

# Neutral oligosaccharides in colostrum in relation to maternal allergy and allergy development in children up to 18 months of age

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Several recent studies have demonstrated a relationship between the composition of the gut microbiota in infancy and subsequent development of allergic disease. Human milk is the major food in infancy and may thus profoundly influence the composition of the gut flora. Oligosaccharides in breast milk survive the passage through the stomach and are utilized by the gut microbiota. As the relationship between breast feeding and childhood allergy is controversial we hypothesized that the composition of oligosaccharides in breast milk might explain the controversy. Nine of the most abundant neutral oligosaccharides in human milk were analysed in colostrum samples from allergic and non-allergic women and related to subsequent development of allergy in their children. The carbohydrate fraction of the colostrum was separated by gel permeation chromatography and neutral oligosaccharides, tri- to hexasaccharides were collected. Neutral oligosaccharides were analysed with high-performance liquid chromatography. There was a large variation in the concentration of neutral oligosaccharides in colostrum, which could not be explained by the allergic status of the women. Allergic children consumed higher amounts of neutral oligosaccharides in total, although not significantly ( $p = 0.12$ ). When different oligosaccharides were analysed separately, there was no significant difference in consumption between the infants who developed atopic allergy later ( $n = 9$ ) and infants who did not ( $n = 11$ ). Thus, the amount of neutral oligosaccharides in colostrum does not directly correlate with maternal allergy, nor with allergy development in children up to 18 months of age.

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**Abbreviations:** 2'-FL, 2'-Fucosyllactose; 3-FL, 3-Fucosyllactose; HPLC, high-performance liquid chromatography;  $\gamma$ -IFN, interferon- $\gamma$ ; LDFT, lactodifucotetraose; LNDFH I, lacto-N-difucohexaose I; LNFP I, lacto-N-fucopentaose I; LNFP II, lacto-N-fucopentaose II; LNFP III, lacto-N-fucopentaose III; LNT, lacto-N-tetraose; LNnT, lacto-N-neotetraose; TFAN, 4-Trifluoroacetamidoaniline.

The intestinal microflora comprises approximately  $10^{14}$  micro-organisms and has been suggested to be important for the development of a normal immune system (1–3). The mucosal epithelium of the gastrointestinal tract is the largest surface of the human body where interaction between microbial antigens and the immune system occurs (4). Böttcher et al. showed that microflora-associated characteristics, as assessed

in the faeces, differ between allergic and non-allergic infants (5). An explanation for these differences could be given from studies showing that allergic children were less often colonized with bifidobacteria, lactobacilli and enterococci but had higher counts of clostridia and *Staphylococcus aureus* (2, 6, 7).

Probiotic bacteria is defined as 'a live microbial feed supplement which beneficially affects the host animal by improving its intestinal balance' (8). Different strains of bifidobacteria and lactobacilli are normal components of the human intestinal flora and the most frequently used probiotics (9). Several studies on diarrhoea have shown that probiotic bacteria could be beneficial (reviewed in 9). Probiotic bacteria might also be able to strengthen the gut defence barrier mechanisms that often are defective in children with atopic eczema and food allergy (3). Furthermore, experimental studies indicate that probiotics induce T-helper cell 1 and T-regulatory cell type of cytokines, such as  $\gamma$ -interferon (IFN- $\gamma$ ), interleukin-12 and interleukin-10 (3, 10–12). Recent investigations also indicated that *Lactobacillus GG*, given to mothers and their infants, prevented atopic eczema in the latter (13).

Human milk is the ideal nutrition for infants. Apart from containing optimal proportions of nutrients, it contains several components which compensate for the developmental delay in the infant's immune system (14). Of special importance are secretory immunoglobulin-A, lactoferrin, lysozyme, oligosaccharides and some cytokines (14). Yet, the reported associations of breast feeding with a protective effect for allergic disease are often contradictory (15). Both protective and allergy-promoting effects of breast feeding have been reported. While potential confounding may account for some of this variation between studies, it seems unlikely that this is the entire explanation. An alternative explanation could therefore be that the immunomodulatory components in breast milk differ between mothers and affects the immune development of the newborn child. Some of these immunomodulatory components, including interleukin-4, seem to vary depending on the allergy status of the mother (16, 17). Moreover, the concentrations of some polyunsaturated fatty acids seem to be lower in the breast milk of allergic mothers (17).

