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Colostrum TGF- β -1 associates with the duration of breast-feeding

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Abbreviations: BF: breast-feeding

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■ **Abstract** *Background* Several stressful environmental factors are associated with short-term breast-feeding. A high concentration of sodium in colostrum has predicted early failure. *Aim of the study* We studied the association of growth factors in colostrum and the length of breast-feeding (BF). *Methods* We measured concentrations of TGF- β 1 and - β 2; epidermal growth factor, total protein, and sodium and compared their concentrations in colostrum samples from mothers who either breast-fed their infants exclusively less than 0.5 months ($n = 109$) or longer than 3.5 months ($n = 119$). *Results* In the short BF group more mothers smoked and were primiparous more frequently and had less often a university education. They also provided the colostrum samples significantly later than did those

with long BF. Geometric mean concentration for TGF- β 1 was 1.9 times as high in the samples from short BF mothers as in those with long BF; significant difference remained in comparisons of samples taken equally long postpartum. Samples from the short BF group showed higher levels for sodium, TGF- β 2 and total protein, whereas concentrations of epidermal growth factor were similar between groups. *Conclusions* We thus infer that concentrations of factors in breast milk with an effect on the development and involution of the mammary gland, like TGF- β 1 in milk, may be one of many biological factors having an impact on the successful initiation of breast-feeding.

■ **Key words** breast-feeding – transforming-growth-factor- β – epidermal growth factor

Introduction

Hormonal changes during late pregnancy responsible for the initiation of lactation are well established [8, 11, 12, 14]. In addition to circulating hormones, locally produced growth factors, epidermal growth factor (EGF), the insulin-like growth factor family, and transforming growth factor- β (TGF- β), play important roles both in maintaining lactation and in the involution of the mammary gland associated with cessation of lactation [5, 15].

Insufficient emptying of the breast, followed by stasis of milk and local production of factors inhibiting milk secretion all lead to involution of the mammary gland at weaning [8, 11, 12, 14]. Several environmental factors causing stress in breast-feeding mothers are implicated in failure of lactation [2, 6]. Such factors lead to diminished secretion of oxytocin and impaired ejection reflex. Milk stasis causes increased paracellular permeability of the epithelial lining, resulting in increased concentration of sodium in the milk [8]. Thus far, an increased level of sodium

in milk is the only change in breast milk predicting short breast-feeding [9, 13], and even it is suggested to be secondary to milk stasis.

In a study on development of cow's milk allergy in healthy newborn babies, we collected colostrum samples from the mothers [16]. In the analysis of breast milk components and of development of allergies by age 4, we noted that TGF- β levels were different in groups with either short or long breast-feeding [17]. Here we show that levels of TGF- β in colostrum samples from mothers breast-feeding their infants for several months are lower than in samples from mothers whose infants required early formula feeding.

Materials and methods

Subjects

We studied the effect of supplementary milks given in the delivery hospital upon development of cow's milk allergy among 6,209 healthy newborn infants. All participants were born at or after 37 weeks' pregnancy [16]. From mothers in this group we collected colostrum samples during 1 to 5 days postpartum. They were urged to express a small quantity of foremilk in a container. These samples were kept at -70°C until processed. The Ethics Committee of Hospital for Children and Adolescents, University of Helsinki, approved the study.

We had complete information on the mode of feeding during the first year of life and on the allergic diseases of parents from 4,674 families; among them four groups were selected based on the presence or absence of a family history of atopy, and on the early milk-feeding pattern of each child. Groups were defined according to family history of atopy in one or both parents. Atopic and non-atopic groups were further divided according to early feeding pattern either into a short (<0.5 month) or long (>3.5 months) exclusive breast-feeding group. Colostrum samples were available from 228 mothers from these groups. These mothers' own allergy had no influence on levels of cytokines or other variables measured [17]. In the present study, the groups compared were those mothers who exclusively breast-fed their infants for less than 0.5 month and those with exclusive breast-feeding exceeding 3.5 months.

