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Evaluation of diet and growth in children with and without atopic eczema: follow-up study from birth to 4 years

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Current research into dietary factors contributing to the development of allergic diseases is directed towards new active approaches instead of passive elimination diets. The present study aimed to investigate the explanatory role of the diet in a probiotic intervention study on the appearance of atopic eczema (AE) in childhood and the safety of perinatal supplementation with probiotics (*Lactobacillus rhamnosus* strain GG; ATCC 53 103). A prospective follow-up study from birth to 48 months of children (*n* 159) with a family history of allergic disease was carried out. Outcome measures included growth, dietary intake assessed with 4 d food diaries and their association with AE by logistic regression models. Increased intakes of retinol, Ca and Zn, with perinatal administration of probiotics, reduced the risk of AE, whilst an increase in intake of ascorbic acid increased the likelihood of AE. Perinatal administration of probiotics was safe, as it did not influence the height (mean difference 0·04 (95 % CI −0·33, 0·40) SD scores, $P=0·852$) or the weight-for-height (mean difference −3·35 (95 % CI −7·07, 0·37)%, $P=0·077$) of the children at 48 months with and without perinatal administration of probiotics. Up to 48 months, AE did not affect height (mean difference −0·05 (95 % CI −0·42, 0·33) SD scores, $P=0·815$), but mean weight-for-height in children with AE was −5·1 % (95 % CI −8·9, −1·2 %) lower compared with children without ($P=0·010$). The joint effects of nutrients and probiotics need to be considered in active prevention and management schemes for allergic diseases.

Atopic eczema: Nutrient intake: Growth: Probiotics

Symptoms compatible with allergic diseases are increasing in industrialized countries (Ninan & Russell, 1992; Aberg *et al.* 1995). Allergic diseases not only hamper the daily life of children but may also influence their long-term health, as indicated by a decline in growth (Chang *et al.* 1982; Ferguson *et al.* 1982; Kristmundsdottir & David, 1987; Massarano *et al.* 1993; Tiainen *et al.* 1995; Isolauri *et al.* 1998; Agostoni *et al.* 2000; Christie *et al.* 2002). Principal theories on the origins of allergic diseases are linked to changes in nutrition along with general environmental changes, which appear to shape the immune responder type of the host during a critical period of life to allergic-type immune responsiveness.

For natural reasons, the main interest in dietary factors with regard to allergic diseases has centred on proteins, the antigens. Food-derived antigens may initiate or exacerbate the symptoms of allergic diseases and consequently various elimination diets, i.e. exclusion of one or more foods from the diet, have been used in attempts to both prevent and manage allergic diseases. In the former case, elimination diets have proved unsuccessful (Zeiger & Heller, 1995), whilst in the latter they form the basis for management of documented food allergies (Fiocchi *et al.* 2004). Both are associated with an increased risk of nutritional inadequacies resulting particularly from the elimination from the diet of key foods such as milk or cereals.

Beyond this, recent research has focused on active prevention schemes as opposed to passive elimination diets. Supplementation with nutrients, including fatty acids (Dunstan *et al.* 2003) and probiotics (Kalliomäki *et al.* 2001, 2003), due to their immunomodulatory properties, may play a role in reducing the risk of allergic disease. However, previous studies do not report the nutrient intakes in subjects' habitual diets. This calls for an evaluation of the contribution of early dietary composition and supplements and their interactions to the risk of allergic disease. We sought to investigate the contribution of early dietary factors in a probiotic intervention study on the appearance of atopic eczema (AE) in childhood. In addition, we report the safety of perinatal administration of probiotics and the dietary intake and growth of children with and without AE.

Subjects and methods

Subjects and study design

The study population consisted of children (*n* 159) with a family history of AE (i.e. mother, father and/or older sibling with atopic

Abbreviations: AE, atopic eczema; CMA, cow's milk allergy; NNR, Nordic Nutrition Recommendations.

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eczema, allergic rhinitis or asthma) who participated in a prospective allergy prevention study described in detail elsewhere (Kalliomäki *et al.* 2001, 2003). Briefly, in a double-blind placebo-controlled trial, *Lactobacillus GG* (*Lactobacillus rhamnosus* strain GG; ATCC 53 103) was administered prenatally to mothers and postnatally for 6 months to their infants. Children were followed for their clinical status during the first four years of life. Study visits took place at 3 weeks and at 3, 6, 12, 18, 24 and 48 months. The present paper reports dietary and growth data on children with and without AE at the ages of 6, 12 and 48 months, and evaluates the explanatory role of diet for AE at 24 and 48 months. The study was approved by the First Ethical Committee of the Hospital District of SouthWest Finland. Informed consent was obtained from the children's parents.

Clinical evaluation

Weight and height were measured at each study visit, and biceps, triceps, subscapular and suprailiac skin fold thicknesses (Holtain Tanner/Whitehouse skinfold calliper; Marsden Weighing Group, Henley-on-Thames, Oxfordshire, UK) and mid-upper arm circumference were measured at the 48-month visit. To standardize data for age and sex, height standard deviation (SD) scores and weight proportional for height (weight-for-height, %) were calculated using Finnish reference values (Sorva *et al.* 1984). Mid-upper arm muscle circumference (mid-upper arm circumference (cm) minus pi times triceps skinfold (cm)) and proportion of body fat were calculated (Siri, 1961; Brook, 1971). Values less than -2 SD scores for height and less than $-20\,\%$ for weight-for-height were considered low and $+20\,\%$ for weight-for-height were considered high.

