Glycosaminoglycan Content in Term and Preterm Milk during the First Month of Lactation.

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Contributors

N.V. developed the applied methodologies. L.Z., T.G., F.M., D.B. and F.B. performed the experimental procedures and analyses. E.B. contributed in milk sample collection. N.V., G.V.C. and O.G. designed and developed the experimental design, performed data analysis and wrote the manuscript.

All authors reviewed and approved the study.

Conflicts of interest

We declare that we have no conflicts of interest.

Grant support

None

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Glycosaminoglycans in human milks

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Abstract

Background. In a recent study, we performed a complete structural characterization of glycosaminoglycans (GAGs) in human mature milk. However, no data are available on the total content of GAGs in human milk from healthy mothers having delivered term or preterm newborns.

Objectives. In this study, we evaluated the total content of GAGs in pooled milk from healthy mothers having delivered term or preterm newborns during the first month of lactation.

Methods. Highly specific and sensitive analytical approaches were used to quantify human milk total GAGs.

Results. Highest GAGs values are present at 4^{th} day (~ 9.3 g/L and ~ 3.8 g/L in preterm and term milk, respectively), followed by a progressive decrease up to day 30^{th} (~ 4.3 g/L and ~ 0.4 g/L). The more remarkable differences are related to the first phases of lactation in which a strong decrease of GAGs was observed between days 4^{th} and 10^{th} (~-73% in term and ~-50% in preterm).

Conclusions. During the first month of lactation the absolute amount of polysaccharides was constantly and significantly higher in preterm milk than in term one, with a similar behaviour in the decrease. These data further indicate that human milk GAGs may have an active role in protecting newborns during the first phases of lactation.

Keywords. Human milk; Glycosaminoglycans; Chondroitin sulfate.

Introduction

In a recent study [1], we performed a complete structural characterization of glycosaminoglycans (GAGs) in human mature milk in comparison with bovine milk. Quantitative analyses yielded 0.41 g/L of GAGs in human milk, ~7 times more than in bovine milk. In particular, chondroitin sulfate (CS) and dermatan sulfate (DS), were found to differ considerably from one type of milk to the other. In fact, in human milk a low-sulfated CS was the main component (~56%), while DS was observed in very low amount (~2%). On the contrary, bovine milk was demonstrated to be composed of ~66% DS and ~34% CS. Structural analysis also showed the presence of fast-moving

heparin (FM-Hep) that account for ~30-40% of total GAGs in both milk samples. The same research [1] offered the first full characterization of GAGs in human milk, providing useful data to gain a better understanding of their physiological role, as well as of their fundamental contribution to the health of the newborn. In fact, several types of microorganisms have also been demonstrated to use CS as receptors for the adhesion to and infection of host cells [1-3]. Furthermore, in human milk, Newburg *et al.* [4] demonstrated that CS or a CS-like moiety is able to inhibit the binding of the HIV envelop glycoprotein gp120 to the cellular CD4 receptor. From these studies it emerges that human milk GAGs, on the contrary of bovine milk, could play a role as soluble receptors. In fact, intact GAG molecules reach the small intestine, as no specific digestive enzyme able to degrade them is present on the intestinal wall.

In this study, we evaluated the total content of GAGs in pooled milk from healthy mothers having delivered term or preterm newborns. To include in our study a relatively homogeneous but representative population we selected women who had both the Secretor and Lewis phenotypes, as determined in their saliva by the hemoagglutination-inhibition test. This is the most common phenotype in Europe, as it is present in about 70% of the general population [5]. Furthermore, we used pooled milk to have a robust characterization of the total content of GAGs. Additionally, we already observed no great variations between different term healthy mothers at the same time of lactation for the quali/quantitative composition of GAGs that was confirmed quite similar in all human Subjects [1].

Results and Discussion

Figure 1 illustrates agarose-gel eletrophoresis utilized to separate and quantify extracted GAGs from different milk samples. Both in term and preterm milk, GAGs showed a constant pattern essentially composed of two main polysaccharides, a low-sulfated CS (~60-70%), FM-Hep (~30-40%) and quite no DS. Quantitative data are shown in Figure 2. As evident, considerable variation of GAGs concentration occurs during the first month of lactation. Highest values are present at 4^{th} day (~ 9.3 g/L and ~ 3.8 g/L in preterm and term milk, respectively), followed by a progressive decrease up to day 30^{th} (~ 4.3 g/L and ~ 0.4 g/L). The more remarkable differences are related to the first phases of lactation in which a strong decrease of GAGs was observed between days 4^{th} and 10^{th} (~-73% in term and ~-50% in preterm). During the first month of

lactation the absolute amount of polysaccharides was constantly and significantly higher in preterm milk than in term one, with a similar behaviour in the decrease.

