

Correlation between early neonatal diet and atopic symptoms up to 5-7 years of age in very low birth weight infants: follow-up of randomized, double-blind study

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The influence of early feeding on the risk of atopic diseases has been studied in full-term newborns, not in very low birth weight infants (VLBW). The study evaluated effect of early feeding of VLBW infants with either cow's milk-based formula (CMF) or extensively hydrolyzed milk formula (HF) on incidence of atopic diseases and markers of atopy at 5-7 years of age. This was a follow-up of the randomized, double-blind study evaluating the influence of different enteral feeding protocols on the early morbidity of VLBW infants. In the original study 80 children were randomly allocated into 2 groups receiving during first month of life HF (experimental group) or CMF (control group). At the age of 5-7 years, 62 children among 74 available (84%) with mean birthweight 1124g were evaluated according to standardized ISAAC (International Study of Asthma and Allergies in Childhood) protocol. Total IgE level, specific IgE, lymphocyte CD4+CCR4+/CD4+CXCR3+ ratio and skin prick tests (SPT) were done. Prevalence of obvious allergic diseases was not significantly different between the studied groups (HF: 12/33; CMF: 6/29; RR [relative risk] HF vs CMF: 1.76; 95%CI [confidence interval]: 0.76–4.09). Comparison of atopic status across groups revealed similar rate of positive markers of atopy: IgE (RR: 2.57 95%CI: 0.91–8.08), SPT (RR: 5.13; 95%CI: 0.93–31.6), lymphocyte CD4+CCR4+/CD4+CXCR3+ ratio (OR: 2.32; 95%CI: 0.78–7.53) in the both studied groups. Based on the carried out follow-up study we were unable to confirm the usefulness of hydrolyzed formula in prevention of allergy in an unselected cohort of very low birth weight infants.

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The development of neonatal intensive care has resulted in an increased survival of very low birth weight (VLBW) newborns. Owing to high degree of biochemical immaturity, fast growth rates, and a greater incidence of medical complications these infants require specialized nutritional support. The first stage of feeding consists in parenteral nutrition and minimal enteral feeding aimed at securing basic caloric intake and stim-

ulating the gastrointestinal tract. It is followed by enteral feeding. Feeding practices in VLBW infants differ among neonatal units. Maternal milk is a milk of choice, but in many cases it is unavailable. In such conditions, formula feeding should be started. Some authors advocate the widespread use of hydrolyzed protein based formulas, suggesting that its introduction can improve feeding tolerance (1).

Atopic diseases pose a serious health problem in children. Genetic and environmental interaction is responsible for the predisposition and expression of allergic disorders and, as it has been recognized recently, factors present in early life can increase the risk of allergic asthma, hay fever, and eczema. Such factors include, among others, low birth weight, pre-term birth, and possibly, early cessation of exclusive breast feeding (2–6). So far, only a few studies conducted on the effect of early feeding on the risk of atopic diseases included pre-term infants (7–9). Most of them, however, assessed the benefits of hydrolyzed protein formulas only in infants with family risk of atopy. Therefore, we decided to carry out a follow-up study of a cohort of unselected pre-term infants. The aim of our study was to evaluate the effect of early feeding of VLBW infants with either cow's milk-based formula or extensively hydrolyzed milk formula on incidence of atopic diseases and other markers of atopy at 5–7 yr of age.

Methods

Patients

This is a follow-up of the study conducted between January 1998 and October 2000 (10), whose main aim was to evaluate the influence of different enteral feeding protocols on the early morbidity of VLBW infants. Inclusion criteria to the original study were: birth weight ≤ 1500 g, age on admission < 72 hours, and negative blood culture on admission. Exclusion criteria included: GI tract anomalies, early-onset gram negative sepsis (before the beginning of enteral feeding), necrotizing enterocolitis (NEC) before the beginning of enteral feeding, Apgar score below three points at five minutes, intraventricular hemorrhage grade IV.

