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Detection of β -lactoglobulin in human breast-milk 7 days after cow milk ingestion

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ABSTRACT

Background: β -lactoglobulin (BLG), a major allergen in cow milk (CM) can be detected in human breast-milk (BM) and is associated with exacerbation of symptoms in breastfed infants with cow milk protein allergy (CMPA). Currently, it is not known how long lactating mothers who consume dairy products need to withhold breastfeeding.

Objective: To elucidate the kinetics of BLG in BM after maternal ingestion of a single dose of CM.

Methods: Nineteen lactating mothers, four of whom had infants with CMPA, were instructed to avoid CM for 7 days before ingesting a single dose of CM and to continue to withhold CM thereafter throughout the study period. BLG was measured by ELISA in BM from 15 mothers of healthy infants before and at 3, 6 and 24 h, and 3 and 7 days after CM ingestion. Four pairs of mothers and CMPA infants were enrolled for BM challenge after the mothers had ingested CM.

Results: After CM ingestion, the level of BLG in BM increased significantly from 0.58 ng/ml (0.58 g/L) (IQR 0.38–0.88) to a peak level of 1.23 ng/ml (IQR 1.03–2.29), $p < 0.001$. The BLG level on day 3 (1.15 ng/ml, IQR 0.89–1.45) and day 7 (1.08 ng/ml (IQR 0.86–1.25) after CM ingestion was significantly higher than baseline ($p = 0.01$ and $p = 0.001$, respectively). BLG was detected in all BM samples from the four mothers of CMPA infants after CM ingestion, and the level was not different from that in the mothers of the 15 healthy infants. Three of the four CMPA infants developed symptoms such as maculopapular rash and hypersecretion in the airways after BM challenge.

Conclusions: BLG can be detected in BM up to 7 days after CM ingestion. Lactating mothers should suspend breastfeeding to CMPA infants more than 7 days after CM ingestion.

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Introduction

Cow milk (CM), the first foreign food introduced to infants, is also the most important food allergen in infants. The prevalence of cow milk protein allergy (CMPA) in infants is 2–5 per cent [1]. Infants may be exposed to CM by direct ingestion or through consumption of human breast milk (BM) that contains CM protein after maternal ingestion of CM or dairy products [2,3]. A birth cohort study has estimated that the incidence of CMPA through mother's milk in exclusively breastfed infants is 0.5% [4]. CM protein in human milk has been shown to aggravate eczematous dermatitis [5] and food protein-induced enterocolitis syndrome [6] in CMPA infants. The current management of CMPA includes the avoidance of CM protein by lactating mothers and their infants, the selection of appropriate milk formulae and monitoring the child's growth [7].

The major CM allergens which cause the symptoms of CMPA are casein, α -lactalbumin and BLG. While casein and α -lactalbumin are natural components of human

milk, BLG is not present in human milk [1]. Therefore, the presence of BLG in human BM is derived from maternal consumption of CM produce. Current guidelines clearly state that nursing mothers of CMPA infants should avoid CM and other dairy produce [1,8]. However, nursing mothers might ingest CM accidentally. There is no clear recommendation on the duration of ceasing breastfeeding after accidental CM consumption by nursing mothers.

The objectives of this study were to evaluate the kinetics of the major CM allergenic protein, BLG, in human BM after ingestion of a controlled amount of CM, and the symptoms in CMPA infants after a BM challenge.

Methods

Subjects

During the 12-month study, 15 healthy lactating mothers of non-CMPA infants and four mothers of infants with CMPA were recruited from the BM clinic of Ramathibodi

Hospital, Bangkok. All participants gave written informed consent. The inclusion criteria were healthy mothers who continued breastfeeding their 0–12-month-old infants and were able to express BM at indicated timed intervals. CMPA infants were diagnosed by clinical histories of symptoms after exposure to CM, improvement of symptoms after elimination of CM from the diet and exacerbation of symptoms after CM challenge. Histories of atopic diseases in the lactating mothers were recorded.

Study protocol

To evaluate the amount and duration of BLG in BM, mothers of non-CMPA infants were instructed to avoid CM and CM products for 7 days before baseline BM collection and throughout study period. Baseline (time 0) BM samples were collected on the morning before mothers consumed 240 ml of pasteurised CM (Foremost, Thailand) in the clinic. BM samples were then collected at 1, 3 and 7 days after CM ingestion. In a number of cases, samples were also collected 3 and 6 h after CM ingestion. BM samples were stored at -70°C for further analysis. Mothers in this group continued to breastfeed their infants throughout the study period.