Comparative analysis of term and preterm milk demonstrate compositional differences in several of their nutritional components [6]. It is well known that several components show different behaviour during lactation. As regard as the carbohydrate component, dynamic variations were observed during the first month. In particular, lactose content increases both in term and preterm milk with higher concentration in the first. On the contrary, oligosaccharides decrease in both types of milk, with higher concentration in preterm milk [7, 8]. Very interestingly, the total amount of GAGs was higher in preterm than term milk, with a strong decrease after the first days of lactation, with the same trend already observed in oligosaccharides.

Recent studies showed that some cell surface receptors are constituted by GAGs [2-4], which in this way directly participate in infective and inflammatory processes. As a consequence, along with oligosaccharides, we can suppose that the high concentration of GAGs could be useful for the preterm newborn in defence processes against several pathogens (viruses, bacteria and their toxins) with a receptor-like mechanism preventing the adhesion of pathogens to epithelial cells. Furthermore, CS (and GAGs) is a well known antioxidant and antiinflammatory agent [9] and remarkably, for preterm infants endowed with an immature antioxidant defense system [10], GAGs may have important antioxidant properties. In fact, babies being exclusively fed with mother's milk develop less oxidative stress than babies nourished with formula [11], and colostrum possesses relevant antiinfective and antioxidant properties contributing to the infant's defense against free radicals generated by oxygen administration, infection, or byproducts of nutrient metabolism. In conclusion, further effords should be addressed to improve our knowledge not only on GAGs composition and structure but also on their role in physiological and pathological conditions.

Materials and methods

A morning milk sample was obtained with an electric breast pump at 4, 10, 20, and 30 post-partum days from 18 women who delivered at term and from 26 women who delivered between 27th and 35th week of gestation.

GAGs were extracted and quantified by analytical procedures reported in details in Coppa *et al.* [1]. 5 mL of milk were defatted with acetone. After centrifugation at

10,000 g for 10 min and drying at 60°C for 24 h, the pellet was solubilized in 20 mL of 100 mM Na-acetate buffer pH 5.5 containing 5 mM EDTA and 5 mM cysteine. 200 mg of papain were added and the solution incubated for 24 h at 60°C in a stirrer. After boiling for 10 min, the mixture was centrifuged at 5,000 g for 15 min, and three volumes of ethanol saturated with sodium acetate were added to the supernatant. After storing at +4°C for 24 h, the precipitate was recovered by centrifugation at 5,000 g for 15 min and dried at 60°C for 6 h. The dried precipitate was dissolved in 20 ml of 50 mM NaCl and after centrifugation at 10,000 g for 10 min, the supernatant was applied to a column (2 cm x 7 cm) packed with QAE Sephadex® A-25 anion-exchange resin equilibrated with the same NaCl solution. GAGs were eluted with a linear gradient of NaCl from 50 mM to 1.2 M from 0 to 150 min using low-pressure liquid chromatography (Biologic LP chromatography system from BioRad) at a flow of 1 ml/min. Fractions positive to uronic acid assay [1] were collected. Three volumes of ethanol saturated with sodium acetate were added to the pooled fractions and stored at +4°C for 24 h. The precipitate was recovered by centrifugation and dried at 60°C for 12 h. The dried precipitate was dissolved in 100 µL distilled water and further analyzed.

Quantitative agarose-gel electrophoresis in barium acetate/1,2-diaminopropane was performed essentially as previously reported elsewhere [1].

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Legends to Figures

Figure 1. Agarose-gel electrophoresis of GAGs from human milk samples at different days of lactation in mothers delivering term and preterm newborns. CS: chondroitin sulfte. DS: dermatan sulfate HS: heparan sulfate. St: mix of CS, DS and HS. The suddivision in GAG species CS, DS and HS is just related to the standard.

Figure 2. Total amount of GAGs in human milk during different days of lactation in mothers delivering term and preterm newborns. A curve illustrating the polysaccharides content trend is also reported for each type of milk. Data are reported as mean ± SD.