Diagnosis of AE was confirmed if the following features were detected: pruritus, facial and/or extensor involvement and chronic course (Hanifin, 1991). The last-mentioned criterion was fulfilled at 6- and 12-month study visits if there had been manifest eczema with a duration of 1 month or longer. At 24 and 48 months the criterion was fulfilled only if eczema evinced a chronic relapsing course, i.e. it had occurred at least twice with a duration of 1 month or longer. Cow's milk allergy (CMA) was diagnosed by doubleblind placebo-controlled cow's milk challenge at the age of 12 months. Clinical evaluation for the manifestation of AE was carried out by the same physician (M. K.).

Dietary assessment

Children who had their dietary intake recorded at the ages of 6, 12 and 48 months and had their clinical status recorded for AE at the same time points and at 24 months were included in the analyses of dietary intake and association between diet and AE. In view of the unknown amounts suckled and the composition of individual breast milks, the food diaries of partially or exclusively breastfed infants were excluded when the intake of energy and nutrients was analysed. Four-day food diaries with household measures were filled in by the parents or by personnel if the child was in daycare. On each occasion the families were requested to keep a food record on certain days and also to include at least one weekend day. Food records were checked item by item for completeness and accuracy with the aid of a portion picture booklet at each study visit. If needed, missing portion sizes, food descriptions, food preparation methods, etc. were added. Energy and nutrient intakes were calculated with the Micro-Nutrica® computer program (version 2.0; Research Centre for Social Insurance Institution, Turku, Finland), which uses the Food and Nutrient Data Base of the Social Insurance Institution and is constantly updated with manufacturers' data including commercial infant formula and foods. The program gives a reasonably good estimate of the intake of energy and most nutrients (Hakala *et al.* 1996).

Statistical analysis

Values are reported as mean with 95 % CI, unless otherwise specified. Differences between children with and without AE were evaluated by the independent-samples *t* test for continuous variables. The χ^2 test or Fisher's exact test was used for dichotomous variables. The influence of AE on longitudinal growth, height (SD score) and weight-for-height (%), separately, was analysed by repeated-measures ANOVA, where the intervention (*Lactobacillus GG v*. placebo) was included as a categorical covariate. At the age of 48 months the effect of AE and *Lactobacillus GG* on growth was analysed by two-way ANOVA. In both analyses the effects of probiotics and AE are given as mean effect with 95 % CI. To characterize dietary factors possibly explaining AE in children at the age of 24 and 48 months, three logistic regression models were formed for both ages, including the dietary variables measured at the age of 6 months (model A), at 12 months (model B) and at 6 and 12 months (model C). Variables introduced into the models included dietary intakes of vitamins and minerals (μg/d or mg/d or per MJ per d) and fat (g/d and % of energy), duration of exclusive (months) or total breast-feeding (months), breastfeeding status at 6 months (yes/no) and 12 months (yes/no), and administration of *Lactobacillus GG* (yes/no). In order to control for the possible confounding effect of maternal and paternal allergic disease, these were introduced into the models separately and together (yes/no). The criteria for entering and removing a variable were probability of *F*-to-enter $\leq 0{\cdot}10$ and probability for *F*-to-remove $\geq 0{\cdot}15$. Odds ratios (OR) and 95 % CI for the final models were computed. A two-tailed *P* value of $< 0{\cdot}05$ was considered statistically significant. All statistical analyses were performed using SPSS for Windows (version 12.0.1; SPSS Inc., Chicago, IL, USA).

Results

Clinical characteristics

AE was diagnosed in 29 % (46/159) of children at the age of 6 months, 46 % (65/140) at 12 months, 35 % (46/132) at 24 months and 36 % (39/107) at 48 months. At the age of 12 months, CMA was diagnosed in eighteen children (14 %). To describe the natural course of AE in the study population (Fig. 1), only children attending all study visits up to 48 months of age were examined to avoid bias caused by patient drop-outs. Forty-one per cent of the thirty-nine children with AE at 6 months persisted with AE during the whole follow-up, up to the age of 48 months. The proportion of children without evidence of AE decreased from 63 % to 39 %, from 6 to 48 months, and 69 % of those sixty-seven children healthy at 6 months were without AE during the whole follow-up. A total of sixty-five (61 %) out of all children were diagnosed with AE during the follow-up, thirty-nine (60 %) of them at the age of 6 months, twelve (18 %) at 12 months, seven (11 %) at 24 months and seven (11 %) at 48 months.

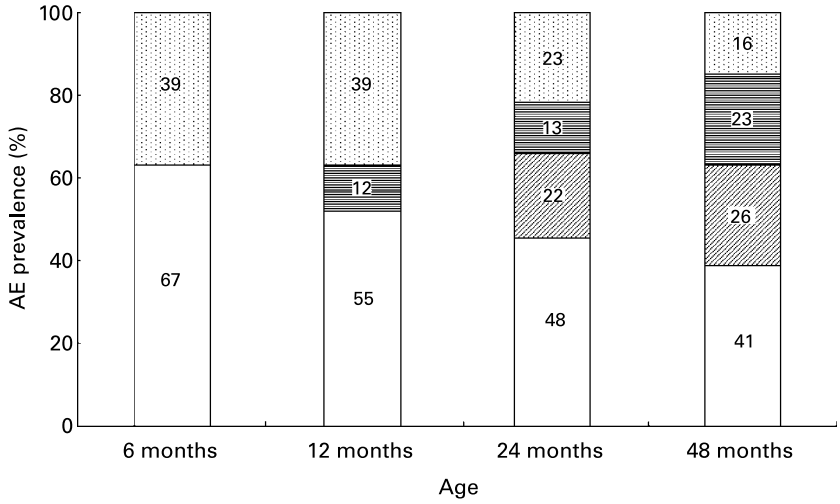

Fig. 1. Natural course of atopic eczema (AE) over the first four years of life. The bars represent the proportion (with numbers included in the bars) of subjects with/without AE at different ages. (□), Without AE persistent from 6 months; (▨), without AE but a history with AE; (▤), with AE but a history without AE; (▩), with AE persistent from 6 months. Only children attending at all study visits were included.

