

## Exercise and aspirin increase levels of circulating gliadin peptides in patients with wheat-dependent exercise-induced anaphylaxis

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### Summary

**Background** Food-dependent exercise-induced anaphylaxis (FDEIA) is an allergic reaction characteristically induced by intense exercise combined with the ingestion of causative food. Recent reports have shown that aspirin intake is a contributing factor in some patients with FDEIA. Wheat is known to be the most frequent causative food, and the IgE-binding epitopes of a major wheat allergen ( $\omega$ -5 gliadin) in wheat-dependent exercise induced anaphylaxis (WDEIA) have already been clarified. However, the mechanism of eliciting the symptom in WDEIA remains not fully understood.

**Objectives** The aim of this study was to examine the relationship of serum gliadin levels and allergic symptoms induced by exercise or aspirin in patients with WDEIA.

**Methods** Six patients with a history of recurrent anaphylaxis associated with wheat ingestion were diagnosed as having WDEIA by the provocation test, which included wheat ingestion, exercise, aspirin intake and a combination of these challenges. During the tests, serum levels of gliadins were monitored by gliadin-specific sandwich ELISA. The effects of exercise and aspirin on serum gliadin levels were also investigated in four healthy subjects.

**Results** Immunoreactive gliadins appeared in the sera of patients during the provocation test with both wheat-exercise and wheat-aspirin challenges in parallel with allergic symptoms. Serum gliadin levels also increased under the two same challenge conditions in the healthy subjects, although they exhibited no allergic symptoms. However, low levels of gliadin were detected in the sera of both patients and healthy subjects when challenged with wheat alone.

**Conclusion** We demonstrated for the first time that blood gliadin levels correlate with clinical symptoms induced by exercise and aspirin in patients with WDEIA. These findings suggest that exercise and aspirin facilitate allergen absorption from the gastrointestinal tract.

**Keywords** allergen, aspirin, exercise, food-dependent exercise-induced anaphylaxis, gliadin, wheat

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### Introduction

Food-dependent exercise-induced anaphylaxis (FDEIA) is believed to be a distinct form of food allergy because the symptoms are characteristically induced by physical exercise after ingestion of causative food [1]. Patients with FDEIA usually have IgE antibodies to the causative food and exhibit symptoms such as urticaria, erythema, pruritus and dyspnea typical to IgE-mediated allergic reactions [2]. Although various foods have been reported to be the cause of FDEIA, wheat is the most frequent [3]. Among wheat proteins, gliadin including  $\alpha$ -gliadin,  $\gamma$ -gliadin and  $\omega$ -5 gliadin, which are components of wheat gluten, was identified as an allergen in patients with wheat-dependent exercise-induced anaphylaxis

(WDEIA) [4–8]. In addition, linear IgE-binding epitopes consisting of seven amino acids were identified in  $\omega$ -5 gliadin and the epitopes were found to be recognized by IgE antibodies in most of the patients with WDEIA [9]. However, the induction mechanism by exercise in this anaphylaxis still remains unclear.

Studies in animal models of food allergy have shown that physical exercise and sensitization affect the absorption of antigen from the gastrointestinal tract because of gastrointestinal mucosal damages [10, 11]. It is well established that strenuous physical exercise may lead to gastrointestinal disorders and increases the intestinal permeability in humans [12]. On the other hand, recent investigations have documented that instead of exercise, aspirin pretreatment also induces allergic symptoms in FDEIA [13, 14]. The mechanism is different from that of aspirin intolerance in that aspirin itself does not induce symptoms in patients with FDEIA. Intake of aspirin as well as physical exercise [15, 16] is known to promote an increase of gastrointestinal permeability.

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Although several studies suggest that exercise and aspirin facilitate the absorption of allergens from the gastrointestinal tract and increased amounts of allergens in the circulation blood cause allergic symptoms in FDEIA have been completed, a study on the blood allergen level has not been conducted.

To clarify the correlation between serum levels of gliadin and allergic symptoms induced by exercise and aspirin in WDEIA, we measured the levels of gliadin in the sera of patients with WDEIA as well as levels in healthy subjects during the provocation tests.

