Turnip rapeseed does not worsen atopic eczema in most sensitized children

To the Editor,

Turnip rapeseed oil – rich in essential fatty acids and vitamin E – is a significant source of vegetable oils in the diet. Its fatty acid composition is ideal, and in addition to its current use in baby foods and margarines, components of turnip rapeseed may be increasingly used in the future. The seeds containing 20% protein are also used to high-protein animal feed and to produce biodiesel. Turnip rape and oilseed rape – sources of *canola oil* – grow in cool temperature and their cultivation is rapidly increasing in the Northern Hemisphere. Turnip rape and oilseed rape pollens may cause hay fever in sensitized individuals, primarily in cultivators.

Turnip rape and oilseed rape have been proposed as potential food allergens. Sensitization to turnip rapeseed was earlier reported to be up to 10% among children with atopic eczema in Finland (1). The clinical importance of the sensitization is, however, remains controversial. Whether purified oil extracted from the seeds by crushing plays any role in the sensitization and in the development of eczema is debated. In an open food challenge performed to 28 Finnish children sensitized to turnip rapeseed, clinical allergy was diagnosed in 22 (89%) with immediate labial/facial urticaria, or delayed flare-up of eczema, asthma, and abdominal complaints (1). An open challenge performed to turnip rapeseed-sensitized children with eczema revealed high rate of clinical allergy in Finland 14/14 (100%). In France, clinical allergy was less frequent 5/14 (36%) (2).

Eczema in infancy is frequently associated with IgE-mediated sensitization to foods (3), but symptomatic food allergy confirmed by double-blind placebo-controlled food challenge (DBPCFC) is less common in eczema patients. To prevent unnecessary avoidance of turnip rapeseed oil or other rapeseed products in the future, we deemed it important to conduct a DBPCFC with turnip rapeseed in sensitized children.

At the Helsinki University Skin and Allergy Hospital, children (aged 0–16 yr) with atopic eczema and strongly sensitized (skin prick test ≥6 mm) to turnip rapeseed were recruited to participate in a study with a crossover DBPCFC. At the hospital, the child received 10 mg, 100 mg, and 1 g of purified and milled turnip rapeseeds, or placebo with 30-min intervals. In the absence of immediate symptoms, the patient was discharged. A daily dose of 1 g continued for 4 days at home. The parents filled in symptom diaries. Between the two 5-day challenges, there was a 7-day washout period. Any known allergy to the ingredients of the vehicle led to exclusion. We assessed the severity score of atopic dermatitis (SCORAD) (4) at baseline and after each challenge, performed skin prick tests to a panel of food allergens and measured specific IgE against oilseed rape, mustard, birch, peanut 2S albumin *Ara h 2*, peanut birch pollen homology *Ara h 8*, profilins in birch *Bet v 2*, and peach *Pru p 4* (ImmunoCAP; Thermo Fisher, Uppsala, Sweden). Sera of fourteen age-matched non-atopic children served as negative controls. The ethics committee at the Helsinki University Central Hospital for Children and Adolescents approved the study protocol. One of the parents signed a written informed consent.

To study cross-reactive allergens of turnip rapeseed, we performed an ISAC® 112 microarray (ThermoFisher, Uppsala, Sweden) inhibition. To inhibit IgE-binding of solid-phase allergens by turnip rapeseed extract (1:3 w/v in phosphate buffer), we used a mixture of 27 sera from patients with specific IgE against 2S albumins (sesame *Ses i 1*, buckwheat *Fag e 2*, peanut *Ara h 2* and *Ara h 6*, walnut *Jug r 1*, and Brazil nut *Ber e 1)*, 7S and 11S globulins, lipid transfer proteins (LTP) (peanut *Ara h 9*, hazelnut *Cor a 8*, walnut *Jug r 3*, peach *Pru p 3*, mugwort *Art v 3*, olive pollen *Ole e 7*, and plane tree *Pla a 3*), and profilins (birch *Bet v 2*, timothy *Phl p 12*, annual mercury *Mer a 1*, and latex *Hev b 8)*. The microarray contains no rapeseed-specific allergens.

Our primary outcome measure was the number of positive DBPCFCs. The estimated 60% positive turnip rapeseed challenges and 5% positive placebo challenges with the 0.8 power and probability of 0.05 gave us a sample of 15 (McNemar test). The sample size calculation was based on the expected 89% rate of positive open challenges among turnip rape-sensitized individuals (1). McNemar test was used to compare the number of positive active and placebo challenges. Related samples Wilcoxon's signed rank test was used to compare SCORAD.

