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Feasibility and compliance in a nutritional primary prevention trial in infants at increased risk for type 1 diabetes

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Abstract

Aim—The international Trial to Reduce IDDM in the Genetically at Risk (TRIGR) was launched to determine whether weaning to a highly hydrolysed formula in infancy reduces the incidence of type 1 diabetes in children at increased genetic disease susceptibility. We describe here the findings on feasibility and compliance from the pilot study.

Methods—The protocol was tested in 240 children. The diet of the participating children was assessed by self-administered dietary forms, a structured questionnaire and a food record. Blood samples were taken and weight and height measured at birth and at 3, 6, 9, 12, 18 and 24 months.

Results—A majority of the subjects (84%) were exposed to the study formula at least for 2 months. Linear growth or weight gain over the first 2 years of life was similar in the two study groups. The levels of IgA and IgG antibodies to cow's milk and casein were higher in the cow's

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CONFLICT OF INTEREST

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milk-based formula group than in the hydrolysed formula group during the intervention period ($p < 0.05$), reflecting the difference in the intake of cow's milk protein.

Conclusion—This randomized trial on infant feeding turned out to be feasible, and dietary compliance was acceptable. Valuable experience was gained for the planning and sample size estimation of the study proper.

Keywords

Compliance; Feasibility; Hydrolysed infant formula; Infants; Primary prevention

INTRODUCTION

Environmental factors such as diet may play an important role in the development of type 1 diabetes (1). Early introduction of complex proteins, such as cow's milk proteins, has been reported to be associated with increased risk for type 1 diabetes among children carrying genetic disease susceptibility, although not consistently so (2).

The Trial to Reduce IDDM in the Genetically at Risk (TRIGR) project was launched to determine whether complete avoidance of cow's milk exposure during at least the first 6 months of life prevents type 1 diabetes in genetically susceptible children (3). We report here findings from the pilot study of the TRIGR project carried out in Finland, Estonia and Sweden. The primary aims of the pilot study were to develop and test protocols for a nutritional intervention trial in infants with increased genetic diabetes risk and to assess the feasibility of such an approach. This work sets out to assess dietary compliance, infant feeding patterns and the feasibility findings in this pilot study of a nutritional primary prevention trial.

SUBJECTS AND METHODS

Recruitment

Between 1995 and 1997, all women with childbirth and their newborn infant known to have a first-degree relative with type 1 diabetes were invited to the study in 15 Finnish hospitals. Written informed consent was obtained from the family. The study was approved by the Joint Ethics Committees of the participating hospitals. The information on diabetes in the families was obtained from maternal health care centres, maternal clinics, or from childhood diabetes clinics, in which an invitation letter was given to the families including a mother, father and / or child with type 1 diabetes. The staff in the maternal health centres were informed about the study at regional training and through an information letter. The personnel at the maternal clinics as well as at paediatric diabetes clinics received both oral and written information about the study through the local TRIGR investigators. Information about the study was also spread nationwide through the journal of the Finnish Diabetes Association. To facilitate recruitment and to minimize inadvertent exposure to cow's milk proteins, every attempt was made to identify eligible families before the child was born.

Altogether, parents of 521 newborn infants consented to participate in the study. Of them, 25 were excluded because they did not fulfil the inclusion criteria. The reasons for exclusion

were prematurity (gestational age < 36 weeks) (n = 20), stillbirth (n = 4) and serious disease in the newborn infant (n = 1). In addition, no blood sample for HLA analysis was received from seven infants, the hospital personnel had forgotten to ask for a research code for five infants, three families withdrew their participation, and one family did not want to know the HLA genotype. The reason for not starting the study remained unknown in the case of four families. Of the 476 infants who received the study code at birth, 230 (48.3%) carried one of the HLA risk genotypes and were asked to continue in the intervention study, as described earlier (3). In addition, four children from Linköping, Sweden, and six children from Tallinn, Estonia, carrying one of the HLA risk genotypes participated in the pilot study.

Randomization and dietary intervention

Immediately after birth, the infants were randomized to receive either the intervention formula (n = 112, Nutramigen®, based on extensively hydrolysed casein) or the control formula (n = 118, whey-adapted cow's milk protein formula) whenever breast milk was not available. Both formulas were provided by Mead Johnson Nutritionals, Evansville, IL, USA. The control formula included 20% casein hydrolysate to eliminate the taste and smell difference between the two study formulas. All formulas were prepared and numbered and colour-coded by the company that kept the codes.