## Methods

### Subjects

Six patients with WDEIA who had recurrent episodes of anaphylaxis and four healthy subjects with no known episodes of food allergy were enrolled in this study. Informed consent was obtained from all patients and healthy subjects. Specific IgE antibodies for gluten in the sera were determined with the CAP-RAST system (Pharmacia, Uppsala, Sweden) [17]. The characteristics of the subjects are shown in Table 1.

### Provocation test

The provocation test included exercise, wheat ingestion, aspirin intake and a combination of these challenges. The challenge protocols and the sampling time of the venous blood are schematically displayed in Fig. 1. Subjects fasted for 6 h before the tests. Treadmill exercise was carried out 20–30 min after wheat ingestion using the standard Bruce protocol as described previously [18]. The kind and the amount of wheat food product challenged to each patient were decided on the basis of clinical history. The noodles and bread used in these tests were made from wheat flour and small amounts of salt. In addition, the bread contained baker's yeast. The amounts of wheat ingested are expressed as weight of wheat flour in Table 2. Aspirin (500 mg) was administered right before wheat ingestion for patients with WDEIA. For the healthy subjects, 1000 mg of aspirin was administered 30 min before wheat ingestion. During the test, heart rate, blood pressure and electrocardiogram were monitored and venous blood was taken at a set time.

### Measurement of serum gliadin levels

The serum concentration of gliadin was measured using a sandwich enzyme-linked immunosorbent assay kit (Morinaga Institute of Biological Science, Yokohama, Japan) with a slight modification. The gliadin-specific antibodies in this kit were established from the purified serum of the immunized rabbits with gliadins extracted with aqueous ethanol from wheat flour. Serum samples (0.5 mL) were mixed with 0.1 mL deionized water and 1.4 mL ethanol (70% of final concentration) and gliadins were extracted for 1 min by vortexing. After the mixture was centrifuged at 20 000 *g* for 5 min at room temperature, the supernatant was transferred to a new tube and evaporated. The residue was dissolved in 0.1 mL of the sample dilution buffer included in the kit. The gliadin concentration in each sample was calculated by using native

**Table 1.** Characteristics of subjects

| Subjects                   | Age (years) | Gender | Total IgE (IU/mL) | RAST (UA/mL) for gluten |
|----------------------------|-------------|--------|-------------------|-------------------------|
| <b>Patients with WDEIA</b> |             |        |                   |                         |
| 1                          | 61          | M      | 44.5              | 1.55                    |
| 2                          | 29          | M      | 146               | <0.35                   |
| 3                          | 15          | M      | 21.9              | <0.35                   |
| 4                          | 56          | M      | 920               | 0.51                    |
| 5                          | 52          | F      | 261               | 0.66                    |
| 6                          | 47          | F      | 104.8             | <0.35                   |
| <b>Healthy subjects</b>    |             |        |                   |                         |
| 1                          | 33          | M      | 57                | <0.35                   |
| 2                          | 35          | M      | 110               | <0.35                   |
| 3                          | 25          | F      | 100               | <0.35                   |
| 4                          | 24          | F      | 500               | <0.35                   |

WDEIA, wheat-dependent exercise-induced anaphylaxis.

