

ORIGINAL ARTICLE

Blackcurrant seed oil for prevention of atopic dermatitis in newborns: a randomized, double-blind, placebo-controlled trial

P. Linnamaa¹, J. Savolainen², L. Koulou¹, S. Tuomasjukka³, H. Kallio³, B. Yang³, T. Vahlberg⁴ and R. Tahvonen^{3,5}

¹Department of Dermatology, ²Department of Pulmonary Diseases and Clinical Allergology, ³Department of Biochemistry and Food Chemistry, ⁴Department of Biostatistics, University of Turku, Turku, Finland and ⁵MTT Agrifood Research Finland, Jokioinen, Finland

Clinical & Experimental Allergy

Summary

Background The present increased incidence of atopic diseases has been associated with an altered intake of essential fatty acids (EFAs). The composition of blackcurrant seed oil (BCSO) corresponds to the recommended dietary intake of EFAs, and as a dietary supplement could, in small doses, modify the imbalance of EFAs in an efficient way.

Objective To assess the effect of dietary supplementation with BCSO on the prevalence of atopy at 12 months of age.

Methods Three hundred and thirteen pregnant mothers were randomly assigned to receive BCSO (151) or olive oil as placebo (162). The first doses were administered at 8th–16th weeks of pregnancy and were continued until the cessation of breastfeeding, followed by supplementation to the infants until the age of 2 years. Atopic dermatitis and its severity (SCORAD index) were evaluated, serum total IgE was measured and skin tests were performed at the age of 3, 12 and 24 months.

Results Parental atopy was common (81.7%) among study subjects, making them infants with increased atopy risk. There was a significantly lower prevalence of atopic dermatitis in the BCSO group than in the olive oil group at the age of 12 months (33.0% vs. 47.3%, $P = 0.035$). SCORAD was also lower in the BCSO group than in the olive oil group at 12 months of age ($P = 0.035$). No significant differences in the prevalence of atopic dermatitis were observed between the groups at the age of 24 months ($P = 0.18$).

Conclusion Dietary supplementation with BCSO was well tolerated and it transiently reduced the prevalence of atopic dermatitis. It could therefore be one potential tool in the prevention of atopic symptoms when used at an early stage of life.

(Registration number SRCTN14869647, <http://www.controlled-trials.com>)

Keywords atopic dermatitis, black currant seed oil, EFA, PUFA

Submitted 5 November 2009; revised 9 April 2010; accepted 16 April 2010

Correspondence:

Johannes Savolainen, Department of Pulmonary Diseases and Clinical Allergology, University of Turku, FI-20520 Turku, Finland.
E-mail: johannes.savolainen@utu.fi

Introduction

Atopic diseases, such as atopic dermatitis, allergic rhinitis and asthma, have become a prevalent public health problem in the industrialized world [1]. The rapid increase in the incidence of atopic diseases has been suggested, in part, to be associated with changing dietary habits such as the intake of essential fatty acids (EFAs) α -linolenic acid (ALA, 18:3n-3) and linoleic acid (LA, 18:2n-6), and with their changed ratios in people's diets [2, 3]. EFAs are an essential part of the diet because they cannot be synthesized in the body, and they are the precursors of other n-3 and n-6 fatty acids (FAs). Δ^6 -desaturase is the regulatory

enzyme that begins the conversion of EFAs in phospholipids into other FAs to be further converted into a series of eicosanoids, such as thromboxanes, prostaglandins and leukotrienes [4]. These play an important role in the regulation of epidermal inflammation and immune function [5, 6]. Arachidonic acid (AA, 20:4n-6)-derived eicosanoids are mostly pro-inflammatory whereas eicosapentaenoic acid (EPA, 20:5n-3)-derived eicosanoids are anti-inflammatory [7]. Eicosanoids derived from dihomo- γ -linolenic acid (DGLA, 20:3n-6), the intermediate metabolite of n-6 series, are either anti-inflammatory or inactive [7]. Both n-6 and n-3 FAs and their metabolites regulate and modulate inflammation and chronic diseases

