

# Effect of Enzymatically Modified Isoquercitrin, a Flavonoid, on Symptoms of Japanese Cedar Pollinosis: A Randomized Double-Blind Placebo-Controlled Trial

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## Key Words

Flavonoid • Quercetin • Pollinosis

## Abstract

**Background:** Flavonoids exert antiallergic and antioxidant effects. We investigated the efficacy of enzymatically modified isoquercitrin (EMIQ), a flavonoid, to relieve symptoms of pollinosis. **Methods:** In a parallel-group, double-blind placebo-controlled study design, 20 subjects with Japanese cedar pollinosis took two capsules daily of 100 mg EMIQ or a placebo for 8 weeks during the pollen season. Subjective symptoms and activities of daily living (ADL) scores were recorded every day, and the quality of life (QOL) score was obtained every 4 weeks. Blood sampling was performed before and after the study to measure serum cytokines, chemokines, IgE, quercetin and oxidized biomarkers. **Results:** During the entire study period, total ocular score and ocular itching score for the EMIQ group were significantly lower ( $p < 0.05$ ) than for the placebo group. When limited to the individual periods, total symptom score for the EMIQ group was significantly lower ( $p < 0.05$ , week 4–5) than that for the placebo group while other scores for the EMIQ group,

such as total nasal score ( $p = 0.06$ , week 4–5), nasal obstruction score ( $p = 0.08$ , week 4–5), lacrimation score ( $p = 0.06$ , week 5–6), ocular congestion score ( $p = 0.08$ , week 4–7) and ADL score ( $p = 0.08$ , week 4–7), all tended to be lower. The levels of serum cytokines such as interleukin (IL)-4, IL-5, IL-12, IL-13, interferon- $\gamma$ , and eotaxin and IgE were not significantly downregulated by the intake of EMIQ but the serum concentrations of oxidized low-density lipoprotein and thymus and activation-regulated chemokine were reduced. **Conclusion:** Intake of the quercetin glycoside EMIQ was safe and influenced ocular symptoms caused by pollinosis.

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## Introduction

The number of patients with allergic diseases, such as allergic rhinitis, asthma and atopic dermatitis, has been increasing all over the world, especially in developing countries [1, 2]. In 1964, the first report of Japanese patients with allergic rhinitis caused by Japanese cedar pollen was published [3], and now about 24–29% of the population is thought to be suffering from this disease [4].

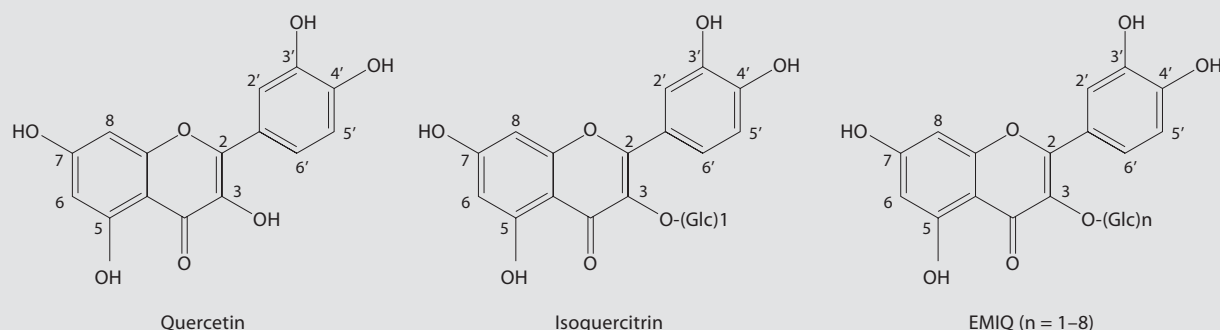


**Fig. 1.** Chemical structures of quercetin, isoquercitrin and EMIQ.

Diet has recently been posited as one of the environmental factors which cause an increase in the prevalence of allergic diseases (the so-called 'dietary hypothesis') [5–9]. Changes in intake of lipid or antioxidant substances during the last 2 decades are thought to have increased the prevalence of and/or to have worsened allergic symptoms. If this is the case, an appropriate daily intake of lipid or antioxidants may reduce the onset of allergic diseases or ameliorate allergic symptoms.

Flavonoids, ubiquitously found in fruits, vegetables or teas, exert antioxidant as well as antiallergic effects [10]. In addition to the inhibitory activity of flavonoids on histamines and other chemical mediators [11, 12], we have demonstrated that flavonoids such as apigenin, fisetin, luteolin and quercetin suppress the production of Th2 cytokines and CD40 ligand expression in activated human basophils [13–15]. Furthermore, oral administration of astragalín, a flavonoid, had a preventative and ameliorative effect on dermatitis in an atopic dermatitis mouse model, NC/Nga [16, 17]. We therefore proposed that an appropriate intake of flavonoids might constitute a dietary preventative or therapeutic strategy for allergic diseases [18–20].