Human breast-milk challenge

Human BM challenge was performed in four CMPA infants. Infants were diagnosed with CMPA by clinical history, improvement of symptoms after the elimination diet and exacerbation of symptoms after CM challenge. Mothers of CMPA infants were instructed to avoid CM and CM produce for 7 days before ingesting 240 ml of pasteurised CM in the clinic. The challenge was undertaken at various time points (3–12 h) after mothers had ingested CM, depending on the time of breastfeeding. BM samples were collected immediately before breastfeeding to measure the BLG level. CMPA infants were then observed after breastfeeding for the presence of symptoms. The human milk challenge was declared positive if infants developed reactions similar to those following direct CM challenge at the time of diagnosis.

Determination of BLG in human breast-milk

A sandwich ELISA for BLG was developed in-house on the basis of a previous report by Makinen-Kijunen et al. [9]. Briefly, plates were coated with anti-BLG antibody (Bethyl Laboratories) and blocked with 1.5% gelatin (Bio-Rad). Standard BLG (Sigma-Aldrich) and BM samples were added in duplicate. Diluent, extensively hydrolyzed formula (Nutramigen, MeadJohnson Nutrition) and pasteurised CM (Foremost, Thailand) were used as blank, negative and positive controls, respectively. Horseradish peroxidase (HRP)-conjugated anti-BLG antibody (Abcam) was subsequently added. Tetramethylbenzidine (TMB) peroxidase substrate (KPL) was then added to each well.

The reaction was stopped using hydrochloric acid and the absorbance was measured at 450 nm (EZ Read 400 Microplate Reader, Biochrom). BLG concentrations in BM were calculated based on the BLG standard curve.

Statistical analysis

Descriptive analysis was used to report mean values and standard deviations (SD) of the data or the median and interquartile range (IQR) if the data were not normally distributed. Student's *t*-test was used to compare the BLG levels in BM from atopic and non-atopic mothers. Comparative analysis of BLG levels before and after consumption of CM was undertaken using paired Student's *t*-test or a Wilcoxon signed-rank test. Comparative analysis of BLG levels at different time points was undertaken using repeated measures ANOVA. A *p*-value < 0.05 was considered statistically significant. Data were analysed using SigmaPlot 12.

Ethics approval

The study protocol was approved by the Research Ethics Committee of the Faculty of Medicine, Ramathibodi Hospital, Mahidol University.

Results

During the 12-month study period, 19 healthy lactating mothers, 15 mothers of non-CMPA infants and 4 mothers of CMPA infants were recruited to the study on a voluntary basis. The mean (SD) age of the mothers was much the same in the two groups [32.73 (3.08) and 33.75 (2.36) years, respectively]. Nine of the 15 (60%) mothers of the non-CMPA infants had a history of atopic diseases and all mothers of the CMPA infants had a history of atopic diseases. All the atopic mothers had allergic rhinitis; there was no history of food allergy. The mean (SD) ages of the CMPA and non-CMPA infants were 9.8 (1.92) months and 5.87 (2.56) months, respectively.

β -lactoglobulin (BLG) level in the breast-milk of healthy lactating mothers of non-CMPA infants after CM ingestion

BLG levels in the BM of the lactating mothers of non-CMPA infants were measured at various time points from time 0 and 3 h to 7 days after ingesting 240 ml of CM. At baseline, after 1 week of a CM-free diet and before CM ingestion, the median level of BLG in their BM was 0.580 ng/ml (0.580 g/L) (IQR 0.38–0.88). After CM ingestion, the level of BLG in BM was significantly increased to a median peak level of 1.23 ng/ml (IQR 1.03–2.29, $p < 0.001$ (Figure 1). The level of BLG in BM significantly increased from baseline in both atopic and non-atopic mothers (Figure 2). However, comparison of the BLG level in BM from atopic and non-atopic mothers found no significant

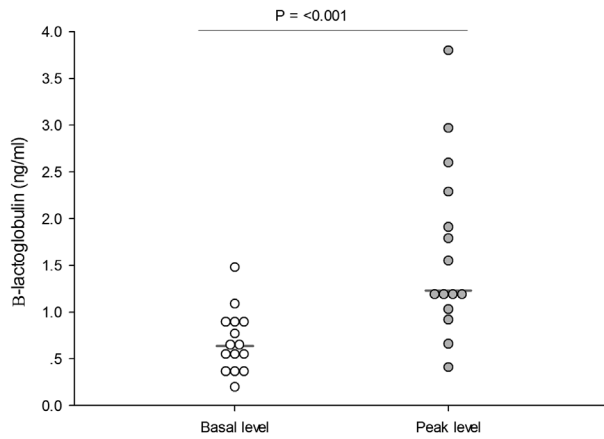


Figure 1. β -lactoglobulin level in breast-milk at baseline and peak level after ingestion of 240 ml of cow milk.

differences mean (SD) neither at baseline 0.8 (0.42) ng/ml vs. 0.6 (0.25) ng/ml nor at peak level 1.87 (0.96) ng/ml vs. 1.45 (0.93) ng/ml (Figure 2).