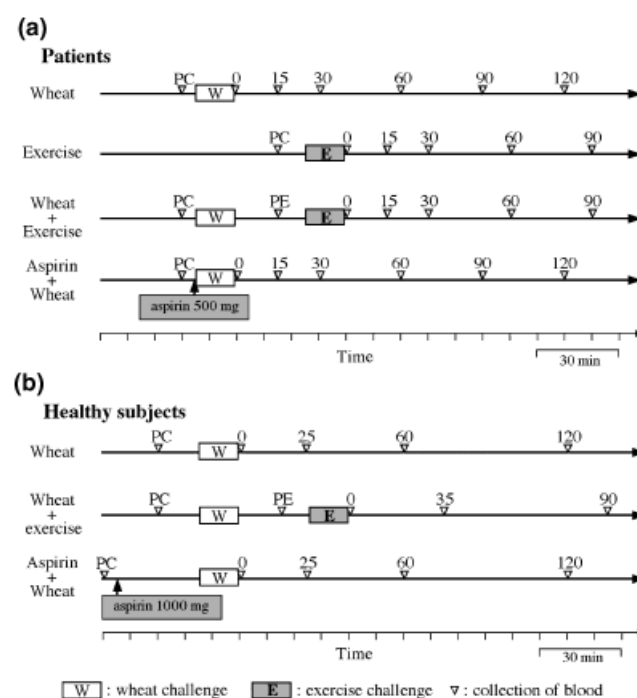


**Fig. 1.** Challenge protocols for (a) patients with wheat-dependent exercise induced anaphylaxis and (b) healthy subjects. PC, pre-challenge; PE, pre-exercise.

gliadin (Tokyo Kasei Kogyo Co., Ltd, Tokyo, Japan) as a standard that was prepared by extraction with aqueous ethanol from wheat flour. The detection limit was 15 pg/mL in this method.

## Results

### Serum gliadin levels during the provocation tests in patients with WDEIA

The results of the provocation test in six patients with WDEIA are shown in Table 2. The exercise challenge test was carried out in five of the six patients (patients 1–5). None of the five patients tested exhibited symptoms in response to either exercise or wheat intake alone. Wheat ingestion

**Table 2.** Results of provocation tests for patients with WDEIA and healthy subjects

|                     |                         |                |              | Symptoms    |                |                |               |               |
|---------------------|-------------------------|----------------|--------------|-------------|----------------|----------------|---------------|---------------|
| Subjects            | Wheat style/amount (g)* | Exercise (min) | Aspirin (mg) | Wheat alone | Exercise alone | Wheat+exercise | Aspirin alone | Aspirin+wheat |
| Patients with WDEIA |                         |                |              |             |                |                |               |               |
| 1                   | Noodles/120             | 15             | –            | None        | None           | U, S           | –             | –             |
| 2                   | Bread/100               | 15             | –            | None        | None           | E, U           | –             | –             |
| 3                   | Bread/70                | 15             | –            | None        | None           | P, E, D        | –             | –             |
| 4                   | Noodles/100             | 15             | 500          | None        | None           | E              | None          | E             |
| 5                   | Bread/70                | 15             | 500          | None        | None           | P, E           | None          | U, N          |
| 6                   | Noodles/135             | –              | 500          | None        | –              | –              | None          | P, E, U       |
| Healthy subjects    |                         |                |              |             |                |                |               |               |
| 1                   | Noodles/120             | 15             | 1000         | None        | –              | None           | –             | None          |
| 2                   | Noodles/120             | 15             | 1000         | None        | –              | None           | –             | None          |
| 3                   | Noodles/120             | 15             | 1000         | None        | –              | None           | –             | None          |
| 4                   | Noodles/100             | 15             | 1000         | None        | –              | None           | –             | None          |

\*Amounts of wheat ingested are expressed as weight of wheat flour.

WDEIA, wheat-dependent exercise-induced anaphylaxis; –, not done; U, urticaria; S, shock; E, erythema; D, dyspnea; P, pruritus; N, nausea.

followed by the exercise challenge induced various symptoms such as urticaria, erythema, pruritus, dyspnea and/or shock in all five of these patients. The aspirin challenge test combined with wheat ingestion was performed in three of the six patients (patients 4–6). As a result, these three patients exhibited symptoms of allergic reactions; however, no reactions were observed when aspirin was solely challenged.

The serum concentrations of immunoreactive gliadin were determined by ELISA. In the patients with WDEIA, serum gliadin remained at low levels (below 30 pg/mL) after either exercise or wheat challenge alone (Table 3). However, when symptoms were provoked by a combination of wheat ingestion and exercise, significant amounts of gliadin were detected immediately after the exercise challenge in patients 1–4 in the range of 53–216 pg/mL. Under the aspirin-challenge condition, serum gliadin levels also increased markedly with the apparent symptoms in patients 4–6 (122, 34 and 132 pg/mL).