In the study presented here, we used enzymatically modified isoquercitrin (EMIQ), which has been recognized as a food additive [21], to investigate, by means of a randomized double-blind placebo-controlled trial, the effect of this flavonoid on allergic symptoms as well as serum parameters in patients with Japanese cedar pollinosis.

## Materials and Methods

### Participants

Twenty adult patients living in various areas in the Osaka Prefecture, who had been suffering from Japanese cedar pollinosis during the pollen season and showed positive blood IgE specific for Japanese cedar pollen, were enrolled. Patients were excluded if they had any systemic diseases such as malignancies, collagen diseases and chronic infections or were pregnant. All participants provided written informed consent. The study protocols were approved by the Ethics Committee of the Osaka University.

### Study Design

A randomized, double-blind, placebo-controlled design was used for the trial performed at the Osaka University Medical Hospital. EMIQ (San-Ei-Gen F.F.I., Toyonaka, Japan) [21] and placebo were supplied in capsule form. Figure 1 shows the chemical structure of EMIQ. The EMIQ capsule contained 50 mg EMIQ plus corn starch and the placebo capsule contained corn starch only but had an identical appearance and flavor to the EMIQ capsule. In this study, we employed EMIQ for the following two reasons. First, EMIQ is more soluble than quercetin and isoquercitrin and is believed to be the highest absorptive flavonoid among quercetin derivatives. Second, since EMIQ has been recognized as a food additive [21], found to be safe by many evaluation tests including acute toxicity test, 4-week toxicity test, 13-week toxicity test, chronic/carcinogenic test [22] and mutagenicity test (Ames assay), its no-observed-adverse-effect level was estimated at approximately 500 mg/kg/day. Therefore the acceptable daily intake for human beings is thought to be 5 mg/kg/day and we selected 100 mg EMIQ for the study.

Patients were randomly assigned to the EMIQ group or the placebo group and took one capsule twice a day for 8 weeks. This study was started on March 4, 2007 and terminated on April 28. In 2007, according to the regional records for the Osaka Prefecture, the onset of cedar pollen release was on February 7 and the amount of pollen showed two peaks on February 22 and at the

beginning of March, and on March 26 another increase in the cedar pollen count was observed. This was followed by the release of Japanese cypress pollen, which began on March 26 and showed two peaks in the count on April 2 and 11. The average values of the Japanese cedar and cypress pollen counts at three locations (Osaka, Toyonaka and Suita City) in the Osaka Prefecture are shown as grains/cm<sup>2</sup>/week in figure 2, which was drawn according to our data and the measurement of the Japan Weather Association.

Since EMIQ is used as an antioxidant in some commercially available food products, participants were not allowed to eat such products during the test period.

#### Outcome Measurements

Severity of subjective symptoms was evaluated by a scoring system based on the slightly modified method by Baba et al. [23]. During the test period, participants were asked to record symptom scores and activities of daily living (ADL) 3 times a day. Symptoms were evaluated with the following scale: sneezing and rhinorrhea (0 = none, 1 = 1–5 episodes/period, 2 = 6–10 episodes/period, 3 = 11–20 episodes/period, 4 = >20 episodes/period), nasal obstruction (0 = none, 1 = little, 2 = difficult to breath through noses, 3 = unable to breath through noses), ocular itching (0 = none, 1 = mild and bearable, 2 = severe but bearable, 3 = unbearable), lacrimation (0 = none, 1 = seldom, 2 = sometimes, 3 = always), and congestion of conjunctiva (0 = none, 1 = little, 2 = mild, 3 = severe). The ADL score used a range from 0 to 3, with 0 = no problem, 1 = mild symptoms not causing difficulties with ADL, 2 = moderate symptoms with some difficulties with ADL, 3 = severe symptoms with major difficulties with ADL. The total symptom score was calculated as the sum of the total nasal and ocular symptom scores. The total nasal score was calculated as the sum of specific nasal scores such as for sneezing, rhinorrhea and nasal obstruction. The total ocular score was shown as the sum of specific ocular scores including those for ocular itching, lacrimation and ocular congestion. Thus, the range of these scores was 0–420/week for the total symptom score, 0–231/week for the total nasal score, 0–189/week for the total ocular score and 0–63/week for the ADL score.

The degree of quality of life (QOL) was assessed by using the Japanese Allergic Rhinitis QOL Questionnaire (JRQLQ) [24] before, in the middle and at the end of the study. The JRQLQ comprises questions about rhinorrhea, sneezing, nasal obstruction, nasal and ocular itching, lacrimation, general fatigue, irritability, depression and difficulties with daily activities such as working, housekeeping, studying, reading, doing sports, going outdoors, sleeping and having conversations.