Oligosaccharides are the third most abundant solid component in breast milk after lactose and lipids (18, 19). A considerable amount of the human milk oligosaccharides reaches the colon intact and is utilized by gut microbes (18). A recent study showed that human milk oligo-

saccharides enhanced the growth of *Bifidobacterium infantis in vitro* to a considerably higher degree than other carbohydrates, such as glucose and inulin (20). However, the growth of *Lactobacilli gasseri* was not induced to the same extent, indicating that human milk oligosaccharides might affect the growth of different gut microbiota. Interestingly, the monosaccharide *N*-acetylglucosamine, which is a component in several neutral oligosaccharides, seems to be essential for the growth of a subspecies of *Bifidobacterium bifidum* (9, 18). This is one of the gut microbiota, which appears to be more abundant in healthy infants than in allergic children (21). About 80–90% of the carbohydrate content of human milk consists of lactose, the rest are neutral and acidic oligosaccharides (18). The neutral oligosaccharides make up the largest proportion of oligosaccharides, about 75–85% (22). The synthesis of milk oligosaccharides takes place in the mammary acinar cells by enzymes which are under genetic control (23). In humans, the synthesis of large and complex oligosaccharides occurs through elongation of lactose (24). The most studied and abundant neutral oligosaccharides found in human milk are fucosylated tri- to hexasaccharides: 2'-fucosyllactose (2'-FL), 3-fucosyllactose (3-FL), lactodifucotetraose (LDFT), lacto-N-fucopentaose I–III (LNFP I–III) and lacto-N-difucohexaose I (LNDFH I) and their precursors: lacto-N-tetraose (LNT) and lacto-N-neotetraose (LNnT) (19, 24, 25). About 20% of the Caucasian population are non-secretors and therefore lack the ability to secrete certain fucosylated oligosaccharides. The  $\alpha$ 1,2-linked fucosylated oligosaccharides 2'-FL, LNDFH I, LDFT and LNFP I are missing in the breast milk of non-secretors (24).

As oligosaccharides in breast milk influence the infant gut flora and as several studies show that allergic children are less often colonized with lactobacilli and bifidobacteria, we hypothesized that the amount of neutral oligosaccharides in breast milk could affect the development of allergy in breast-fed babies. We therefore analysed the neutral oligosaccharide content in colostrum samples, relating the amounts to the allergy status of the mother and to the allergy development in children up to 18 months of age.

## Subjects and methods

### Study population

Mothers attending the Antenatal Health Care Centres in Linköping August 1993 to March 1996 and in the beginning of 2004 were invited to

participate in a prospective study of the development of atopic symptoms in relation to environmental factors and maternal immunity. The children were born between January 1994 to July 1997 and June to October 2004. All children were delivered at term and they had an uncomplicated perinatal period. Mothers who breastfed their babies for < 3 months were excluded.

Clinical examinations and skin prick tests (SPT) against fresh hen's egg, milk and extracts from cat and peanut (ALK, Hørsholm, Denmark) were carried out at 6, 12 and 18 months of age, and whenever allergic symptoms were suspected. At these appointments the parents also completed a questionnaire regarding clinical symptoms, nutrition and allergen exposure to pets on behalf of their babies.

The diagnosis of atopy in the parents was based on a convincing clinical history of bronchial asthma, allergic rhinoconjunctivitis, atopic eczema and food allergy. The mothers were classified as allergic if they showed clinical symptoms of allergic disease and had circulating immunoglobulin-E (IgE) antibodies against a panel of common allergens, measured with an allergy screen test (Magic Lite™; ALK, Hørsholm, Denmark) or Phadiatop (Pharmacia CAP System RAST FEIA; Pharmacia Diagnostics AB, Uppsala, Sweden). Mothers not reporting clinical symptoms of allergy and having a negative allergy screen test or Phadiatop were classified as non-allergic.

Children were classified as non-allergic when they did not show any symptoms of allergy and had a negative SPT. Children were classified as allergic if they showed clinical symptoms of allergic disease.

The SPT was considered to be positive if the mean diameter of the wheal reaction was  $\geq 3$  mm. Atopic eczema was defined as pruritic, chronic or chronically relapsing dermatitis with typical morphology and distribution. Food allergy was defined as a positive SPT, combined with a positive clinical history of immediate skin and/or gastrointestinal reactions or atopic symptoms upon exposure to a certain food and clinical remission of atopic symptoms on an exclusion diet. Breast feeding was defined as exclusive when all cows milk formula, except for extensively hydrolysed formula (i.e. Nutramigen™; Bristol Meyers, New York, NY, USA), were avoided.

