Sialic acid content of infant saliva: comparison of breast fed with formula fed infants

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Abstract

Sialic acid is found in especially high concentrations in brain gangliosides, and supplementary sialic acid is associated with increased learning behaviour in animals. It was hypothesised that breast fed infants may have higher concentrations of sialic acid in body fluids and tissues because human milk is a rich source of sialylated oligosaccharides, while formulas contain very little. The aim therefore was to compare the sialic acid content of saliva collected from full term infants who were either solely breast fed or formula fed until weaning at 3–5 months of age. Thirty three infants, 18 breast fed and 15 formula fed, were studied at a mean (SD) age of 5 (2) months. The breast fed infants, when compared with formula fed infants, were found to have almost two times more free sialic acid in saliva (mean (SE) 16.0 (2.7) *v* 8.2 (2.1) mg/l, p < 0.036) and nearly 50% more total sialic acid (47.3 (3.9) *v* 32.2 (4.4) mg/l, p<0.014). The findings provide a preliminary indication that an exogenous source of sialic acids derived from human milk may contribute to higher concentrations of sialic acid in body fluids. There are important implications for the formulation of human milk substitutes.

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Keywords: sialic acid; saliva; human milk; infant formula

Preterm infants who consume breast milk in the early weeks of life have been shown to have significantly higher developmental scores at 18 months of age and higher IQ scores at 8 years than those who are formula fed, even after controlling for confounders such as socioeconomic status.[1–4] The distinction becomes more pronounced as the duration of breast feeding increases.[5] Human milk contains many factors that may promote the development of the nervous system, including long chain polyunsaturated fatty acids. The results of supplementation, however, have yielded conflicting findings.[6] We believe that a sugar called sialic acid, present in human milk and brain gangliosides in large amounts, but virtually absent in some infant formulas,[7–9] may be another factor which promotes brain development. In a previous study, we showed that human milk contains between 256 and 879 mg/l sialic acid derived from the human milk oligosaccharides.[10]

N-acetylneuraminic acid is the predominant and perhaps only form of sialic acid occurring in humans.[11] It is commonly present as a component of oligosaccharide chains of mucins, glycoproteins, and gangliosides. Especially high concentrations of gangliosides (a large group of sialic acid containing glycolipids, composed of sialyloligosaccharide and lipid moieties) are found in the cerebral cortex of the human brain.[12] Studies have shown that intraperitoneal and oral administration of free *N*-acetylneuraminic acid (= sialic acid) during the early postnatal period in the rat,[13] results in a significant increase in the concentration of sialic acid in brain ganglioside and glycoprotein. Addition of gangliosides to cell cultures was also shown to enhance neurite outgrowth and to stimulate regeneration of neurons in the central nervous system.[7 12] In rats during the early postnatal period, a decrease in exogenous sialic acid concentration leads to irreversibly impaired learning behaviour. Conversely, supplementary sialic acid improves learning.[13]

These findings suggest that the normal development of the infant brain may also be dependent on the sufficient production and maintenance of brain gangliosides and therefore dependent on an adequate supply of sialic acid. Mammals can synthesise *N*-acetylneuraminic acid from simple sugar precursors, however, the human infant liver may not have the full adult capacity for synthesising sialic acids during the early postnatal period.[7] This was shown in young rats where the activity of uridine diphosphate *N*-acetylglucosamine-2-epimerase (responsible for catalysing the first step to the biosynthesis of sialic acids) was low.[14]

Studies comparing sialic acid concentrations in human infants have not been reported. This study was initiated to determine if there were any differences in the sialic acid content of saliva collected from full term breast fed and formula fed infants. Although preterm infants show greater brain development ex utero than term infants, there is still significant accretion of brain tissue in the first few months of life of term infants. Saliva was chosen as an indicator fluid that is known to have a high concentration of sialic acid (from which the word saliva is derived) and which could be collected non-invasively from healthly young infants. Adult saliva was also studied to allow comparison with previous reports of sialic acid concentration in saliva. The findings provide a preliminary indication that an exogenous source of sialic acids derived from human milk may con-

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tribute to higher concentrations of sialic acid in body fluids.

Methods

Thirty three healthy, full term infants, mean (SD) age 5 (2) months were recruited for the study. Eighteen were solely breast fed and 15 were solely formula fed with cows' milk based preparations until weaning at 3–5 months of age. At the time of study, 10 of the 18 breast fed infants and seven of the 15 formula fed infants received small amounts of other foods in addition to breast milk or formula. Milk contributed the majority of energy intake and, to our knowledge, it was the only significant source of sialic acid in the diet. None of the subjects was receiving antibiotics. Infant saliva (1–1.5 ml) was collected using a sterile pasteur pipette under the supervision of a mothercraft nurse and analysed within one hour of collection. Twenty four adults, mean (SD) age 23 (2) years were studied for comparison. Their saliva was collected by expectorating into a sterile container (2–4 ml) at least two hours after food ingestion. The adult saliva was analysed immediately.

