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Original Article

Differences in serum biochemistry between breast-fed and formula-fed infants

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Abstract

Background: We investigated the differences in serum biochemistry levels between breast-fed and formula-fed infants. We provide reference values of serum biochemistry levels for 4-week-old (w/o) and 8 w/o infants for future clinical applications.
Methods: Sixty healthy infants were enrolled in this study (30 infants were included in the breast-fed and formula-fed groups, respectively). During hospital visits at 4 and 8 w/o, several parameters, including body weight, body length, and head circumference were recorded. Blood was drawn to measure 14 serum biochemistry markers.
Results: There were no significant differences between the two groups in terms of growth or anthropometric measurements. Serum cholesterol, triglyceride (TG), alanine aminotransferase (ALT), aspartataminotransferase (AST), gamma glutamyl transferase (GGT), total bilirubin (T-bil) and direct bilirubin (D-bil) levels were significantly higher in the breast-fed group compared with those measured in the formula-fed group at both 4 and 8 w/o. Serum blood urea nitrogen (BUN) and inorganic phosphate (IP) levels were significantly lower in the breast-fed group compared with the formula-fed group at 4 and 8 w/o. In the formula-fed group, serum total protein (TP), albumin, cholesterol, uric acid (UA), ALT, and AST levels were significantly higher in 8 w/o infants compared with 4 w/o infants; in addition, serum GGT, T-bil, D-bil, IP, and iron (Fe) levels were significantly lower in 8 w/o infants compared with 4 w/o infants. In the breast-fed group, serum TP, albumin, and UA levels were significantly higher in 8 w/o infants compared with 4 w/o infants; in addition, serum BUN, GGT, T-bil, D-bil, IP, and Fe levels were significantly lower in 8 w/o infants compared with 4 w/o infants.
Conclusion: Different sources of nutrition may result in different metabolic responses; these differences are reflected by different serum biochemistries. The reference values for serum biochemistry levels also differ according to the infant's postnatal age.

Keywords: breast-fed; formula-fed; infant; serum biochemistry

1. Introduction

Nutritionally, human breast milk is considered the best food for infants under normal circumstances. However, there has been a significant decline in the incidence and duration of breast-feeding in many industrialized countries. Although many adjustments have been made to render formula as close to human milk as possible, there are still differences in the composition of human milk and various infant formulas.[1] Although some studies have measured the reference values of various biochemical constituents found in the sera of healthy infants,[2,3] little is known about the influence of different diets on the concentrations of biochemical analytes in infants. Therefore, the present study was designed to investigate the differences in the serum biochemistry levels of breast-fed and formula-fed infants and establish reference values for 4-week-old (w/o) and 8 w/o infants that can be used in clinical applications.

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2. Methods

2.1. Study population

This 8-week metabolic study was conducted on apparently healthy, full-term infants who were being fed either human breast milk or formula. Infants were eligible for enrollment in this study if they were delivered at full-term with a gestational period of 38–42 weeks, had a 5-minute Apgar score of 8 or higher, were in apparently in good health with no evidence of cardiac, respiratory, gastrointestinal, or other systemic diseases, had no history of birth asphyxia or clinically apparent blood group incompatibilities, and if the mother had no history of diabetes, tuberculosis, or perinatal infection with proven effects on the fetus. In addition, the parents were required to breast feed or provide their infants with the study formula as the sole source of nutrition for the duration of the study, voluntarily provide written informed consent, and adhere to the design of this study. The study was approved by the Institutional Review Board of the Taipei Veterans General Hospital, Taiwan.

2.2. Study procedure

Thirty eligible infants (15 males and 15 females) of mothers who chose to breast feed were assigned to the human breast milk group. These infants were exclusively breast fed until 8 weeks of age. No other foods except water were allowed during the study period. Another 30 eligible infants (15 males and 15 females) from mothers who chose not to breast feed were assigned a standard cow milk-based formula (Similac® with Iron infant formula, Abbott Laboratories, Illinois, USA) and assigned to the formula-diet group. Infants who failed to complete the study were replaced by an infant of the same sex.

