

Peanut oil in vitamin A and D preparations: Reactions to skin test and manifestation of symptoms

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The aim of this study was to establish whether there is a link between sensitisation to peanut and exposure to peanut oil in vitamin A and D preparations. Forty-one children with a positive *in vivo* or *in vitro* test towards peanut were included. Twenty-one children had consumed vitamins A and D in oil solution, 14 in water solution, and 6 both types. Refined and unrefined peanut oils were obtained and skin prick test extracts were prepared. None of the children exhibited a positive SPT in response to the refined peanut extract. In contrast, 15 children exhibited a positive SPT to the unrefined extract. There was no significant difference in the number of children reacting clinically to peanut exposure who had received vitamins A and D in oil-based or water-based formulations. However, children with clinical allergy to peanut and who had exclusively consumed vitamin A and D in peanut oil, exhibited a greater number of different allergic symptoms upon consumption of peanut compared with clinical allergic children who had consumed the vitamins in water solution or both types ($p < 0.01$). This study indicates that sensitisation to peanut during childhood through consumption of vitamins A and D in oil-based solution seems unlikely, but its consumption may contribute to the development of a wider range of clinical symptoms due to peanut exposure.

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The incidence of peanut allergy is reported to have increased in recent years and is also seen in children under three years of age who have never consumed peanuts (1–2). Allergy to peanut may cause serious allergic symptoms and account for the majority of food-related anaphylactic fatalities (3–5). The routes of sensitisation, apart from direct exposure through the consumption of peanuts, are not fully known. The possibility of intrauterine exposure to peanut-allergens has been discussed (6–7), as well as exposure through breast-milk (2). Sensitisation to food-stuffs through airborne exposure (8–9) can occur and such exposure could theoretically contribute to the increased sensitisation to peanuts among small children. Moreover, peanuts and peanut-containing products are increasingly used in food

products because of their value as a source of easily digested protein, versatility and their low price (5). This is also true for peanut oil, which is used in both the food and pharmaceutical industries (10–12).

One use for peanut oil is in vitamin A and D solutions, which are recommended to all children under the age of 6 years in Sweden. Vitamin A and D preparations have been universally administered since 1940. Since 1968 they have been available in two types of preparations: oil- and water-based solutions. Sales of the two preparations have been equal. The oil-based solution in the commercially available vitamin A and D preparations has until now been based on refined peanut oil. To guarantee that the product is free from proteins and other by-products, a three-step

refining process is used: neutralization with elimination of free fatty acids and impurities, bleaching the oil and boiling the oil at 230°C. No immunochemical method is used to test that the product is protein free (13).

In a French study, a possible connection between the consumption of D vitamins in peanut oil solution and sensitisation to peanuts was reported (14). In the present study, the aim was to establish whether there is a link between sensitisation to peanut and exposure to peanut oil in vitamin A and D preparations.

Materials and methods

During the autumn of 1995, 45 children were identified as sensitive to peanuts at the pediatric out-patient clinics at Jakobsberg Hospital and at Sach's Children's Hospital in Stockholm. Allergy testing, performed over the last two years, in either of the two clinics, showed evidence of sensitisation to peanuts ($\geq 2+$ in SPT /44 children or RAST-class ≥ 2 /one child to peanut) in all patients. All children had one or more allergic manifestation.

During the winter of 1995–1996, the 45 children and their parents were asked to visit the allergy out-patient clinic for renewed SPT. After obtaining informed consent from the parents, such a test was performed by nurses with many years of experience in skin testing. Refined and unrefined peanut oil preparations, one production lot of each type, were obtained from Karlshamns Oils and Fats AB, Sweden. The unrefined oil is the source for making the refined oil preparation, in which the vitamins A and D are dissolved.

The peanut oil extracts were prepared by diluting the oils 1/10 (volume/volume) with a 0.9% saline solution, containing 0.3% phenol as conservation agent. The mixture was cooled and sonicated, rotated end-over-end for 1 hour at room temperature and finally centrifuged at 1250 *g* for 10 minutes. The water phase was collected and sterile-filtered (0.22 μ m, Millipore AB, Stockholm, Sweden) before use.