Dietary intake

The mean duration of total breast-feeding was 6·8 (95 % CI 6·1, 7·5) months and exclusive breast-feeding 2·8 (95 % CI 2·5, 3·1) months. At 6 months infants consumed 457 (95 % CI 400, 514) ml and at 12 months 371 (95 % CI 317, 426) ml of infant formula daily, when only infants consuming infant formula were accounted for. The mean daily intakes of energy and nutrients in the children with and without AE are shown in Tables 1 and 2. As no statistical difference was observed in dietary intake between sexes, males and females were combined for analysis. Children with AE had a lower intake of retinol at 6 months compared to those without. The intakes of protein and Ca were lower, and the intake of ascorbic acid higher, in children with AE than in those without at 12 months. At 48 months, the intakes of protein and Ca were lower, and the intake of cholecalciferol higher, in children with AE compared with those without.

The mean intake of energy per kg body weight was 97 % (95 % CI 86, 109 %) and 89 % (95 % CI 84, 95 %) of the reference intake (Nordic Nutrition Recommendations (NNR); Nordic Working Group on Diet and Nutrition, 1996) at 6 months ($P=0·149$), 97 % (95 % CI 91, 103 %) and 90 % (95 % CI 85, 96 %) at 12 months ($P=0·086$) and 84 % (95 % CI 79, 88 %) and 81 % (95 % CI 77, 84 %) at 48 months ($P=0·320$), in children with and without AE, respectively. The mean intakes of macronutrients were within the range of recommended intake (NNR 1996) in both groups at 6 months (Table 1). At 12 months, the mean intake of protein was higher and the mean intake of fat lower, and at 48 months the intake of protein was higher and that of carbohydrates lower than the recommended intake in both groups (Table 1). The intakes of vitamins and minerals from the diet as a proportion of the recommended intake (NNR 1996), and a comparison between children with and without AE, are shown in Fig. 2(a to c). At 6 months the mean intakes of Zn and Fe (% of recommended) were higher in children with AE than in those without. The mean intakes of Fe, ascorbic acid and vitamin E (% of recommended) were higher and that of Ca lower in children with AE at 12 months; the mean intakes of ascorbic acid and cholecalciferol were higher in children with AE at 48 months than in those without. The intakes of individual nutrients were below the recommendations for several nutrients, the most notable being Zn, Fe, Ca and cholecalciferol at 6 months; Fe, Ca and cholecalciferol at 12 months; and Fe, vitamin E and cholecalciferol at 48 months. In children with AE the intake of vitamin A ($P=0·044$) at 6 months and that of Ca ($P=0·021$) at 12 months were less than recommended more frequently compared with children without AE. Fe intake was less than recommended more frequently in children without AE compared with those with AE at 6 months ($P=0·004$) and at 12 months ($P=0·020$), whilst no difference was observed between the groups at 48 months.

Children diagnosed with CMA (*n* 14) at 12 months had higher intakes of energy (4525 (95 % CI 4140, 4910) kJ *v.* 3687 (95 % CI 3538, 3836) kJ, $P<0·001$) and fat as a proportion of total energy (33·0 (95 % CI 30·4, 35·7)% *v.* 28·2 (95 % CI 26·9, 29·5)%, $P=0·004$) but a lower intake of protein as a proportion of total energy (11·1 (95 % CI 10·3, 11·8)% *v.* 15·4 (95 % CI 14·7, 16·2)%, $P<0·001$) than did infants without CMA (*n* 81). Children with CMA had higher intakes of cholecalciferol, vitamin E, ascorbic acid and Fe and a lower intake of Ca than infants without CMA (data not shown). The intake of infant formula was higher (658 (95 % CI 543, 774) ml/d) in children with CMA than without (238 (95 % CI 188, 288) ml/d, $P<0·001$).

Growth and body composition

The presence of AE did not affect height SD score or weight-for-height at different ages (Fig. 3(a and b)), except that children with AE (0·40 (95 % CI 0·17, 0·65) SD scores) were shorter than children without (0·73 (95 % CI 0·51, 0·96) SD scores, $P=0·048$) at 24 months. One child (2 %) with AE at 6 months and one (2 %) at 12 months had height less than −2 SD scores. The weight of one child (2 %) with AE at 6 months, one (1 %) without AE at 12 months and one (1 %) at 24 months was less than 20 % of the population mean. One child with AE (2 %) at 6 months and three (3 %) without AE at 6 months, one (1 %) at 12 months, two (2 %) at 24 months and five (8 %) at 48 months

Table 1. Intake of energy and energy-yielding nutrients from the diet in children with and without atopic eczema (AE) at the ages of 6, 12 and 48 months†
(Mean values and 95 % CI in parentheses)