Kinetics of BLG in breast-milk after CM ingestion

The median BLG level was significantly higher 3 and 7 days after CM ingestion than at baseline, rising from 0.58 ng/ml at baseline (IQR 0.38–0.88) to 1.15 ng/ml (IQR 0.89–1.45, $p = 0.01$) and 1.08 ng/ml (IQR 0.86–1.25, $p = 0.001$) (Figure 3). However, there was great variability in the kinetics of BLG in BM in the study population. Peak levels of BLG were demonstrated at 3 h ($n = 1$) and 24 h ($n = 3$) and at 3 days ($n = 7$) and 7 days ($n = 5$) after CM ingestion (Figure 4).

Breast-milk challenge to CMPA infants

BLG was detected in BM from the four mothers of CMPA infants after ingestion of CM. After challenge with BM, three of four CMPA infants developed symptoms of CMPA (Table 1). However, one infant (C1) had no symptoms after BM challenge (Table 1). This was consistent with the infant's clinical history of being able to tolerate breastfeeding despite the mother's consumption of CM before breastfeeding.

Discussion

Most guidelines for CMPA recommend that lactating mothers of CMPA infants should avoid CM and dairy produce [1,8], but none advise on the duration of BM avoidance after accidental CM exposure in nursing mothers. This study demonstrated significantly increased BLG in human BM up to 7 days after maternal consumption of CM. In addition, breastfeeding with milk containing BLG elicited symptoms of CMPA in three of the four CMPA infants.

This study demonstrates the variation in the peak level of BLG in BM after CM consumption in each individual,

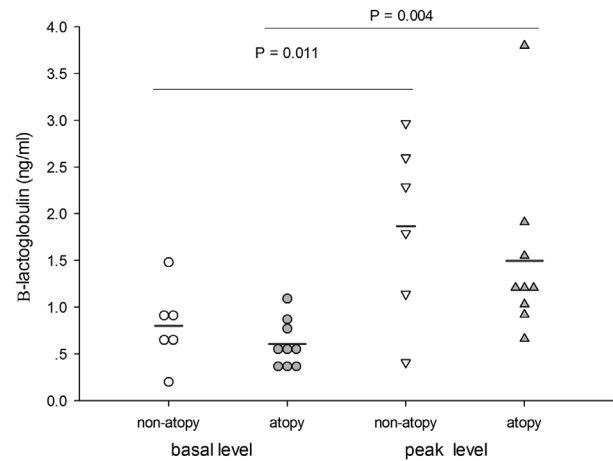


Figure 2. Comparison of β -lactoglobulin levels in breast-milk of atopic and non-atopic mothers at baseline and peak level after ingesting cow milk.

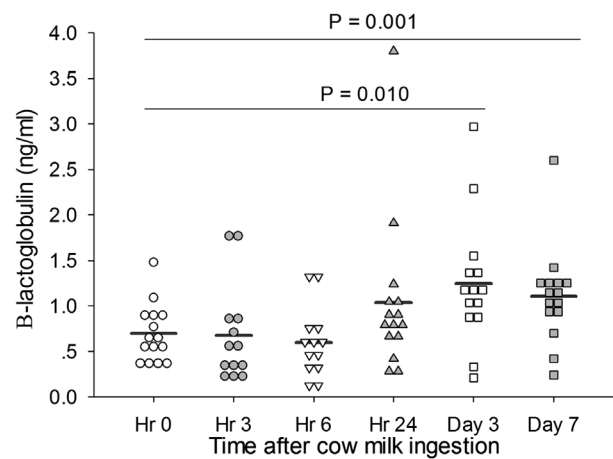


Figure 3. Comparison of β -lactoglobulin levels in breast-milk at baseline and at 3, 6 and 24 h and 3 and 7 days after cow milk ingestion.

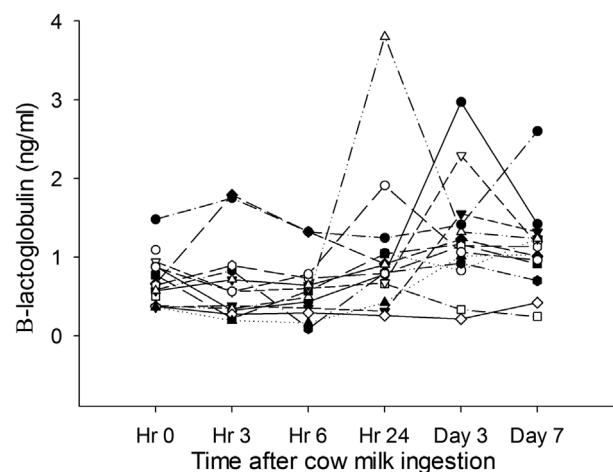


Figure 4. β -lactoglobulin levels in breast-milk of each subject at various time points after cow milk ingestion. Each symbol represents each subject.

