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ORIGINAL ARTICLE

Plasma amino acids in preterm infants fed different human milk diets from a human milk bank

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Summary
Background & aims: The enzymatic immaturity of the amino acid metabolic pathways in preterm infants makes these children vulnerable to deficiencies or amino acid excess. The purposes of this study was to evaluate the plasma amino acid profile of preterm infants fed one of three diets, and then compare them to the reference standards for preterm infants.
Methods: Thirty low-birthweight preterm infants were randomly assigned to diets: unmodified banked human milk ($n = 10$), banked human milk evaporated to 70% ($n = 10$), and banked human milk fortified with a bovine whey protein hydrolysate ($n = 10$). Amino acid concentrations were analyzed by high efficiency liquid chromatography.
Results: No significant statistical differences in the amino acid profiles were found across groups. With few exceptions (arginine and glutamic acid), plasma amino acid concentrations in the three groups were lower than or reached the minimum values of references found in the literature for preterm infants.
Conclusions: The diets utilized led to deficiencies in amino acids, relative to the reference standards. It can be concluded that the supplies of these nutrients were below the needs of the infants in all groups.

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Introduction

Scientific and technological advances in medicine, particularly in perinatal intensive care, have reduced the mortality rate among preterm infants, especially in those with birth-

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weights of less than 1500 g. This achievement has led to the investigation of a number of related issues, particularly in the area of nutritional support.[1] In 1985, the American Academy of Pediatrics[2] stated that the main objective of preterm infant nutrition was to achieve postnatal growth rates similar to those found *in utero*. In 1992, Steer et al.[3] pointed out that nutrition should also target the long run to normal neurological development. The concept of optimal protein nutrition emerges when the adjusted protein intake has to be not only quantitative, but also qualitative, taking into account the enzymatic immaturity of the amino acid metabolic pathways, which are peculiar in preterm infants, making these children vulnerable to deficiencies or toxicity resulting from any amino acid excess.[4] Research has provided evidence of the inadequacy of human milk as the sole nutritional source for preterm infants.[5] An alternative to this inadequacy is to fortify human milk, which significantly increases growth rates, as expressed by weight gain, linear length, and head circumference.[3] Another option is to increase human milk concentration by controlled evaporation, resulting in a diet that provides much higher protein and energy intake. The purpose of this study was to evaluate the plasma amino acid profile of preterm infants fed one of three diets: banked human milk (BHM), banked human milk evaporated to 70% (EBHM), and banked human milk fortified with FM85 (FBHM) and to compare them with conventional reference standards for preterm infants, in order to identify toxicity risks and/or nutritional deficiencies.

Patients and methods

Thirty preterm infants of both genders at 34 weeks or less of gestational age (GA), as evaluated by the Ballard method,[6] with birthweights of up to 1750 g were included in the study. Birthweight was appropriate for GA according to the curves of intrauterine growth designed by Bataglia and Lubchenco.[7] The infants were admitted to the Neonatal Intensive Care Unit of the Teaching Hospital of Universidade Federal de Mato Grosso do Sul (UFMS), Campo Grande, MS, Brazil, in the period from March 2000 to June 2002. Written informed consent was obtained from parents. The study protocol was approved by the local Ethics Committee. Carriers of congenital pathologies or intercurrent diseases (infectious and/or metabolic disorders) were excluded, in accordance with election criteria. The patients were randomly assigned to three groups of 10 infants, each group receiving one of three diets, all processed by the local milk bank:

(a) Diet BHM was obtained from milk donors in different periods of lactation, pasteurized, and frozen.
(b) Diet EBHM was prepared by concentrating banked human milk under controlled evaporation to 70% of its initial volume using a Buchi Rotavapor RE vacuum rotating evaporator. Each milk sample to be evaporated (400 mL at a time) was maintained at 56 °C under a vacuum pressure ranging from 62 to 63 cmH_2O. The 70% reduction in sample volume (from 400 to 280 mL) was achieved in about 30–40 min, and the resulting milk was frozen and stored. The procedure was always carried out by the same investigator.
(c) Diet FBHM was prepared from banked human milk with the addition of protein supplied as a bovine whey protein hydrolysate associated with a fortifier containing maltose–dextrine and minerals (FM85, Nestlé, Switzerland).

Before the period of study, the infants were fed banked human milk associated with small volumes of their own mothers' milk, whose production was not yet sufficient to meet daily requirements. The children were included in the study after 11 ± 1 days of life, when they had already been on full enteral feeding for at least 72 h. Each group received the assigned diet for a period of 10–12 days. According to their individual ability, the children were fed orally or by gavage. Daily intakes were of 170–180 mL/kg, in 3-h feeding intervals, for all groups. Intake volume was defined according to the protocol of the service, without researchers' interference. The macronutrient and energy content of the banked breast milk fed to the infants was not analyzed. After the period of 10–12 days, preprandial venous blood samples of 1 mL were taken 150–180 min after the last feeding. Plasma was separated by centrifugation, identified, and frozen at −20 °C. Plasma amino acids were determined by High-Efficiency Liquid Chromatography in a high-pressure liquid chromatograph (Shimadzu, model LC-10, Japan).

The amino acids analyzed were arginine, alanine, aspartic acid, glutamic acid, glutamine, serine, histidine, glycine, threonine, tyrosine, methionine, valine, phenylalanine, leucine, isoleucine, and lysine. The reagent used in the pre-column reaction was orthophthaldialdehyde (OPA), a compound that reacts with the amino group of amino acids yielding a colored molecular complex (isoindole) that can be detected by a fluorescence reader. The compound used in the pre-column reaction does not react with the amino acids proline, cystine, or tryptophan, none of which were quantified by reason of this technical limitation.

Body weights were measured with an electronic balance of 5-g sensitivity (Filizola, Brazil) immediately before and after the period of study—i.e., at 10–12 days and at 22 ± 2 days of age.

Descriptive statistics was expressed as medians, minimums, and maximums for the variables that characterize the population studied. No sample size calculation was performed; the power achieved with the number of infants included in the study was not estimated. Medians and first and third quartile values were taken into account for analysis of weight variation and plasma amino acid levels. The differences were tested with nonparametric procedures, Mood's median test[8] for the quantitative variable and with Fisher's exact test[9] for the qualitative variable. No correction for multiple testing was performed. A p-value of less than 0.05 was considered significant.

Results

Table 1 reports the variables that characterize the population studied: GA, gender, birthweight, length, head circumference, and type of delivery. Statistical analysis showed that the groups were similar ($p > 0.05$).

Table 2 shows that no significant statistical differences in plasma amino acid concentrations were found across groups,