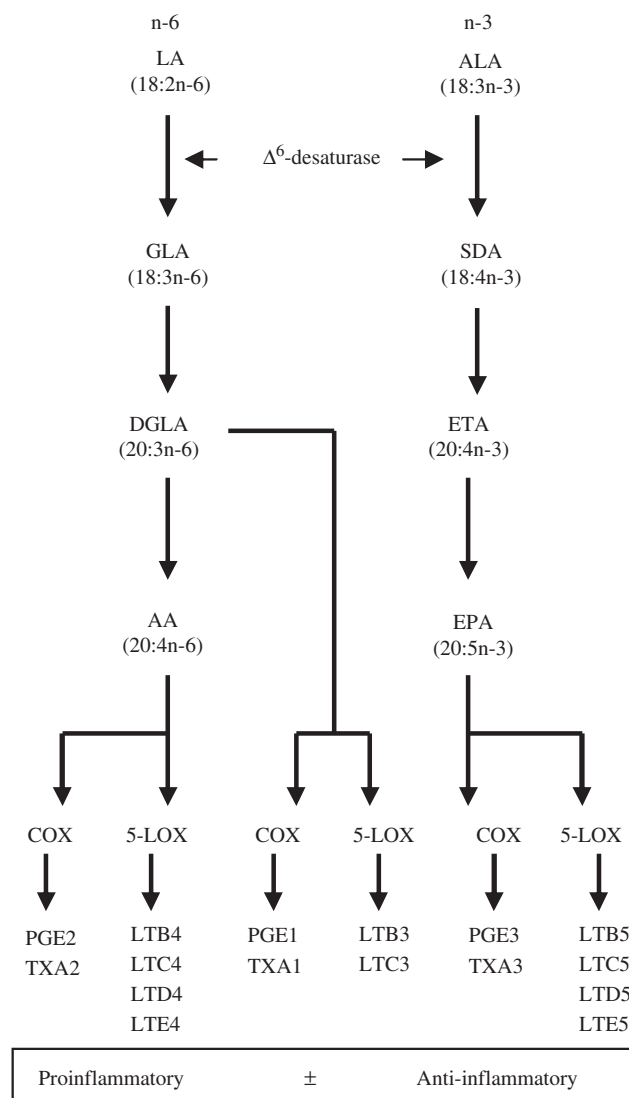


Fig. 1. The metabolism of n-6 and n-3 fatty acids and the synthesis of their eicosanoids presented together with the effects of different eicosanoids on inflammatory response (LA, linoleic acid; ALA, Δ -linolenic acid; GLA, γ -linolenic acid; SDA, stearidonic acid; DGLA, dihomo- γ -linolenic acid; ETA, eicosatetraenoic acid; AA, arachidonic acid; EPA, eicosapentaenoic acid; COX, cyclo-oxygenase pathway; 5-LOX, 5-lipo-oxygenase pathway; PGE, prostaglandin E; TXA, thromboxane; LTB, leukotriene B; LTC, leukotriene C; LTD, leukotriene D and LTE, leukotriene E). Modified from Calder et al. 2002 [6].

via several pathways [4, 6]. Fig. 1 depicts the metabolism of n-6 and n-3 FAs and the synthesis of eicosanoids, and summarizes the effects of different eicosanoids on inflammatory response.

Atopics have been found to have reduced levels of EFAs in some studies, with considerable variations in the plasma levels of n-3 and n-6 FA [8]. Several types of FA composition abnormalities have been found in atopic disease; however, any really clear pattern of altered status of a particular FA or a particular FA family seems not to exist [9]. These abnormalities may be due to dietary

factors and/or polygenic factors. It has also been hypothesized that atopic persons may have a lower apparent activity of the Δ^6 -desaturase enzyme [10, 11]. Recently, polymorphisms in the FADS gene cluster (producing Δ^5 - and Δ^6 -desaturases) have been associated with atopic diseases [12]. The first year of infancy is crucial for the development of immune function, with a deviation towards an atopic immune response being the result of an inadequate early T-helper type 1 (Th1) response [13, 14]. Recently, fetal period has also been suggested to be important for EFAs and their metabolites in programming or imprinting the development of the immune system [15, 16]. Therefore, early intervention concerning EFAs could be immunomodulative and have an influence on the development of atopic dermatitis. The first manifestation of atopic allergy in infants is very often atopic dermatitis.

Blackcurrant (*Ribes nigrum*) seed oil (BCSO) is rich in both n-6 FAs [LA >40% and γ -linolenic acid (GLA, 18:3n-6) >10%] and n-3 FAs [ALA >15% and stearidonic acid (SDA, 18:4n-3) ~3%]. The content of oleic acid (OA, 18:1n-9) in BCSO is typically around 10% [17]. The composition of BCSO corresponds to the recommended optimal dietary intake of n-3 and n-6 FAs; in other words, the oil has a ratio of n-3 FAs to n-6 FAs from 1/3 to 1/4 [18, 19]. We have shown that dietary supplementation of BCSO increased the content of DGLA in plasma phospholipids, which is an indication of the increased capacity of precursors of anti-inflammatory eicosanoids [20]. It should thus be possible to modify the imbalance of polyunsaturated fatty acids (PUFAs) and eicosanoids in an efficient way with small doses of supplementation of BCSO, independently of the function of Δ^6 -desaturase. Furthermore, blackcurrant is one of the most common cultivated berries, especially in Europe, and has commercial potential and high availability.

BCSO could be beneficial if used at the crucial age for the development of the immune system and atopic immune response. In the present double-blind placebo-controlled (DBPC) trial, we assessed the effect of dietary supplementation with BCSO on the development of atopic dermatitis and atopic sensitization during the fetal period and infancy.

Methods

Patients

This randomized double-blind placebo-controlled intervention trial was carried out during the years 2004–2008 at public health care centres in the Salo and Kaarina-Piikkiö regions in South western Finland. The trial, which started before July 2005, was registered retrospectively at the ISR-CTN register (ISRCTN14869647, <http://www.controlled-trials.com>). The trial was conducted according to the principles expressed in the Declaration of Helsinki. The study was approved by the Ethical Committee of the Hospital District of South West Finland.

Public-health nurses informed all pregnant mothers about the study when they first visited the maternity care units, and the mothers were asked for permission to be contacted by the study nurse. All pregnant mothers willing to participate were included in the study, the only inclusion criterion being less than 16 weeks of gestation. The study nurse sent an information leaflet about the study to those mothers who had given their consent and contacted them by telephone. Around 500 mothers were interested in the study and 322 of them wanted to participate in it. The mothers provided their written informed consent for the study.

Altogether, 322 mothers were randomly assigned by a random number list to receive either BCSO or olive oil as a placebo immediately after the mother was enrolled. The random allocation sequence was concealed until interventions were assigned. Recruitment and subsequent contacts with the study subjects were carried out by different personnel than randomization. Nine of the mothers cancelled their participation, leaving 313 mothers participating in the study. Of the 313 mothers, 151 were assigned to receive BCSO and 162 to receive the placebo.

Blackcurrant seed oil preparations

BCSO, manufactured by supercritical CO₂ extraction technology (Aromtech Ltd, Tornio, Finland), and olive oil (Santagata Luigi s.r.l., Genova, Italia) as a reference oil (placebo) were raffinated in an identical way. The peroxide values of the raffinated oils were <1.0 mEq O₂/g and the acid values were <0.5 mg KOH/g. The FA compositions (expressed as a wt% of total) of the oils are presented in Table 1. Both oils were encapsulated in soft gelatine capsules for supplementation to the mothers and bottled as loose oil for delivery to the infants.

