CHARACTERIZATION OF OLIGOSACCHARIDES IN MILK AND FECES OF BREAST-FED INFANTS BY HIGH-PERFORMANCE ANION-EXCHANGE CHROMATOGRAPHY

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1. ABSTRACT

Human milk contains a large amount of oligosaccharides, which represent its third largest solute. Nevertheless, both the metabolism and the role of these substances are still largely unknown. A previous study we conducted documented that the amount of oligosaccharides excreted in the feces varies from 6% to 13% of the 24-hour ingested oligosaccharides. The aim of this study was to characterize the pattern of oligosaccharides in the feces compared with the pattern of the ingested milk. Six term newborn infants were studied at the end of the first month of life. A 7:00 AM milk sample was obtained with an electric breast pump. Feces were collected during the day of milk sampling. Analyses of oligosaccharides were performed using high-pH anion-exchange chromatography with pulsed amperometer detection. Pure milk oligosaccharides were used as reference standards. The chromatographic profile of the oligosaccharides present in the feces and in the milk samples showed more than 40 peaks, 20 of which have been identified. The oligosaccharide profile observed in the feces was similar to the pattern of oligosaccharides present in the milk ingested. A significant difference was represented by the almost complete absence of lactose in the feces of all infants and of sialyllacto-N-tetraose a and disialyllacto-N-neotetraose in 3 samples. A substantial reduction of lacto-N-tetraose was observed in 5 samples.

Our results demonstrate that the oligosaccharide profile in the feces is similar to that of the ingested milk. Approximately 40% to 50% of the total ingested oligosaccharides can be found in feces of breast-fed infants.

2. INTRODUCTION

Quantitatively, oligosaccharides are the third most representative component of human milk (Newburg & Neubauer 1995). The quantity of oligosaccharides is 20 to 23 g/L in colostrum and 10 to 13 g/L in mature milk (Montreuil & Mullet 1960; Viverge et al. 1985; Coppa et al. 1993). Higher values have been detected recently in milk samples of women delivering before term (Coppa et al. 1997). Therefore, breast-fed infants daily ingest several grams of oligosaccharides.

Knowledge of the amount of each single oligosaccharide in human milk, variations during the different phases of lactation, and the metabolic fate of oligosaccharides is minimal. The aim of this study was to identify the main oligosaccharides in human milk and to evaluate their presence in the feces of breast-fed infants.

3. MATERIALS AND METHODS

Six mothers with blood types secretor A, B, H, and Lewis, who had delivered infants at term, were studied. Milk samples were collected as previously described (Coppa et al. 1993) and preserved at -80°C until use. The amount of ingested milk was calculated by weighing the infant before and after each feeding. During the same 24-hour period feces were collected from the 6 infants and from 1 formula-fed infant and stored at -80°C until analysis.

3.1. Sample Preparation

One milliliter of milk was added to 1 mL of acetonitrile. After stirring, the sample was centrifuged at 4000 rpm for 15 min. The supernatant was then diluted to 1:24 (v/v) with deionized water and filtered through a 0.22-μm membrane (Millipore, Bedford MA); the feces were treated according to Sabharwal et al. (1984) and lyophilized; 3 mg of dry ultrafiltrate was dissolved in 1 mL of deionized water.

3.2. Analytical Method

A 25-μL milk or feces sample was injected by autosampler (AMS Dionex, Sunnyvale CA) in a Dionex HPLC A1 450 system. Such a system consists of a CarboPac PA-1 (4 × 250 mm) column, a CarboPac PA (3 × 25 mm) pre-column and a pulsed amperometric detector (PAD II) with a gold electrode.

Separation was obtained according to the method of Townsend et al. (1989) modified as follows: eluent 1, a 100-mM solution of NaOH; eluent 2, a 100-mM solution of NaOH with 1 M sodium acetate.