Colostrum collection

Samples of colostrum were frozen within 12 h of collection and kept frozen at -70°C . After thawing, the sample was centrifuged at 10,000 g for 30 min, the densest layer that contains cells and membranes and

fat layer were discarded and the clear middle layer was used for analyses. We had the date of birth and of the collection of colostrum samples; the time of collection could be calculated only in days.

Measurements of TGF- β 1 and - β 2, epidermal growth factor (EGF), total protein, and Na

Concentrations of TGF- β 1 and - β 2 in colostrum were measured with the Quantikine[®] Human TGF- β 1 and TGF- β 2 Immunoassays (R&D Systems, Inc. Minneapolis, Minn, USA). Activation of TGF- β 1 in colostrum was performed as described for cell culture supernatants with 1N HCl (1/5 the sample volume) for 10 min and neutralized with 1.2 N NaOH/0.5 M HEPES (11, 12). Quantikine, EGF was measured with Human EGF Immunoassay[®] produced by R&D Systems Europe Ltd. (Abingdon, UK)

Statistical analysis

All measurements were transformed to logarithmic values to correct for the non-normal distribution. Geometric means and their 95% confidence intervals were calculated for the study groups. Short and long breast-feeding groups were compared with the *t*-test by use of the SPSS version 12.0.1 (SPSS Inc., Chicago, IL, USA). Levels of TGF- β 1 and -2 of mothers giving the colostrum sample on 1–2, 3 or 4–5 days postpartum were compared in the short and long BF groups by oneway ANOVA test.

Results

Because groups were based on length of breast-feeding; the lengths of both exclusive and total breast-feeding were significantly longer in the long breast-feeding group (Table 1). In the long BF group, the mothers smoked less frequently, they had more fre-

Table 1 Characteristics of mothers in groups with short and long breast-feeding

	Short BF (<i>n</i> = 109)	Long BF (<i>n</i> = 119)	<i>P</i>
Mean duration of exclusive BF (months)	0.2	4.4	0.0001*
Mean duration of total BF (months)	5.5	10.3	0.0001*
Smoking mothers	17 (16%)	4 (3.4%)	0.001†
Symptoms of atopy	52 (48%)	57 (48%)	NS
Asthma	20 (18%)	16 (13%)	NS
University education	28 (28%)	50 (46%)	0.02†
Primiparous	29 (27%)	16 (13%)	0.03†

* *T*-test; † Chi-square test

Table 2 Collection days of colostrum samples and geometric mean concentrations (ng/ml) of TGF- β 1 in samples collected 1–2, 3, or 4–5 days postpartum; in those from primiparous or multiparous, and in those from smoking and non-smoking mothers

	Short BF	Long BF	P
Collection of colostrum sample, 1–2 days postpartum	19 (18%)	63 (53%)	0.0001*
3 days postpartum	57 (52%)	42 (35%)	
4–5 days postpartum	32 (30%)	14 (12%)	
TGF- β 1 1–2 days postpartum; geo-metric mean (95% confidence interval) <i>n</i>	630 (497;799) ^a 19	313 (246;398) ^b 63	0.0001†
TGF- β 1, 3 days postpartum	540 (402;725) ^a 57	373 (301;463) ^b 42	0.052†
TGF- β 1, 4–5 days postpartum	660 (508;860) ^a 32	242 (149;391) ^b 13	0.002†
TGF- β 1, primiparous	512 (337;780) 29	288 (207;401) 16	0.041†
TGF- β 1, multiparous	616 (509;746) 79	331 (277;396) 102	0.001†
TGF- β 1, smokers	692 (432;1108) 17	355 (156;808) 4	0.2†
TGF- β 1, non-smokers	575 (474;699) 91	324 (275;381) 114	0.001†

* Kruskal-Wallis test, † T-test

Values on the same column with the same superscript did not differ by oneway ANOVA test (for ^a $P = 0.6$, for ^b $P = 0.3$)

quently higher education and were more frequently multiparous. The mothers with short breast-feeding gave the colostrum samples significantly later than those who subsequently breast-fed their infants for a long period (Table 2).