Study design

The doses were first administered to the mothers during 8th to 16th weeks of pregnancy and then continued until the end of the exclusive breastfeeding period. The oil was

given in the form of capsules, six capsules per day, all in all 3 g/day. The infants started receiving the same oil in the form of drops, dose 1 mL/day, when exclusive breastfeeding was ended, and the children continued receiving the oil until 2 years of age. The sensory properties of the oils were close to each other. They were packed in plastic bottles with a volume of 15 mL. The bottles were stored in the freezer and only bottles in use were stored in the refrigerator. The treatment codes were not opened until the study material had been analysed in March 2008. The study physician or the study nurse met with the mothers mostly every 3 months, and the mothers received a 3-month batch of capsules at a time. During the mothers' first visit, the study physician, a dermatologist, or the nurse filled in a preliminary information form that contained evaluations of, among other things, the mothers' residential environment, pets, smoking, usage of vitamins and additional nutrients and tendency for atopic diseases in the family. Blood samples for plasma FA analyses were taken after 9 months of gestation. The children were examined at the ages of 3, 12, and 24 months, at which points any occurrences of atopic dermatitis were evaluated, skin prick testing (SPT) was carried out and blood was drawn for a serum sample (serum stored at -20 °C). In addition, a query form was filled in at each evaluation point to identify any previous occurrences of atopic dermatitis and signs of allergies, as well as the child's general health, use of supplements and any changes in living environment.

Study end-point

The study endpoint was the prevalence of atopic dermatitis by the age of 12 months as the prevalence was predicted to be highest at this time-point. The secondary endpoint was the prevalence of atopic dermatitis by the age of 24 months. The prevalence of atopic dermatitis by the age of 3 months was predicted to be too low to detect significant difference. Further, it was anticipated that the compliance with the protocol would be highest when the infants still had a limited diet (<12 months of age), and the outcome of dermatitis at the age of 24 months would be affected considerably by the children's habitual diet and environmental factors.

Clinical evaluation

A specialist in dermatology evaluated the skin of each child at each visit. Atopic dermatitis was defined as a chronic or relapsing itchy dermatitis with a characteristic morphology and distribution [21]. The SCORAD index, an internationally used batch of criteria for atopic dermatitis, was used to assess dermatitis severity [22, 23]. Atopic dermatitis was also recorded if there was a history of chronic or relapsing itchy dermatitis with a typical

Table 1. Major fatty acids in blackcurrant seed oil and olive oil (weight% of total)

Fatty acids	Blackcurrant seed oil	Olive oil
Palmitic acid (16 : 0)	6	11
Palmitoleic acid (16 : 1n-7)	0	1
Stearic acid (18 : 0)	2	3
Oleic acid (18 : 1n-9)	14	73
<i>cis</i> -Vaccenic acid (18 : 1n-7)	1	0
Linoleic acid (LA, 18 : 2n-6)	48	9
γ -Linolenic acid (GLA, 18 : 3n-6)	13	0
α -Linolenic acid (ALA, 18 : 3n-3)	14	0
Stearidonic acid (SDA, 18 : 4n-3)	3	0

localization. At each follow-up visit, a data form was filled in, which charted the skin symptoms observed in the children, atopic dermatitis, food allergies, allergic nose or eye symptoms, asthma symptoms, any other diseases and the use of vitamins and additional nutrients by the children and their mothers. Any changes in the residential environment, household animals and smoking were also recorded in the forms.

Skin prick testing and serum total immunoglobulin E

The skin tests were carried out by an experienced study nurse at the 3-, 12- and 24-month follow-up visits of the children according to the EAACI guidelines [24]. The tested allergens were standardized allergen extracts of birch, cat, dog, cow's milk, egg, wheat, gliadin and house dust mite (SoluPrick, ALK-Abellø, Horsholm, Denmark). Histamine hydrochloride (10 mg/mL) was used as a positive control and the ALK-Abello NSA-diluent was used as a negative control. In addition, 12- and 24-month testing included *Candida albicans* (Soluprick, ALK-Abellø). Half or more of the histamine reaction size was regarded as a positive reaction. Serum total IgE was analysed by Pharmacia CAP System (Phadia, Uppsala, Sweden).

Fatty acid analyses

FAs of plasma triacylglycerols (TAG) were analysed as described earlier [20]. Briefly, lipids were extracted, and TAG were separated with silica columns and trans-esterified with sodium methoxide. The FA methyl esters were dissolved in hexane and analysed with gas chromatography using reference compounds.

Statistical analyses

We predicted the prevalence of atopic dermatitis at the age of 12 months to be 34% in the olive oil group and 17% in the BCSO group. Based on an 80% power to detect a significant difference ($P=0.05$, two-sided χ^2 -test) and a 30% early discontinuation rate, a sample size of 147 subjects was required for each study group. In a Per-protocol analysis, the difference in proportions between the groups was tested using the χ^2 -test. Graphical methods were used for testing the normality of the continuous variables. In the case of normally distributed variables, a two-sample t -test was applied to test the mean differences between the groups; otherwise, a non-parametric Mann-Whitney U -test was used. P -values <0.05 were considered to be statistically significant. The statistical analyses were carried out using SAS/STAT(r) software, Version 9.2 of the SAS System for Windows (SAS Institute Inc., Cary, NC, USA).

Results

Demographics

The flow chart of the participants is presented in Fig. 2. Of the babies of the 322 randomized mothers, 241 babies were examined at the age of 3 months (112 BCSO, including a pair of twins, 129 placebo). At 12 months of age, 210 babies were examined (100 BCSO, 110 placebo), of which altogether 177 babies continued to the end and were examined at the age of 24 months. Of those children, 85 belonged to the BCSO group and 92 to the placebo group.