From the original study population, we randomly selected 20 women (11 allergic and nine non-allergic) and 20 children. Eleven of these children were non-allergic and nine showed symptoms of atopic eczema, food allergy or both (Table 1).

Table 1. Clinical description of the allergic mothers and the allergic children included in the human milk oligosaccharide study. Clinical history of atopic disease, allergens inducing allergic symptoms upon exposure and appearance of IgE antibodies are given

| No.               | Sex  | Clinical history | Allergens    | IgE antibodies* |
|-------------------|------|------------------|--------------|-----------------|
| Allergic mothers  |      |                  |              |                 |
| 1                 |      | AE, ARC, AB      | Pets         | Positive        |
| 2                 |      | AE, ARC          | Pollen       | Positive        |
| 3                 |      | AB               | Pets         | Positive        |
| 4                 |      | ARC              | Pollen       | Positive        |
| 5                 |      | ARC              | Pollen       | Positive        |
| 6                 |      | ARC              | Pets, pollen | Positive        |
| 7                 |      | AE, ARC          | Pets, pollen | Positive        |
| 8                 |      | AE, ARC          | Pollen       | Positive        |
| 9                 |      | ARC              | Pollen       | Positive        |
| 10                |      | AB, AE           | Pets, horse  | Positive        |
| 11                |      | AB               | Pets, pollen | Positive        |
| Allergic children |      |                  |              |                 |
| 1                 | Boy  | AE, FA           | Egg, milk    | Positive        |
| 2                 | Girl | FA               | Egg          | Positive        |
| 3                 | Boy  | AE               | Cat, egg     | Positive        |
| 4                 | Boy  | AE               |              | Negative        |
| 5                 | Girl | AE               |              | Negative        |
| 6                 | Boy  | AE, AB           |              | Negative        |
| 7                 | Girl | AE               |              | Negative        |
| 8                 | Girl | AE               |              | Negative        |
| 9                 | Girl | FA               | Egg          | Positive        |

AB, asthma bronchiale; AE, atopic eczema; ARC, allergic rhinoconjunctivitis; FA, food allergy.

\*IgE antibodies in mothers were detected by Allergy Screen or Phadiatop and in children by skin prick test.

#### Collection of milk samples

Breast milk (5–20 ml) was collected by a mechanical breast pump (Arta Plast, Stockholm, Sweden) in conjunction with the second meal of the day, i.e. late in the morning at 2–4 days postpartum. The baby was allowed to suckle the nipple for about 2 min before collecting the breast milk sample. The milk samples were frozen at  $-20^{\circ}\text{C}$  and stored until transport. The samples were transported on dry ice. All analyses were blinded.

#### Extraction of carbohydrates from human milk

The milk samples were thawed, shaken and centrifuged at 680 *g* for 10 min followed by a 30-min centrifugation at 10,000 *g* to remove fat. Extraction of carbohydrates from human milk was performed, in part, as described by Sumiyoshi et al. (25). A portion of each milk serum (1 ml) was extracted with 4 ml of chloroform-methanol (2:1). The emulsion was centrifuged at 1500 *g* for 30 min at room temperature and the upper layer, containing the carbohydrates and the salts, was collected. The carbohydrate fraction was evaporated and dissolved in 3 ml of  $\text{H}_2\text{O}$ . A mixed bed ion exchange resin (Amberlite monobed resin MB-1 and MB-3; BDH Ltd, Poole, UK)

was added to remove salts and acidic oligosaccharides. The resin was filtered off and the remaining neutral carbohydrate fraction was freeze-dried.

#### Gel permeation chromatography

The carbohydrate fraction obtained by the treatment described above was separated into five pools by gel filtration as follows: Freeze-dried carbohydrates were dissolved in H<sub>2</sub>O, filtered and applied to a size exclusion column (2.6 × 100 cm) (Bio Gel P-2, 45–90 µm; Bio-Rad, Hercules, CA, USA). The column was eluted with H<sub>2</sub>O at a flow rate of 28 ml/h and fractions of 4.7 ml were collected. To avoid bacterial growth in the column, 0.05% trichlorobutanol was added to the water prior to elution. Aliquots (200 µl) of each fraction were analysed for carbohydrates by a modified Anthrone method (26) as follows: Each aliquot was diluted with 0.8 ml H<sub>2</sub>O and 2 ml anthrone reagent (1 g/500 ml conc. H<sub>2</sub>SO<sub>4</sub>) was added. The samples were heated for 5–10 min in a water bath at 100°C and cooled to room temperature. The absorbance was measured at 610 nm. Peak fractions, containing tri- to hexasaccharides were pooled and freeze-dried.