The geometric mean concentration of TGF- β 1 was 1.9 times as high in the colostrum samples from mothers in the short BF group ($P = 0.0001$) (Fig. 1). The difference for TGF- β 2 was smaller; 1.5 times as high in colostrum samples in the short BF group ($P = 0.002$) (Fig. 1). Groups' mean concentration of EGF was similar.

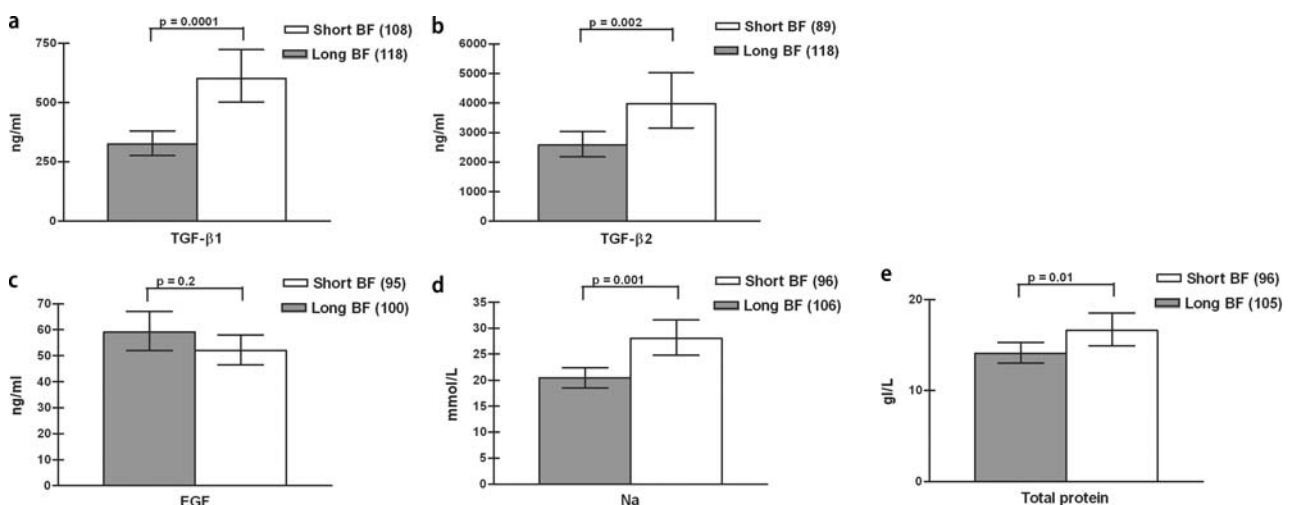
Sodium concentration was significantly higher in the short BF group ($P = 0.001$) (Fig. 1). Concentrations of all measurements were similar in the samples from smoking and non-smoking mothers in short and long BF groups (data for TGF- β 1 in Table 2, other data not shown).

To rule out the possibility that the differences in TGF- β 1 or TGF- β 2 were due only more concentrated

secretion, the relation of TGF- β 1/total protein and TGF- β 2/total protein were compared for groups; those differences were significant ($P = 0.0001$ and 0.02, respectively).

When the time of collection of colostrum samples differed between groups, we compared samples taken during the same post-delivery day in the groups. : The mean level of TGF- β 1 was higher in the short breast-feeding group also in the samples collected at the same time postpartum (Table 2). A similar difference between samples taken equally late post-partum remained for sodium (data not shown), but was lost for TGF- β 2.

Levels of TGF- β 1 and -2 (data not shown) in colostrum samples taken 1–2, 3 or 4–5 days postpartum similar did not differ in the short and long BF groups as tested by oneway ANOVA (Table 2). Mean levels of TGF- β 1 and - β 2 (data not shown) were similar in primiparous and multiparous mothes in long and short BF groups (Table 2).