	Age							
	6 months		12 months		48 months		NNR‡	
Nutrient	With AE (*n* 23)	Without AE (*n* 37)	With AE (*n* 41)	Without AE (*n* 54)	With AE (*n* 38)	Without AE (*n* 67)	6/12 months	48 months
Energy								
(kJ)	3267 (2901, 3632)	2962 (2797, 3127)	3952 (3695, 4209)	3702 (3524, 3882)	5893 (5567, 6218)	5648 (5423, 5874)	400	385 (M), 370 (F)
(kJ/kg body weight)	389 (343, 435)	356 (334, 378)	389 (364, 415)	361 (340, 382)	335 (316, 354)	322 (306, 338)		
Protein								
(g/MJ)	6·0 (5·4, 6·6)	5·9 (5·6, 6·2)	8·1** (7·4, 8·7)	9·2 (8·6, 9·7)	9·3* (8·8, 9·7)	9·9 (9·5, 10·2)	4–6	
(% of energy)	10·2 (9·2, 11·2)	10·1 (9·6, 10·6)	13·7** (12·6, 14·7)	15·6 (14·7, 16·5)	15·8* (15·0, 16·6)	16·8 (16·2, 17·3)	7–10	10–15
Fat								
(g/MJ)	9·1 (8·4, 9·8)	9·2 (8·8, 9·6)	7·6 (7·2, 8·1)	7·6 (7·2, 8·0)	8·0 (7·6, 8·5)	7·8 (7·5, 8·1)	9–12	
(% of energy)	34·5 (31·9, 37·2)	34·9 (33·4, 36·4)	29·0 (27·2, 30·8)	28·9 (27·2, 30·5)	30·6 (28·9, 32·2)	29·7 (28·7, 30·7)	35–45	30
Carbohydrates								
(g/MJ)	32·6 (31·4, 33·8)	32·4 (31·6, 33·2)	33·8 (32·9, 34·6)	32·7 (31·9, 33·5)	31·6 (30·4, 32·8)	31·5 (30·9, 32·1)		
(% of energy)	55·4 (53·3, 57·5)	55·1 (53·7, 56·5)	57·4 (55·9, 58·9)	55·6 (54·2, 57·0)	53·7 (51·7, 55·7)	53·6 (52·5, 54·6)	45–60	55–60

M, males; F, females.
† Breast-fed infants excluded from the analysis.
‡ NNR, Nordic Nutrition Recommendations (Nordic Working Group on Diet and Nutrition, 1996).
Mean values were significantly different from those of children without AE (independent samples *t* test): *$P<0·05$, **$P<0·01$.

Table 2. Intake of nutrients from the diet in children with and without atopic eczema (AE) at the ages of 6, 12 and 48 months† (Mean values and 95 % CI in parentheses)

	Age					
	6 months		12 months		48 months	
Nutrient	With AE (*n* 23)	Without AE (*n* 37)	With AE (*n* 41)	Without AE (*n* 54)	With AE (*n* 38)	Without AE (*n* 67)
Retinol (μg/MJ)	172·0** (141·4, 202·5)	237·0 (205·6, 268·5)	161·6 (134·4, 188·8)	159·1 (144·0, 174·3)	141·2 (101·5, 180·8)	113·3 (95·5, 131·1)
Cholecalciferol (μg/MJ)	2·8 (2·4, 3·3)	3·1 (2·8, 3·5)	1·7 (1·4, 2·0)	1·7 (1·4, 2·0)	0·4* (0·3, 0·5)	0·3 (0·3, 0·4)
Vitamin E (mg/MJ)	1·9 (1·6, 2·2)	1·9 (1·7, 2·1)	1·5 (1·3, 1·7)	1·3 (1·2, 1·5)	1·0 (0·9, 1·1)	1·0 (0·9, 1·0)
Ascorbic acid (mg/MJ)	32·2 (28·3, 36·1)	29·8 (28·2, 31·3)	25·7** (23·3, 28·1)	21·6 (19·4, 23·8)	15·5 (12·8, 18·2)	12·7 (11·4, 14·0)
Ca (mg/MJ)	121·3 (108·3, 134·4)	123·6 (117·2, 129·9)	140·2** (123·4, 157·0)	175·9 (157·2, 194·6)	148·0* (130·6, 165·4)	168·6 (158·6, 178·5)
Fe (mg/MJ)	2·3 (2·1, 2·4)	2·1 (2·0, 2·2)	2·0 (1·9, 2·1)	1·9 (1·7, 2·0)	1·3 (1·3, 1·4)	1·4 (1·3, 1·5)
Zn (mg/MJ)	1·4 (1·3, 1·6)	1·3 (1·2, 1·4)	1·6 (1·5, 1·7)	1·6 (1·5, 1·7)	1·3 (1·3, 1·4)	1·4 (1·3, 1·4)

† Breast-fed infants excluded from the analysis.
Mean values were significantly different from those of children without AE (independent samples *t* test): *$P<0·05$, **$P<0·01$.

had weight above the 20 % of the population mean (all dichotomic analyses between groups, NS).

According to longitudinal analysis (ANOVA for repeated measures from 6 to 48 months) using AE (at least one AE diagnosis during follow-up) and probiotics as explaining factors, neither AE nor probiotics significantly affected height. The mean difference for height during the follow-up from 6 to 48 months between children with (*n* 60) and without (*n* 38) AE was 0·02 (95 % CI −0·30, 0·35) SD scores ($P=0·890$). The mean weight-for-height was −3·5 % (95 % CI −6·5, −0·5 %, $P=0·025$) lower in children with AE compared with those without. In the probiotic group the mean height was −0·1 SD scores (95 % CI −0·4, 0·2, $P=0·532$) lower and the mean weight −2·9 % (95 % CI −6·0, 0·1, $P=0·056$) lower compared with the children with perinatal administration of placebo.