At the delivery hospital, all mothers received practical advice and a booklet about the use of the study formula and foods to be avoided. The study dieticians and nurses gave further dietary counselling at the 3- and 6-month follow-up visits. The dietary intervention lasted until the infant reached at least the age of 6 months. If the mother chose to exclusively breastfeed up to that age, she was asked to give the study formula for 2 months thereafter, at most until the age of 8 months. The aim was that all subjects would use study formula for at least 2 months. The families were advised to completely avoid foods containing cow's milk or beef in the infant diet during the intervention period. Otherwise, the families were encouraged to follow the national nutritional recommendations for infants (4). Breastfeeding was encouraged and no intervention was applied in terms of maternal diet during pregnancy and lactation.

Among the Swedish children, one was randomized to receive the hydrolysed infant formula and three the cow's milk-based formula. The respective figures among the Estonian children were two and four.

Training of the study personnel

The local study nurses, dieticians and paediatricians were trained for the field study at national training meetings twice a year. The coordinating study dietician and nurse made site visits to each centre at least twice during the study period. They went carefully through the study protocol and gave advice on counselling of the families, giving dietary advice, completing questionnaires and forms, taking blood and breast milk samples, as well as on checking food records and other questionnaires. Further information and education was given via regular newsletters (3–4 per year). Local study personnel were encouraged to consult the coordinating centre in case of any uncertainties. The field manual contained a detailed description of the background, aims, target population, exclusion criteria, design,

timetable, invitation procedures, study material, study codes (for infants, blood samples, breast milk samples and study personnel), procedures at the delivery hospital, contents of the follow-up visits and calls, measurement of growth, distribution of the study formula and withdrawal from the study.

Delivery of study formula

If the infants needed additional feeding to breast milk in the delivery hospital, they received their own study formula. Newborn infants requiring supplemental feeding prior to randomization (e.g. subjects born at night or on weekends) received banked breast milk or casein hydrolysate formula. When discharged from the delivery hospital, the families received 2.4 kg of the study formula powder (equals to 18 L of milk). Those who continued in the study received additional study formula from the local study centre or by mail whenever needed.

Follow-up of the diet of the infant and the mother

The families completed a dietary form at home on which they were instructed to record all deviations from the advised diet (type and amount of food and date) and the age at introduction of the following foods or food groups: potato and vegetables; fruits and berries; cereals; pork or chicken; fish; egg; dietary fats; and soy-based infant formula. The dietary form was reviewed at the visits to the study centre and the study dieticians and nurses recorded the information on the child's follow-up chart. In addition, they asked the families about the duration of exclusive and total breastfeeding and the age at introduction and end of the study formula feeding. The amount of study formula delivered and returned, as well as the feeding at the delivery hospital was also recorded on the follow-up chart. For Swedish and Estonian children, only information on breastfeeding and study formula use was recorded.

The diet of the participating children was studied by a 2-day food record at the age of 6 months and by a 3-day food record at the age of 1 and 2. Maternal diet was studied by a pre-tested and validated food frequency questionnaire (5). The questionnaire focused on the diet of the mother during the 8th month of pregnancy. If the mother was breastfeeding when the child was 3 months old, her diet during the preceding month was studied with the same food frequency questionnaire.

Blood samples

Venous blood samples were taken from the infants' arm after applying anaesthetic cream by experienced paediatric nurses / laboratory technicians at the study centre visits at 3, 6, 9, 12, 18 and 24 months of age. At birth, the blood sample was taken from cord blood.

Monitoring of growth

Length and weight were measured at birth and at the study centre visits at the age of 3, 6, 9, 12, 18 and 24 months.

Measurement of dietary compliance

The age at the start and end of study formula feeding was recorded. The compliance to the advised diet was assessed by self-reported deviations from the advised diet and by analysing the levels of circulating antibodies to cow's milk proteins at the age of 3 and 6 months.

HLA genotyping

HLA-DQB1 genotyping was performed on cord blood specimens to define selected alleles (DQB1*02, DQB1*0301, DQB1*0302 and DQB1*0602 / 3) known to be significantly associated with either susceptibility to or protection against type 1 diabetes in the Finnish population (6), as described earlier (3).

Measurement of cow's milk protein antibodies

Cow's milk antibodies (IgG, IgA and IgM), β -lactoglobulin antibodies (IgG and IgA) (7), α -casein antibodies (IgG and IgA) (8) and bovine serum albumin antibodies (IgG and IgA) (9) were measured with modifications of the original ELISA techniques as described (10).