From 29 January 1998 to 31 October 2000 89 newborns were enrolled into the original study. After an introductory observation for exclusion criteria, enteral feeding was introduced between the seventh and tenth day of life. During the pre-study period, nine children were excluded due to lack of tolerance of minimal feeding and finally, 80 newborns were included into the main part of the study. Infants were randomly assigned into two groups: Group A – received hydrolyzed protein based formula (casein hydrolyzate formula – HF; Pregestimil, Mead and Johnson) ($n = 40$), and Group B – received cow's milk based formula (CMF; Bebilon Nenatal, Nutricia) ($n = 40$). Daily feeding increments and daily

doses of protein and energy did not differ between the groups. During the first month of life only the studied formulas were used. After one month of life the parents had the possibility to decide to continue study feeding or switch their baby to other type of feeding. The study formulas were prepared by the hospital pharmacy in the blind manner. Allocation to the group was concealed. Protocol of the original study was approved by the Ethical Committee for Clinical Investigations of Collegium Medicum, Jagiellonian University.

Study design

All patients included into the original study were invited to participate in the follow-up study. The follow-up study was approved by the Ethical Committee for Clinical Investigations of Collegium Medicum, Jagiellonian University.

After signing the informed consent, parents were asked to complete two questionnaires:

- (i) including demographic variables, family characteristics (education level, health status), nutrition during the first year of life, environmental risk factors of atopy (parents smoking habits, siblings, day care attendance); and
- (ii) validated, standardized ISAAC (International Study of Asthma and Allergies in Childhood) questionnaire assessing past and current health status of the child (11, 12).

All questions were verified by physicians during face-to-face discussion. Afterwards, all children were examined by an investigator in the blinded fashion for the presence of atopic eczema, (rhino)conjunctivitis, wheezing, and other clinical signs of allergy.

Laboratory evaluation

After examination, a venous blood sample (5 ml) was taken for the assessment of:

- (i) absolute eosinophil count,
- (ii) total IgE and specific IgE against cow's milk, hen's egg, and soy, and
- (iii) lymphocyte $CD4 + CCR4 + /CD4 + CXCR3 +$ ratio.

Automated blood count was used to establish white cell count. Next, to identify the amount of different white cells, a blood film was made, and 100 white cells were counted. The percentage of eosinophils was established, and by multiplying

the percentage with the total number of white blood cells, the absolute number of eosinophils was obtained.

Quantitation of serum IgE was based on commercial technique Phadia ImmunoCAP System (Phadia AB, Uppsala, Sweden) with the use of micro-sponge capable of fixing a large amount of allergen using radio-labeled anti-IgE antibodies (13).

The following process was used to determine the ratio of lymphocytes CD4 + CCR4 + to lymphocytes CD4 + CXCR3 +. Blood taken from the patients was diluted with phosphate buffered saline (PBS) with 2% fetal bovine serum (FBS). Cells were treated with human monoclonal antibodies anti-CD4 +, anti-CCR4 +, anti-CXCR3 + (Becton Dickinson, San Jose, CA, USA). The blood was incubated in 4°C for 30 min. FACS Lysing Solution was added to eliminate erythrocytes, and then the material was centrifuged and washed with PBS. The samples were analyzed with the use of Fluorescence Activated Cell Sorter (FACS). Lymphocytes were separated based on preselected characteristic ('lymph-gate') including cell size and density of the cell. As each of the antibodies was conjugated with a different fluorochrome, multivariate analysis was possible. A three-color analysis was performed. First, CD4 + cells were found among these included in the 'lymph-gate', and then we noted the percentage of CCR4 + and CXCR3 + cells.

