

Content of β -casomorphins in milk of women with a history of allergy

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The prevalence of food allergies increased over the past decade. Most symptoms of food allergy appear during the first 2 yr of life. The aim of this study was to determine the β -casomorphin-5 and -7 (BCMs) in colostrum and milk of 12 breast-feeding women with a history and clinical manifestation of food allergy. The results were compared with the data obtained from a control group of healthy age-matched breast-feeding women. The level of BCM in women with food allergy was constant during lactation, whereas the highest level of opioid peptides was found in colostrums of healthy women with a subsequent rapid decrease in mature milk. These differences in BCMs profile between allergic and healthy breast-feeding women suggest that BCM content in the human milk may be an indicator of allergic conditions.

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An increased risk of the development of atopic disease has been reported in infants whose parents had an evidence of atopy. Similarly, food allergy in a sibling or the parental history of allergy contributes to the risk in a child; however, the causal pathways of atopic dermatitis are unclear (1–3). Although human milk is considered as a hypoallergenic food, the proportions of breast-fed children demonstrating symptoms of atopic dermatitis are increasing (4). The allergy preventing effect of breastfeeding remains controversial and there are no consistent data regarding the degree to which breastfeeding prevents, reduces, delays or increases the development of allergic disease (2, 5). A number of studies have shown that, except for genetic factors, the composition of human milk was also associated with the development of atopic disease in breast-fed infants (6). Some reports suggested differences between allergic and non-allergic mothers in relation to milk concentration of immunologic agents involved in allergic reactions (7, 8).

β -Casomorphins are a specific group of biologically active milk peptides with opioid activity (9). Exogenous opioid peptides can exert their

physiologic effects by interactions with opioid receptors. The influence of exogenous opioid peptides on nervous, immune and cardiovascular systems has been widely described (9–12). Their content has been demonstrated in human milk (13, 14), bovine milk and milk products (15), but some of the mechanisms by which the exogenous opioid peptides can exert the physiologic significance still remain unclear (9). β -Casomorphins in gastrointestinal tract can exert a variety of functions, including motility, secretion and transport of electrolytes and fluids by the reaction with μ -opioid receptor (9, 10, 16). Some research suggests that the short opioid peptides are able to pass the immature gut-blood barrier (13, 17–19). Goody and Kitchen imply that the milk-derived β -casomorphin-like peptides could be responsible for the regulation of opioid receptor development and could elicit centrally mediated behavioral effects in rats pups (20). It has been demonstrated that the high concentration of β -casomorphin-7 stimulate the proliferation of the human peripheral lymphocytes (21). These data suggest that casein-derived opioid peptides content in the diet of newborn could play an important role in the regulation and

development of infants' gastrointestinal immunologic and neurologic functions (9, 17, 22). Different studies indicate that β -casomorphins could be a milk ingredient associated with pseudo-allergic reactions (23).

The aim of this study was to investigate the β -casomorphin-5 and -7 content in breast milk of mothers with history of allergy and to compare the result with healthy control group.

Materials and methods

Study participants

Samples of human milk were collected from 12 volunteer breast-feeding women with a mean age 26.7 years and with a documented history of allergy (skin tests, spirometry, total and specific IgE). In the allergic group, nine of 12 women had asthma, four were diagnosed with an allergy to bovine milk proteins and one had both symptoms. All women for a lengthy period were patients of the Allergy Clinic of the Independent Public Teaching Hospital of the Medical University of Biaystok. The control group stated 30 healthy age-matched women without any symptoms of allergy. Milk samples were collected in two phases of lactation – the first sample was colostrums (between 2nd and 3rd day from delivery) and the second sample was milk obtained 1 month after delivery.

All women gave written informed consent and the study was conducted with approval of the local ethical committee. Recruitment of women to this project was conducted before delivery during a class of prenatal education training. Data of their health (including prevalence of allergic disease) were assessed by a questionnaire filled out during the collection of the first milk sample at the Department of Obstetrics.

All women participating in the study gave term-birth and postpartum period without complications, lactation started 1–2 days after delivery. None of the infants was formula-fed.

All children in the control group were exclusively breast fed from 0 to 4 months of life; whereas in the group with a history of allergy, nine stopped breastfeeding before the fourth month of the child's life. The main reason was the onset of atopic dermatitis (AD) in infants. The symptoms were typical for AD. The characteristic lesions (papules with vesicles) were found on the face, trunk, arms and legs. The clinical features of AD in children were evaluated using the SCORAD index. The mothers from the allergic group tried a hypoallergenic milk-free diet before they discontinued breastfeeding but it

did not stop the symptoms. Hydrolyzed milk formulas (Nutramigen/Mead Johnson; Bebilon pepti 1/Ovita Nutricia) were used as a supplement to breastfeeding.