Statistical methods

The t-test was used for comparison of duration of breast-feeding and body mass index between the intervention groups. Duration of exclusive breastfeeding and results on study formula use were analysed by Mann–Whitney test. Dietary deviations were analysed by chi-square test and Mann–Whitney test. For comparison of cow's milk antibody levels, area under the curve (AUC) (11) was calculated and the results were analysed with Mann–Whitney test. The association between dietary deviations and levels of cow's milk antibodies was analysed by Mann–Whitney test. Statistical analyses were performed by SPSS for Windows 11.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Dropout rate

Fifty (22%) of the 230 Finnish infants who started the intervention withdrew from the study by the age of 2 (Fig. 1). About one-third of the dropouts occurred before the age of 3 months, i.e. before the first follow-up visit, and half by the age of 6 months. The reasons for loss to follow-up were mostly related to family circumstances, e.g. moving to another location or disease in the family. In 16 families, the dropout was related to study formula: six infants refused to have the study formula, seven families did not want to give it to their infant, two families suspected that the formula induced diarrhoea, and one family thought that the formula had caused constipation. Four children dropped out from the study at the time of diagnosis of cow's milk allergy and one child when cow's milk allergy was suspected, although according to the protocol they should have continued in the follow-up. Two families did not want the child to be pricked for blood testing, one family did not want to know the genetic risk, and two families could not be reached. In addition, one family had been erroneously invited to the study; there was no family member with type 1 diabetes, but the mother had had gestational diabetes.

Breastfeeding

All of the Finnish children participating in the study were breastfed. The duration of total and exclusive breastfeeding was similar in the two study groups (Table 1). The total duration of breastfeeding was shorter among mothers with type 1 diabetes than among those without type 1 diabetes (6.6 months, $n = 72$ vs. 8.6 months, $n = 117$, $p = 0.005$). The duration of exclusive breastfeeding did not differ between non-diabetic and affected mothers ($p = 0.188$). Among the four Swedish infants, the median duration of exclusive breastfeeding was 6 (range 4–8) months and that of total breastfeeding 9 (range 5–13) months, and among the six Estonian children 1.7 (range 0.2–4.0) months and 2.5 (range 0.5–19.0) months, respectively.

Use of study formula

Among the Finnish infants, the median age at introduction of study formula was lower in the cow's milk-based group than in the hydrolysed formula group, but the age at the end of study formula use did not differ between the groups (Table 1). Consequently, the duration of study formula feeding was longer in the cow's milk-based group than in the hydrolysed formula group.

The majority of the Finnish participants received study formula according to the protocol, i.e. for at least 2 months by 6 months of age and the use was stopped then, or the use of study formula was stopped between the age of 6 and 8 months when study formula had been used for 2 months (Table 2). One-third of the children received study formula after the use should have stopped. Ten per cent did not receive study formula at all. In total, 85% in the hydrolysed formula group and 83% in the cow's milk-based formula group received study formula for at least 2 months.

Of the Swedish children, only one used study formula (from the age of 4 months to 8 months). The other three infants did not receive study formula at all. The Estonian infants started to receive study formula at the median age of 1.7 months (range 0.2–4.0 months) and received it for a median duration of 7 months (range 5.0–7.8 months).

Age at introduction of new foods

The age at introduction of complementary foods was similar in the two study formula groups. Potatoes and vegetables as well as fruits and berries were introduced to the Finnish infants at a median age of 4 months. The median age at introduction of cereal products and meat was 5 months. Fish was introduced at a median age of 7 months. The majority (56%) of the infants were exposed to study formula before any other complementary food. In 37% of the infants, the first complementary food introduced was vegetables or fruits.

Deviations from the advised diet

Of the Finnish infants, 18% were reported to have deviated from the dietary restrictions at least once during the intervention period (Table 3). No continuous deviations were reported. There were no differences in the dietary deviations between the two study groups.

Cow's milk protein antibody levels during the intervention

At birth, the median levels of cow's milk IgG antibodies were slightly positive in both the hydrolysed and cow's milk-based formula groups (Fig. 2). This reflects the fact that IgG is transmitted to the infant from the mother via cord blood. The IgG antibody levels decreased during the first 3 months of life and remained low up to the age of 6 months in the hydrolysed formula group. After 3 months of age, the median levels of IgA and IgG antibodies to cow's milk and casein started to increase in the cow's milk-based formula group. The levels of the antibodies, measured as AUC (11), were higher in the cow's milk-based formula group than in the hydrolysed formula group during the intervention period ($p < 0.05$), reflecting the difference in the intake of cow's milk protein. The reported single deviations from the dietary restrictions were not reflected in the levels of cow's milk protein antibodies (data not shown).