Perinatal administration of probiotics proved safe, as it also did not significantly influence the height or weight-for-height of the children as measured at 48 months. According to two-way ANOVA with AE (at least one AE diagnosis during follow-up) and probiotics as explaining factors, the effect of perinatal administration of probiotics on height was 0·04 SD scores (95 % CI −0·33, 0·40, $P=0·852$) and on weight-for-height −3·35 % (95 % CI −7·07, 0·37, $P=0·077$). However, the effect of AE at 48 months was significant with respect to weight, −5·1 % (95 % CI −8·9, −1·2, $P=0·010$), but not height, −0·05 SD scores (95 % CI −0·42, 0·33, $P=0·815$).

The weight-for-height and height SD score of the children were not significantly affected by age at diagnosis (at 6 months or at 12, 24 or 48 months) or age at appearance of symptoms (data not shown). The height (0·5 (95 % CI 0·08, 1·0) SD scores) and weight-for-height (−3 (95 % CI −6, 0·4)%) of eighteen children with CMA at 12 months were within the population reference range.

Mid-upper arm muscle circumference (14·3 (95 % CI 14·0, 14·6) cm and 14·8 (95 % CI 14·4, 15·1) cm with and without AE, *n* 31 and 49, respectively, $P=0·041$) and proportion of body fat (14·1 (95 % CI 12·7, 15·4)% and 16·6 (95 % CI 15·4, 17·7)% with and without AE, *n* 29 and 45, respectively, $P=0·007$) were lower in children with AE compared with those without at 48 months.

Association of dietary intake with atopic eczema

According to the final logistic regression models (Table 3) the dietary factors, along with perinatal administration of *Lactobacillus GG*, which reduced the likelihood of AE at 24 months were increases in intake of retinol and Ca at all ages studied. A higher intake of fat at 6 months as well as maternal AE, but not paternal AE, increased the likelihood of AE in the child at 24 months. The likelihood of AE at 48 months was explained by increases in ascorbic acid intake at all ages studied, whilst higher intakes of retinol and Zn reduced the likelihood of AE in the child.

Discussion

Previous nutritional approaches attempting to reduce the risk of allergic diseases have mainly involved the elimination of foods or environmental factors, based on the conception that allergen avoidance results in prevention of allergic disease. Current research is focusing more on active prevention schemes identifying factors