Participants were allowed to take antiallergic drugs and to use ocular drops and nasal sprays, depending on the severity of their symptoms, but the usage of these drugs was recorded in the symptom records and was evaluated by the medication score [23] defined by the Japanese Society of Allergology with the following scale: 0 = none, 1 = oral antihistamines, oral histamine release inhibitors, nasal sprays excluding focal steroids and ocular drops excluding focal steroids, 2 = focal administration of steroids, and 3 = oral administration of steroids.

#### Blood Sampling

Blood sampling was performed before and at the end of the study. Serum concentrations of total IgE antibody, specific IgE for

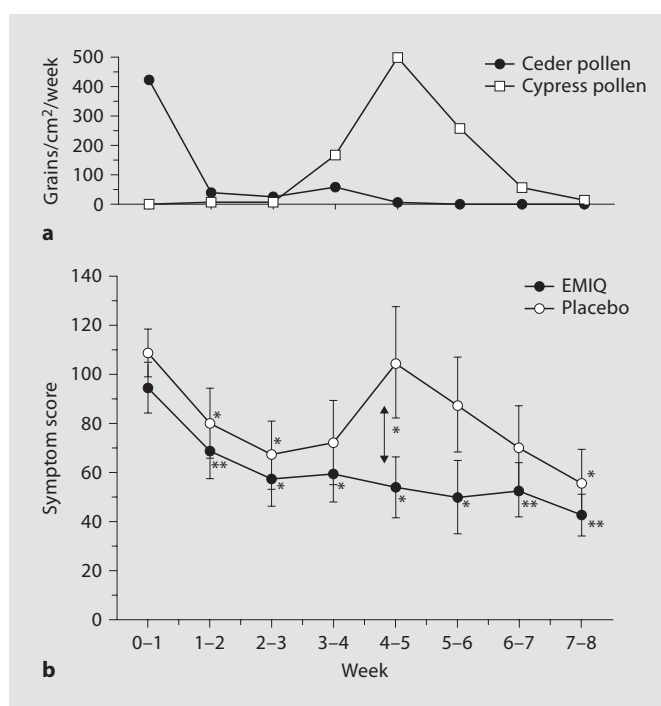


**Fig. 2.** The changes of total symptom scores in the EMIQ and the placebo group. **a** Changes of total amounts of cedar and cypress pollen scattered. **b** Changes of total symptom score. Each value represents the mean  $\pm$  SE. \*  $p < 0.05$ , \*\*  $p < 0.01$  compared with the 1st week in each group; arrow with \*  $p < 0.05$  between the two groups in the same week.

Japanese cedar and cypress pollen and thymus and activation-regulated chemokine (TARC) were analyzed by Shionogi Biomedical Laboratories (Osaka, Japan). Serum levels of quercetin were measured by HPLC as described elsewhere [25] after hydrolyzation of the samples. The serum activity of ferric reducing antioxidant power (FRAP) was determined as described previously [26]. Oxidized biomarkers such as hexanoyl-lysine (HEL) and oxidized low-density lipoprotein (LDL) were analyzed by means of ELISA by using a commercially available ELISA kit (HEL ELISA kit, Japan Institute for the Control of Aging, Nikken Seil, Shizuoka, Japan, and The Mercodia Oxidized LDL Competitive ELISA assay kit, Mercodia AB, Uppsala, Sweden). Serum levels of cytokines and chemokines such as interleukin (IL)-4, IL-5, IL-12, IL-13, eotaxin and IFN- $\gamma$  were measured with the Bio-Plex System (Bio-Rad Laboratories, Hercules, Calif., USA) [27].

#### Statistical Analysis

Symptom scores were analyzed with two-way ANOVA (analysis of variance) for repeated measurements with multiple comparisons. The data of serum cytokines, chemokines, quercetin and oxidized parameters are expressed as mean  $\pm$  SD and compared using a two-tailed Student *t* test. In both tests,  $p < 0.05$  was considered statistically significant.

**Table 1.** Characteristics of the subjects

|                                            | Placebo    | EMIQ       |
|--------------------------------------------|------------|------------|
| Participants                               | 10         | 10         |
| Age, years                                 | 35.1 ± 8.5 | 39.2 ± 9.5 |
| Gender                                     |            |            |
| Male                                       | 8          | 8          |
| Female                                     | 2          | 2          |
| Total IgE, IU/ml                           |            |            |
| At the beginning                           | 233 ± 272  | 133 ± 139  |
| At the end                                 | 287 ± 289  | 147 ± 135  |
| Specific IgE for cedar pollen (RAST score) |            |            |
| At the beginning                           | 3.4 ± 1.3  | 2.6 ± 0.8  |
| At the end                                 | 4.2 ± 1.2  | 3.3 ± 0.9  |
| Specific IgE for cypress pollen            |            |            |
| Sensitization, %                           | 90         | 90         |
| At the beginning (RAST score)              | 2.0 ± 1.3  | 1.3 ± 1.0  |
| At the end (RAST score)                    | 2.4 ± 1.2  | 1.8 ± 0.8  |