Growth during the first 2 years of life

The growth of the Finnish participants in the hydrolysed and cow's milk-based formula groups did not differ: there were no differences in the development of length, weight or body mass index at any time point during the first 2 years of life (Fig. 3).

DISCUSSION

The present study design of a double-blind, randomized dietary intervention trial turned out to be feasible with a relatively low dropout rate, 22% by 2 years of age. The study formula was well tolerated, and dropouts because of the study formula were quite few. In other infant feeding trials using hydrolysed and long-chain polyunsaturated fatty acid containing infant formulas, dropout rates of 16–30% by 2 years have been reported (12–16) compared to 22% in the present study.

The compliance with the use of study formula was high in the current study. Of the participants, 89% were exposed to the study formula and 84% for a minimum of 2 months. Of the participating families, 18% made deviations from the dietary restrictions. However, the deviations were mostly limited to single occasions. The dietary non-compliance could be anticipated to be higher in a trial of this type, where all subjects randomized were observed according to the intention-to-treat principle, regardless of compliance, compared to trials with a per-protocol approach. Reports on infant feeding interventions during the last decade are mainly available in two fields: prevention of allergy with hydrolysed formulas and safety and efficacy of adding long-chain polyunsaturated fatty acids to infant formulas. The results regarding compliance with the study diet are seldom described in these studies, while the number of dropouts is usually reported.

In studies where dietary compliance is assessed, comparable levels of deviations from dietary restrictions as those seen in our study have been observed. In a Danish study on 550 infants at high risk for allergy, 6.2% of the children received other infant formulas than the one originally randomized to (17). In addition, 2.3% received solid food before 4 months of age against the dietary recommendations. A Swiss population-based study to assess the health benefits of an allergen-reduced nutritional programme in 1130 newborn infants

included exclusive breastfeeding, use of moderate whey hydrolysate formula and delayed introduction of low-allergenic weaning foods (18). The control group was advised to follow national infant feeding recommendations consisting of 3 months of exclusive breastfeeding and introduction of weaning foods thereafter. If infant formula was needed, conventional cow's milk-based formula was used. A dietary non-compliance rate of 8.4% was reported, including use of other infant formulas and weaning foods than recommended. In a study with a similar design from the United States (13), the diet in the intervention group was stricter: the mothers also avoided certain foods during the last trimester and lactation, and no solid foods were to be introduced to the infant before 6 months of age. About 11% of the families left the study because of difficulties with the dietary regimen, and an additional 9% in the active intervention group were exposed to another infant formula than the study formula. In a German study on allergy prevention, 2252 infants were randomized to receive one of four study formulas from weaning up to 6 months of age (19). Of the infants, 58% received study formula, and of them, 11% made deviations from the advised diet.

The dietary intervention in the present study did not interfere with breastfeeding nor with practices of introducing complementary foods. The median duration of total breastfeeding was 8 months in the current study population, which is somewhat higher than the median duration of 7 months observed in a population-based Finnish study conducted close to the same time period (10). In another Finnish intervention study (Special Turku Coronary Risk Factor Intervention Project) performed some years before the present study, the median duration of breastfeeding as the only milk source was 4 months among the control children (20). In a nationwide study on infant feeding conducted in Finland in 1995, 52% of the children were breastfed up to the age of 5 months (21), whereas in the present study this proportion was 74%. We observed a somewhat shorter duration of breastfeeding among mothers with type 1 diabetes compared to mothers without type 1 diabetes, which is in accordance with previous findings (22–25).

The median age at introduction of infant formula in the present study, 1.9 months, was comparable to a population-based Finnish study (10). In a Danish infant intervention trial (17), the median age at introduction of formula feeding was 1.0 month. In a nationwide Finnish study, 54% of the children received infant formula at the age of 3 months, whereas the respective proportion was 58% in this study. The age at introduction of the first complementary foods, i.e. vegetables, fruit and berries, was younger than recommended in the nutritional guidelines in Finland (4), but comparable to other Finnish observations (10).

During the intervention period, the AUC of four cow's milk antibodies measured was higher in the cow's milk-based formula group than in the hydrolysed formula group. This indicates that measuring cow's milk antibodies can provide a useful tool when assessing dietary compliance at a group level. Single deviations from a milk-free diet cannot be detected by measuring cow's milk antibodies, but the lack of immunological response might also indicate that these deviations are not important.

When planning the study proper, we used the information gained in this pilot study on the feasibility and compliance in such an infant intervention trial. The follow-up of the subjects was intensified with more frequent study visits and calls. The dietary interview forms were

further developed based on the experience from the present pilot study. In the study proper, the dietary compliance was continuously monitored, and the study centre personnel informed and trained at regular intervals.

