milk samples after the ingestion of MOM HA, we detected β -lactoglobulin peptides but not intact form (data not shown). The peptides in breast milk derived from ingested MOM HA whose proteins have been already hydrolyzed is probably less allergenic than the intact protein.

The consumption of eggs in the MOM and COW groups from birth to one week before the first sampling was 4.0 ± 3.0 and 4.0 ± 1.3 eggs per week, respectively. There were no differences in the intake of eggs between the two groups. The consumption of eggs in the MOM and COW groups was 3.2 ± 1.7 and 4.0 ± 1.8 eggs for the week just before the first sampling, and 3.9 ± 2.0 and 3.7 ± 2.4 eggs for the week just before the second sampling, respectively. At the first sampling, ovalbumin was detected in two subjects (17.6%) in the MOM group and two subjects (15.4%) in the COW group (Fig. 2). At the second sampling, there were three (25.0%) and four (30.8%) ovalbumin-detected subjects of the MOM and COW groups, respectively. The percentages of subjects with detectable ovalbumin in the two groups were essentially the same. The low frequency of appearance of ovalbumin in the breast milk of both groups suggests that the high β -lactoglobulin levels in some women were not caused by illness like food intolerance or diarrhea,

Fig. 2. Relationship between ovalbumin and β -lactoglobulin levels in breast milk.

which may increase non-specific permeation of molecules through intestinal damage (15). In the MOM group at the second sampling, ovalbumin was detected in three subjects, of which two subjects were β -lactoglobulin-negative even after they equally ingested cow's milk prior to the sampling. These results imply that the effect of long-term consumption of MOM HA in lowering β -lactoglobulin transfer into breast milk occurs antigen-specific.

The strict restriction of allergenic food is effective to decrease the incidence of allergy in infants by reducing the amount of allergens in the breast milk of lactating women (7–9). However, it should not be recommended for every lactating woman, because much nutrition is required for milk production during lactation. A nutritious and hypoallergenic formula can possibly replace the maternal restriction of cow's milk for an allergy prevention program in risk infants. In a previous trial, allergic sensitization to cow's milk occurred significantly less in infants whose mothers consumed MOM HA as compared to mothers who consumed cow's milk during lactation (13). In this study, we showed that the long-term consumption of MOM HA lowers the level of β -lactoglobulin in breast milk and that the low level can be maintained even after inadvertent ingestion of cow-milk protein. A hypoallergenic formula for lactating women may help in preventing allergy development in breast-fed infants by reducing the exposure to cow-milk allergens via breast milk in a safer way.

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