Sailva was centrifuged at 4°C, 11 000 *g* for 10 minutes and the supernatant removed for analysis. The remaining sediment was washed with saline (7%, pH 7.0) and centrifuged at 4°C, 11 000 *g* for three minutes. The saline supernatant fraction was discarded, and the wash procedure was repeated. The sediment was then resuspended to the original volume with saline (7%, pH 7.0). Sialic acid content was determined by a combined modification of the thiobarbituric acid method of Aminoff[15] and the method of Skoza and Mohos.[16] The thiobarbituric assay is considered to be more precise and subjected to the least interference than other colorimetric methods for the detection of sialic acid content in saliva.[17]

N-acetylneuraminic acid was used as the standard. Aliquots (140 µl) of standard, blank, or sample were added to an equal volume of water. The samples were treated with 70 µl of periodate reagent (25 mM periodic acid in 0.125 N sulphuric acid) and incubated at 37°C for 30 minutes. The reaction was terminated through the addition of 70 µl of sodium arsenite (2% sodium arsenite in 0.22 M hydrochloric acid). Once the yellow colour of liberated iodine had disappeared, 140 µl of thiobarbituric acid (0.1M, pH 9.0) was added and the solution was heated in a boiling water bath for 7.5 minutes then cooled in ice water. Dimethyl sulphoxide (560 µl) was added and the solutions were measured at 549 nm using plastic microcuvettes. In cases where the solution was turbid due to the presence of precipitates, the solution was centrifuged for three minutes before measuring on the spectrophotometer.

Because the method measures free sialic acid only, the salivary supernatant and sediment samples (140 µl) were hydrolysed with 0.05M sulphuric acid (140 µl) at 80°C for 60 and 70 minutes respectively, to determine the total (free plus released sialic acid) content. The bound sialic acid content was determined by substracting the value of free from total. The final values for sialic acid content of saliva were determined by summing the values obtained for the supernatant and sediment.

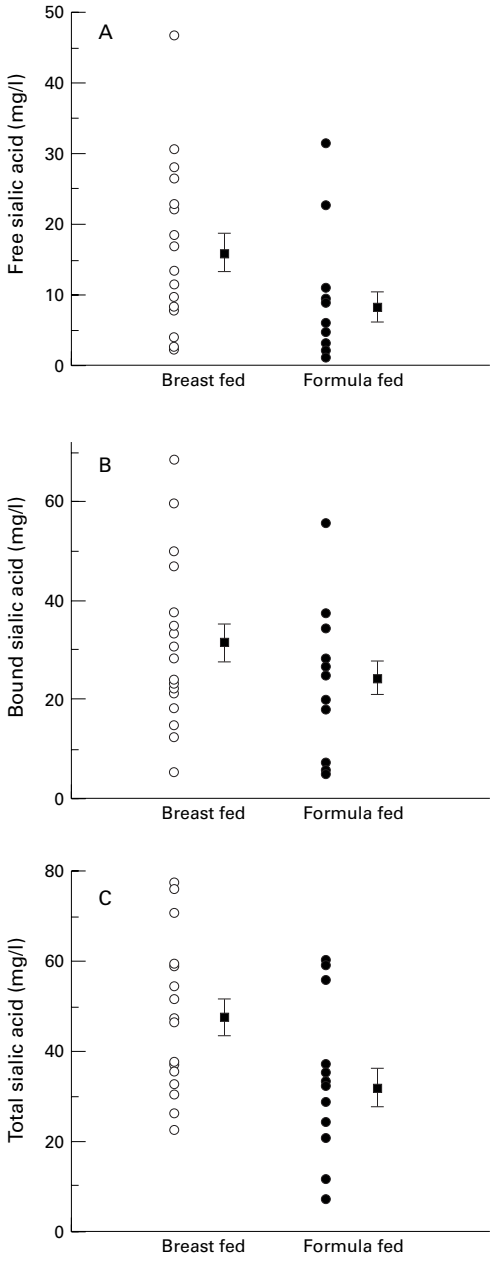

Figure 1 (A) free, (B) bound, and (C) total sialic acid content of infant saliva according to method of feeding.

All results are expressed as mean (SE) with $p<0.05$ taken as the level of significance using unpaired Student's *t* test and one way analysis of variance.

Results

Saliva collected from breast fed infants was found to have approximately double the amount of free sialic acid and nearly 50% more total sialic acid content than that from formula fed infants (fig 1, table 1).

By comparison, adult concentrations of bound and total sialic acid in saliva (table 2) were higher than that of breast fed and formula fed infants ($p<0.05$). Free sialic acid concentrations, however, were similar in adults and

Table 1 Mean (SE) sialic acid content in saliva of breast fed and formula fed infants.

	Breast fed (n=18)	*Formula fed (n=15)*	*p*
Free (mg/l)	16.0 (2.7)	8.2 (2.1)	0.036
Bound (mg/l)	31.5 (3.9)	24.3 (3.4)	0.175
Total (mg/l)	47.3 (3.9)	32.2 (4.4)	0.014

breast fed infants and both groups were significantly higher than that of formula fed infants ($p<0.05$). Adult men had more bound and more total sialic acid content than adult women (table 2).