## Results

### Characterization of Participants

Table 1 shows the characteristic features of participants in both groups. The baseline characteristics were similar for the two groups, and all participants showed positive serum IgE for Japanese cedar pollen, while 90% did so for Japanese cypress pollen. Total IgE levels for the EMIQ and the placebo group were 233 or 133 IU/ml, respectively. There was no significant difference in age, sex distribution, total IgE or sensitization to the two types of pollens between the two groups.

### Symptom Assessment

Figure 2 show changes in the total symptom scores. The total symptom score for the placebo group gradually decreased until week 2–3 but during week 3–5 it was elevated, whereas the EMIQ group did not show this elevation during the same period. At week 3–4 an increase in the cedar pollen count was observed and at week 3–6 there was a marked increase of the cypress pollen count. There was no significant difference in the total symptom score ( $p = 0.15$ ) for the entire period between the two groups, but for restricted periods (week 4–5) a significant therapeutic effect of EMIQ was observed ( $p < 0.05$ ). The changes in the total nasal and ocular scores are shown in figure 3. Although the beneficial effect of EMIQ on nasal symptoms (fig. 3a) was weak, it was statistically significant for total ocular symptoms and ocular itching during the whole period ( $p < 0.05$ ) (fig. 3b). The changes in the QOL score, ADL score and medication score are shown

in figure 4. During the restricted periods (weeks 4–5, 5–6 and 6–7) the ADL score for the EMIQ group tended to be lower than that for the placebo group. There were no differences in the QOL and medication scores between the two groups.

All participants completed the study, and no side effects, such as gastrointestinal symptoms, allergic reactions, or cardiovascular symptoms, were observed during the entire period. The mean percentage of intake for the EMIQ and placebo groups was 98.4 and 91.5%, respectively.

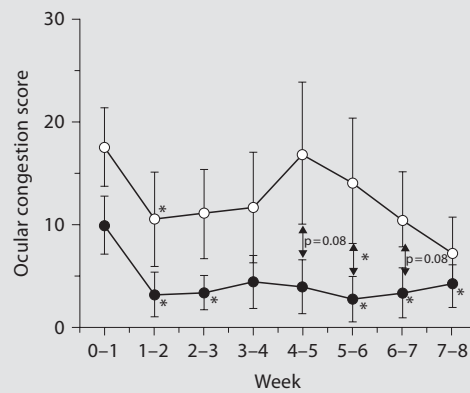
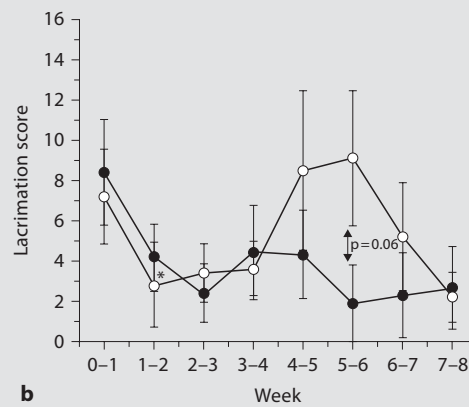
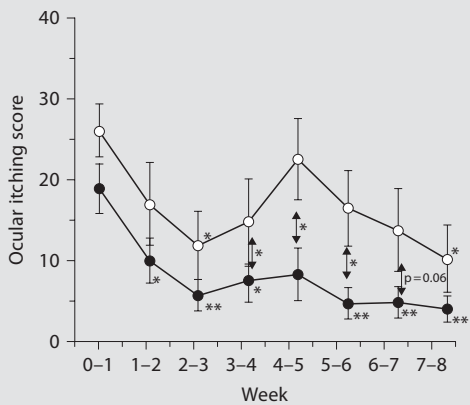
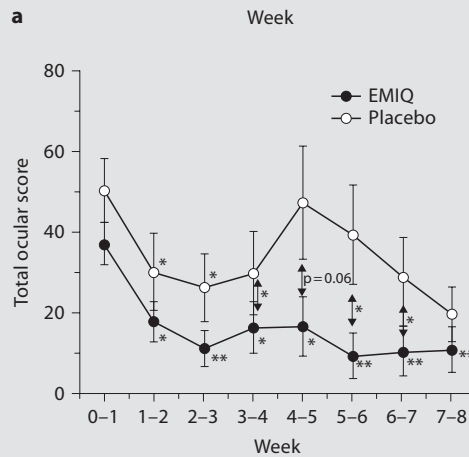
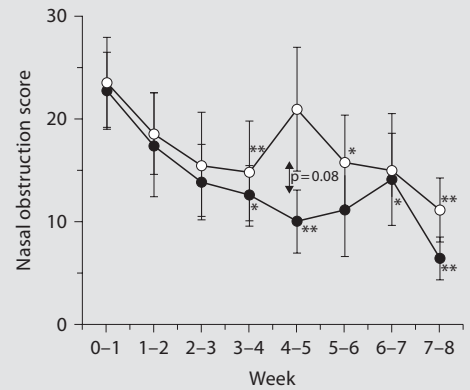
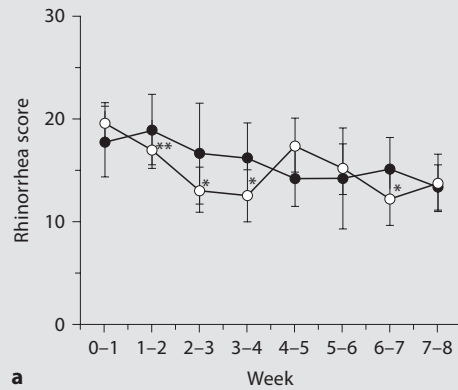
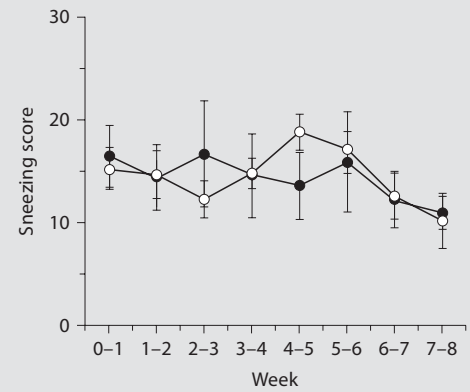
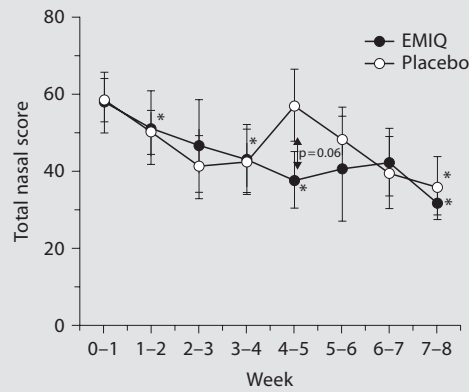
### Blood Parameters