Sick children and those born prematurely (a total of 8), who required more intensive care, naturally dropped out and were excluded from the study. Twenty-nine mothers from the BCSO group withdrew from the study during pregnancy due to miscarriage, problems with swallowing the capsules, pregnancy-related nausea, stomach problems, abortion or poor compliance. Post-birth reasons for dropping out included premature birth, cholic and poor compliance. Twenty-eight mothers withdrew from the placebo group during pregnancy due to pregnancy-related nausea, problems with swallowing the capsules, miscarriage or poor compliance. Post-birth reasons for interruption included premature birth, cholic and poor compliance. After the 3-month follow-up visit, 12 children withdrew from the blackcurrant group and 18 from the placebo group before the 12-month visit. The most common reasons for withdrawal were post-partum tiredness of the mother, other worries in the family and poor compliance. After the 12-month examination, 15 withdrew from the blackcurrant group and 19 from the placebo group before the end of the study. The most common reasons were again other worries in the family and poor compliance. There were no significant differences in the baseline characteristics between the BCSO and placebo groups (Table 2). The compliance of the mothers was confirmed with plasma TAG composition at 9 months of gestation. The levels of LA ($P=0.028$), GLA ($P=0.026$) and ALA ($P=0.0042$) FA levels and the GLA/LA ratios ($P=0.032$) in the TAG fractions were significantly higher in the BCSO group when compared with the placebo group.

Atopic dermatitis and sensitization

In the total study population, in 81.7% of the cases, one or both of the parents had atopy, thus redefining the study subjects as infants with increased risk of atopy. Based on these figures, the predicted prevalence of atopy was 49% in the total study population. The Per-protocol analyses of the proportions of children with a recurrent atopic dermatitis or atopic dermatitis with a duration longer than 1 month in each group at the age of 3, 12 and 24 months are presented in Fig. 3. At the age of 3 months, the prevalence of atopic dermatitis was low and no significant differences were observed between the treatment groups (BCSO

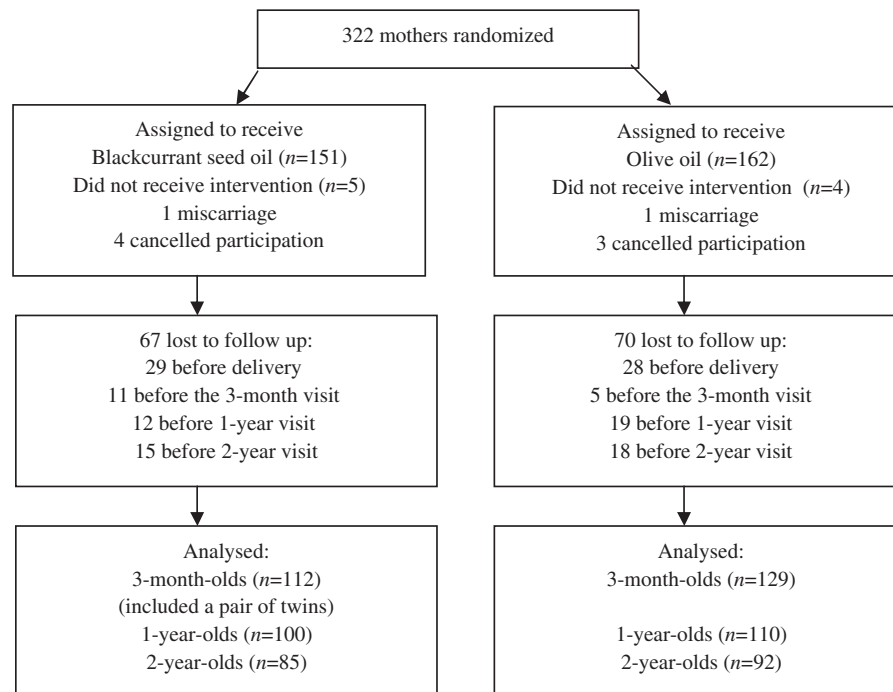


Fig. 2. The flow chart of the participants enrolled in the study.

Table 2. Baseline demographic and clinical characteristics

	Blackcurrant seed oil	Olive oil	P-value
Number	112	129	
Gender; n (%)			0.70*
Male	54 (48.2)	59 (45.7)	
Female	58 (51.8)	70 (54.3)	
Parental atopy; n (%)			0.87*
Both	29 (25.9)	34 (26.4)	
One	61 (54.5)	73 (56.5)	
None	22 (19.6)	22 (17.1)	
Birth weight (g); mean (SD)	3595 (461)	3599 (468)	0.94 [†]
Parental smoking; n (%)	28 (25.0)	40 (31.0)	0.30*
Breastfeeding (weeks); mean (SD)	33.8 (22.7)	30.8 (18.0)	0.50 [‡]
Any pets at home; n (%)	48 (42.9)	52 (40.3)	0.69*
Older siblings (n); mean (SD)	0.78 (0.87)	0.71 (0.90)	0.45 [‡]

*Chi-square test.

[†]Student's two-sample *t*-test.

[‡]Mann-Whitney *U*-test.

group: 13.4%; placebo group: 12.4%; $P = 0.82$). At the age of 12 months, there was a significant ($P = 0.035$, χ^2 -test) difference in favour of the BCSO group, with only 33 children out of 100 (33.0%) with atopic dermatitis, while atopic dermatitis was diagnosed in 52 children out of 110 (47.3%) in the olive oil group. At the age of 24 months, the difference was no longer statistically significant ($P = 0.18$), with atopic dermatitis in 38.8% of the children in the BCSO group as compared with 48.9% in the placebo

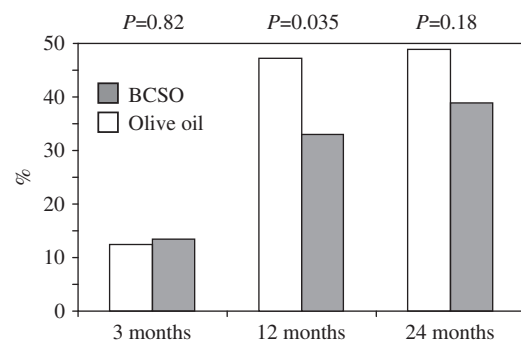


Fig. 3. Prevalence of atopic dermatitis for 1 month or longer in the blackcurrant seed oil (BCSO) intervention group (grey bar) and the placebo group (white bar) at three different study time-points: 3, 12 and 24 months. Statistical comparison with the χ^2 -test.

group. According to SCORAD, atopic dermatitis was less severe in atopic children in the BCSO group when compared with those in the placebo group at the age of 12 months ($P = 0.035$, Mann-Whitney *U*-test, Table 3). When the atopic status of the mothers was included in the analysis, it appeared that there was a trend of a decrease of atopic dermatitis in the BCSO group in children of non-atopic mothers, which was not observed in children of atopic mothers. The prevalence of atopic dermatitis of infants of non-atopic mothers at age of 12 months was 23.1% in the BCSO group and 44.8% in the placebo group ($P = 0.058$). The corresponding numbers of infants of atopic mothers were 39.3% and 48.2% ($P = 0.30$).