It is known that restricted chemokine receptor patterns are expressed on the Th₁ and Th₂ cells. In particular, it is evident that CCR5 and CXCR3 are expressed primarily on Th₁ cells, whereas CCR3, CCR4 and CCR8 expression predominates on Th₂ cells (14, 15). Evaluation of CD4 + CCR4 + to CD4 + CXCR3 cells ratio was used as an extrapolated model to illustrate a Th₁ to Th₂ shift. The immunological findings suggest that increased antibiotic use and alteration in endogenous gut microflora (very common in pre-term infants) can disturb instructing Th₁/Th₂ responses in the mucosal lymphoid tissues (16–18). It is thought that the immune system of neonates is biased toward Th₂ immunity. In non-allergic individuals this Th₂ predominance gradually decreases during the first 2 yr of life. By comparison, Th₂ response can persist and even increase in allergic individuals (17). Moreover, Ahern et al. suggest that allergic inflammation is driven by the activation of Th₂ helper type 2 CD4 T cells producing IL-4 and IL-13, which contributes to IgE production, mucus hypersecretion and airway hyper-responsiveness, and IL-5 that promotes eosinophilic

inflammation (18). Based on the data cited above, we assumed that lymphocytes CD4 + CCR4 + /CD4 + CXCR3 ratio below 1.0 can be used as a marker of lymphocyte Th₂ predominance.

During the same visit skin prick tests (SPTs) were performed by an experienced nurse with Allergopharma kits (Allergopharma Joachim Ganzer KG, Reinbeck, Germany), including cow's milk, house dust mite (*Dermatophagoides pteronyssinus*), cat dander and dog dander, grass pollen (a mix of *Phleum pratense* and *Dactylis glomerata*), tree pollen (a mix of birch, alder, hazel, and willow) and moulds (a mix of *Aspergillus fumigatus*, *Alternaria alternata*, *Cladosporium herbarum*, and *Penicillium notatum*).

Inhalant allergens were chosen for SPTs to detect sensitization to the most common allergens in the environment. SPTs are considered the most sensitive diagnostic procedure in IgE-mediated allergy, so there was no rationale to use both methods for inhalant allergens. To evaluate sensitization to cow milk allergens, both serum specific IgE measurement and SPTs were performed, while egg white and soy allergy was diagnosed only by serum IgE detection. Our choice of food allergens was due to their well known high allergenic potential (19).

Examination, blood sampling, laboratory analyses and SPT were performed by personnel blinded to study group allocation.

Outcome variables

The primary outcome at 5–7 yr of age was the presence of atopic disease according to the following categories: obvious atopic disease, possible atopic disease, no atopic disease. Secondary outcome variables were subject's sensitization status (atopic status) determined by IgE, SPT or by CD4 + CCR4 + /CD4 + CXCR3 + lymphocyte ratio (Table 1).

Statistical methods

The comparison between the groups utilized Student's *t*-test, Kruskal–Wallis test or Fisher's exact test, as appropriate. Relative risk (RR) and 95% confidence interval (CI) were calculated for the risk of atopic disease. Next, different factors (sex, family history of atopy, breast feeding) associated with obvious allergic disease in univariate analyses were entered as covariates for logistic regression analysis. Then logistic regression was used to estimate odds ratios for developing allergic disease, depending on the type of feeding during the

Table 1. Definitions of primary and secondary outcomes

Primary outcomes:

Obvious atopic disease is recognized if one of the following symptoms is present:

- (i) wheezing in the previous year;
- (ii) sneezing with itchy-watery eyes in the previous year; itchy rash in the previous year with any flexural involvement or around the neck, ears or eyes.

Possible atopic disease is recognized if one of the following symptoms is present:

- (i) an affirmative answer to at least one of the questions: Has your child ever had asthma? Has your child ever had hay fever? Has your child ever had eczema?;
- (ii) sneezing in the previous year;
- (iii) itchy rash in the previous year

Secondary outcomes:

Definition of positive atopic status determined by IgE:

- (i) subject's serum total IgE level above upper limit of serum IgE for age of the patient; or
- (ii) specific serum IgE >2.5 kU(A)/L for any tested allergen.

Definition of positive atopic status determined by SPT:

- (i) positive SPT for at least one allergen (mean wheal diameter ≥ 3 mm larger than the negative control).