Dietary intake was recorded for 3 days prior to study visits that were conducted at 4 and 8 w/o. The mothers of the formula-fed infants provided information on the amount of formula and other liquids ingested by their babies. Blood samples were obtained at 4 and 8 w/o. Milk was the sole source of nutrition for all infants during the first 8 weeks of this study.

2.3. Anthropometric and laboratory measurements

During the hospital visits at 4 and 8 w/o, several parameters, including body weight, body length, and head circumference, were recorded. Venous blood samples (3 mL) were drawn from each infant between the hours of 8:00 AM and 12:00 PM, approximately 2–3 hours after feeding. Blood was collected in order to determine several biochemical parameters, including total protein (TP), albumin, cholesterol, triglyceride (TG), blood urea nitrogen (BUN), uric acid (UA), alanine aminotransferase (ALT), aspartate aminotransferase (AST), Gamma-glutamyltransferase (GGT), total bilirubin (T-bil), direct bilirubin (D-bil), total calcium (Ca), inorganic phosphate (IP), and iron (Fe) levels using a Hitachi 736-30 system (Boehringer Mannheim Diagnostics [BMD], Indianapolis, IN, USA).

2.4. Statistical analyses

Clinical and laboratory data were entered into a computer data base for analysis using SPSS (SPSS Inc., Chicago, IL, USA). Categorical data were analyzed using the Chi-square or Fisher exact test. Continuous variables were compared using the t test or the Wilcoxon sign rank test. For all tests, results with p-values < 0.05 (two-tailed) were considered statistically significant.

3. Results

There were no significant differences between the two groups in terms of mean changes in body weight, length, or head circumference that were measured between birth and 4 to 8 w/o, for either male or female infants.

As shown in Table 1, serum cholesterol, TG, ALT, AST, GGT, T-bil, and D-bil levels were significantly higher in the breast-fed group compared with the formula-fed group, both at 4 and 8 w/o. Serum BUN and IP levels were significantly lower in the breast-fed group compared with the formula-fed group at both 4 and 8 w/o.

In the formula group, Serum TP, albumin, cholesterol, UA, ALT, and AST levels were significantly higher at 8 w/o compared with 4 w/o infants; serum GGT, T-bil, D-bil, IP, and Fe levels were significantly lower at 8 w/o compared with 4 w/o infants. In the breast-fed group, serum TP, albumin, and UA levels were significantly higher in 8 w/o infants compared with 4 w/o infants; serum BUN, GGT, T-bil, D-bil, IP, and Fe levels were significantly lower in 8 w/o infants compared with 4 w/o infants.

Fig. 1 illustrates the differences in serum cholesterol, TG, and total bilirubin levels between formula-fed and breast-fed infants at different weeks of age. In 4 w/o infants, serum cholesterol, TG and T-bilirubin levels were significantly higher in the breast-fed infants compared with formula-fed infants by 35.91%, 30.27%, and 213.40%, respectively. When comparing 8 w/o infants, serum cholesterol, TG and T-bilirubin levels were higher in breast-fed infants than formula-fed infants by 34.10%, 34.28%, and 182.05%, respectively.

4. Discussion

The concentration of urea in serum is inversely correlated to the net utilization of proteins and reflects the balance between intake, usage, and degradation of proteins and the renal excretion of protein metabolites.[4,5] Increased protein intake induces an increase in amino acid oxidation and the subsequent excretion of nitrogen, mainly as urea.[4,6] The concentration of nitrogen in the urine and serum increased linearly with the amount of metabolizable protein that is fed to lactating dairy cows, indicating the decreased efficiency of nitrogen utilization.[7] In this study, the reason for lower concentrations of serum urea measured in 4 and 8 w/o infants

Table 1
Serum biochemistry levels measured in 60 healthy infants (30 infants in the breast-fed group; 30 infants in the formula-fed group) at different ages.