We observed no differences in growth over the first 2 years of life between the children weaned to the extensively hydrolysed formula and those weaned to the cow's milk-based formula. Rzehak et al. (26) reported recently a transient lower weight gain during the first year of life among infants weaned to an extensively hydrolysed formula compared to subjects weaned to a conventional cow's milk-based formula or to hydrolysed whey formulas in the German Infant Nutritional Intervention Study. That study recruited newborn infants with atopic heredity, while the present pilot trial targeted infants with at least one family member affected by type 1 diabetes and an HLA genotype conferring increased disease susceptibility.

In conclusion, the present intervention study design and implementation turned out to be feasible, and the dietary compliance was acceptable. The pilot study gave us valuable experience in the planning and sample size estimation for the full-scale trial.

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Figure 1.
Flow chart of the Finnish study population.

Figure 2.

(A–D) Median levels of (A) cow's milk-based formula IgG antibodies, (B) cow's milk-based formula IgA antibodies, (C) casein IgG antibodies and (D) casein IgA antibodies in the Finnish children in the hydrolysed and cow's milk-based formula groups. Up to the ages of 6 and 9 months, the area under the curve (AUC) was smaller in the hydrolysed than in the cow's milk-based formula group for all four antibodies ($p < 0.05$). Up to the age of 12 months, the AUC was smaller for cow's milk-based formula IgG, casein IgG and casein IgA, and up to the age of 18 months, the AUC was smaller for cow's milk-based formula IgG in the hydrolysed than in the cow's milk-based formula group ($p < 0.05$).

Figure 3. Body mass index [weight (kg) / height (m)²] up to the age of 2 years in the Finnish children in the hydrolysed and cow's milk-based formula groups.

Table 1

Infant feeding characteristics in the hydrolysed and cow's milk-based formula groups (Finnish infants observed at least up to the age of 9 months included)

Infant feeding variable, months	Hydrolysed formula group, n = 88	Cow's milk-based formula group, n = 101	Difference p value
Duration of total breast feeding, mean (SD)	8.6 (4.8)	7.3 (4.9)	0.059
Duration of exclusive breast feeding, median (range)	3.0 (0.0–6.0)	1.5 (0.03–6.0)	0.086
Age at introduction of study formula, median (range)	3.0 (0.0–8.0)	1.1 (0.0–8.0)	0.028
Age at end of study formula feeding, median (range)	7.4 (0.2–11.8)	6.5 (0.5–12.0)	0.149
Duration of study formula feeding, median (range)	3.5 (0.3–11.5)	5.4 (0.2–9.9)	0.038

Table 2

Use of study formula in the hydrolysed and cow's milk-based formula groups (Finnish infants observed at least up to the age of 9 months included)

Use of study formula	Hydrolysed formula group, n (%)	Cow's milk-based formula group, n (%)
According to protocol		
End at 6 months [*]	28 (31.8)	43 (42.6)
End between 6 and 8 months [†]	18 (20.5)	11 (10.9)
Too late end [‡]	30 (34.1)	33 (32.7)
Too early end [§]	2 (2.3)	3 (3.0)
No use at all	10 (11.4)	9 (8.9)
Missing data	0 (0.0)	2 (2.0)
Total	88 (100.0)	101 (100.0)

^{*} Study formula use ended at 6 months of age, length of study formula use = 2 months.

[†] Length of study formula use <2 months at 6 months of age, study formula use ended between 6 and 8 months of age as soon as length of use was 2 months or study formula use ended at 8 months of age even if length of use was <2 months.

[‡] Use of study formula ended after 6 months of age even if the length of study formula use was = 2 months at 6 months of age or study formula use ended after 8 months of age.

[§] Use of study formula ended before 6 months of age or use of study formula ended before 8 months of age in case the length of study formula use was <2 months.

Table 3

Proportion of infants whose parents reported dietary deviations during the dietary intervention period and median number of deviations and median age of the infant at the time of deviation (Finnish infants observed at least up to the age of 9 months included)

Dietary deviation variable	Hydrolysed formula group, n = 88	Cow's milk-based formula group, n = 101
Infants with at least one deviation, n (%)	15 (17.0)	19 (18.8)
Cow's milk products	8 (9.1)	12 (11.9)
Beef products	8 (9.1)	7 (6.9)
Infants with at least two deviations, n (%)	4 (4.5)	7 (6.9)
Number of deviations, median (range)	1 (1–2)	1 (1–4)
Age at the time of first deviation, median months (range)	5.5 (3.2–7.5)	5.5 (1.7–7.0)