Definition of positive atopic status determined by lymphocyte CD4 + CCR4 + /CD4 + CXCR3 + ratio:

- (i) peripheral blood lymphocyte CD4 + CCR4 + /CD4 + CXCR3 + ratio <1.0.

first month of life; controlling for confounding variables.

Statistical significance was defined at the two sided $p = 0.05$ level. Data was analyzed using SAS Software (SAS Institute Inc., Cary, NC, USA, 2006).

The episode of food intolerance in VLBW children was the end-point of the original study. It was assumed that to demonstrate the reduction of food intolerance frequency by 20% (supposing this frequency in the cow's milk exposed group is 35%) with the power of 80% (two sided test, $\alpha = 0.05$) 80 children had to be included into the study (if randomization was to be 1:1). Seventy-four children who completed the original study and were discharged from the neonatal unit were available as the initial population for the present study. It was assumed that the follow up may include 68 infants (92% of the available population, 34 in each group), and that the frequency of atopic disease in the control group (fed with cow's milk formula) will amount to 40%. Such frequency was adopted on the basis of a study presented by Lucas et al., in which the incidence of any allergic reaction ranged from 42% to 46% of the cohort of 777 pre-term infants (8). Assuming that the risk of type I error equaled to 5% (two sided test) the study with the power of 80% might demonstrate the reduction of 21% (absolute risk reduction – ARR) or the increase of the risk of atopic disease in the experimental group by 23%. Sample size calculation was

performed using PS Power and Sample Size Calculations software (Version 2.1.30, February 2003, <http://www.mc.vanderbilt.edu/prevmed/ps/index.htm>).

Results

From among 80 infants participating in the double-blind original study, 74 children were available for the present study (six newborns died during early infancy). From February 1, 2005 to August 31, 2006, 62 newborns (84% of the available population) agreed to participate in the study. Study design and follow-up rate are presented in the Fig. 1.

Both groups were compared with reference to various perinatal parameters as well as major known risk factors of allergy. The characteristics of the study populations are shown in the Tables 2 and 3. Data about parental education are missing in some children, as the parents were unwilling to disclose such information. The hydrolyzate and cow's milk formula groups did not differ in terms of demographic characteristics and perinatal risk factors.

Table 4. presents the results of comparison between both formula groups in terms of prevalence of allergic diseases. The prevalence of obvious allergic diseases was not significantly different between the studied groups. The detailed analysis of the incidence of wheezing, atopic dermatitis or hay fever failed to show a significant difference between the groups. Comparison of atopic status across groups revealed no significant difference for positive markers of atopy (IgE, SPT, CD4 + CCR4 + /CD4 + CXCR3 + ratio) between the studied groups (Table 5). Serum total IgE levels, as well as absolute eosinophils count were similar in both groups. The difference in lymphocyte CD4 + CCR4 + /CD4 + CXCR3 + ratio was the only statistically significant difference (Table 6). Logistic regression was used to estimate odds ratios for developing obvious allergic disease, depending on type of feeding during the first month of life; controlling for confounding variables. It was found that family history of atopy is the only independent risk factor for developing obvious allergic disease (Table 7).

Discussion

To our knowledge, there have been no studies on the influence of early nutrition in VLBW infants on development of allergy. Past studies included premature infants at later gestational age and these with family history of allergy (8, 9). Ours is