Discussion

To our knowledge, we are the first to report the sialic acid content of infant saliva. Our results show that breast fed infants, when compared with formula fed infants, have almost two times more free sialic acid in saliva (16.0 *v* 8.2 mg/l respectively) and nearly 50% more total sialic acid (47.3 *v* 32.2 mg/l). While the mean differences are clearly biologically important, there was considerable overlap between the two groups in all three measures of sialic acid concentration. This suggests that infants may vary markedly in their capacity to synthesise their own sialic acid and/or to absorb it from the diet. Alternatively, infants may show variation in other attributes of their saliva, such as its protein or water content, which ultimately impacts on the final concentration of sialic acid. Clearly, further studies are needed to confirm these provocative findings.

We found no significant difference between the sexes with the mean total sialic acid content being 39.2 and 42.1 mg/l for boys and girls respectively. No correlation was detected between the age of the infant and sialic acid content of their saliva. In fact, the concentrations of free sialic acid in the saliva of breast fed infants, although not formula fed infants, approached that of adults.

It has been shown that the measurement of substances in saliva might be a reliable indicator of plasma and body concentrations of these agents.[18] The amount of nitrite in saliva, for example, was found to depend directly on the amount of nitrate and nitrite ingested.[18 19] Dietary supplementation with β carotene (provitamin A) in human subjects results in significantly higher concentrations of β carotene in whole saliva compared with a control group.[20] Hence it is highly plausible that the higher value for total sialic acid content in the saliva of breast fed infants reflects the high concentration of sialic acid in breast milk which augments uptake and incorporation into salivary glycoproteins. Like nitrates and nitrites,[18] ingested sialic acid is probably absorbed by the upper gastrointestinal tract, concentrated from the plasma and excreted into the saliva by salivary glands. Thus sialic acids in breast milk may supplement a limited capacity for endogenous synthesis of sialic acid in the human infant.

Table 2 Mean (SE) sialic acid content in the saliva of adults

	Adults (both sexes) (n=24)	*Men (n=12)*	*Women (n=12)*	*p (men v women)*
Free (mg/l)	13.5 (2.1)	15.4 (3.6)	11.5 (2.4)	0.369
Bound (mg/l)	52.0 (4.3)	59.3 (6.4)	44.6 (5.3)	0.090
Total (mg/l)	65.2 (4.2)	74.5 (5.3)	55.9 (5.4)	0.023

Key messages

- Sialic acid is found in especially high concentrations in the gangliosides of the cerebral cortex of the human brain
- Human milk contains significant amounts of sialic acid but infant formulas contain very little
- In rats, supplementary sialic acid has been shown to increase learning and the concentration of sialic acid in brain gangliosides
- In this study, breast fed infants, compared with formula fed infants, were found to have higher concentrations of sialic acid in saliva
- An exogenous source of sialic acids derived from human milk may contribute to higher concentrations of sialic acid in other body fluids, including brain gangliosides

Many researchers have assumed that the gastrointestinal tract of suckling mammals contains significant amounts of sialidase activity that can cleave sialic acid residues from the oligosaccharides in the milk. The extent to which this occurs will influence the amount of sialic acid absorbed in the small intestine and the amount of the exogenous sialic acid available for brain ganglioside synthesis. Dickson and Messer found a direct correlation between intestinal sialidase activity and the sialic acid content in milk of suckling rats and 12 other species.[14] Sialidase activity has also been shown to occur in human adult intestinal mucosa, and in gastric and intestinal secretions[21] and human milk.[22] If substantial amounts of sialic acids derived from human milk are incorporated, then the developmental and intellectual scores of human infants and children may be critically influenced by the nature of feeding in the first few months of life.

It is interesting that in the present study, free sialic acid concentration of breast fed infants was similar to that of adults (16.0 *v* 13.5 mg/l, respectively) while formula fed infants achieved about half this concentration (8.2 mg/l). This observation supports the view that formula fed infants may neither have a sufficient dietary supply of sialic acid, nor are they able to synthesise sufficient amounts endogenously. Previous studies in adults have documented similar concentrations of sialic acid in saliva to those found in the present study,[23] with a range from 45 mg/l[24] to 63 mg/l[25] for total sialic acid.

There are other implications of a higher sialic acid content in saliva of breast fed infants. It has been shown that sialic acid contributes to the viscosity of saliva[26] and that the viscosity of saliva and the mucin content are positively correlated.[27] The main function of mucins is to

protect the mucosal surfaces by forming a coating layer thereby acting as a lubricative gel, protecting the oral surfaces against mechanical disruption or abrasion. Thus, it is also possible that the breast fed infant has enhanced physical protective mechanisms as a result of the greater sialic acid content in their saliva.

Further research is needed to determine whether other fluids and tissues such as serum, cerebrospinal fluid, and brain gangliosides of breast fed infants have higher concentrations of sialic acid and, importantly, that this can be related to higher intake from exogenous sources. Any difference may contribute to our understanding of why intelligence scores are higher in breast fed infants. If sialic acid from dietary sources can be shown to influence the development of the human brain, this will have important implications for the formulation of human milk substitutes.

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