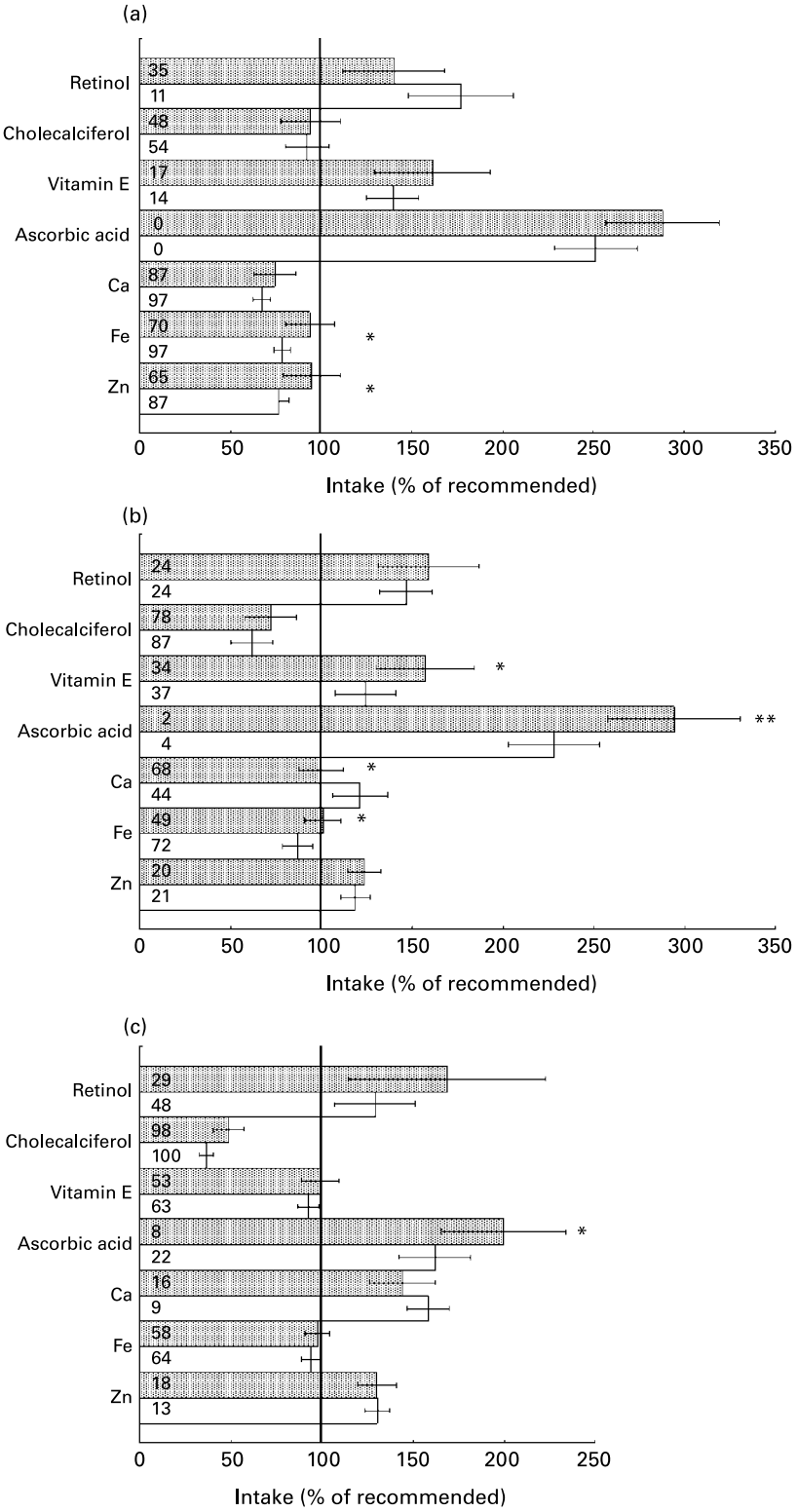

Fig. 2. Intake of nutrients from the diet (percentage of recommended intake; Nordic Working Group on Diet and Nutrition, 1996) in children with (▨) and without (□) atopic eczema (AE) at the age of (a) 6 months (*n* 23 and *n* 37, respectively), (b) 12 months (*n* 41 and *n* 54, respectively) and (c) 48 months (*n* 38 and *n* 67, respectively). Breast-fed infants are excluded from the analysis. Values are means with their 95% CI shown by error bars and the numbers represent the proportion of individuals below 100% of recommended intake. Mean values were significantly different from those of children without AE (independent-samples *t* test): $^{*}P<0{\cdot}05$, $^{**}P<0{\cdot}01$.

possibly influencing immunoregulatory pathways in early life, for example those promoting tolerance against potentially harmful agents, including supplementation of nutrients or probiotics. We aimed to broaden the view of exclusion on one hand, and sole supplementation on the other, by providing a unifying approach in identifying the contribution of early dietary factors in a probiotic intervention study on the appearance of AE in childhood.

We showed that with careful monitoring of growth and management of AE in early childhood, the growth of patients remained within population reference values and only 2 % of children with AE at different ages were seen to evince poor growth. However, in the longitudinal analysis the mean weight of the children with AE was lowered by 3·5 %, and at 24 months they were shorter compared with those without AE. Additionally, they had less skeletal muscle protein mass at 48 months, as indicated by a lower mid-upper arm muscle circumference than children without AE, which may reflect the lower dietary intake of protein or relate to AE itself through inflammation.

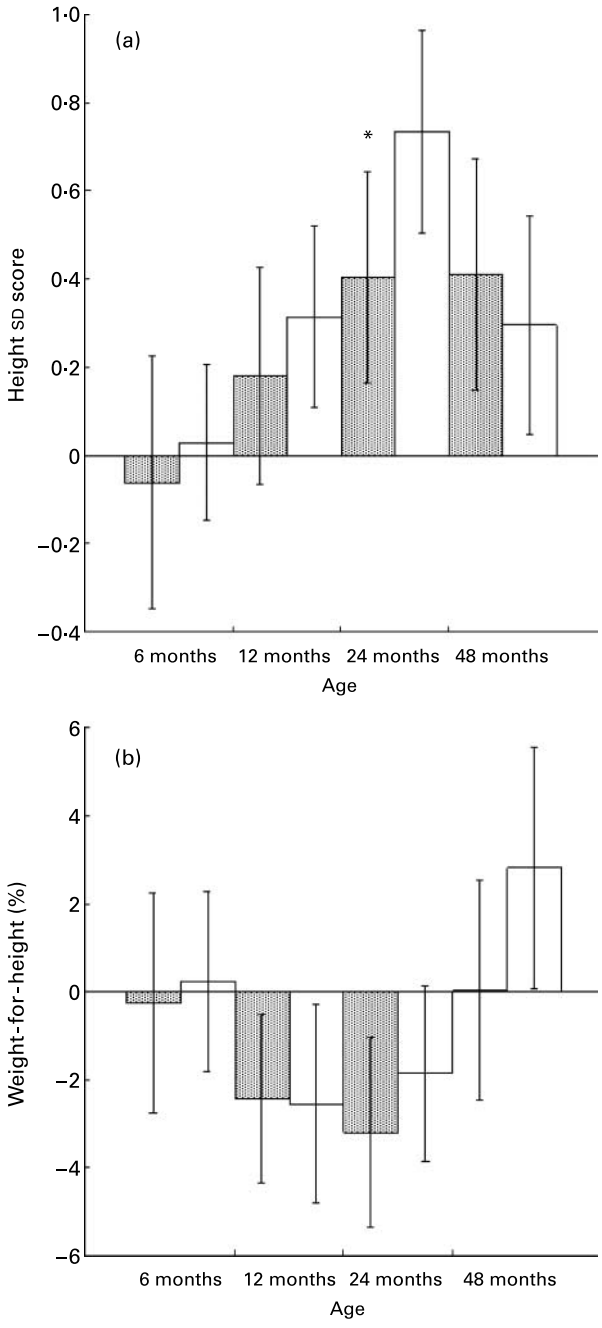

Fig. 3. (a) Height SD score and (b) weight proportional for height (weight-for-height, %) in children with (▨) and without (□) atopic eczema (AE) at 6 (*n* 46 and 87, respectively), 12 (*n* 61 and 70, respectively), 24 (*n* 41 and 83, respectively) and 48 months of age (*n* 38 and 64, respectively). Values are means with their 95 % CI shown by error bars. Mean values were significantly different from those of children without AE (independent-samples *t* test): **P*<0·05.