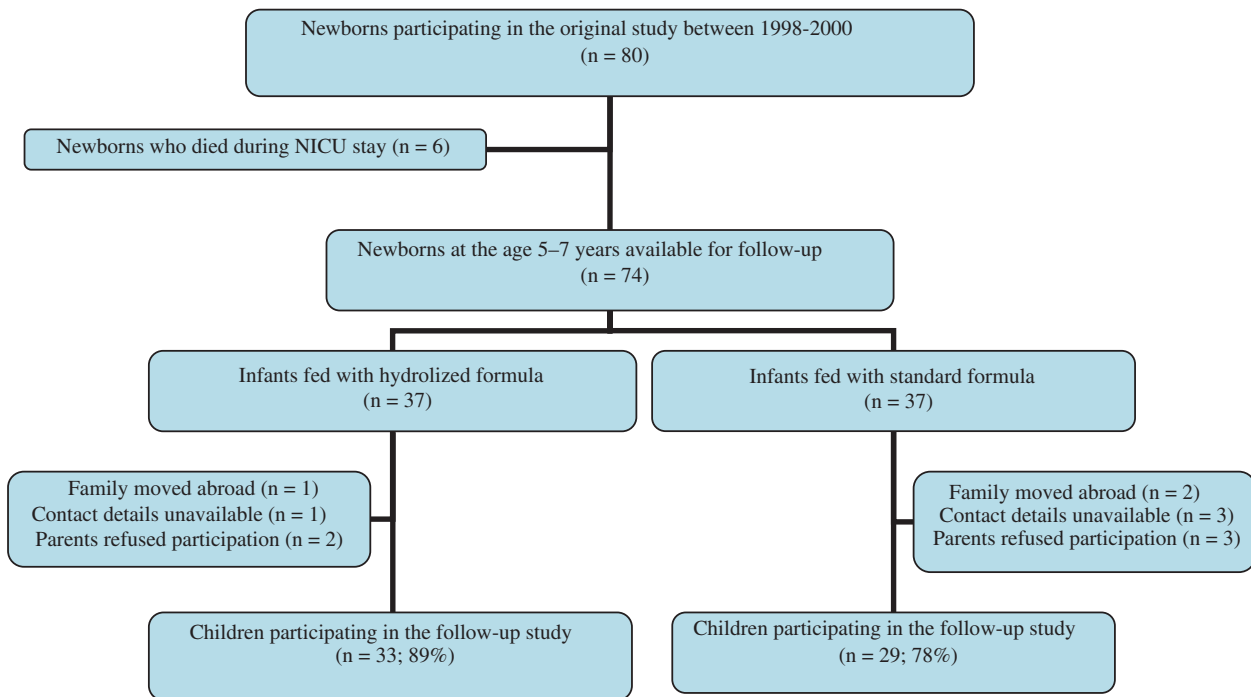


Fig. 1. Study design and follow-up rate.

Table 2. Comparison of the selected demographic variables and environmental risk factors of atopy between the studied groups

	Cow's milk formula group (n = 29)	Hydrolyzed formula group (n = 33)	p
Sex (male/female)	15/14	17/16	0.99*
Age on evaluation (yr; mean; 25th–75th percentile)	6.2 (5.84–6.64)	6.1 (5.49–6.74)	0.42†
Weight on evaluation (kg; mean ± SD)	18.8 ± 2.8	19.1 ± 2.7	0.65‡
Height on evaluation (cm; mean ± sd)	108.6 ± 21	115 ± 5.2	0.1‡
Head circumference on evaluation (cm; mean ± SD)	50.7 ± 1.1	50.6 ± 1.6	0.73‡
Birth weight (g; mean ± sd)	1156 ± 258	1097 ± 217	0.32‡
Gestational age (wks; mean ± SD)	28.7 ± 2.2	28.6 ± 2.4	0.8‡
Family history of atopy	5 (17%)	3 (9%)	0.46*
Pets at home	22 (76%)	21 (64%)	0.41*
Passive smoking	20 (69%)	19 (58%)	0.43*
Parental education			
(i) Less than 10 yr (primary)	6	4	0.55§
(ii) 10–13 yr (secondary)	18	23	
(iii) More than 13 yr (high)	2	4	
Day care attendance	22 (76%)	22 (67%)	0.58*
Siblings at home	24 (83%)	26 (79%)	0.76*

*Fisher's exact test.

†Kruskal–Wallis test.

‡Analysis of variance (anova).