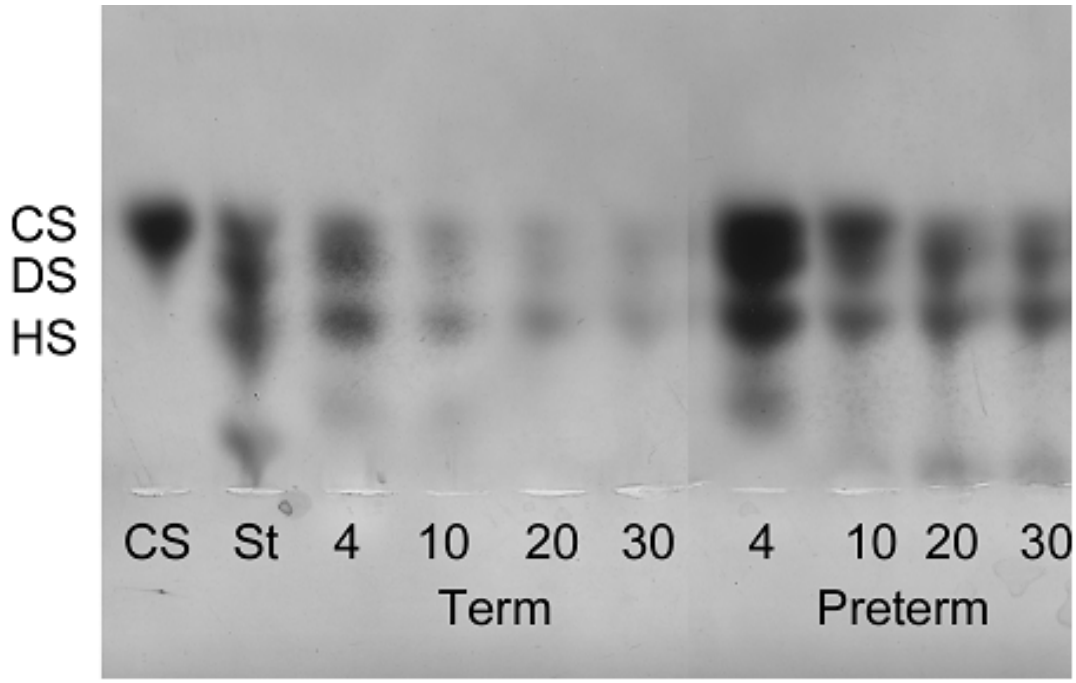

Figure 1

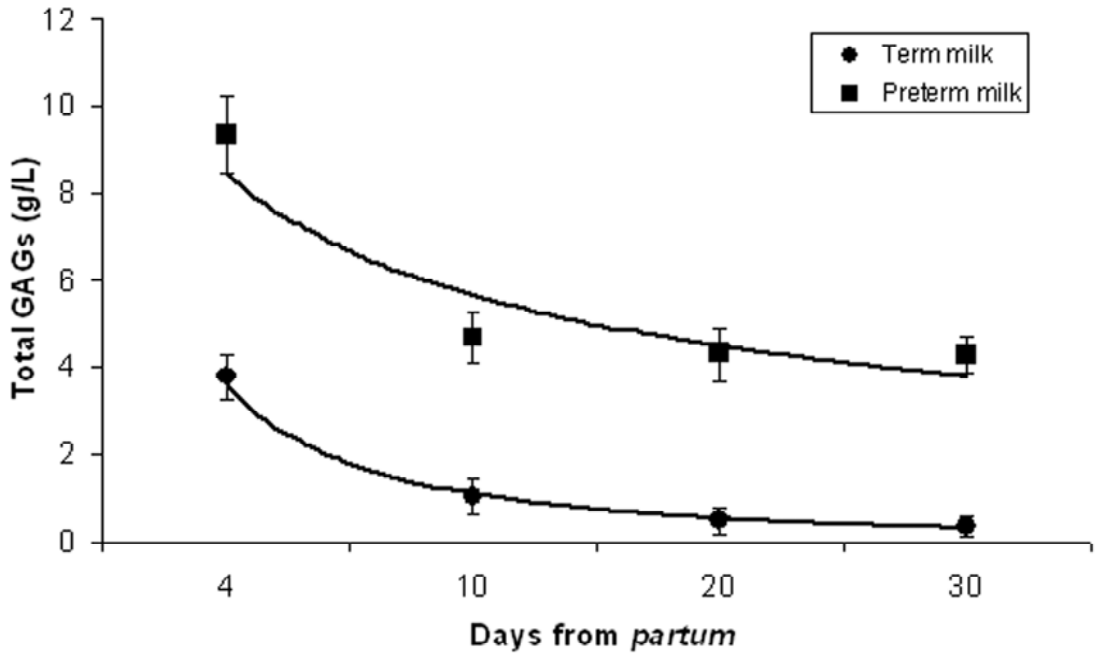
12
10
8
6
4
2
0
Total GAGs (g/L)
Term milk
Preterm milk
4
10
20
30
Days from *partum*

Figure 2