#### Quantification of neutral oligosaccharides

High-performance liquid chromatography (HPLC) was used for the quantification of neutral oligosaccharides. A HPLC gradient system (Waters 600; Waters, Milford, MA, USA) equipped with a UV-Vis variable wavelength detector (Waters 448; Waters) was used for analysis of derivatized samples. For analysis of underivatized samples the same pump was used equipped with a differential refractometer (Waters 410; Waters).

A chromophoric group, 4-trifluoroacetamido-aniline (TFAN) was introduced to yield a better quantification of the small amount of oligosaccharides. We mainly followed the procedures of Kallin et al. (27). This method gives a relatively high yield, 76–88% for different oligosaccharides from human milk. The freeze-dried fractions containing oligosaccharides were dissolved in 0.5 ml H<sub>2</sub>O. Aliquots corresponding to approximately 1 mg of oligosaccharides were mixed with 100 µl TFAN reagent (10.2 mg TFAN in 1 ml ethanol) and 100 µl of the reducing agent sodium cyanoborohydride (6.3 mg NaCNBH<sub>3</sub> in 1 ml ethanol). Two microlitres of concentrated acetic acid was added to obtain pH 6, and the mixture was stirred at ambient temperature for 15 h. To

obtain a more stable product 5 µl of acetic acid anhydride was added. The mixture was stirred again at room temperature for 3 h. After evaporation, the derivatives were dissolved in 400 µl H<sub>2</sub>O, filtered and 10 µl was injected into a reverse-phase HPLC (Genesis, Grace Vydak, Hesperia, CA, USA, C18 column, 4 µ, 4.6 × 250 mm). The derivatives were eluted, at a flow rate of 1 ml/min, with 13–14% acetonitrile (isocratic system) as eluent. The detection was observed at 254 nm. Each component was identified by comparison with an authentic derivatized reference oligosaccharide. Quantification of each oligosaccharide was done by calculating the ratio of the peak area with that of an internal standard, maltotetraose. The maltotetraose standard (100 µl) (5 mg/ml) was added to the oligosaccharides prior to derivatization.

*Reference oligosaccharides.* The reference oligosaccharides 2'-FL, 3-FL, LDFT, LNT, LNnT, LNFP I, LNFP II, LNFP III and LNDFH I were purchased from Isosep AB (Tullinge, Sweden). Lactose was also used as reference and maltotetraose (Sigma, St Louis, MO, USA) was used as the quantification standard.

The oligosaccharides LNT and LNnT have the same molecular weight and are hard to separate from each other when derivatized with TFAN. Therefore, we did quantification with the non-derivatized oligosaccharides. We used the same reverse-phase HPLC column (see above). Twenty microlitres was injected into the HPLC and the oligosaccharides were eluted, at a flow rate of 1 ml/min, with water as eluent. A differential refractometer was used for detection of oligosaccharides. The identification was performed by comparing the relative retention time of each component with that of an authentic reference oligosaccharide. The ratio between LNT and LNnT was calculated and a quantitative value was obtained. The same procedure was repeated to separate LNFP I from LNFP II and LNFP III.

#### Statistics

For independent, nonparametric comparison of oligosaccharides in samples from allergic and non-allergic mothers as well as from allergic and non-allergic children, the Mann-Whitney *U*-test was used. Values of *p* < 0.05 were considered significant.

#### Ethical considerations

The local Ethics Committee at the University Hospital, Linköping, Sweden, approved the study.