Among three children of the allergic mothers that were breastfed, two still suffered from atopic dermatitis. The alleviation to these symptoms was observed when mothers used hypoallergenic diets. In order to determine immunoglobulin E (total IgE) and specific antibodies (sIgE) against casein, α -lactoglobulin and β -lactoglobulin blood samples were taken from children of studied women.

Methods

Isolation of a peptide fraction. Peptides were extracted from milk according to the method of Harwalkar and Elliot. Milk was mixed with a methanol/chloroform mixture (2:1 v/v), left for 1 h on gentle shaking and centrifuged ($5000 \times g$ for 30 min). Peptide rich water/methanol layer was collected, evaporated and lyophilized.

Purification of a peptide extract. Peptide extracts were purified by solid phase extraction (SPE) with the use of C_{18} columns (STRATA C-18T, 140 Å, 50 μ , Phenomenex, Torrance, CA, USA). Peptide extracts were dissolved in 0.1% TFA and applied on columns previously equilibrated by aspiration of methanol and 0.1% TFA. Loaded column was washed with 10% acetonitrile in 0.1% TFA and peptides were eluted with 50% acetonitrile in 0.1% TFA. The eluate was collected, lyophilized, dissolved to a concentration of 10 mg/ml for HPLC analysis.

HPLC of purified peptide extract. Analysis was performed on the Shimadzu SCL-10Avp HPLC system with SPD-M10Avp diode-array detector (both from Shimadzu Corporation, Kyoto, Japan) and Phenomenex Jupiter Proteo (250 $\times$ 4.6 mm, 4 μ , 90 Å, Phenomenex, Torrance, CA, USA) column. Gradient elution was used with 0.1% TFA as phase A and acetonitrile with 0.1% TFA as phase B. Peaks were detected at a wavelength of $\lambda = 280$ nm. Qualitative and quantitative determination of β -casomorphins was performed by comparison with the standards (custom synthesized).

Results

The standard level (no more than 15 IU/ml) of IgE against casein, α -lactoalbumin and β -lactoglobulin has been determined in all blood samples from children from allergic group.

Table 1. Summary of results

Group	Control				Allergic			
Peptide	BCM-5		BCM-7		BCM-5		BCM-7	
Phase of lactation	C	M	C	M	C	M	C	M
No. of samples	30	30	30	30	12	12	12	12
Mean (μg/mL)	5.03	0.98	3.10	0.39	1.58	1.63	1.67	1.26
SEM	1.02	0.35	0.89	0.07	0.40	0.64	0.42	0.64

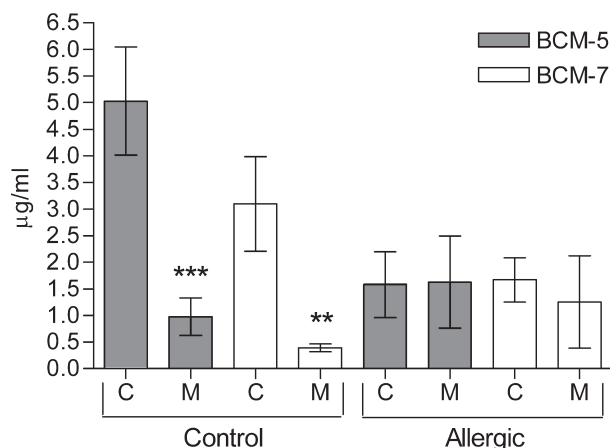


Fig. 1. Content of β-casomorphin-5 and -7 in colostrum and milk in allergic and control group (C, colostrum; M, milk; BCM-5, β-casomorphin-5; BCM-7, β, casomorphin-7).

Casomorphins were determined in triplicate for each sample in both groups (Allergic and Control) and both lactation phases (Colostrum and Milk). Summary of the results obtained is shown in Table 1 and Fig. 1. β-Casomorphins content in the allergic group was roughly similar (1.26–1.67 μg/ml) and there was no statistically significant difference in β-casomorphins content between phases of lactation for both investigated peptides. Results in the control group were more diversified – the content of β-casomorphin-5 and -7 in colostrum was significantly higher than in milk ($p < 0.001$ and $p < 0.05$ respectively; ANOVA, Statistica 6.0PL). Content of β-casomorphin-5 in colostrum in the control group was three times higher than in the allergic group.

It can be clearly seen that in the control group the content of both β-casomorphins is decreasing during the two investigated phases of lactation, when in allergic group β-casomorphins content is roughly constant.

Typical HPLC with overlaid standard was shown in Fig. 2.