	4 wk old		8 wk old	
	Formula-fed	Breast-fed	Formula-fed	Breast-fed
TP	5.25 ± 0.49	5.45 ± 0.45b	**5.43 ± 0.31	**5.64 ± 0.39
Albumin	3.77 ± 0.28	3.85 ± 0.29	**3.86 ± 0.26	**4.04 ± 0.27
Cholesterol	108.70 ± 21.3 *	147.73 ± 32.3	**111.48 ± 20.2 *	**149.50 ± 26.8
TG	90.43 ± 37.7 *	117.80 ± 43.6	100.66 ± 51.3 *	135.17 ± 61.0
BUN	9.17 ± 2.71 *	8.27 ± 2.30	8.79 ± 2.64*	**6.00 ± 2.11
UA	2.77 ± 0.76	2.87 ± 0.92	**3.40 ± 1.03	**3.80 ± 1.2
ALT	17.13 ± 14.1 *	34.07 ± 21.0	**24.93 ± 18.2 *	40.00 ± 22
AST	32.20 ± 17.31 *	47.32 ± 23.25	**38.66 ± 14.9 *	54.24 ± 19
GGT	46.13 ± 27.5 *	70.62 ± 42.24	**23.45 ± 11.5 *	**37.53 ± 17.3
T-bil	0.97 ± 0.45 *	3.04 ± 2.19	**0.39 ± 0.23 *	**1.10 ± 0.70
D-bil	0.14 ± 0.15 *	0.28 ± 0.27	**0.01 ± 0.1 *	**0.15 ± 0.14
Ca	10.28 ± 0.66	10.33 ± 0.73	10.16 ± 0.46	10.38 ± 0.4
IP	7.27 ± 1.09 *	6.53 ± 0.77	**6.67 ± 0.83 *	**6.13 ± 0.72
Fe	112.93 ± 27.39	111.23 ± 26.18	**74.00 ± 24.2	**76.52 ± 24.6

*$p < 0.05$ compared to breast-fed infants of the same age.
**$p < 0.05$ compared to 4 w/o infants for the same biochemical item.
ALT = alanine aminotransferase; AST = aspartataminotransferase; BUN = blood urea nitrogen; Ca = total calcium, D-bil = direct bilirubin; Fe = iron; GGT = gamma glutamyl transferase; IP = inorganic phosphate; T-bil = total bilirubin; TG = triglyceride; TP = total protein; UA = uric acid.

in the breast-fed groups may be the lower intake and presumably more efficient use of proteins in the breast-fed infants. Growth (e.g., weight, length, head circumference) and albumin levels were similar between the two groups. Therefore, these differences give evidence that the formulas that are now in common use may provide a protein intake that is in excess of the amount of protein required by infants. In addition, in contrast to serum BUN levels at 4 w/o, the serum BUN levels measured in 8 w/o infants were lower in both the breast-fed and formula-fed groups. This decrease in the serum BUN level could have resulted from the maturation of the kidneys. This could also be the reason why the inorganic phosphate (IP) value in infants was lower at 8 w/o compared with 4 w/o.

In this study, serum ALT, AST and GGT levels were higher in infants enrolled in the breast-fed group than infants enrolled in the formula-fed group. This correlation was also reported in a study performed in Denmark, which found that serum AST levels are significantly higher in breast-fed infants because of the induction of hepatocytes by factors found in human breast milk.[8] Human breast milk contains a variety of hormone and

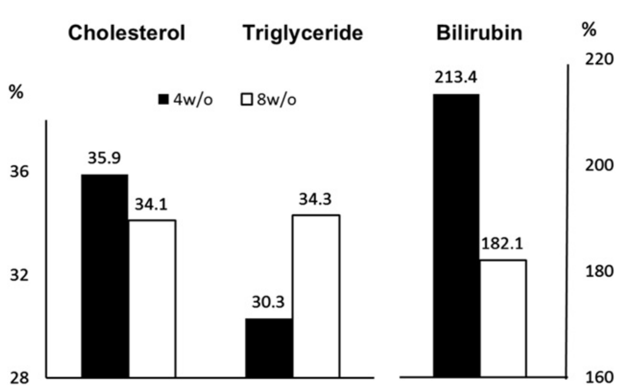

Fig. 1. Differences in serum cholesterol, triglyceride, and total bilirubin levels in breast-fed infants compared with formula-fed infants.