Western blot analysis of purified gliadin proteins,  $\alpha$ -,  $\beta$ -,  $\gamma$ -,  $\omega$ -1, 2 and  $\omega$ -5 gliadin, with the antibodies used for measurement of serum gliadin levels revealed that these antibodies bound to all of those gliadins (data not shown).

#### *Effect of exercise and aspirin on serum gliadin levels in healthy subjects*

To probe whether the increase of the serum gliadin level induced by exercise or aspirin is particular to patients with WDEIA, we carried out the same tests on four healthy subjects. For the healthy subjects, in order to maximize the effects of aspirin on increase of serum gliadin levels, 1000 mg of aspirin, double dose for patients with WDEIA, was administered 30 min before wheat ingestion. In contrast to patients with WDEIA, the four healthy subjects did not exhibit any allergic symptoms under any challenge condition including the wheat alone, the wheat-exercise and the wheat-aspirin challenges (Table 2). However, the levels of gliadins in sera increased soon after exercise following the ingestion of wheat in subjects 1, 3 and 4, (Table 4). The highest concentration of gliadins in the serum of healthy subjects was found immediately after exercise, as found in the results

of the patients with WDEIA. The gliadin levels were somewhat lower in the healthy subjects (88, not detected, 15 and 49 pg/mL) than those in the patients with WDEIA (216, 150, 96, 53 and 17 pg/mL). Intake of aspirin before wheat ingestion also enhanced serum levels of gliadin in the three healthy subjects (subjects 1, 3 and 4). The serum concentrations of gliadin were higher in the case of aspirin induction than those after exercise induction. In subject 2, no serum gliadins were detected throughout the tests.

#### **Discussion**

In the present study, we clearly demonstrated that serum gliadin levels increase well in accordance with the allergic symptoms in patients with WDEIA. This result strongly indicates that the blood gliadin level is a key factor in the induction of symptoms in WDEIA. Previous studies of the role of exercise and aspirin in provoking symptoms of FDEIA have hypothesized that exercise and aspirin facilitate allergen absorption from the gastrointestinal tract or cause a decrease of the threshold for degranulation of mast cells [13, 19, 20]. Hanakawa et al. [21] reported that the development of allergic reactions depends on the amount of allergen ingested in FDEIA. Our findings, together with this result, strongly support the hypothesis that exercise and aspirin facilitate allergen absorption. Palosuo et al. [22] speculated that tissue transglutaminase, which could be activated by exercise and aspirin, forms a complex with  $\omega$ -5 gliadin, a major allergen in WDEIA, and the resulting large complexes possibly elicit allergic reactions in patients with WDEIA. However, they did not carry out the *in vivo* experiment of confirming the existence of gliadin–transglutaminase complexes in the circulating blood. In the present study, we detected the immunoreactive gliadin in the sera of patients WDEIA, but could not demonstrate the molecular size or the form of the gliadin. Further *in vivo* studies are needed to elucidate whether tissue transglutaminase is involved in eliciting symptoms of WDEIA.

Several studies of food allergy have clearly shown that the existence of a threshold of IgE levels and the quantification of