Of 1751 children screened with a panel of skin prick tests between 2009 and 2011, 11.8% was sensitized (≥3 mm) to turnip rapeseed. Of the 62 strongly sensitized (≥6 mm) children, 16 (10 males and 6 females) entered the study. Their median (range) age was 3.15 (1.18–13.6) yr, and SCORAD was 19.5 (3.5–83.5) at baseline. Fourteen children underwent the DBPCFC. No difference occurred in the number of positive reactions between active 1/14 (7%) and placebo 3/14 (21%) challenges ($p = 0.4795$). One child had immediate skin symptoms at both challenges, and two experienced worsening of eczema during placebo. The median (range) SCORAD was 12.9 (3.6–83.5) after active and 13.5 (3.6–57) after placebo challenge. Parentally reported symptoms did not differ between active and placebo challenges (data not shown). The children had multiple sensitizations: mustard 13/14 (93%), peanut 11/14 (78%), soy 10/14 (71%), egg white 11/14 (78%), wheat 5/14 (36%), cow's milk 5/14 (36%), and turnip rapeseed oil 0%. Serum-specific IgE analyses appear in Table 1.

In the microarray analysis, turnip rapeseed extract strongly inhibited IgE-binding of the profilins. Bet v 2 was inhibited 97%, Hev b 8 95%, Mer a 1 98%, and Phl p 12 100%. No inhibition was detected in the 2S albumins (0%),

Abbreviations

DBPCFC, Double-blind placebo-controlled food challenge; SCORAD, Severity score of atopic dermatitis; LTP, Lipid transfer protein.

Table 1 Sensitization of the study patients and controls

Specific IgE ≥0.35 kU/l	Patients N = 14 (%)	Range kU/l	Controls N = 14 (%)
Birch pollen	10 (71)	0.7–635	0 (0)
Oilseed rape	11 (78)	0.43–23.0	0 (0)
Mustard	13 (93)	0.36–15.0	0 (0)
Profilin Bet v 2*	4 (28)	0.82–15.3	0 (0)
Profilin Pru p 4*	4 (28)	0.54–12.2	0 (0)
2S albumin Ara h 2	6 (43)	0.4–51.43	0 (0)
PR-10 Ara h 8	7 (50)	0.67–182	0 (0)

*The same children were sensitized to both of the profilins.

other storage proteins, or PR-10 proteins LTPs were not significantly inhibited, however, low IgE binding to LTPs in the non-inhibited array did not allow reliable comparison.

Our results show that clinical turnip rapeseed allergy among sensitized individuals is uncommon. Although sensitization to turnip rapeseed may be relatively frequent in children with atopic eczema (12%), most of the children seem to be tolerant and demonstrate no symptoms at a double-blind placebo-controlled food challenge.

Our finding disagrees with the previous trials. In an open labial challenge, most (89%) sensitized individuals were reported to have an immediate allergic reaction. In another open challenge, 14/14 (100%) of sensitized children with atopic eczema were symptomatic (1, 2). An open food challenge may overestimate the role of a food allergen when symptoms are difficult to interpret, such as in atopic eczema characterized by regular unexplained flare-ups seldom due to food ingestions. It is vital to perform DBPCFC in clinical trials, especially when defining clinical significance of new allergens.

Our sample size was based on the previous trials (1, 2). We aimed at 20 patients to allow 25% drop out and 14 children went through both challenges. Our small sample size does not exclude the possibility that turnip rapeseed causes symptoms in some rare cases.

Turnip rape belongs to the highly cross-reactive Brassicaceae family with oilseed rape, mustard, cabbage, and turnip. Binding of IgE to the major allergenic proteins from oilseed rape and turnip rapeseed is very similar (5–8). In our study, 93% of turnip rape-sensitized children were sensitized to mustard and 78% to oilseed rape, indicating a cross-reaction between the species.

In the microarray inhibition used to further investigate turnip rapeseed cross-reactive allergens at molecular level, turnip rapeseed extract strongly inhibited *in vitro* IgE-binging of profilins. Profilins are highly cross-reactive and labile proteins, which do not resist heat or gastric digestion. Present in all plant tissues and conserved throughout the plant kingdom, they are responsible for multiple cross-sensitizations without any clinical relevance. Oilseed rape pollen profilin shares IgE epitopes with plant profilins such as birch pollen Bet v 2 (6). In a case–control study among children with atopic eczema, turnip rapeseed-sensitized children were significantly more often sensitized to pollens than the age- and sex-matched controls. Oilseed plant sensitization was associated with multiple sensitizations (9).