hormone-like peptides, such as epidermal growth factor (EGF), insulin-like growth factor-1 (IGF-1), and hepatocyte growth factor (HGF), which are low or undetectable in infant formula.[9–11] Previous studies have shown that EGF is transported intact across the gastrointestinal mucosa during the suckling period where it retains its influence on the development of hepatic microcirculation and the phagocytic functions of Kupffer cells in the postpartum liver.[9,12–15] Recent evidence also suggests that EGF receptor-dependent signaling contributes not only to liver cell proliferation but also the regulation of hepatic regeneration.[16] Also, HGF is abundant in human breast milk and is involved in the proliferation of hepatocytes.[17–19] Supported by this evidence, we believe that the most likely explanation for the elevated AST, ALT, and GGT values in the breast-fed infants is the stimulation of liver metabolism through one or several growth factors that are naturally found in human breast milk.

It is well established that breast-fed infants are at a higher risk of developing hyperbilirubinemia. Although the pathogenesis of hyperbilirubinemia is not well understood, the inhibition of hepatic UDP-glucuronyltransferase, increased enterohepatic recirculation of bilirubin, and low levels of intestinal bacteria capable of converting bilirubin to non-resorbable derivatives are possible causes.[20] A recent study suggested that neonatal hyperbilirubinemia is significantly correlated with the concentration of EGF, which is more abundant in breast milk.[21] This disparity between serum bilirubin levels was also found in this study. The concentrations of total and direct bilirubin were higher in breast-fed infants at 4 and 8 w/o than those that were fed formula. The mean serum level, however, was well below the threshold for pathologic jaundice (<15 mg/dL). The relatively elevated level of bilirubin in the breast-fed infants may also have long-term favorable effects. Bilirubin has been recently recognized for its cytoprotective and anti-oxidant properties. Reduced serum bilirubin levels are associated with a higher prevalence of coronary artery disease, and several groups have suggested the protective effects of serum bilirubin against malignancies.[22,23]

Cholesterol is an essential component of the brain, accounting for 2–3% of its weight and 20–30% of its total lipids.[24] Higher concentrations of serum cholesterol were measured in the breast-fed infants in this study, in accordance with the results of previous studies.[25–28] This difference has been attributed to the greater cholesterol content, usually between 10–20 mg cholesterol/dL, found in human milk, whereas infant formulas in the US contain less than 10% of this amount of cholesterol.[28–30] Furthermore, several of the long-chain (i.e., n-3) and polyunsaturated fatty acids (i.e., n-6) present in human milk, including arachidonic acids, are absent in formula. The lipid content of human milk could reflect the needs of the brain during this active period of growth. Our previous study showed that the levels of linoleic acid, alpha linoleic acic, arachidonic acid, and docosahexaenoic acid in Taiwanese infants are the highest among several countries that were studied.[31] Many studies have shown that adolescents and adults who were breast-fed during infancy have lower serum levels of cholesterol and LDL.[32,33] The high intake of cholesterol by breast-fed infants may downregulate hepatic hydroxymethyl glutaryl coenzyme A reductase, thereby resulting in the reduced endogenous synthesis of cholesterol in later life.[26] Whether or not breast feeding has a profound impact on future cardiovascular events is still controversial.

Human breast milk contains a considerable amount of nucleotides, the possible biological effects of which, such as the enhancement of immune functions, increase in the available iron, modification of intestinal microflora, changes in plasma lipids, and promotion of gut growth and maturation, have been reviewed. The nucleotide content of bovine milk is less, but also of a different composition.[34,35] Human breast milk and fetal mucosal enterocyte contain the complete sequence of enzymes necessary to completely convert purine nucleotides to uric acid.[36] This is the possible reason why there were higher levels of serum uric acid in breast-fed children at 4 and 8 w/o, though this finding was not statistically significant.

In conclusion, different types of nutrition intake may result in different metabolic responses, which are reflected by different serum biochemistries. Not only does breast milk provide the ideal nutritional content for newborns, but it also contains a variety of substances that may actively influence the growth and development of infants. The reference values for serum biochemical levels also differ depending on the infant's age (e.g., from birth to 8 w/o).

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