

Impact of early life nutrition on growth and intestinal microflora composition in low-birth-weight infants

By Anne Ormisson, Epp Sepp,
Urmas Siigur, Heili Varendi and Marika Mikelsaar

Received February 11, 1997; Accepted May 15, 1997

Abstract

We investigated the effect of early life nutrition on growth and intestinal microflora in low-birth-weight infants. Eleven preterm newborns (birth weight 1456 ± 293 g, gestational age 30.6 ± 1.2 weeks) received special preterm infant formula as a sole diet from the first enteral feeding until the end of the second month of life (preterm infant formula group (PF group)). Thirteen infants (birth weight 1728 ± 276 g; gestational age 32.6 ± 1.4 weeks) received standard casein-dominant infant formulas available in Estonia (standard formula group (SF group)). Growth parameters were measured at the age of 1, 2, 3, 4 weeks and 2 months. Faecal samples for short chain fatty acids (SCFAs) and bacterial cultures were collected at the age of 6 days and 2 months.

Two results have been received: better growth and better microbial colonization in the group of preterm infant formula. There was significantly higher weight gain in the PF group at the age of 2 months (1814 ± 566 g) than in the SF group (1255 ± 365 g).

The relative amount of coagulase negative staphylococci was significantly higher in the SF group at the age of 6 days in comparison with the PF group. At the age of 6 days the prevalence of faecal acetic acid was 98% of total concentration of short chain fatty acids in the PF group and 87% in the SF group ($p < 0.05$), which shows the higher prevalence of anaerobic microbes in the PF group. Thus, a nutrient-enriched preterm formula has a positive effect on the intestinal bacterial colonization and growth of low-birth-weight infants.

Introduction

Development of intestinal microflora and respectively the profile of faecal SCFAs, as well as infant growth depend on the food character (breast-feeding, formula-feeding), immunological and environmental factors (1-5). Insufficient contact with the mother and the usual formula feeding have been altering the composition of microflora of preterm neonates: the delayed colonisation with lactobacilli and bifidobacteria, on one hand, and the high counts of opportunistic microorganisms like coagulase negative staphylococci (CONS), enterococci, enterobacteria and candida, on the other hand, have been described. The latter microorganisms are also the most frequent etiological agents of neonatal infections (6,7). The idea of influencing the infants' welfare, e.g. quick weight gain of the newborn through nutrition and intestinal microflora is not a new one (8,9). However, there is a lack of data whether different

nutrition can influence the health of the preterm infant through microbial ecology.

This study was designed to compare the body weight gain and composition of intestinal microflora and faecal SCFAs of preterm infants receiving different formulas during their first two postnatal months.

Material and Methods

Subjects

The study was performed in Tartu University Children's Hospital from November 1991 until July 1993. Twenty-four preterm infants (birth weight < 2100 g, gestational age of 28 to 34 weeks), admitted to the neonatal ward were recruited to the study if enteral feeding was introduced within the first days of life and breast milk was not available. Oral informed consent was obtained from the parents and the study was approved by the local Ethics Committee. Of the 24 studied infants, 11 received special preterm infant formula (PF group) (Valio Ltd., Finland) since the first enteral feeding up to the age of 2 months. The remaining 13 infants were fed by standard casein-dominant term formulas (SF group) (Detolakt, Russia) during the same period. The infants were selected for the PF and SF groups according to transferring hospitals. PF group neonates were transferred from Tartu Maternity Hospital (taking care over risk pregnancies and births) which was equipped with preterm infant formula (PF) to be given to

the infants from their first enteral feeding. The control group (SF group) infants were referred from other maternities that were not equipped with PF. The amount of the formula given to the infants was calculated according to the infants' weight and age needs.

The nutrient composition of PF does not markedly differ from standard formula (SF) in the protein or fat content (Table 1). However, the carbohydrate content was quite different and besides a similar amount of lactose, PF contained maltodextrine. All formulas were fortified with minerals like calcium, phosphorus, iron, and different vitamins (vitamin E, C, B₁, B₂, B₆, B₁₂, folic acid; SF with and PF without vitamin D).

The clinical characteristics of the studied newborns are presented in Table 2. All infants were ill and received treatment. Most of the infants received antibiotics either for prevention or treatment of related infections (omphalitis, conjunctivitis; two infants had neonatal septicaemia, no microorganisms were detected in the blood). Parenteral antibacterial therapy (Ampicillin, Gentamycin, followed by Cefotaxim in most cases) in ordinary neonatal doses lasted from 5 days to several weeks. There were no reliable statistical differences in clinical characteristics between the studied groups, except the birth weight with lower figures for the PF group.

Anne Ormisson¹ Associate Prof., Epp Sepp² MD, Master of Science, Urmas Siigur³ MD, PhD, Heili Varendi¹ MD, Marika Mikelsaar² Prof.

¹ Dept of Pediatrics, Univ. of Tartu, Tartu, Estonia.
² Institute of Microbiology, Univ. of Tartu, Tartu, Estonia.

³ Laboratory Department, Tartu Univ. Hospital, Tartu, Estonia.

Correspondence: Anne Ormisson, Dept of Pediatrics, Children's Hospital, Univ. of Tartu, Lunini str. 6, EE-2400 Tartu, Estonia.

Table 4. Differences in faecal flora of preterm infants at the age of 6 days and 2 months (log cfu/g and frequency of occurrence - %).