Turnip rapeseed, oilseed rape (7), and mustard (8) contain 2S albumins, which are potent allergens. Patients primarily sensitized to turnip rapeseed 2S albumins are therefore expected to be symptomatic. In the microarray inhibition, cross-reactivity of 2S albumins in turnip rapeseed and other seeds and nuts was low. Avoiding turnip rapeseed products in patients with some other 2S albumin sensitization (such as Ara h 2 in peanut) may be unnecessary. Lipid transfer proteins (LTP) have been suggested as the stable cross-reactive allergens in Brassicaceae family, especially in mustard (8). LTP sensitization is common in the Mediterranean area (10). LTP allergy is much more rare in northern Europe and North America. Our data imply that allergens other than LTP are responsible for turnip rapeseed sensitization in our patients.

We conclude that although sensitization to turnip rapeseed is common among children with atopic eczema, symptomatic food allergy is rare. Some labile cross-reactive allergen, such as profilin, may explain asymptomatic sensitization. Turnip rapeseed oil has known health benefits and also the use of the seeds in foods may increase in the future. Screening for turnip rapeseed sensitization without a history of an immediate reaction is not useful and may lead to unnecessary avoidance. Furthermore, consumption of turnip rapeseed in oil should not be blamed for inducing atopic eczema in childhood.

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Conflicts of interest.

The authors declare no conflict of interest.

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Is 30 minutes between doses long enough in oral food challenges?

To the Editor,

In clinical practice, the double-blind, placebo-controlled food challenge (DBPCFC) is considered the gold standard for diagnosing food allergy (1). This test is performed with gradual increases in the dose of the culprit food at regular time intervals to reduce the adverse events. When performing the DBPCFC, an interval of 30 min is often used between the doses (1, 2). However, it is currently unknown whether this interval is long enough to avoid reactions to accumulated doses, which might be more severe than reactions to individual doses.

The objective of this study was to investigate if an accumulation of doses in the DBPCFC occurs in patients who report longer time intervals (≥30 min) between ingestion of the culprit food and the subsequent reaction, as these patients could receive a subsequent dose before reacted to the previous dose. This was done by dividing patients into two groups based on reaction time and comparing the eliciting dose (ED) and the cumulative dose (CD) as well as the severity of the challenge reaction, corrected for confounders for these outcomes. As patients with longer reaction times by history (≥30 min) might be expected to accumulate doses during challenge testing, they could have higher EDs and CDs as well as more severe reactions than those with shorter reaction times (<30 min).

A database analysis of positive DBPCFCs (2002–2011), performed at University Medical Center Groningen was carried out. Children were challenged as a part of routine care for suspected food allergy. Unstable comorbidity or unwillingness to undergo the test were the only reasons to forego challenge testing, which was the case in <2% of the individuals. No children were excluded because of a history of previous anaphylaxis. Challenges were excluded from the present analysis when multiple food challenges to the same food were performed; in which case only the first challenge to each food was included (90 cases excluded). Patients were also excluded when there was no medical history for the tested food (103 cases) or if there was incomplete data for the relevant variables (92 cases). The exclusion of patients with only subjective symptoms in the DBPCFC (28 cases) had no influence on the outcome. Results from the five most commonly challenged foods were used for the analysis: milk, egg, peanut, hazelnut, and cashew nut.

The DBPCFCs were performed according to previously published procedures and protocols (2, 3). In brief, a food matrix was used to mask the suspected allergenic food. Administration of placebo and allergenic food using an incremental, 'semi-logarithmic' scale occurred on separate days, at least a week apart in random order. Double blinding was maintained until 48 h after the second test day. A food challenge was considered positive when objective symptoms occurred on the allergenic food-test day but not on the placebo day (2–5).

A scoring system was used to determine the severity of the allergic reaction during the oral challenge test (6). The patients' symptoms were allocated to different categories and received a corresponding score. The categories used and their score were the following: skin (1), upper airways (3), lower airways (3), gastrointestinal (2) and cardiovascular/neurological symptoms (3). The scores of each category were summed and expressed as a severity index. The severity index ranges from 0–12 and is divided into groups. Scores from 0–2 are considered mild reactions. A score of 3–6 is regarded as a moderate reaction. The third group with scores of 7–12 is classified as severe reactions.

The statistical analysis of the data was performed using the statistics software package SPSS Statistics 17.0 for Windows. Reaction severity, ED, and CD were compared in patients with long (≥30 min) and short (<30 min) reaction times by history using the Mann–Whitney *U*-test. Possible confounders to the analysis were pre-selected through Spearman's correlation test and multiple linear regression by adopting a liberal p-value cut off (>0.2). Confounders reported in other studies were also included as well as biologically plausible confounders and general demographics (age, sex). Subsequently, multiple linear regression analysis was used to assess any confounding by evaluating whether the coefficient of the

Abbreviations:
CD, cumulative dose; DBPCFC, double-blind, placebo-controlled food challenge; ED, eliciting dose.