The inhibition test was performed as follows; oil extracts were serially diluted and incubated for 1 hour at room temperature, in equal volumes with a pool of 7 peanut-positive sera with a peanut-specific IgE concentration of 3.1 kU/l, as measured by the Pharmacia CAP test (Pharmacia & Upjohn, Uppsala, Sweden). The remaining free IgE in the mixture was measured with the peanut CAP test according to instructions from the manufacturer. The inhibition level of the IgE test reflects the allergen content in the extract. An

inhibition level of at least 50% is required for quantitation, according to the standard method (15).

Double skin pricks were performed on the volar aspects of both left and right arms with a commercial, standardised extract containing peanut (Soluprick 1 HEP, ALK, Copenhagen, Denmark) along with extracts from refined and unrefined peanut oil. Histamine hydrochloride (10 mg/ml) was used as a positive control and the solvent (ALK) was used as the negative control. Test results were read after 15 minutes; the wheals were encircled and the mean diameter was calculated. A SPT was considered positive if the diameter of the wheal was ≥ 3 mm and was greater than or equal to half the diameter of the wheal of the histamine control $\geq 2+$ (16). The accompanying parent was asked to fill in a questionnaire to determine whether the child had been exposed to peanuts, the clinical symptoms upon consumption of peanuts, whether the child had consumed A and D vitamins and which preparation had been used. No food challenges were performed. Questions regarding maternal peanut consumption during pregnancy and breast-feeding were also included in the questionnaire.

Statistics

Symptom scores were used for grading the reactions upon the consumption of peanuts. We asked about eight different allergic symptoms: asthma, coughing, rhinoconjunctivitis, swollen face, swollen throat, eczema, urticaria and gastrointestinal symptoms. If the child had one symptom one point was scored, two symptoms scored two points, etc. There was no weighting of scores between different symptoms.

For comparisons of distributions between different groups the chi-square test were used and Fisher's exact test when appropriate. For all other comparisons between groups, paired and non-paired *t*-tests were used, since the diameters of the wheals were assessed to be normally distributed. Trend tests were performed using "Bartholomew's test for order" (17). Two-tailed tests were used and a *p*-value < 0.05 was considered significant.

Results

Peanut allergens in peanut oil

Refined peanut oil extracts did not contain any measurable amounts of peanut allergen, as judged by the inhibition test. The unrefined oil

extract showed significant inhibition, but it was too small to be quantified.

Subjects

All 45 children had a renewed SPT performed and 4 of them showed a wheal $< 2+$ and a diameter < 3 mm with the standardised peanut extract (ALK) and they were excluded from further evaluation. Two of the excluded children had consumed vitamins A and D in oil-based solution, one had consumed vitamins A and D in water solution and another child had received both preparations.

Of the remaining 41 children, 30 were boys and 11 were girls with ages ranging between 1 and 15 years (mean age 6.3 years). Upon compilation of the answers from the questionnaires it was found that 21 children had received vitamins A and D in oil-based solution, 14 children had received water-based solution and 6 had received both types at different times. A family history of allergy was present in 82% of the children (single heredity in 55% and double heredity in 27%).

Of the 14 children who had received only water-based vitamins A and D, 10 had allergic rhino-conjunctivitis, as compared with 6 out of the 21 children who had consumed oil-based vitamins A and D (χ^2 ; $p < 0.05$). Allergic rhinoconjunctivitis was also common among the children who had received both vitamin A and D preparations (5 out of 6 children). Otherwise, there was no statistically significant difference between the groups for other confounding factors for atopy, such as gender, family history of atopic disease, duration of breastfeeding or number of positive SPT to inhalation allergens (data not shown).

Clinical presentation upon exposure to peanuts

Fifteen children (37%) had evidence of sensitisation to peanuts (positive SPT), despite the fact that they had never knowingly consumed peanuts or any peanut-containing food product. Of the 26 children who had eaten peanuts or peanut-containing food products, 21 children (80%) reported clinical symptoms after consumption. Itching and swelling of mucosal membranes in the mouth were the most prevalent symptoms (Table 1). No case of anaphylactic reaction after consumption of peanuts was reported or had been recorded in the medical records of the children.