Table 3. SCORAD classes

	Class*	Blackcurrant seed oil	Olive oil	P-value†
3 months	1	91 (81.3%)	107 (83.0%)	0.65
	2	7 (6.3%)	11 (8.5%)	
	3	11 (9.8%)	9 (7.0%)	
	4	3 (2.7%)	2 (1.6%)	
12 months	1	80 (80.0%)	74 (67.3%)	0.035
	2	9 (9.0%)	13 (11.8%)	
	3	8 (8.0%)	18 (16.4%)	
	4	3 (3.0%)	5 (4.6%)	
24 months	1	70 (83.3%)	68 (76.4%)	0.24
	2	6 (7.1%)	8 (9.0%)	
	3	8 (9.5%)	12 (13.5%)	
	4	0 (0.0%)	1 (1.1%)	

*1 = 0–5 pts, 2 = 6–10 pts, 3 = 11–20 pts, 4 ≥ 20 pts.

†Mann-Whitney's *U*-test.

Table 4. Total IgE antibodies and skin prick test results

		Blackcurrant seed oil	Olive oil	P-value
Total IgE (kU/L)	<i>n</i>	Mean (SD)	<i>n</i>	Mean (SD)
3 months	71	4.6 (7.0)	65	2.0 (2.2)
12 months	68	20.5 (37.2)	68	14.8 (17.5)
24 months	26	19.2 (17.8)	38	24.1 (27.3)
Positive egg skin test	<i>n</i> (%)		<i>n</i> (%)	
3 months	112	1 (0.9%)	126	1 (0.8%)
12 months	98	14 (14.3%)	104	18 (17.3%)
24 months	79	4 (5.1%)	87	7 (8.1%)

*Student's two-sample *t*-test.

†Chi-square test.

The serum total IgE levels in 3-, 12- and 24-month serum samples and SPT results with egg at the age of 3, 12 and 24 months in both study groups are presented in Table 4. Over 90% of all sensitization in skin tests was to egg. There were no significant differences in serum IgE levels or skin test results between the BCSO and placebo groups.

Discussion

In this double-blind placebo-controlled trial, the first large study of FA intervention during pregnancy, we demonstrated that the use of BCSO in pregnant and breastfeeding mothers and their infants after breastfeeding resulted in a reduced outcome of atopic dermatitis at the age of 12 months. According to SCORAD, atopic dermatitis was also less severe in the blackcurrant group when compared with the placebo group at the age of 12 months.

In this study, the fat supply of the fetus and the infant was affected by both the diet of the pregnant and breastfeeding mother and the supplementation. The fact that no difference was found between the prevalence at 3

months of age could result from the variation in the starting point of the supplementation, varying lengths of sole breastfeeding and low prevalence of atopic allergy at the age of 3 months in general. An intention-to-treat (ITT) analysis was not performed as it was regarded as unreliable and misleading because children who discontinued between 3 and 12 months of age were seen only at the age of 3 months. Therefore, the problem is that an ITT analysis would have judged all discontinued children based on dermatitis status at 3 months of age, although the prevalence of atopic dermatitis later increased fivefold (from 8.7% to 40.5%) from the age of 3 to 12 months.

The difference in the prevalence of atopic dermatitis was no longer significant at the age of 2 years ($P = 0.18$) due to the increase of dermatitis in the BCSO group. It is possible that the positive effect of BCSO on the EFA balance is disturbed after the first year of life as a less healthy diet begins to be adopted. According to a recent Finnish report [25], the diet of 1-year-old children is close to recommendations, but subsequently worsens rapidly when children begin to eat the same food as the rest of the family. By the time the child has reached 2 years of age, the saturated-to-polyunsaturated fat ratio of the diet is similar to the ratio for adults. It remains to be investigated in further studies as to what kind of additional interventions are required to exert a longer-lasting effect. It is interesting to note that in the children of non-atopic mothers, there was a trend of a preventive effect of BCSO on atopic dermatitis, not seen in the children of atopic mothers. This may indicate that FAs have a direct effect on the development of dermatitis, and the effect is more evident when the maternal atopic immunity is less modulatory.

The symptoms of atopic dermatitis (SCORAD) were less severe in the BCSO group than in the olive oil group. These results are in line with those of our previous DBPC randomized parallel study, in which the intensity of the dermatitis symptoms, including itching, of atopic children aged between 6 months and 4 $\frac{1}{2}$ years were reduced significantly more with alpine currant seed oil than with the rapeseed oil placebo [26]. The composition of alpine currant seed oil is close to that of blackcurrant [17]. Blackcurrant is one of the most common cultivated berries in the Western countries and can offer proper availability, whereas alpine currant only grows wild and in the Scandinavian countries.

PUFA are precursors for several lipid mediators involved in the resolution of inflammation [27]. The role of these mediators in atopic dermatitis has not been studied as yet, but changes in their profile towards more beneficial ones might be one of the mechanisms induced by BCSO supplementation. Large amounts of saturated fats in the mother's diet have been linked to atopic sensitization in the child [28]. A low proportion of n-3 FAs in breast milk and a large ratio of saturated to PUFAs have also been found to be connected to atopic dermatitis in children [29]. Controversial results have been observed in earlier

interventional, post-natal, EFA supplementation studies [30]. Supplementations of fish oil for infants with a high risk of atopy have been disappointing with regard to prevention of asthma [31, 32]. However, a study by Van Gool et al. [33] found that the supplementation of GLA tended to reduce the severity of atopic dermatitis in infants. Further on, Dunstan et al. [34] found that if mothers were given fish oil substitution during pregnancy, the children had, at the age of 1 year, three times less positive prick reactions to egg in comparison with placebo, and atopic dermatitis was less severe. In addition, probiotics have been effective in reducing atopic dermatitis, especially when administered during pregnancy [35–38]. According to Enke et al. [15], fetal immune development commences in midterm, and the physiological balance from midterm onwards is important.