Table 1. Human breast-milk challenge in four infants with CMPA.

Case no.	Presenting symptoms	Timing of challenge after maternal ingestion of cow milk, hrs	BLG levels in challenged breast-milk, ng/ml	Results	Breast-milk challenge reaction
C1	Urticaria	3	1.04	Pass	No reaction
C2	Hypersecretion* and eczema	12	1.47	Fail	Hypersecretion*
C3	Hypersecretion* and eczema	3	3.91	Fail	Hypersecretion*
C4	Hypersecretion* and atopic dermatitis	4	2.02	Fail	Maculopapular rash

*Excessive secretion in the airways.

ranging from 3 h to 7 days after CM consumption. Previous reports of the kinetics of BLG in BM after CM consumption examined the presence of BLG only up to 24 h [3,10–13]. While they found a similar variation in the timing of peak BLG among studied participants during their study period, BLG were detected in only 60–75% of BM samples. In this study, the majority of lactating mothers had a peak BLG level later than 24 h after CM ingestion which might partly explain the lack of BLG detection in some cases before 24 h in previous studies.

Previous studies have demonstrated that the level of BLG in BM after CM consumption is highly variable [3,10–13] and depends on study protocols including the CM wash-out time before CM exposure, volume of CM load and timing of BM collection. In this study, the peak BLG level in BM collected over a 7-day period was 1.23 ng/ml (range 0.41–3.80) after 1 week of a CM-free diet, followed by a single 250-ml CM consumption. Sorva et al. found increased BLG in BM of 0.12 ng/ml (range 0.01–7.84) from baseline of 0.01 ng/ml (0–3.5) after a 24-h wash-out period, followed by a 400-ml CM load [13]. Other studies with no wash-out period [4,11,12] with the volume of CM consumed varying from 236 ml to 500 ml demonstrated BLG in BM of 0–800 ng/ml at various time-points. The variability in BLG secretion in BM could be owing to the different efficiency and rate of CM digestion, CM absorption and BLG secretion in BM among different lactating mothers. Furthermore, the frequency of feeding and volume of BM fed at each feed might also affect the kinetics of BLG secretion in BM after CM ingestion. One possible explanation for the variation in intestinal absorption might be the mothers' atopic status as it has been previously reported that atopic individuals have increased intestinal permeability to macromolecules compared with non-atopic controls [14,15]. However, in this study, there was no significant difference in the levels of BLG in BM from atopic and non-atopic lactating mothers. This observation is consistent with previous studies that failed to observe significant differences in BLG and other food allergens in breast-milk between atopic and non-atopic lactating mothers [3,11].

The study has demonstrated that, in some mothers, BLG was detected in BM at least 7 days after CM ingestion. It is possible that BLG may be detected even after 7 days in these mothers, but BLG in BM beyond 7 days was

not measured. This may explain the small but detectable amount of BLG in BM at baseline before CM ingestion even after 7 days of avoiding CM. Further studies are needed to better determine the duration of BLG secretion in BM after CM ingestion by extending the study period beyond 7 days, until BLG is undetectable in all study participants. In addition to BLG, other major CM protein allergens such as casein can also be a causative allergen for CMPA infants. Recent studies using proteomic methods have detected casein in BM [16,17].

The level of BLG in BM which causes the symptoms of CMPA varies between infants. In the BM challenge study in the four CMPA infants, it was demonstrated that BLG of 1.47–3.91 ng/ml could exacerbate the symptoms of CMPA in infants C2, C3 and C4, which is comparable with a previous study that reported that a BLG range of 0.01–11.54 ng/ml could exacerbate symptoms [5]. CMPA infant C1 was the only subject who passed the BM challenge, perhaps because the level of BLG in the BM (1.04 ng/ml) was lower than the threshold to cause symptoms in this infant. Alternatively, the infant might be allergic to another CM allergenic component which is not secreted in a sufficiently high amount in BM and was not measured in this study.

The limitations of this study include the small sample size and that the study period was limited to 7 days. Furthermore, other CM allergens were not measured in the BM and component resolved diagnostics was not performed on CMPA infants to delineate which CM allergens cause their CMPA symptoms.

In conclusion, BLG can be detected in BM of lactating mothers for at least 7 days after a single ingestion of 240 ml of cow milk. To avoid exacerbating symptoms, lactating mothers should not breastfeed infants allergic to CM for at least 7 days after ingesting CM.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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