Microorganisms	Age	PF group			SF group		
		Median	Range	% of infants colonised	Median	Range	% of infants colonised
Total aerobes	6 d	10.2	8.1-11.7	100	9.6	6.4-11.7	100
	2 m	7.9	5.6-10.1	100	10.9	8.6-11.6	100
<i>S.aureus</i>	6 d	0	0-8.3	17	0	0-5.1	8
	2 m	0	0-4.6	14	4.3	0-7.3	60
CONS	6 d	0 **	0-10.6	42 **	6.6 **	0-9.9	100 **
	2 m	4.6	0-7.8	57	5.3	0-10.6	60
Streptococci	6 d	9.1	0-11.4	58 *	0	0-11.3	39 *
	2 m	4.8	0-9.3	57	5.3	0-11.6	60
Enterococci	6 d	0	0-11.6	33	5.14	0-11.7	54
	2 m	3.8	0-7.6	57	8.3	0-10.6	60
Lactobacilli	6 d	0	0-8.3	17	0	0-7.3	23
	2 m	4.3	0-7.3	57	7.8	0-11.1	60
Enterobacteria	6 d	8.3	0-11.4	68	7.1	0-11.2	69
	2 m	0 ***	0-6.3	29 *	8.6 ***	6.8-11.4	100 *
Candida	6 d	0	0-10.7	33	4.3	0-11.6	54
	2 m	0 ***	0-5.3	29 *	6.6 ***	4.6-8.6	100 *
Total anaerobes	6 d	10.3	0-12.0	92	8.9	0-11.8	92
	2 m	7.8	5.6-10.7	100	10.3	9.0-11.1	100
Bifidobacteria	6 d	0	0-11.0	17	0	0	0
	2 m	0	0-7.4	29	0	0-8.6	0
Anaerobic cocci	6 d	0	0-11.3	42	0	0-10.3	46
	2 m	0	0-10.7	43	10.3	0-12.0	80
Clostridia	6 d	0	0	0	0	0-7.3	30
	2 m	0	0-5.3	29	0	0	0
Bacteroides	6 d	8.2	0-11.5	68	8.14	0-11.8	77
	2 m	7.0	0-8.3	86	6.9	0-11.1	60

PF = preterm infant formula; SF = standard formula; 6 days : PF group: n= 11; SF group: n= 13; 2 months: PF group: n= 7; SF group: n= 5
* p<0.05; ** p<0.01; *** p<0.001

SCFAs measurements

The samples were thawed at room temperature and the aliquots (0.4-0.6 g) homogenised in 2 ml of distilled water containing 3 mmol/l of aethylbutyric acid as internal standard and 0.5 ml of H₂SO₄ (0.5 mmol/l). The homogenate was vacuum-distilled and analysed for SCFAs as described by Zijlstra with modifications by Hoverstad (13). Chromatograms were recorded, peak areas determined and concentrations calculated with the Shimadzu Data Processor Chromatopac C-R3A.

Statistics

Wilcoxon's rank sum test for paired data and Mann-Whitney's rank sum test for unpaired data, Student's t-test and Fisher's exact test for comparing the groups and Kendall rank correlation analyses for discrete data were used. For comparison of members of staphylococci and streptococci in different types of feeding the Spearman nonparametric correlation index was calculated.

Results

Infants' growth

Changes in body weight of preterm infants during the first 2 months of life are

presented in Table 3. The infants of the PF group had statistically significant (p<0.03) lower birth weight than the infants of the SF group.

The weight loss did not differ and the infants of both groups regained birth weight within 10-12 days.

There were no reliable differences in the increase of body weight within the first month of life. At the age of 2 months body weight gain was significantly higher in the PF group than in the SF group (p<0.05). At the age of 2 months body weight of the PF group was 226±36 % of their birth weight and in the SF group it was only 174±22 % (p<0.001). Increase of the other growth parameters (head circumference, body length) didn't show differences (data not shown). All infants studied had anaemia of prematurity at the age of 2 months. There were no differences found in the mean haemoglobin values nor in the count of erythrocytes between the studied groups (data not shown).

Bacteriological data

At the end of the first week of life the intestinal tract of all preterm newborns was colonised with aerobes and their counts varied in wide ranges, Table 4. The majority of newborns were also colonised

with anaerobes, only one newborn in both groups did not show colonisation with any anaerobes investigated. We could rarely trace bifidobacteria and lactobacilli in studied newborns. Concerning opportunistic microorganisms the intestinal tract of infants of the PF group was more rarely colonised with CONS in comparison with the infants of the SF group. Also the counts of CONS (log cfu/g) in the PF group were in a lower level than those of the SF group. There was noticed a tendency that streptococci had been more frequently colonizing the newborns of the PF group. At the same time there was a significant negative correlation between the occurrence of CONS and streptococci in both groups at the age of 6 days (r=0.5546, n=24, p<0.03).

At the end of the second month of life all the observed infants were colonised with aerobic and anaerobic microorganisms. The frequency of occurrence of enterobacteria and candida was increased in infants fed by SF compared to the infants of the PF group.

Faecal short chain fatty acids

The SCFAs' profiles did not differ between the PF and SF groups as a whole at the age of 6 days and 2 month, Table 5. In the analyses of individual fatty acids