Human intervention studies concerning FA and fetal immune development are so far rare, and the results are inconclusive. However, the results of both prenatal and antenatal studies suggest that for successful modulation of the immune system, a prenatal supplementation of diet is required. Our results support the need for balance of n-6 and n-3 FAs during pregnancy and infancy.

The BCSO used in the present study contained 14.5% ALA, 12.6% GLA, 47.5% LA and 2.7% SDA. We have previously shown that BCSO supplementation increased the proportion of GLA in TAG and cholesteryl esters and DGLA, in addition, in glycerophospholipids in young healthy volunteers [20]. In another study, serum levels of GLA and DGLA were increased significantly with BCSO supplementation with encouraging, yet not significant, healing process of canine atopic dermatitis [39].

Reduced GLA levels have been found in sera of atopic dermatitis patients as have low GLA levels in the breast milk of allergic mothers when compared with healthy mothers [40]. In addition, children with atopic dermatitis have significantly lower levels of GLA and DGLA, and these correlate with the severity of atopic dermatitis [41]. It is, therefore, likely that the beneficial effect on atopic dermatitis achieved by dietary BCSO supplementation is due to increased GLA and DGLA and DGLA-derived neutral or anti-inflammatory eicosanoids [7]. BCSO supplementation leads to increased levels of GLA and DGLA, but not AA [20, 42]. In agreement with this, BCSO has been shown to reduce the production of prostaglandin E_2 (PGE_2) [43]. This may explain why supplementation with n-6 FAs can be beneficial in preventing atopic dermatitis. On the other hand, in murine models of asthma, PGE_2 has been shown to reduce allergic inflammation and expression of Th2-type cytokines IL-4 and IL-5, thus showing anti-inflammatory properties [44, 45]. In addition, a novel AA-derived eicosanoid, lipoxin A4, has been found to possess anti-inflammatory properties [27]. Thus, there are several indications that the n-6 FAs traditionally considered to be pro-inflammatory may not always be so.

In addition to GLA, ALA and SDA are also important in controlling inflammation. In an earlier study by us, oral supplementation of sea buckthorn seed oil increased the plasma levels of ALA and EPA in patients with atopic dermatitis, and the incorporation of ALA into plasma phospholipids correlated positively with the reduction in SCORAD values of atopic dermatitis [46]. However, the SCORAD value did not differ significantly between the sea buckthorn and the placebo groups. In the metabolic chain, a significant part of the FAs (15%) of BCSO are metabolites beyond the Δ^6 -desaturase step, whereas in sea buckthorn seed oil, no FAs are Δ^6 -desaturase-derived. Therefore, it is possible that the presumed low activity of Δ^6 -desaturase in atopics can be compensated for with BCSO supplementation [47]. The presence of SDA in BCSO may play a role in controlling inflammatory response by regulating the release of AA from phospholipids by the activity of phospholipase A2 and by inhibiting the 5-lipoxygenase pathway [48–50]. Olive oil contained only 9% of n-6 FAs and no n-3 FAs at all, and was therefore a suitable control for investigating the potential effects of n-3 and n-6 FAs.

The prevalence of atopy in the olive oil group was 49%, which is considerably higher than what we predicted and what would be expected in randomly chosen healthy infants in Finland. The reason for this is that the study attracted families with predisposition to atopic allergy. Over 80% of the children had at least one atopic parent, and in 25% of the families, both parents were atopic. This was also the case in another intervention study with probiotics performed in the Turku region, where a high prevalence of atopic dermatitis was seen in the placebo group and the dropout rate was comparable to ours [35].

BCSO has been used in different kinds of foods and dietary supplements in Europe and the United States for the last 20 years. In addition to this, dietary supplements made of blackcurrant have been tested in various long-term clinical trials without side-effects [20, 43, 51–54], rendering BCSO a safe and convenient source of rare and EFAs.

In conclusion, dietary supplementation with BCSO was well tolerated by the pregnant mothers and their infants. It significantly reduced the prevalence of atopic dermatitis in infants at the age of 1 year. The effects were comparable to those of probiotics administered during pregnancy. BCSO offers an interesting safe alternative for re-balancing the profile of EFAs in atopic patients and can potentially be a tool for preventing atopic dermatitis at an early stage of life.

Acknowledgements

This study was funded by the Ministry of Agriculture and Forestry of Finland, and was additionally supported by grants from the Finnish Allergy Research Foundation, the Finnish Society of Allergology and Immunology, the

Turku University Foundation, the Finnish Dermatological Society and the EVO – Government Grant of the Turku University Central Hospital. We thank Janette Räikkönen, Raija Nurmi, Anja Pirinen, Tuire Palokangas and Marjukka Sillanpää for excellent technical assistance as well as the personnel in the public health care centres of the Salo and Kaarina regions for their help and co-operation.