**Table 3.** Serum gliadin levels in provocation tests for patients with WDEIA

| Patients              | Gliadin (pg/mL) |          |          |     |     |          |
|-----------------------|-----------------|----------|----------|-----|-----|----------|
|                       | 1               | 2        | 3        | 4   | 5   | 6        |
| <b>Wheat</b>          |                 |          |          |     |     |          |
| Pre-challenge         | nd              | nd       | nd       | nm  | nd  | nd       |
| Post-challenge (min)  |                 |          |          |     |     |          |
| 0                     | –               | –        | –        | –   | –   | –        |
| 15                    | nd*             | –        | –        | nm  | nd  | nd       |
| 30                    | –               | nd       | nd       | nm  | nd  | 30       |
| 60                    | –               | 29       | nd       | nm  | nd  | nd       |
| 90                    | –               | 20       | 16       | –   | –   | nd       |
| 120                   | –               | –        | –        | –   | –   | nd       |
| <b>Exercise</b>       |                 |          |          |     |     |          |
| Pre-challenge         | nd              | 23       | nd       | nd  | nd  |          |
| Post-challenge (min)  |                 |          |          |     |     |          |
| 0                     | –               | 16       | nd       | –   | nd  |          |
| 15                    | nd*             | nd       | –        | nd* | nd  | Not done |
| 30                    | –               | nd       | nd       | nd  | nd  |          |
| 60                    | –               | nd       | nd       | nd  | nd  |          |
| 90                    | –               | nd       | nd       | –   | –   |          |
| <b>Wheat+exercise</b> |                 |          |          |     |     |          |
| Pre-challenge         | nd              | nd       | nd       | nd  | 16  |          |
| Pre-exercise          | nd              | nd       | nd       | 18  | nd  |          |
| Post-exercise (min)   |                 |          |          |     |     |          |
| 0                     | –               | 150      | 96       | –   | 15  |          |
| 15                    | –               | 108      | 76†      | 53* | 16  | Not done |
| 30                    | 216             | 72       | 63       | 41  | 16  |          |
| 60                    | –               | 36       | 26       | 47  | 17  |          |
| 90                    | –               | –        | 36       | –   | –   |          |
| <b>Aspirin+wheat</b>  |                 |          |          |     |     |          |
| Pre-challenge         |                 |          |          | nd  | nd  | nd       |
| Post-challenge (min)  |                 |          |          |     |     |          |
| 0                     |                 |          |          | –   | –   | –        |
| 15                    |                 |          |          | –   | nd  | 65       |
| 30                    | Not done        | Not done | Not done | 38  | nd  | 132      |
| 60                    |                 |          |          | 122 | 33  | 76       |
| 90                    |                 |          |          | –   | 34‡ | 23       |
| 120                   |                 |          |          | 42  | –   | 61       |

\*The venous blood was taken 10 min after wheat or exercise challenge.

†The venous blood was taken 20 min after exercise challenge.

‡The venous blood was taken 80 min after wheat challenge.

WDEIA, wheat-dependent exercise-induced anaphylaxis; nd, not detected; nm, not measured; –, a blood sample was not taken.

food allergen-specific IgE is a useful tool in predicting symptomatic allergy to egg, milk, peanut and fish [23]. However, gluten-specific IgE values were negative ( $<0.35$ ) or very low (1.55, 0.51 and 0.66) in the patients with WDEIA enrolled in our study, even though they had a positive provocation test. Asero et al. [24] have hypothesized that FDEIA represents the clinical symptoms of food allergy in patients whose immunologic reactivity is low and who need an additional trigger for their symptoms to appear. Our findings support their hypothesis and indicate that because of low gluten-specific IgE levels in WDEIA, a higher amount of gliadin must be absorbed in a short time to cause the degranulation of mast cells and basophils, and that such amounts are reached by increasing intestinal permeability induced by exercise or aspirin intake.

Orally ingested proteins are transported across an epithelial layer of the small intestine by two routes: through the extracellular spaces between the epithelial cells (paracellular

pathway) and across both the luminal and basolateral membranes (transcellular pathway). Transport of the protein through the paracellular pathway is regulated by the tight junction and is restricted based on its size and charge [25, 26]. Normally, a small amount of allergens is transported across the intestinal epithelium by the transcellular route, which is thought to be important in the generation of oral tolerance [27, 28]. It has been shown that prolonged or intense exercise increases small intestinal permeability of macromolecules through a paracellular route in humans by the lactulose and rhamnose differential urinary test [12]. This phenomenon may be related to the small intestinal tract ischaemia because exercise causes epithelial barrier dysfunction following the reduction of splanchnic blood flow levels [29]. In addition, acute exercise induces gastrointestinal leakage of allergen in lysozyme-sensitized mice [10]. These previous data also support our findings that exercise after wheat ingestion increases blood gliadin levels.