Table 2. Concentrations of neutral oligosaccharides in colostrum from allergic and non-allergic mothers

|              | Non-allergic mothers<br>(n = 9) |            | Allergic mothers<br>(n = 11) |            | p-value |
|--------------|---------------------------------|------------|------------------------------|------------|---------|
|              | Median (g/l)                    | Range      | Median (g/l)                 | Range      |         |
| In total     | 7.53                            | 4.90–13.90 | 8.67                         | 5.97–11.86 | 0.34    |
| LNDFH I      | 0.99                            | 0.00–2.45  | 1.22                         | 0.65–2.63  | 0.27    |
| LNFP I       | 1.37                            | 0.00–5.32  | 1.46                         | 0.74–3.30  | 0.43    |
| LNFP II, III | 0.69                            | 0.39–2.17  | 0.57                         | 0.29–1.44  | 0.47    |
| LNT          | 0.80                            | 0.18–2.22  | 0.58                         | 0.18–1.59  | 0.45    |
| LNnT         | 0.39                            | 0.11–0.58  | 0.28                         | 0.11–0.55  | 0.29    |
| 3-FL         | 0.07                            | 0.02–0.27  | 0.10                         | 0.03–0.18  | 0.73    |
| 2'-FL        | 2.99                            | 0.00–5.24  | 3.33                         | 2.24–5.05  | 0.79    |
| LDFT         | 0.13                            | 0.00–0.39  | 0.26                         | 0.06–0.66  | 0.27    |

LNDFH I, lacto-N-difucohexaose I; LNFP I, lacto-N-fucopentaose I; LNFP II, lacto-N-fucopentaose II; LNFP III, lacto-N-fucopentaose III; LNT, lacto-N-tetraose; LNnT, lacto-N-neotetraose; 3-FL, 3-fucosyllactose; 2'-FL, 2'-fucosyllactose; LDFT, lactodifucotetraose.

## Results

In order to investigate whether maternal allergy influences the neutral oligosaccharide content in breast milk, we analysed the content of LNDFH I, LNFP I, LNFP II, LNFP III, LNT, LNnT, 3-FL, 2'-FL and LDFT in colostrum samples from allergic (n = 11) and non-allergic (n = 9) women. The colostrum samples were collected at the same time of the day and at the same time in the lactation period, 2–4 days postpartum. Results are shown as median (range) values and a great variation between individuals (Table 2). In accordance with previous studies (19, 24, 25), 2'-FL and LNFP I were the most abundant neutral oligosaccharides. The levels were similar in colostrum from allergic and non-allergic women. One non-allergic mother was a non-secretor, lacking the oligosaccharides LNDFH I, LNFP I, 2'-FL and LDFT. This mother had the highest values of the neutral oligosaccharides LNFP II, III and LNT.

The children who had developed allergic symptoms at the age of 18 months tended to have consumed colostrum with higher concentrations of oligosaccharides in total, although the difference did not reach statistical significance (p = 0.12) (Table 3). This tendency could however not be attributed to any specific oligosaccharide (Table 3). One child had a non-secreting mother and this child developed food allergy with positive SPT for egg.

## Discussion

Both genetic and environmental factors seem to be involved in the development of atopic disorders (1). We have investigated a connection

Table 3. Concentrations of neutral oligosaccharides in colostrum consumed by infants who later developed allergic disease compared to children who did not develop allergic disease

|              | Non-allergic children<br>(n = 11) |            | Allergic children<br>(n = 9) |            | p-value |
|--------------|-----------------------------------|------------|------------------------------|------------|---------|
|              | Median (g/l)                      | Range      | Median (g/l)                 | Range      |         |
| In total     | 7.53                              | 5.94–11.01 | 9.88                         | 4.90–13.90 | 0.12    |
| LNDFH I      | 1.22                              | 0.00–2.45  | 1.10                         | 0.00–2.63  | 0.76    |
| LNFP I       | 1.37                              | 0.00–3.30  | 2.37                         | 0.00–5.32  | 0.36    |
| LNFP II, III | 0.64                              | 0.39–1.94  | 0.85                         | 0.29–2.17  | 0.24    |
| LNT          | 0.58                              | 0.00–1.83  | 0.73                         | 0.18–2.95  | 0.52    |
| LNnT         | 0.30                              | 0.02–0.60  | 0.30                         | 0.04–0.58  | 0.85    |
| 3-FL         | 0.11                              | 0.05–0.71  | 0.11                         | 0.04–0.27  | 0.73    |
| 2'-FL        | 2.99                              | 0.50–4.74  | 4.41                         | 0.00–5.05  | 0.47    |
| LDFT         | 0.17                              | 0.00–0.68  | 0.11                         | 0.00–0.61  | 0.36    |