Table 3. Presence of atopic eczema at 24 and 48 months of age explained by diet at 6 (Model A), 12 (Model B) and at 6 and 12 months of age (Model C). Results are based on stepwise logistic regression analysis, where dietary variables, *Lactobacillus GG* (*Lactobacillus rhamnosus* strain GG; ATCC 53 103) administration, parental allergy and breast-feeding status were entered in the stepwise logistic regression model as potential explanatory variables

	B	Odds ratio (95 % CI)	*P* value
Atopic eczema at 24 months			
Model A (*n* 108)			
Lactobacillus GG (yes/no)	−1·182	0·31 (0·12, 0·78)	0·013
Maternal allergy	1·398	4·05 (1·17, 14·02)	0·028
Fat intake at 6 months (g/d)	0·061	1·06 (0·99, 1·14)	0·092
Retinol intake at 6 months (μg/MJ per d)	−0·005	0·99 (0·99, 1·00)	0·047
Ca intake at 6 months (mg/MJ per d)	−0·038	0·96 (0·94, 0·99)	0·003
Constant	2·459		0·114
Model B (*n* 96)			
Lactobacillus GG (yes/no)	−1·150	0·32 (0·13, 0·80)	0·015
Retinol intake at 12 months (μg/MJ per d)	−0·008	0·99 (0·98, 1·00)	0·062
Ca intake at 12 months (mg/MJ per d)	−0·008	0·99 (0·98, 1·00)	0·053
Constant	2·270		0·023
Model C (*n* 81)			
Lactobacillus GG (yes/no)	−1·344	0·26 (0·08, 0·82)	0·021
Maternal allergy	1·455	4·29 (0·90, 20·41)	0·068
Retinol intake at 6 months (μg/MJ per d)	−0·007	0·99 (0·99, 1·00)	0·042
Ca intake at 6 months (mg/MJ per d)	−0·048	0·95 (0·92, 0·99)	0·005
Retinol intake at 12 months (μg/MJ per d)	−0·010	0·99 (0·98, 1·00)	0·052
Constant	7·349		0·003
Atopic eczema at 48 months			
Model A (*n* 88)			
Ascorbic acid intake at 6 months (mg/MJ per d)	0·059	1·06 (1·00, 1·12)	0·039
Constant	−2·207		0·010
Model B (*n* 81)			
Ascorbic acid intake at 12 months (mg/MJ per d)	0·015	1·01 (1·00, 1·03)	0·051
Retinol intake at 12 months (μg/MJ per d)	−0·019	0·98 (0·97, 0·99)	0·002
Zn intake at 12 months (mg/MJ per d)	−3·092	0·05 (0·00, 0·63)	0·021
Constant	5·903		0·035
Model C (*n* 67)			
Ascorbic acid intake at 12 months (mg/d)	0·021	1·02 (1·00, 1·04)	0·023
Retinol intake at 12 months (μg/MJ per d)	−0·018	0·98 (0·97, 1·00)	0·007
Zn intake at 12 months (mg/MJ per d)	−3·168	0·04 (0·00, 0·87)	0·040
Constant	5·383		0·086

Failure to thrive may culminate in patients not being regularly monitored, as the reasons for growth failure in AE are various. A sustained inflammation may result in reduced bioavailability or loss of nutrients (Ukabam *et al.* 1984), while metabolic requirements may be increased and the symptoms of the disease may be regulated by elimination of foods (Eggesbo *et al.* 2001). Patients may also develop food aversions, this resulting in inadequate dietary intake or replacement of foods by others and by infant formula, the use of which may extend to toddler age. Dietary adaptations may also explain the differences observed in nutrient intake between children with and without AE. In the present study the low energy intake compared with dietary reference values (NNR 1996) and figures in other studies (Räsänen & Ylönen, 1992; Lagström *et al.* 1997) was not reflected in growth, but may equally be due to inaccurate or under-reporting of the diets. Also, other well-known limitations in reporting the nutrient intake from the food diary method need to be considered in interpretations of the results (Hakala *et al.* 1996). Dietary intake studies in children with AE are scant, but compared with figures for healthy children (Räsänen & Ylönen, 1992; Lagström *et al.* 1997), the intakes of Zn, Fe, Ca (at 8 months) and cholecalciferol (Lagström *et al.* 1997), but also vitamin E, were lower than reference nutrient intakes (NNR 1996) in a notable proportion of the children. The low intake of cholecalciferol at 48 months was particularly striking. At the time the study was undertaken, children were recommended to use vitamin D supplements up to the age of 24 months, which would have compensated for the low dietary intake. Since the study was conducted new recommendations on vitamin D supplementation for children and on fortification of dairy products have been given, the effects of which on intake and serum concentrations as well as on health outcomes should be evaluated. The early dietary intake of cholcecalciferol did not emerge as an explanatory factor for AE in the present study, but may none the less have immunomodulatory properties influencing later risk of diseases also, other than bone-related (Mathieu *et al.* 2004). Although nutritional consequences reflected in growth and dietary intake may be transient (Patel *et al.* 1997), the health consequences may be of long-lasting nature (Barker, 1995).