§Chi-square test.

thus the first one to provide overall data for VLBW infants regardless of their family atopic status. Noteworthy is also a very long follow-up

Table 3. Comparison of the type of feeding during the first year of life between the studied groups

	Cow's milk formula group (n = 29)	Hydrolyzed formula group (n = 33)	p
Feeding after the first month of life (until discharge)			
(i) Cow's milk formula	27	26	0.15*
(ii) Hydrolyzed formula	2	7	
Breast feeding	5 (17%)	4 (12%)	0.72*
Post-discharge formula			
(i) Pre-term milk	20	20	0.66‡
(ii) Standard formula	5	6	
(iii) Special formula	3	6	
Additional foods (soup, etc.) (month of life, mean ± sd)	6.8 ± 2.6	6.5 ± 2.8	0.66†
Solid foods (month of life; mean ± SD)	10.2 ± 3.8	9.9 ± 3.9	0.77†
First gluten foods (month of life; mean ± sd)	11.1 ± 2.4	10.1 ± 2.2	0.3†
Anti-allergenic diet during first year of life	4 (14%)	3 (9%)	0.7*

*Fisher's exact test.

†Analysis of variance (anova).

‡Chi-square test.

period of our patients, by far exceeding the majority of trials performed in pre-mature babies.

We found no significant difference of prevalence of allergic diseases or positive markers of atopy between children fed with cow's milk formula or fed with the hydrolyzates. To validate

Table 4. Comparison of the presence of atopic disease between the groups

	Cow's milk formula group (n = 29)	Hydrolyzed formula group (n = 33)	RR (95% CI)	p
Obvious allergic disease	6 (20.7%)	12 (37%)	1.76 (0.76–4.09) for obvious allergic disease vs. no allergic disease	0.26
Possible allergic disease	6 (20.7%)	5 (15%)		
No allergic disease	17 (58.4%)	16 (48%)		
<i>Obvious allergic disease</i>				
Wheeze in the previous year	4 (14%)	10 (30%)	2.2 (0.77–6.26)	0.14
Sneezing with itchy-watery eyes in the previous year	6 (21%)	9 (27%)	1.32 (0.53–3.26)	0.57
Itchy rash in the previous year with any flexural involvement or around the neck, ears or eyes.	3 (10%)	1 (3%)	0.29 (0.03–2.66)	0.33

RR, relative risk; 95% CI, 95% confidence interval.

Table 5. Comparison of the subject's sensitization status between the groups

	Cow's milk formula group	Hydrolyzed formula group	RR (95% CI)	p
Positive IgE evaluation	3 (14%)	11 (37%)	2.57 (0.91–8.08)	0.11
Positive SPTs	1 (4.5%)	7 (23%)	5.13 (0.93–31.6)	0.12
Lymphocyte CD4 + CCR4 + /CD4 + CXCR3 + <1.0	3 (15%)	8 (35%)	2.32 (0.78–7.53)	0.17

RR, relative risk; 95% CI, 95% confidence interval; SPT, skin prick test.

Table 6. Comparison of the results of laboratory tests between the groups

	Cow's milk formula group	Hydrolyzed formula group	p for anova
Total IgE (kU/l) (geometric mean, SD)	31.5 (3.22)	43 (3.97)	0.3
Absolute eosinophils count (/mm ³)(mean, SD)	209 (219)	293 (395)	0.3
Lymphocyte CD4 + CCR4 + /CD4 + CXCR3 + ratio (mean, SD)	1.99 (0.55)	1.2 (0.4)	0.016

Table 7. Logistic regression with obvious allergic disease as the outcome variable

Factor	OR (95% CI)	p
Birthweight (100 g)	1.07 (0.82–1.41)	0.62
Sex (male)	2.17 (0.58–8.13)	0.25
Family history of atopy	6.16 (1.02–37.2)	0.03
Breast feeding	2.74 (0.47–15.9)	0.26
Hydrolyzed formula feeding during first month of life	3.43 (0.9–13.1)	0.08

OR, odds ratio; 95% CI, 95% confidence interval.

our findings we managed to prove that both groups did not differ significantly in terms of growth parameters, gender, gestational age, risk factors for atopy (family history of atopy, pets at home, passive smoking, post-discharge feeding, including introduction of solid foods, gluten food, use of anti-allergenic diet).