During the pollen season, exposure to allergens affects serum levels of various cytokines and chemokines. EMIQ intake in comparison with placebo did not significantly affect serum concentrations of these measured cytokines and chemokines except for TARC (fig. 5). Serum concentrations of TARC in the EMIQ group significantly decreased whereas those in the placebo group tended to decrease. Figure 6 shows the changes in serum levels of quercetin, FRAP, indicating antioxidant activity, and oxidized biomarkers such as HEL and oxidized LDL. Serum quercetin levels in the two groups at the end of the study were not significantly different and did not increase significantly in the EMIQ group. FRAP tended to increase in the EMIQ group whereas oxidized LDL significantly decreased in the EMIQ group. During the study period, serum levels of total IgE and specific IgE for Japanese cedar and cypress pollen increased in both groups (table 1).

## Discussion

Recently oral intake of extracts derived from tomato and hop water have been shown to be effective for perennial and seasonal allergic rhinitis [28, 29]. The main constituents in these extracts responsible for the antiallergic clinical effect are thought to be flavonoids such as naringenin, quercetin, and kaempferol, but it remains unknown what the direct effect of flavonoids by themselves is on symptoms of allergic rhinitis. To the best of our knowledge, this report represents the first demonstration

**Fig. 3.** The changes of nasal scores (a) and ocular scores (b) in the EMIQ and the placebo group. Each value represents the mean ± SE. \*  $p < 0.05$ , \*\*  $p < 0.01$  compared with the 1st week in each group; arrow with \*  $p < 0.05$  between the two groups in the same week.