In the present study, additionally to the previously observed protective effect of the administration of probiotics (Kalliomäki *et al.* 2001, 2003), intakes of retinol comprising β-carotene, as well as Ca and Zn, reduced the risk of AE, whilst ascorbic acid intake increased the risk of AE. Dietary antioxidant vitamins have raised interest in allergic disease as they counteract oxidative stress and damp the inflammatory response, and may thus be important in implementing the ability of the individual to restrain the inflammatory response and allergic disease (Omata *et al.* 2001). Previous studies have, however, yielded contradictory results on the associations between dietary intake or serum concentrations of antioxidant vitamins and minerals and allergic disease (Powell *et al.* 1994; Soutar *et al.* 1997; Bodner *et al.* 1999; Hijazi *et al.* 2000; Picado *et al.* 2001; Arora *et al.* 2002; Nagel *et al.* 2003; Harik-Khan *et al.* 2004), which discrepancies may be explained by the different role of nutrients depending on the end-organ manifestation. Subjects in the present study evinced cutaneous involvement of the disease, whilst previously a low ascorbic acid intake (Soutar *et al.* 1997) or serum levels (Bodner *et al.* 1999; Harik-Khan *et al.* 2004) have been shown to relate to airway allergies. The disagreements may also result from the differences between the effects of dietary and supplemental nutrients arising from amounts consumed and compositional differences, as shown for vitamin E (Stone *et al.* 2003). In like manner, dietary intakes of nutrients may not reflect serum levels (Kardinaal *et al.* 1995), which on the other hand may not be a good indicator of body status. In one experimental study, although no difference was observed in serum retinol concentrations, the liver retinol concentration was lower in rats subjected to repeated allergen inhalation challenges compared with naïve rats (Shoseyov *et al.* 2002). The diet may also play a differential role in management and risk reduction in allergic disease. Most studies have been conducted in patients with existing disease and until now the role of nutrients in the prevention of AE has remained to be elucidated. One previous study has shown that low intake of ascorbic acid, via breast milk in infants, was associated with an increased risk of atopy (Hoppu *et al.* 2005). Another indication of the effects of maternal diet on the neonatal immune response was reduced cord blood IL-13 levels in neonates whose mothers received fish oil supplementation during pregnancy (Dunstan *et al.* 2003).

The mechanisms by which the nutrients retinol, Ca and Zn, identified in the present study, may reduce the risk of AE remain unresolved, but their role may arise from the anti-oxidative properties of β-carotene, and of Zn as it acts as a cofactor in superoxide dismutase. Ca is an important intracellular messenger, required for the activation of phospholipase A_2 and thus production of prostaglandin E_2 with known effects on the modulation of cytokine and Ig profiles (Newberry *et al.* 1999). Retinol is also an important constituent for cell differentiation and thus for mucosal function above all in patients with gastrointestinal involvement of the disease. Retinoids have been found to inhibit IgE production in mouse peripheral blood mononuclear cells (Worm *et al.* 2001). Additionally, supplementation with vitamin A in mice resulted in depression of interferon-γ and IL-4, the potential cytokines in allergic disease, and in increase in mucosal IgA, with the capacity to protect mucosal surfaces (Albers *et al.* 2003). β-Carotene and retinol may also alter the activation of the arachidonic acid cascade and hence suppress prostaglandin E_2 production *in vitro* (Halevy & Sklan, 1987). This is a conflicting observation as in experimental studies prostaglandin E_2 has been shown to up-regulate IL-10 production (Harizi *et al.* 2002), important for the maintenance of healthy intestinal immune homeostasis, and to suppress antigen-specific T cell proliferation in gut-associated lymphoid tissue, thus contributing to an antiinflammatory intestinal environment (Newberry *et al.* 1999). Together with this, the sensitivity and the number of prostaglandin E_2 receptors in the effector T cells may be reduced in AE (Rocklin *et al.* 1985; Rocklin & Thistle, 1986), suggesting a beneficial role for prostaglandin E_2. Alternatively, the effects of retinol in AE may arise from its contribution to the innate immunity. Indeed, a deficiency of vitamin A impairs innate immunity by diminishing the function of neutrophils, macrophages and natural killer cells as well as antibody-mediated responses by T helper cells (reviewed in Stephensen, 2001). Finally, one explanation for the protective role of retinol observed in the present study could be that the efficacy of innate immunity requires an adequate intake of retinol, which strengthens the host–microbe interaction previously shown to control intestinal epithelial homeostasis (Rakoff-Nahoum *et al.* 2004).

It appears from the present study that the previously observed protective effect of probiotics (*Lactobacillus GG*; Kalliomäki

et al. 2001, 2003) evolves in joint action with the dietary intake of particular nutrients, thus reducing the later risk of AE in a child. This finding calls for further evaluation of the interactions between nutrients and probiotics, the first attempt at which was made by evaluating the influence of PUFA on growth and adhesion of probiotics (Kankaanpää *et al.* 2001). Equally, the levels of dietary intakes of nutrients with potential capacity to reduce the risk of disease need to be re-evaluated bearing in mind the risk of using supplements at high doses or under certain conditions (Rietjens *et al.* 2002; Bjelakovic *et al.* 2004; Miller *et al.* 2004; Milner *et al.* 2004). The same holds true for weights to be lower in the group receiving probiotics perinatally, a circumstance not detected in a previous shorter study (Saavedra *et al.* 2004). Keeping in mind the need for further evaluations with subject numbers sufficiently sampled on these parameters, early dietary modification towards a balanced dietary intake of nutrients supplemented with probiotics may offer a preventive tool in the increasing problem of AE.

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