In view of the small sample size in the study, and given the possibility of type II error, possi-

bilities of interpreting the insignificant results should be considered.

The observed frequency of obvious allergic diseases in the control group (infants fed with cow's milk formula) equaled 21% and was lower than values used to assess the sample size. The size of the population would allow for demonstrating the difference in the incidence of atopic disease if the prevalence in the experimental group (hydrolyzed formula fed group) was lower than 6% or higher than 42% (two-sided test; $\alpha = 0.05$; Power 80%), or, in other words, for showing that the relative risk of atopic disease in the experimental group when compared to the control group is lower than 0.23 or higher than 2.53. The failure to prove a statistically significant difference between the groups indicates that if there exists any difference, it is smaller than the values given above. Even with the 'best possible scenario', hydrolyzed diet may decrease the risk of atopic disease in neonates with very low birth

weight only by less than 15%. The beneficial effect of the hydrolyzed diet would be even smaller if other end points were assessed: with positive SPTs the best scenario risk reduction would be 4.5% and in case of positive IgE the best scenario risk reduction would equal 10%. The question arises if this intervention is important clinically, and the answer depends not only on the kind of intervention but also on the risk of adverse effects and their character.

Moreover, negative results may also originate from the fact that dietary intervention lasted only one month. At the time when the original study was implemented, hydrolyzates were not routinely used. Moreover, the data then available suggested that prolonged use of hydrolyzates in VLBW neonates may impair their growth and nitrogen balance. The length of intervention was discussed in detail among the research team and ethics committee, and it was decided that one-month diet was long enough to verify the hypotheses that the frequency of episodes of food intolerance in the group of children fed with a hydrolyzate is similar to the frequency of such episodes in children receiving the standard formula for pre-term neonates and safe enough for the patients.

The results of a systematic review presented by A. Host indicate that, if breast feeding is not possible, a hydrolyzed formula is recommended for the first four months of life for primary allergic diseases prevention in high-risk infants (20).

Although the study included only 62 children, the results obtained seem to be interesting. The main rationale behind the use of hydrolyzates is reduction of atopic disease risk. Obtained confidence intervals allow us to state that such diet at best only slightly reduce the risk of atopy. The results point the fact that it is necessary to conduct studies including a high number of patients to credibly assess usefulness of hydrolyzed formulas in the prevention of atopic diseases. Such studies, however, are difficult to conduct.

Pregestimil is a lactose-free casein hydrolyzate infant formula, containing, unlike many commonly used hydrolyzates, maize syrup and glucose polymers as carbohydrates as well as medium-chain triglyceride oil as fat source. Hydrolyzate and standard formulas differ in their specific taste and odor. It could have affected results of the primary studies but rather unlikely these of the follow up study, since the investigators evaluating our patients had no chance to know the assignment. In the primary study, formulas were prepared by nutrition staff and administered by nurses who did not partic-

ipate in the follow-up study. Frequently raised issue concerns tolerance of hydrolyzed formulas. In our study it is not an issue, because all children were initially fed by a gastric tube.

It could be doubted if premature infants are capable of eliciting allergic reactions, but Lucas et al. (21) provided evidence of early sensitization in pre-mature infants fed with cow's milk formula in early neonatal period.

It is possible that early exposure to an antigen in VLBW infants would lead to tolerance and lower risk of sensitization (22). This is contrary to events observed usually in term infants, where some investigators have suggested that brief, neonatal exposure to cows' milk formulae may increase the risk of developing atopic disease (23, 24). These studies, however, did not enroll premature infants. Szajewska et al. (9) performed a RCT which enrolled pre-term infants. The subjects had a positive family history of atopy, and received either extensively hydrolyzed or standard pre-term formula. Overall incidence of atopic diseases did not differ between the groups after five and twelve months, except for atopic dermatitis (AD) that by twelve months was more prevalent in the standard formula group. This was the case only in intention-to-treat analysis, because per-protocol analysis eliminated this difference. Likewise, there were no differences between groups as regards total IgE and cow's milk specific IgE. This study differs from ours in terms of cohorts enrolled (family history of atopy, degree of pre-maturity) and the duration of follow-up.