The type of vitamin A and D preparation in relation to a positive or a negative clinical history of allergy to peanuts among the 26 children who had eaten peanuts is shown in Table 2. There was

Table 1. Symptoms after consumption of peanuts among the children with clinical peanut allergy

	n=21	%
Swollen throat	12	57
Swollen face	11	52
Gastro-intestinal symptoms	9	43
Rhinoconjunctivitis	7	33
Urticaria	7	33
Asthma	6	29
Coughing	6	29
Eczema	1	5

no statistically significant difference in presence or absence of symptoms after exposure to peanuts in relation to the use of different A and D vitamins. However, children with a clinical history of reaction after consumption of peanuts and who had consumed A and D vitamins only in oil solution had a significantly greater number of different symptoms (Table 3). Furthermore, these children had a significantly higher symptom score compared with those children who had received vitamins A and D only in water solution (Trend test; $p < 0.01$).

Of the 15 children who were sensitised to peanuts, despite the lack of known exposure, eight had received oil-based A and D vitamins, five children had received water-based vitamins and two had consumed both types (NS).

Skin-prick tests with refined and unrefined peanut-oil

Fifteen children (37%) had a positive SPT to the unrefined oil extract. Only one weak positive, 1+, SPT to the refined peanut oil extract was seen. The same child had a weak positive SPT, 1+, to the unrefined extract.

Connection between wheal size (refined/unrefined peanut oil) and clinical reaction to peanuts

Among the 26 children who had eaten peanuts, eleven had a positive SPT ($\leq 2+$) to the unrefined peanut extract. Of these, ten children (91%) reacted clinically to consumption of peanut containing food products. By comparison, the 15 children who showed weaker SPT ($< 2+$) of unrefined peanut oil extract, eleven (73%) had clinical signs of allergy after eating peanuts (NS).

Table 2. Presence or absence of symptoms after exposure to peanuts in the 26 children sensitised to peanut (SPT+) in relation to use of oil- and water-based A and D vitamin preparations

	n	Oil	Water	Oil and water
Clinical symptoms	21	10	8	3
No clinical symptoms	5	2	2	1

Table 3. Symptoms among 18 children with clinical peanut allergy after consumption of peanut containing food products in relation to the different A- and D-vitamin preparations. Differences in proportions are tested with two-tailed chi square tests

	Oil (n=10)		Water (n=8)		difference	p
	n	per cent	n	per cent		
Swollen throat	4	40%	7	88%	-48%	0.04*
Swollen face	8	80%	2	25%	55%	0.02
Gastro-intestinal symptoms	7	70%	0	0%	70%	<0.01
Rhinoconjunctivitis	3	30%	3	38%	-8%	0.74*
Urticaria	6	60%	0	0%	60%	<0.01
Asthma	3	30%	2	25%	5%	0.81
Coughing	4	40%	1	13%	27%	0.20
Eczema	1	10%	0	0%	10%	0.36

*Oil<Water

Those children (n=21) who had clinical reactions after consumption of peanuts had somewhat larger wheals (mean wheal diameter 2 mm) at SPT with unrefined peanut oil extract, compared with the children (n=5) who showed no clinical reaction (mean wheal diameter 1 mm). This difference was, however, not significant (p=0.19). The child with a weak positive SPT, 1+, to both the refined and unrefined peanut oil extracts, did not get any symptoms after exposure to peanuts.

Association between SPT with ALK peanut extract and extract of unrefined peanut oil

There was a significant association in the wheal size between the two different types of extracts (p < 0.02) (Table 4). The wheal size with the commercial peanut extract (ALK) varied from 2+ to 5+, corresponding to a mean wheal diameter of 8.9 mm. For the unrefined peanut oil extract, the wheal diameter varied between 0 and 3+ with a mean wheal diameter of 1.6 mm.

Routes of sensitization

Peanuts, or peanut-containing food products, were a part of the ordinary diet of only two out of 41 families in the study. Thirty-five of the families reported that they had consciously avoided such products. Peanut consumption during pregnancy and during breast-feeding had occurred in 21 out of 40 mothers (53%). No connection was found between maternal consumption of peanuts during pregnancy or breast-feeding and the degree of skin prick reaction or symptoms after consumption of peanuts. This was true for the commercial, the unrefined and the refined peanut oil extract.