LNDFH I, lacto-N-difucohexaose I; LNFP I, lacto-N-fucopentaose I; LNFP II, lacto-N-fucopentaose II; LNFP III, lacto-N-fucopentaose III; LNT, lacto-N-tetraose; LNnT, lacto-N-neotetraose; 3-FL, 3-fucosyllactose; 2'-FL, 2'-fucosyllactose; LDFT, lactodifucotetraose.

between the genetics and the environment by analysing oligosaccharides in the breast milk (genetic factor), which stimulate the gut bacteria (environmental factor). However, we could not observe any difference in the concentration of neutral oligosaccharides in the colostrum from allergic and non-allergic mothers. This might suggest that the production of oligosaccharides is not influenced by the allergic status of the mother.

The concentration of neutral oligosaccharides in human milk varied considerably, which is in accordance with previous findings (24, 25). The oligosaccharide profile varies according to the secretor status, the ABO blood group type and the Lewis blood group type (23). The enzymes, glycosyltransferases including fucosyltransferases, which are necessary for the production of breast milk oligosaccharides, are also involved in the production of the cell surface glycolipids that determine blood group type in erythrocytes (23). In other cell types these enzymes are responsible for the production of cell surface glycoconjugates, which can bind particles and pathogens. Blood group phenotype and secretor status have been associated with certain diseases, i.e. urinary tract infection and asthma (23, 28). In addition, the breast milk oligosaccharide profile with a high amount of  $\alpha$ 1,2-linked fucosylated oligosaccharides appears to protect the infant from several types of diarrhoea (19). As the oligosaccharides in human milk share structural similarity with cell surface glycoconjugates, pathogens bind to the free oligosaccharides in breast-fed infants and are less able to infect the baby. Consequently, the variation in breast milk oligo-

saccharides could have important medical implications.

The relationship between consumption of breast milk oligosaccharides and allergy development in children has not been studied previously. No significant difference could be shown regarding the oligosaccharide content in colostrum consumed by infants who later developed allergic symptoms compared with those who remained non-allergic. However, there appeared to be a trend towards higher consumption of neutral oligosaccharides in those infants who later developed allergic symptoms. The fact that oligosaccharides in human milk act as receptor homologues (19, 23) and thus prevent pathogens from binding to the cells in the mucosa implies that a high amount of neutral oligosaccharides protects against orofecal pathogens. Interestingly, it has been shown that food and orofecal pathogens were able to reduce the risk of atopy by 60% (29). Accordingly, the ability of human milk oligosaccharides to bind pathogens and serve as anti-infective agents might influence the development of the immune system more than their ability to stimulate gut microbiota-immune interactions.

Human milk oligosaccharides can be absorbed across the intestinal brush border membrane (30). Oligosaccharides might therefore have effects outside the intestine. For example, neutral oligosaccharides inhibit the binding of neutrophils to tumour necrosis factor- $\alpha$ -stimulated endothelium (30) and might thus serve as anti-inflammatory agents. They might also influence cytokine production of cord blood-derived T cells as shown by Eiwegger et al. (31). Furthermore, the neutral human milk oligosaccharides LNFP III and LNnT are found in eggs of the helminth *Schistosoma mansoni* (32). Okano et al. showed that LNFP III conjugated with human serum albumin induced a stronger T<sub>H</sub>2 response in BALB/c mice compared to free serum albumin (32). Significantly higher levels of total IgE were produced.

To minimize the influence of diurnal variation as well as the difference between colostrum and mature milk, milk was collected at the same time of the day and at the same time, 2–4 days postpartum, in the lactation period. The sample size is relatively small but should be sufficient to detect differences with any major biological significance. We analysed the amount of neutral oligosaccharides in colostrum and not in mature milk for several reasons: The amount of oligosaccharides is higher in colostrum compared to mature milk (18) and a possible difference would therefore be more pronounced in colostrum.

Moreover, the gut of the unborn child is germfree but colonization starts immediately after birth (1) and the gut microbiota-stimulating factors would be even more important around this time. The amounts of oligosaccharides should not be considered as exact values, as we used a derivatization method with TFAN that gave a yield of 76–88% for different neutral oligosaccharides (27).

In conclusion, we have shown that the content of neutral oligosaccharides in colostrum does not depend on maternal allergy, nor does consumption of breast milk with high amounts of oligosaccharides protect children against the development of allergy. Nonetheless, human milk oligosaccharides could modulate infant immunity and play a role in prevention of infections.

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