Pediatrics and Neonatology (2011) **52**, 93—97

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ORIGINAL ARTICLE

Profile of Nucleotides and Nucleosides in Taiwanese Human Milk

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Received Mar 29, 2010; received in revised form Jun 18, 2010; accepted Jul 5, 2010

Key Words
human milk
nucleoside;
nucleotide;
Taiwanese

Background: Human milk—borne nucleotides and their related metabolic products have been reported to have important physiological roles in breast-fed infants. The purpose of this study was to measure the concentrations of free nucleotides and nucleosides in human milk from Taiwanese women.
Methods: A total of 24 individual milk specimens were collected from women in Taipei and Kaohsiung, at four stages of lactation. Vegetarian or non-vegetarian dietary patterns were recorded. The samples were analyzed for nucleotides and nucleosides by high-performance liquid chromatography.
Results: The mean (±standard deviation) free nucleotide and nucleoside concentrations in Taiwanese human milk were 213.13 ± 76.26 μmol/L and 16.38 ± 7.11 μmol/L. The predominant nucleotide was cytidine diphosphate for almost all samples, regardless of the location, stage of lactation, or dietary status of the subjects. Overall, the mean concentrations of cytidine diphosphate, cytidine monophosphate, uridine monophosphate, guanosine monophosphate, adenosine monophosphate, and inosine monophosphate, in milk samples were 129.86 μmol/L, 49.10 μmol/L, 5.60 μmol/L, 0.82 μmol/L, 2.96 μmol/L, and 25.25 μmol/L, respectively (equivalent to 61.0%, 23.1%, 2.6%, 0.4%, 1.0%, and 11.9% of free nucleotide composition). In free nucleosides, cytidine and uridine were predominant during all stages of lactation. The average concentrations of cytidine, uridine, adenosine, guanosine, and inosine, in milk samples were 9.25 μmol/L, 6.33 μmol/L, 0.18 μmol/L, 0.36 μmol/L, and 0.23 μmol/L,

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doi:10.1016/j.pedneo.2011.02.012

respectively (equivalent to 56.5%, 38.7%, 1.1%, 2.2%, and 1.4% of free nucleoside composition). Comparing vegetarian and non-vegetarian statuses, it was found that the total free nucleotide concentration was high in the vegetarian group ($p = 0.037$).
Conclusion: Our data showed a wide range of concentrations of individual nucleotides and nucleosides in Taiwanese human milk. Unique dietary status could affect the nucleotide and nucleoside levels in human milk, especially the nucleotides in our study. However, the mechanism of modulation of nucleotide and nucleoside levels in human milk is not clear.

1. Introduction

Approximately 15—30% of the total nitrogen in human milk is nonprotein nitrogen and is made up of substances, including urea, free amino acids, nucleic acids, nucleotides, peptides, creatine, uric acid, ammonia, carnitine, and polyamines.[1] At least 13 separate nucleotides have been found, accounting for 2—5% of the total nonprotein nitrogen.[2] Nucleotides are made up of three components: (1) a nitrogenous heterocyclic base derivative of either a pyrimidine or purine; (2) a pentose (ribose or deoxyribose); and (3) one to three phosphate groups.[3] Nucleotides and their related metabolic products play an essential role in cell replication and metabolism. They store cellular energy, mediate intracellular metabolic processes, and support metabolism and protein synthesis. Their functions may be particularly important in rapidly growing infants.[4]

Nucleotide requirements are met by *de novo* synthesis and by a salvage pathway that recovers metabolized nucleotides and nucleosides originating from dietary sources and intermediary metabolism.[5] Polymeric forms of nucleotides (DNA and RNA) are generally the primary dietary sources of nucleotides. RNA and DNA are digested by ribonucleases and deoxyribonucleases to nucleotides and then broken down by phosphatases to nucleosides, the preferred form for absorption in the small intestine.

It is estimated that an infant consuming human milk as a primary nutrition source would ingest 1.4—2.1 mg of nucleotide nitrogen per day.[2] Human milk is a rich source of nucleotides for young infants, whereas cow's milk lacks in nucleotide content.[4] Our study was designed to determine the concentrations of free nucleotides and nucleosides in Taiwanese human milk. We also evaluated the relationship among diet, lactation stage or geographical area, and nucleotide and nucleoside concentration.

2. Methods

2.1. Human milk specimens

The analysis of free nucleotides and nucleosides was done on 24 individual milk specimens. Each milk specimen containing 30 mL of breast milk was collected at home and was immediately put in the freezer, with temperature less than −4°C. Then, the specimen was sent to our laboratory and put into another freezer at less than −70°C. The milk specimens were classified by the subject's lactation stage, geographical area, and whether they were vegetarian or not. Twelve specimens were collected from Taipei and another 12 specimens from Kaohsiung. Eight subjects were vegetarians. The milk samples were categorized into one of four lactation stages: Stage 1 of lactation was first week postpartum, Stage 2 was first month postpartum, Stage 3 was second month postpartum, and Stage 4 was third to ninth month postpartum. Written informed consent was obtained from each subject before enrollment.

2.2. Sample preparation for high-performance liquid chromatography analysis

The frozen milk specimens were thawed at room temperature, and 5 mL of each specimen was mixed with 10 mL of 10% (w/v) trichloroacetic acid (TCA) solution. After allowing the mixture to stand on ice for 30 minutes, it was centrifuged at 30,000 × *g* for 15 minutes. The aqueous layer was recovered, and the residual cream and precipitate were washed twice with 5 mL of 7.5% (w/v) TCA solution. The aqueous layers were combined, and TCA was removed by adding 20 mL of diethyl ether. The solution was then lyophilized and stored at −30°C until analysis. Just before analysis, the lyophilized samples were dissolved in water and then brought to a volume of 1.5 mL with water. After filtering through a 0.45-μm Chromatodisk (Kurabo, Osaka, Japan), the nucleotides and nucleosides were analyzed by high-performance liquid chromatography.

2.3. High-performance liquid chromatography analysis

The nucleotides and nucleosides were analyzed using a Hewlett Packard LC1050 system (Palo Alto, California, USA) equipped with a Capcellpak C18, type AG (Ø4.6 × 500 mm; Shiseido, Tokyo, Japan) at 20°C. Twenty-five millimolar tetrabutylammonium hydrogen sulfate—50 mM potassium phosphate (pH 3.5) was used as a solvent, and the flow rate was 0.75 mL/min. Nucleotides and nucleosides were detected by the absorbance at 254 nm. After each analysis, the column was washed with methanol for 15 minutes and equilibrated for 1 hour with initial conditions. Appropriate standards were used to establish the retention time of each nucleotide and nucleoside.

2.4. Chemicals

Nucleotides and nucleosides for the standards were purchased from Yamasa Co. (Chiba, Japan). All other chemicals were purchased from Wako Pure Chemical Industries Ltd (Osaka, Japan).