References

- 1 The International Study of Asthma and Allergies in Childhood (ISAAC) Steering Committee. Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema: ISAAC. *Lancet* 1998; **351**:1225–32.
- 2 Sanders TA. Polyunsaturated fatty acids in the food chain in Europe. *Am J Clin Nutr* 2000; **71** (Suppl.):176–8.
- 3 Kankaanpää P, Nurmela K, Erkkilä A et al. Polyunsaturated fatty acids in maternal diet, breast milk, and serum lipid fatty acids of infants in relation to atopy. *Allergy* 2001; **56**:633–8.
- 4 Das UN. Essential fatty acids: biochemistry, physiology and pathology. *Biotechnol J* 2006; **1**:420–39.
- 5 Ziboh VA, Miller CC, Cho Y. Metabolism of polyunsaturated fatty acids by skin epidermal enzymes: generation of antiinflammatory and antiproliferative metabolites. *Am J Clin Nutr* 2000; **71** (Suppl.):361–6.
- 6 Calder PC, Yaqoob P, Thies F, Wallace FA, Miles EA. Fatty acids and lymphocyte functions. *Br J Nutr* 2002; **87** (Suppl.):31–48.
- 7 Nakamura MT, Nara TY. Structure, function, and dietary regulation of delta6, delta5, and delta9 desaturases. *Annu Rev Nutr* 2004; **24**:345–76.
- 8 Devereux G, Seaton A. Diet as a risk factor for atopy and asthma. *J Allergy Clin Immunol* 2005; **115**:1109–17.
- 9 Sala-Vila A, Miles EA, Calder PC. Fatty acid composition abnormalities in atopic disease: evidence explored and role in the disease process examined. *Clin Exp Allergy* 2008; **38**:1432–50.
- 10 Burton JL. Essential fatty acids in atopic eczema: clinical studies. In: Horrobin DF, ed. *Omega6 essential fatty acids: pathophysiology and roles in clinical medicine*. New York: Alan R. Liss Inc., 1990; 67–73.
- 11 Horrobin DF, Manku MS. Clinical biochemistry of essential fatty acids. In: Horrobin DF, ed. *Omega6 essential fatty acids: pathophysiology and roles in clinical medicine*. New York: Alan R. Liss Inc., 1990; 21–50.
- 12 Lattka E, Illig T, Heinrich J, Koletzko B. FADS gene cluster polymorphisms: important modulators of fatty acid levels and their impact on atopic diseases. *J Nutrigenet Nutrigenomics* 2009; **2**:119–28.
- 13 Prescott SL, Macaubas C, Holt BJ et al. Transplacental priming of the human immune system to environmental allergens: universal skewing of initial T cell responses toward the Th2 cytokine profile. *J Immunol* 1998; **160**:4730–7.
- 14 Prescott SL, Macaubas C, Smallacombe T, Holt BJ, Sly PD, Holt PG. Development of allergen-specific T-cell memory in atopic and normal children. *Lancet* 1999; **353**:196–200.
- 15 Enke U, Seyfarth L, Schleussner E, Markert UR. Impact of PUFA on early immune and fetal development. *Br J Nutr* 2008; **100**: 1158–68.
- 16 Prescott SL, Dunstan JA. Prenatal fatty acid status and immune development: the pathways and the evidence. *Lipids* 2007; **42**:801–10.
- 17 Johansson A, Laakso P, Kallio H. Characterization of seed oils of wild, edible Finnish berries. *Z Lebensm Unters Forsch* 1997; **204**:300–7.
- 18 The British Nutrition Foundations Task Force. *Unsaturated fatty acids: nutrition and physiological significance – the report of the British Nutrition Foundations Task Force*. London: Chapman & Hall, 1992; 152–63.
- 19 Recommendations for intake of polyunsaturated fatty acids in healthy adults (revised in accordance with the recommended changes agreed by the ISSFAL Board at ISSFAL 2004). Issfal Board Statement No.3; 2004. [Cited: 2004 June 28]. Available at <http://www.issfal.org.uk/> (accessed 18 May 2010).
- 20 Tahvonen RL, Schwab US, Linderborg KM, Mykkänen HM, Kallio HP. Black currant seed oil and fish oil supplements differ in their effects on fatty acid profiles of plasma lipids, and concentrations of serum total and lipoprotein lipids, plasma glucose and insulin. *J Nutr Biochem* 2005; **16**:353–9.
- 21 Hanifin J, Rajka G. Diagnostic features of atopic dermatitis. *Acta Derm Venereol (Stockholm)* 1980; **92**:42–7.
- 22 Severity Scoring of Atopic Dermatitis: The SCORAD Index. Consensus report of the European task force on atopic dermatitis. *Dermatology* 1993; **186**:23–31.
- 23 Pucci N, Novembre E, Cammarata MG et al. Scoring atopic dermatitis in infants and young children: distinctive features of the SCORAD index. *Allergy* 2005; **60**:113–6.
- 24 European Academy of Allergology and Clinical Immunology. Allergen standardization and skin tests. Position paper. *Allergy* 1993; **48** (Suppl. 14):48–82.
- 25 Kytälä P, Ovaskainen M, Kronberg-Kippilä C et al. *The diet of Finnish preschoolers, Vol. B32*. Helsinki, Finland; The National Public Health Institute, 2008.
- 26 Johansson A, Isolauri E, Salminen S et al. Alpine currant seed oil as a source of polyunsaturated fatty acids in the treatment of atopic eczema. In: Lásztity R, Pfannhauser W, Simon-Sarkadi L, Tömösközi S, eds. *Functional foods – a new challenge for the food chemists*. Budapest, Hungary: Publishing Company of TUB, 1999; 530–6.
- 27 Serhan CN, Yacoubian S, Yang R. Anti-Inflammatory and pro-resolving lipid mediators. *Annu Rev Pathol Mech Dis* 2008; **3**:279–312.
- 28 Hoppu U, Kalliomäki M, Isolauri E. Maternal diet rich in saturated fat during breastfeeding is associated with atopic sensitization of the infant. *Eur J Clin Nutr* 2000; **54**:702–5.
- 29 Hoppu U, Rinne M, Lampi AM, Isolauri E. Breast milk fatty acid composition is associated with development of atopic dermatitis in the infant. *J Pediatr Gastroenterol Nutr* 2005; **41**:335–8.
- 30 van Gool CJ, Zeegers MP, Thijs C. Oral essential fatty acid supplementation in atopic dermatitis – a meta-analysis of placebo-controlled trials. *Br J Dermatol* 2004; **150**:728–40.
- 31 Marks GB, Mihrshahi S, Kemp AS et al. Prevention of asthma during the first 5 years of life: a randomized controlled trial. *J Allergy Clin Immunol* 2006; **118**:53–61.
- 32 Almqvist C, Garden F, Xuan W et al. CAPS team Omega-3 and omega-6 fatty acid exposure from early life does not affect atopy and asthma at age 5 years. *J Allergy Clin Immunol* 2007; **119**:1438–44.