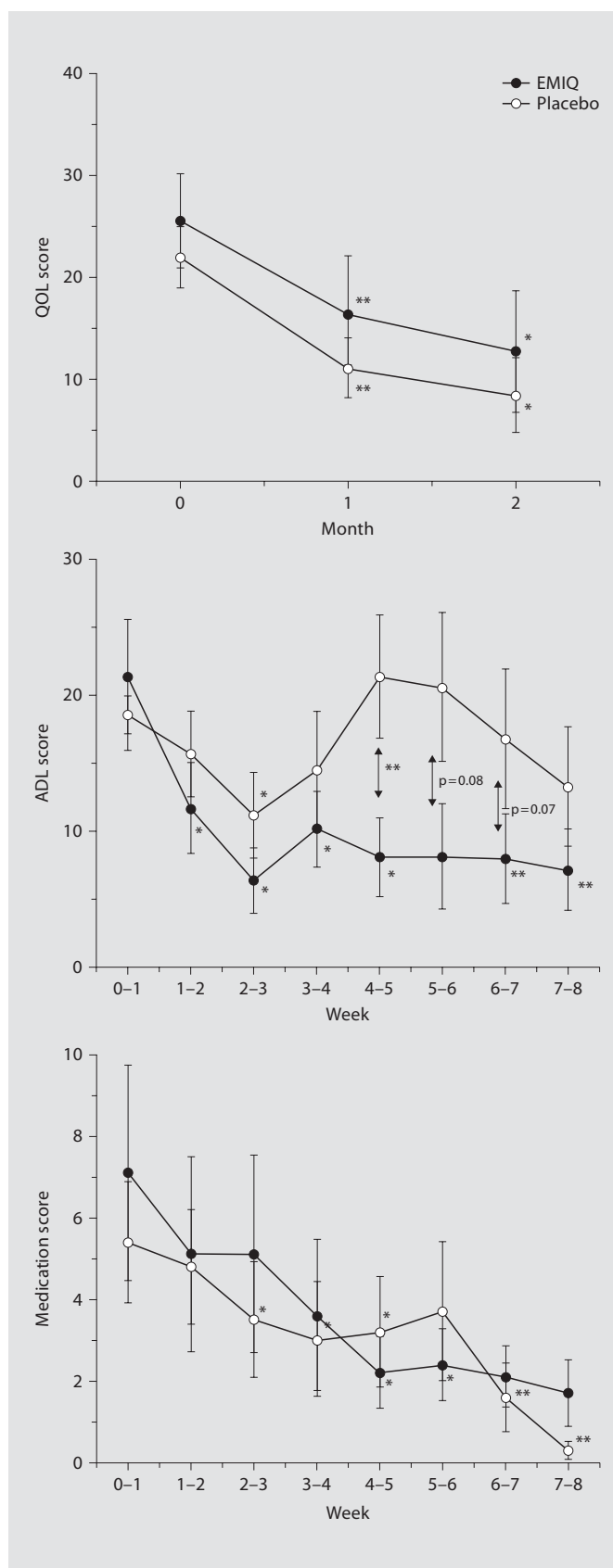
**b**

that oral intake of a flavonoid (EMIQ) leads to an alleviation of symptoms of pollinosis, especially ocular symptoms.

The ameliorative effect of EMIQ was on ocular symptoms and was to a large extent mediated through its antiallergic, antioxidant [30] or other activities [10]. It has been demonstrated that flavonoids inhibit release of histamine [11] and other chemical mediators [12]. We and others have shown that some kinds of flavonoids including apigenin, fisetin, luteolin and quercetin suppress the expression of Th2-type (IL-4, IL-5 and IL-13) [13, 14], proinflammatory cytokines [31] and CD40 ligand [15] in basophils or in mast cells. In addition to these in vitro antiallergic activities, studies with allergic animal models have demonstrated that flavonoids exert preventative and therapeutic effects by modifying Th2-type cytokine expression and suppressing IgE elevation [16, 17, 32–37]. In our study we measured the serum levels of cytokines and chemokines before and after the study but found no significant reduction in Th2 cytokines (IL-4, IL-5 and IL-13), chemokines (eotaxin) or IgE concentration as a result of EMIQ intake except for TARC (fig. 5). The serum levels of TARC were shown to be positively associated with the severity of symptoms of Japanese cedar pollinosis [38]. Since the serum concentrations of TARC tended to decrease in both groups in parallel to the severity of symptoms, whether EMIQ intake primarily reduced TARC production needs to be clarified. On the other hand, the antioxidant activity represented by FRAP tended to increase and one oxidized product, oxidized LDL, decreased in the EMIQ group, suggesting that the ameliorative effect of EMIQ was due to its antioxidant activity. However, due to the small sample size, further studies will be essential to reveal the precise mechanism(s) through which EMIQ influenced ocular symptoms.

Pollen grains themselves also carry flavonoids. For instance the pollen of *Cryptomeria japonica* contains nine flavonoids including quercetin [39], which may be antiallergic [40], but actually causes pollinosis. This contradictory phenomenon simply indicates that the suppressive effect of flavonoids on the sensitization to the pollen and allergic symptoms is marginal. However, we would like to point out that the effects of flavonoids are thought to

**Fig. 4.** The changes of QOL score, ADL score and medication score in the EMIQ and the placebo group. Each value represents the mean  $\pm$  SE. \*  $p < 0.05$ , \*\*  $p < 0.01$  compared with the 1st week in each group; arrow with \*\*  $p < 0.01$  between the two groups in the same week.