**Fig. 1** Geometric means and 95% confidence intervals of concentrations of TGF- β 1 (A), - β 2 (B), EGF (C), sodium (D), and total protein (E), in the colostrum samples of mothers with short or long breast-feeding. Statistical significance of differences between groups and numbers of samples measured indicated

Discussion

To our knowledge, we for the first time report that a concentration of a major growth factor in human milk, TGF- β 1, in early colostrum samples associated with the length of breast-feeding. Colostrum samples from mothers able to exclusively breastfeed their infants for over 3.5 months contained significantly less TGF- β 1 than did samples from mothers whose exclusive breast-feeding duration was less than 0.5 months. Earlier, persistently high concentration of sodium in colostrum has been associated with impeding cessation of lactation and thus short breast-feeding [9]. Similarly, in the present study sodium in colostrum was significantly higher in samples from mothers who breast-fed their infants for only a short time. Mean levels of both groups fall well within the reference values for milk sodium at 2–4 days postpartum [13]. In some mothers with initially high colostrum sodium levels rigorous regimen of breast-pumping reduced levels to normal [13]; whether such intervention could be effective for mothers with high TGF- β 1 levels and prevent early cessation of breast-feeding, should be studied. Mothers who were able to breast-feed their infants for a long period, provided their samples in the delivery hospital significantly earlier than did those whose exclusive breast-feeding time was short. This could have affected lower levels of sodium in the short breast-feeding group, as the sodium concentration falls with duration after delivery [11], but when we compared samples taken at the same time after delivery, the difference remained between short and long breast-feeding group. Levels of TGF β -1 were stable during the first 5 days postpartum, when the collection of samples was completed. Smoking mothers on the average breast-feed for a shorter time than non-smokers [18]. We also had more smoking mothers in the short breast-feeding group. In that group, however, smoking was not associated with any change in the measured parameters. Suboptimal breast-feeding was also associated with primiparity [2]; parity did not associate with any of our measured parameters, we found their levels to be similar in primiparous and multiparous mothers. In cows, experimental bacterial mastitis caused an increase in TGF-beta1 and -2 con-

centrations [1]. Mastitis occurred in 24% of Finnish mothers [3], but if properly treated, seldom results in preterm cessation of breast-feeding [19]. We do not have knowledge on the occurrence of mastitis in our study group, but the possibility that bacterial mastitis had affected TGF-beta concentrations is unlikely as samples in the present study collected early, within 5 days of delivery.

TGF β isoforms, particularly 1 and 3, are important both in developing and in involution of the mammary gland [10, 15]. In the developing mammary gland, TGF- β s inhibit ductal development. During lactation they are down-regulated, and the expression of TGF- β 1 increases with the rate of involution and apoptosis of alveolar epithelium [10, 15]. TGF- β 1 and 2 are also important for suckling offspring; in an lethal model for TGF- β knockout mice, suckling mice were rescued by receiving breast milk containing TGF- β [7].

In human studies the levels of TGF- β has been associated with development of immune responses to food antigens [4]. Our present finding suggests that colostrum TGF- β 1 contributes to the ability to initiate and successfully continue breast-feeding; high concentrations associate with failure to established long, exclusive breast-feeding. The great overlap of our extreme groups for length of breast-feeding in a large cohort shows, however, that TGF- β 1 may be one of many biological factors leading to failure of established breast-feeding.

Epidermal growth factor is a survival factor for mammary epithelium in in vitro studies [5, 20]. Concentration of one growth factor, EGF, known to enhance epithelial growth of mammary gland and thus contrasting with TGF- β ; behaved in a opposite way from that of TGF- β , being insignificantly higher in those mothers whose breast-feeding time was long. Up to now, failure to establish and continue breast-feeding has been associated with stressful experiences of the mother which lead to diminished oxytocin secretion, stasis of milk, with subsequent diminished prolactin secretion, and failure to satisfy the needs of the baby [6]. We infer that primary biological differences between mothers may also play a role in the success or failure of breast-feeding.

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