Discussion

This retrospective study does not show any clear evidence that sensitisation to peanut occur via exposure to refined peanut oil used in vitamin A and D preparations. The number of children with clinically significant peanut allergy (reaction after consumption of peanuts) was not statistically more common in children who had received vitamins A and D in oil-based solution compared with children who had received vitamins A and D in water-based solution. The results of our study are in agreement with the observations by Taylor et al. (18) and by Hourihane et al. (19). In the latter study 60 patients, mean age of 24 years, with a known peanut allergy, underwent a double-blind provocation test. None of the adults showed a reaction with clinical symptoms after exposure to the refined peanut oil.

In a study by de Montis et al. (14), comprising 122 children aged between 7 months and 5 years, a relationship between the daily dose of vitamin D in peanut oil solution and the number of positive SPT against peanut (extract) was observed. In our study we found a dose-response relationship. The children who reacted clinically after consuming peanuts and who had received vitamins A and D only in oil-based solution, had higher symptom scores compared with the children who had received both types of preparation or who had received water-based vitamins A

Table 4. Association between SPT with ALK peanut extract of unrefined peanut oil

	Commercial extract		≥3+
	<3+	≥3+	
Unrefined extract			
<2+	17	3	
≥2+	10	11	

p=0.02

and D only. Thus, exposure to refined peanut oil in vitamin A and D preparations for a long period of time may be a risk factor for the development of clinical peanut allergy in those already sensitised to peanut. This may be explained by the fact that the risk for accidental exposure to incompletely refined peanut oil increases the longer the child receives vitamins A and D in peanut oil.

Eight different peanut oils were investigated in a study by Hoffman et al. (20), four hot-pressed and four cold-pressed. High levels of peanut-allergen were found in two out of four cold-pressed oils and traces of allergen were found in one cold-pressed oil. In contrast, no peanut allergen was found in the four oils that had been refined by heating. It should be noted that in the present study one of the children showed a weak positive reaction to refined as well as to unrefined peanut oil. It has also been speculated that the levels of peanut allergen that are required to sensitise an individual are below the detection limits of current available bioassays (21). These observations further emphasise the possibility that the extract of refined peanut oil can contain enough traces of peanut protein to induce sensitisation to peanuts. De Montis et al. also suggest the need to avoid all medications containing peanut oil in infancy (14).

Fifteen (37%) of the children in our study showed a positive SPT with the unrefined peanut oil extract. A rather weak positive correlation between the mean wheal diameters in SPT with the commercial peanut extract (ALK) and with the unrefined peanut oil extract could be verified (Fig. 1). This indicates that unrefined peanut oil contains sufficient protein for sensitised children to react in SPT. In a recently published study from Southampton (19), it was found that 10% (6/60) of adults reacted to unrefined peanut oil in a provocation test. That study indicates that unrefined peanut oil should be avoided by persons with peanut allergy.

In our study, 38% (15/41) show sensitisation to peanuts despite the fact that they had never eaten peanuts. We could not, in this retrospective study, record at which age the peanut allergy of the child became clinically manifest, nor relate the allergy to consumption of peanuts or peanut-containing food products. This emphasises a need for increasing knowledge regarding different ways of early sensitisation to peanuts. There have been reports of suspected sensitisation via infant milk formulas containing peanut oil (3, 6). From 1958 to 1975 in Sweden, milk formulas were enriched by vitamins dissolved in peanut oil as well as other substances. From 1975 onwards

other vegetable oils (palm, rape and corn) were used in milk formulas.

Topical application of creams and lotions containing peanut oil on children with damaged skin (eczema) has also been suggested as causing sensitisation (10, 12). Whether intrauterine sensitisation to peanuts is a reality is still an open question (6–7).

Even if our study is retrospective and therefore has its limitations, the results of the study have important clinical implications. Why the number of children sensitised to peanuts is increasing remains unknown, but parents and other interested parties are questioning why they should give children vitamin A and D preparations containing peanut oil. However, the observations from our study have already resulted in the Swedish pharmaceutical industry considering abstaining from using peanut oil in vitamin A and D preparations in the future. To answer all the remaining questions about early sensitisation to peanut among children, prospective studies are necessary. These should be carried out on a national basis in order to recruit a sufficiently large patient material for statistically significant conclusions to be drawn.

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