- 33 van Gool CJ, Thijs C, Henquet CJ *et al.* Gamma-linolenic acid supplementation for prophylaxis of atopic dermatitis – a randomized controlled trial in infants at high familial risk. *Am J Clin Nutr* 2003; **77**:943–51.
- 34 Dunstan JA, Mori TA, Barden A *et al.* Fish oil supplementation in pregnancy modifies neonatal allergen-specific immune responses and clinical outcomes in infants at high risk of atopy: a randomized, controlled trial. *J Allergy Clin Immunol* 2003; **112**:1178–84.
- 35 Kalliomäki M, Salminen S, Arvilommi H, Kero P, Koskinen P, Isolauri E. Probiotics in primary prevention of atopic disease: a randomised placebo-controlled trial. *Lancet* 2001; **357**:1076–9.
- 36 Kukkonen K, Savilahti E, Haahtela T *et al.* Probiotics and prebiotic galacto-oligosaccharides in the prevention of allergic diseases: a randomized, double-blind, placebo-controlled trial. *J Allergy Clin Immunol* 2007; **119**:192–8.
- 37 Taylor AL, Dunstan JA, Prescott SL. Probiotic supplementation for the first 6 months of life fails to reduce the risk of atopic dermatitis and increases the risk of allergen sensitization in high-risk children: a randomized controlled trial. *J Allergy Clin Immunol* 2007; **119**:184–91.
- 38 West CE, Hammarström ML, Hernell O. Probiotics during weaning reduce the incidence of dermatitis. *Pediatr Allergy Immunol* 2009; **20**:430–7.
- 39 Noli C, Carta G, Cordeddu L, Melis MP, Murru E, Banni S. Conjugated linoleic acid and black currant seed oil in the treatment of canine atopic dermatitis: a preliminary report. *Vet J* 2007; **173**:413–21.
- 40 Laitinen K, Sallinen J, Linderborg K, Isolauri E. Serum, cheek cell and breast milk fatty acid compositions in infants with atopic and non-atopic eczema. *Clin Exp Allergy* 2006; **36**:166–73.
- 41 Yen CH, Dai YS, Yang YH, Wang LC, Lee JH, Chiang BL. Linoleic acid metabolite levels and transepidermal water loss in children with atopic dermatitis. *Ann Allergy Asthma Immunol* 2008; **100**:66–73.
- 42 Nakamura MT, Nara TY. Essential fatty acid synthesis and its regulation in mammals. *Prostaglandins Leukot Essent Fatty Acids* 2003; **68**:145–50.
- 43 Wu D, Meydani M, Leka L *et al.* Effect of dietary supplementation with black currant seed oil on the immune response of healthy elderly subjects. *Am J Clin Nutr* 1999; **70**:536–43.
- 44 Gauvreau GM, Watson RM, O'Byrne PM. Protective effects of inhaled PGE₂ on allergen-induced airway responses and airway inflammation. *Am J Respir Crit Care Med* 1999; **159**:31–6.
- 45 Martin JG, Suzuki M, Maghni K *et al.* The immunomodulatory actions of prostaglandin E₂ on allergic airway responses in the rat. *J Immunol* 2002; **169**:3963–9.
- 46 Yang B, Kalimo K, Tahvonen R, Mattila LM, Katajisto JK, Kallio HP. Effect of dietary supplementation with sea buckthorn seed and pulp oils on the fatty acid composition of skin glycerophospholipids of patients with atopic dermatitis and measurement of skin surface roughness. *J Nutr Biochem* 2000; **11**:338–40.
- 47 Manku MS, Horrobin DF, Morse N *et al.* Reduced levels of prostaglandin precursors in the blood of atopic patients: defective delta-6-desaturase function as a biochemical basis for atopy. *Prostaglandins Leukot Med* 1982; **9**:615–28.
- 48 Chilton FH, Rudel LL, Parks JS, Arm JP, Seeds MC. Mechanisms by which botanical lipids affect inflammatory disorders. *Am J Clin Nutr* 2008; **87**:498S–503S.
- 49 Finnen MJ, Horrobin DF., inventors; Efamol Holdings [GB], assignee. Stearidonic acid and/or eicosatetraenoic acid for the treatment of inflammations. European Patent 0460484 A1. 11 December 1991.
- 50 Guichardant M, Traitler H, Spielmann D, Sprecher H, Finot PA. Stearidonic acid, an inhibitor of the 5-lipoxygenase pathway. A comparison with timnodonic and dihomo-gammalinolenic acid. *Lipids* 1993; **28**:321–4.
- 51 Watson J, Byars ML, McGill P, Kelman AW. Cytokine and prostaglandin production by monocytes of volunteers and rheumatoid arthritis patients treated with dietary supplements of blackcurrant seed oil. *Br J Rheumatol* 1993; **32**:1055–8.
- 52 Spielmann D, Traitler H, Crozier G *et al.* Biochemical and bioclinical aspects of blackcurrant seed oil: 3–6 balanced oil. *NATO ASI Ser A* 1989; **171**:309–21.
- 53 Sori S, D'Alessandro A, Acitelli S, Giammaria U, Cocchi M, Noble RC. Effect of dietary supplementation by alternative oils on blood lipid levels on haemodialysed patients. *Med Sci Res* 1993; **21**:315–6.
- 54 Deferne JL, Leeds AR. Resting blood pressure and cardiovascular reactivity to mental arithmetic in mild hypertensive males supplemented with blackcurrant seed oil. *J Hum Hypertens* 1996; **10**:531–7.