Mihatsch et al. have performed a study assessing gastrointestinal transit time, in which VLBW infants were fed with either hydrolyzed or standard formula. Transit time in infants fed with hydrolyzed formula was significantly shorter than in the standard formula group (25). Such difference could be responsible for longer contact of intestinal mucosa with milk proteins in non-hydrolyzed formula group, and the question remains whether such contact would produce sensitization or tolerance to milk antigens.

There are some factors that could possibly affect the atopic status by modifying the Th-cell balance, for example, exposure to other allergens, microbial colonization, bacterial infections. So, the presence of bacteria could produce a shift towards a Th₁ response, on the other hand, the use of antibiotics, by modifying gut flora, could prevent the Th₁ cell maturation (26). There are however no grounds to suspect that such differences were responsible for results in our cohorts, given a balanced outcome of randomization.

After literature search on prevalence of allergic diseases in pre-term infants, we have concluded that there is few data applicable for comparison with our results. The prevalence of atopic diseases in our group was 32% for an obvious allergic disease, 19% for a possible atopic disease and 49% for no allergic disease. Elevated IgE levels were detected in 30.4%, positive SPTs occurred in 13%, and lymphocyte CD4 + CCR4 + / CD4 + CXCR3 + ratio < 1 in 18.5% of VLBW infants. These data are consistent with the data on the VLBW infants population presented in other studies (27, 28). Additionally, Agosti et al. have demonstrated that the prevalence of atopy in premature infants did not change significantly with age within the range 2–6 yr.

Moreover, we compared the prevalence of allergic disorders among VLBW infants to ISAAC regional data (11, 12). The prevalence of wheezing ever (48.8% vs. 28.6%) and wheezing last year (26.4% vs. 13.6%) were higher in the VLBW infants. The number of children with asthma diagnosis was higher in the VLBW group (23.6% vs. 5.8%). The prevalence of AR ever symptoms (34.5% vs. 33.6%) and established AR diagnosis (19.1% vs. 21.1%) were similar in the VLBW group and regional ISAAC cohort. These findings suggest that symptoms of asthma in 5–7-yr-old VLBW infants are associated with persistent bronchial hyper-reactivity rather than atopic asthma. Similar results have been presented by other authors (29–31). Additionally, a follow-up study of a group of 454 newborns have demonstrated that neither gestational age nor birth weight were risk factors for AD or AR at 6 yr of age and for positive skin prick test at 2 yr of age (32).

However, the exclusion of the incidence of asthma from the present study and focusing on atopic dermatitis, allergic rhinitis and atopic status does not change the results of the study significantly.

An excellent comparison of the allergic diseases in pre-term and term infants has been presented by Siltanen in her dissertation (26). What is relevant to our study is the finding that early introduction of cow's milk based formula had a somewhat protective effects in terms of lower cow's milk antibodies, in contrast to term infants, where early introduction of cow's milk based formula resulted in higher antibody titers.

Because the existing data supports neither better tolerance nor lower allergenicity of hydrolyzed formulas, a question arises if there are some growth and development benefits. In a small RCT, pre-term infants were fed with extensively hydrolyzed cow's milk formula or standard

isocaloric and isonitrogenous pre-mature formula. After a mean follow-up of about 24 days, head circumference gain and weight gain were significantly better in the standard formula group (33). Picaud et al. have demonstrated that pre-mature infants fed hydrolyzed formula exhibited lower nitrogen absorption than these fed standard pre-mature formula. However, there was no negative effect on infant growth (34). So, it seems that feeding with hydrolyzed formula brings no marked benefits.

Conclusion

Based on the carried out follow-up study we were unable to confirm the usefulness of hydrolyzed formula in prevention of allergy in unselected cohort of VLBW infants.

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