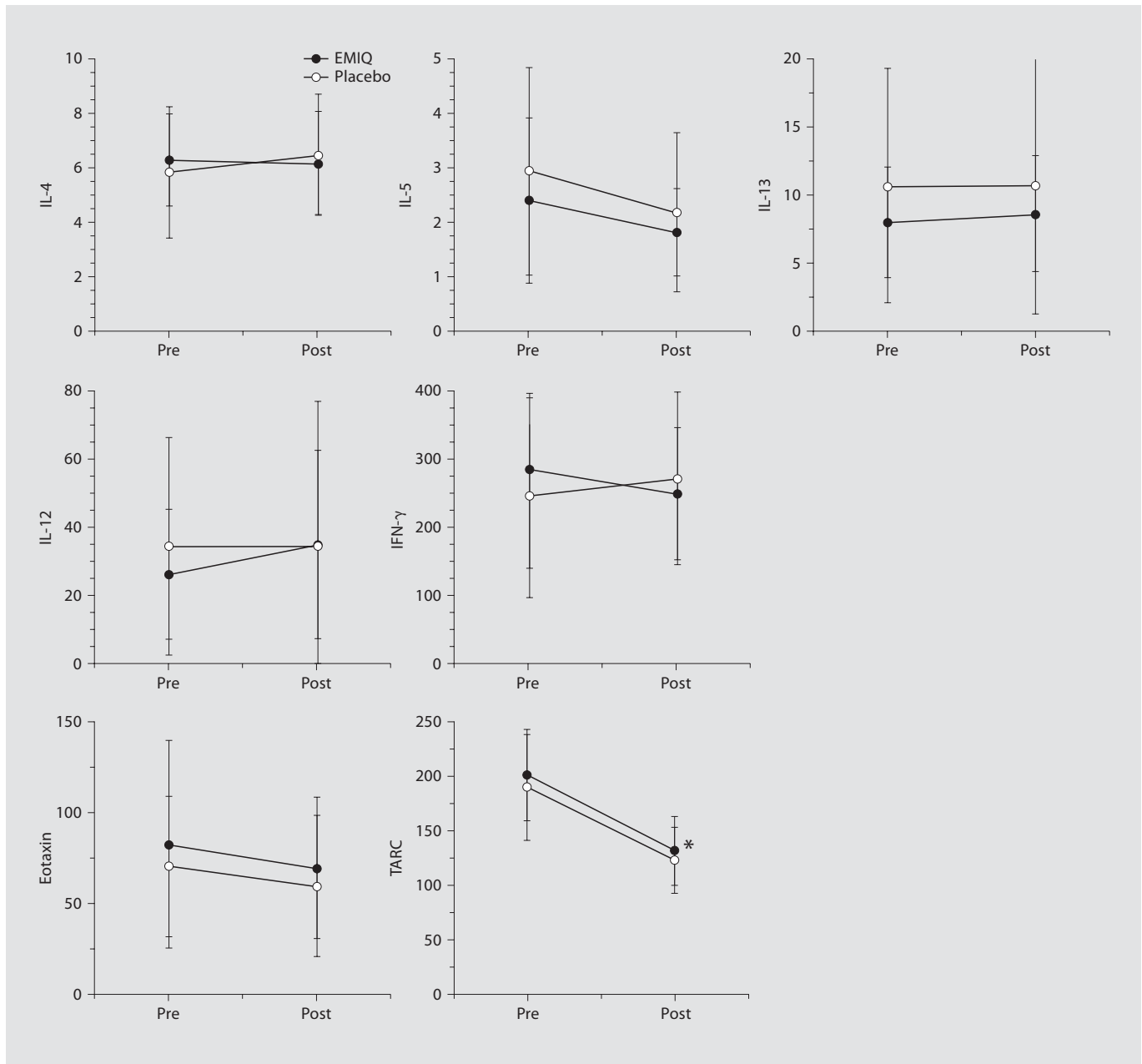


**Fig. 5.** The changes of serum concentrations of cytokines and chemokines in the EMIQ and the placebo group. The data presented are the mean  $\pm$  SD (pg/ml). \*  $p < 0.05$  compared with the value at the beginning of the study.

depend on their quantity, quality and the administration route and indeed daily intake of 100 mg EMIQ led to a reduction in ocular symptoms.