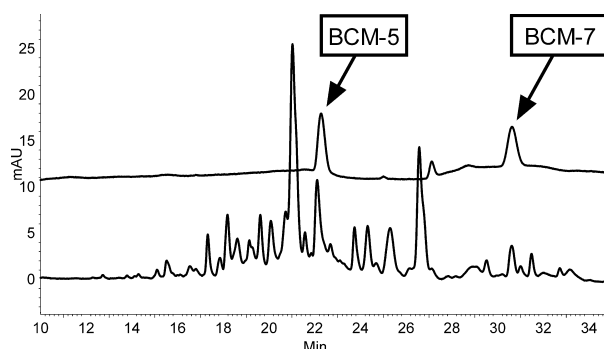


Fig. 2. Typical HPLC of peptide extract (Top line, standards; bottom line, sample; BCM-5, β-casomorphin-5; BCM-7, β-casomorphin-7).

Discussion

The conducted research has confirmed the presence of β-casomorphin-5 and -7 in colostrum and milk of women - both healthy (control group) and with history of allergy (allergic group). An interesting observation is that the content of both investigated casomorphins in the control group is decreasing during lactation but remains roughly constant in the allergic group (visible differences are not statistically significant).

Zandaro, Nicolussi et al. have determined a high level of β-endorphin in colostrum (from 4 to 7 pmol/l) and a lower level in mature milk (about 2 pmol/l). Those results are correlated with a high level of β-casomorphin in control group, but are inconsistent with results from allergic group. The concentration of β-casomorphin-5 in colostrum from the control group (7.69 pmol/l) was similar to the β-endorphin level in the colostrum of mothers after vaginal delivery (6–8 pmol/l). Whereas the concentration of β-casomorphin-7 in colostrum from control group (3.66 pmol/l) correlated with β-endorphin content in colostrum from mothers after cesarean section (about 4 pmol/l). The level of β-casomorphins in colostrum and milk of mothers with AD (mean 2.09 pmol/l) was significantly lower than β-endorphin content in colostrum irrespective of the method of delivery, whereas corresponding with β-endorphin level in mature milk (from 2 to 3 pmol/l). High level of both endo- and exogenous opioid peptides in colostrum may have functional role of postpartum adaptation of infants, lowering stress of birth and soothing down (24). The lower level of opioid peptides in colostrum of women with symptoms of atopic diseases may cause anxiety and weeping, which are also accompanied by allergic symptoms.

Elitsur and Luk have shown that β-casomorphins may also influence the function of intestine lamina propria lymphocytes. *In vivo*

incubation of stimulated lymphocytes with bovine β -casomorphin-7 caused naloxon-reversible inhibition of DNA synthesis. High contents of β -casomorphins in colostrum in the control group suggest that β -casomorphins may play a role of modulating the immune system of intestines by local inhibition of lymphocyte proliferation. Elitsur and Luk suggest that β -casomorphins inhibition of lymphocytes is result of their involvement in protein kinase C metabolic pathway (25).

The presented research indicates an important role of exogenous peptides of colostrum and milk, both by interaction with mi-opioid receptors of the immune system of intestines but also after crossing intestine-blood barrier – with receptors of many other cells in infant organism. Koch et al. have demonstrated the presence of β -casomorphin-8 immunoreactive material in plasma of lactating women (from 295 to 2020 fmol/ml), which has depended on the time from onset of nursing (19). These data suggest the ability of opioid peptides to pass the physiologic barriers, which corresponds with data concerning β -casomorphin-7 transport across the Caco-2 monolayer *in vitro* (26). One of the possible physiologic functions of β -casomorphin in neonate body is an exert in the pseudoallergic reactions. It has been found that the dose is dependent on *in vitro* histamine release from peripheral leukocytes of healthy adults and children after β -casomorphin-7 intradermal injection. The observed wheal and flare reactions in the skin were similar to test reactions with histamine or codeine. Oral pre-treatment with the H1 antagonist significantly inhibited the skin responses to β -casomorphin-7 that prove that histamine is the most important mediator of these reactions (27, 28). The involvement of opioid receptors seems not to be clear and needs further study (29). The same authors have shown secretagogue effects on rodents' mast cells after preincubation with synthetic β -casomorphin-7 as well as histamine release from peripheral blood leukocytes *in vitro* (23, 30). The mechanism of interactions between opioid peptides and the cells of the immune system requires further investigation. In the case of children suffering from allergy disease the gut epithelial barrier is more permeable for food compounds (31, 32). These data suggest that after crossing the gut-blood barrier β -casomorphins can exert an effect as a direct histamine releaser and intensify the skin reactions.

In this study, we have demonstrated the lower content of β -casomorphins content in colostrum

and milk of mothers with history of allergy and no protecting effects of exclusively breastfeeding in infants with the history of atopic. Breastfeeding is widely accepted as the best source of nutrition for newborns, hence further studies are necessary to evaluate the dependencies between nutritional and inflammatory factors in milk of healthy and allergic women.

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