During the recent few years, other antiallergic dietary substances have been shown to alleviate symptoms of pollinosis [41–43]. In these studies, the clinical trials

started 2–4 weeks prior to the onset of Japanese cedar pollen release, so that they were designed to determine their preventative effect. We also intended to ascertain the preventative effect of EMIQ on symptoms, but the release of Japanese cedar pollen in 2007 started earlier than we expected, so that this study had to be restricted

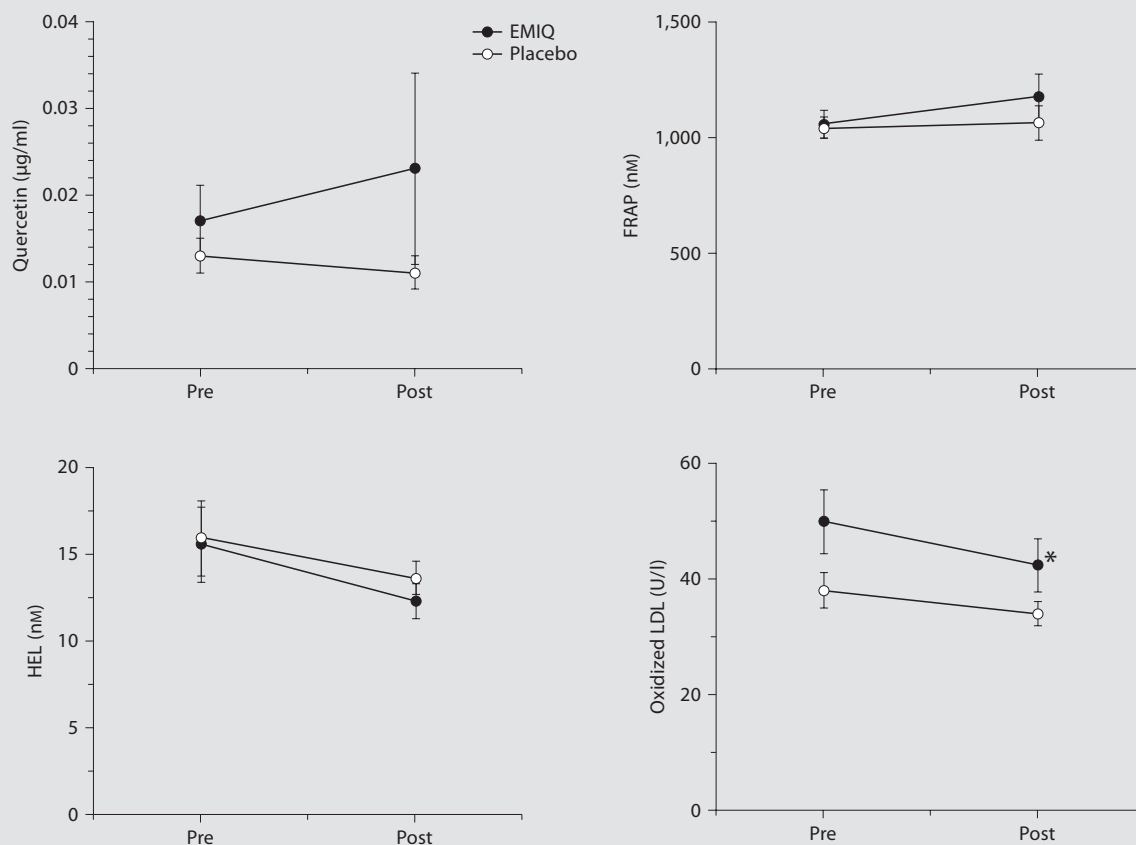


**Fig. 6.** The changes of serum concentrations of quercetin, and oxidized markers in the EMIQ and the placebo group. Each value represents the mean  $\pm$  SE. \*  $p < 0.05$  compared with the value at the beginning of the study.

to the therapeutic effect of EMIQ. Symptoms of pollinosis in the placebo group were exacerbated at the end of March and beginning of April (week 3–5), but not in the EMIQ group. The amount of the cedar pollen scattered was substantially elevated at the end of March and that of the cypress pollen was highly elevated during week 3–6, so that the exacerbation of symptoms in the placebo group can be considered to have been due to exposure to the cedar pollen or more likely to the cypress pollen. It should be pointed out, however, that there was no difference in sensitization to either type of pollen between the two groups, leading us to conclude that EMIQ was effective for the relief of ocular symptoms. The reason why EMIQ was ef-

fective for ocular symptoms but not for nasal symptoms remains unclear but it is hoped that a future study of a preventative clinical design or a large-scale design will provide clarification.

In the dietary hypothesis, it has been proposed that diet is one of the environmental factors that contribute to an increase in the prevalence of allergic diseases and/or worsening of allergic symptoms [6–9]. Furthermore, an epidemiologic study found that a higher intake of flavonoids was associated with a lower incidence of asthma [44]. In experimental models, oral administration of flavonoids has been demonstrated to prevent allergic diseases including asthma, anaphylaxis, food allergies and

atopic dermatitis [16, 17, 32–37]. This study showed that the ocular symptoms were reduced in the EMIQ group. Because of the small number of participants, the result is limited. Therefore, a large-scale clinical study will be required to evaluate its effectiveness. But all these findings suggest that an appropriate intake of flavonoids can be a dietary means for the prevention of and therapeutic approach to allergic diseases.

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