**Table 4.** Effect of exercise and aspirin on serum gliadin levels in healthy subjects

| Healthy subjects      | Gliadin (pg/mL) |    |    |     |
|-----------------------|-----------------|----|----|-----|
|                       | 1               | 2  | 3  | 4   |
| <b>Wheat</b>          |                 |    |    |     |
| Pre-challenge         | nd              | nd | nd | nd  |
| Post-challenge (min)  |                 |    |    |     |
| 0                     | nd              | nd | nd | nd  |
| 25                    | nd              | nd | nd | nd  |
| 60                    | nd              | nd | nd | nd  |
| 120                   | 23              | nd | nd | nd  |
| <b>Wheat+exercise</b> |                 |    |    |     |
| Pre-challenge         | nd              | nd | nd | nd  |
| Pre-exercise          | nd              | nd | nd | nd  |
| Post-exercise (min)   |                 |    |    |     |
| 0                     | 88              | nd | 15 | 49  |
| 35                    | 52              | nd | nd | 17  |
| 95                    | 18              | nd | nd | nd  |
| <b>Aspirin+wheat</b>  |                 |    |    |     |
| Pre-challenge         | nd              | nd | nd | nd  |
| Post-challenge (min)  |                 |    |    |     |
| 0                     | nd              | nd | 49 | nd  |
| 25                    | 93              | nd | 80 | 50  |
| 60                    | 70              | nd | 56 | 175 |
| 120                   | 42              | nd | 42 | 120 |

nd, not detected.

Several studies reported that pre-treatment with aspirin induces allergic symptoms in FDEIA [13, 14]. In these studies, the mechanism was speculated to be that aspirin stimulates histamine release from mast cells and basophils by the suppression of prostaglandin E<sub>2</sub> production, which regulates the release of histamine [30, 31]. The most striking finding in this study is that aspirin facilitates the absorption of wheat allergens when administered with wheat. Aspirin, which is an anti-inflammatory, analgesic, antipyretic agent inhibiting cyclooxygenase, causes damage to the intracellular tight junction in the gastrointestinal mucosa as an adverse effect that could lead to change of gastrointestinal permeability [32]. In addition, Lambert et al. [15] reported that intake of aspirin combined with exercise promotes increased gastrointestinal permeability in the human gastrointestinal tract. Thus, the facilitation of gliadin absorption from the gastrointestinal tract damaged by aspirin may be the cause of the increase of serum gliadin levels in WDEIA.

Specific sensitization is known to affect the increase in gastrointestinal allergen uptake across the intestinal epithelial cells in several animal models of allergy [10, 11, 33, 34]. It is thought that there are two phases in the enhancing of allergen transport: an early phase before the hypersensitive reaction and a later phase subsequent to activation of mast cell [35, 36]. In the early phase, antigen is transported via the transcellular pathway through epithelial cells, and in the later phase the paracellular pathway is used after activation of mast cells. This is followed by the absorption of non-specific molecules such as allergens occurring with the disruption of the epithelial barrier function. This gastrointestinal allergen transport via the paracellular route is enhanced by specific sensitization in a mast cell-dependent manner [34, 35]. These findings are also supported by the data reported by Sakamoto

et al. [11] that the absorption of  $\beta$ -lactoglobulin from the intestine was accelerated after oral ovalbumin challenge in ovalbumin-sensitized rats. We found that the serum gliadin levels were somewhat higher in the patients with WDEIA than in the healthy subjects in the wheat-exercise and wheat challenge tests. In addition aspirin-induced increases of serum gliadin levels in patients and healthy controls were quite similar, despite the fact that a double dose of aspirin was given to the healthy controls. These findings together with results of previous animal studies strongly suggest that allergic sensitization may also play a role in the absorption of food allergens.

This study showed that exercise- and aspirin-induced increases of the serum gliadin level occur not only in patients with WDEIA but also in healthy subjects, even though the allergic symptoms are observed only in the patients. This result strongly suggests that facilitation of gliadin absorption into blood circulation by intensive exercise and intake of aspirin is not specific for patients with WDEIA. Food allergy including FDEIA is now increasing in prevalence in most developed countries [37]. Wide use of medicines such as aspirin, which has an intestinal disorder as a side effect, might be related to the increase. However, further research is required to prove a causal relationship between aspirin use and the development of food allergies.

In conclusion, this is the first study in humans to demonstrate the increases of serum gliadin levels by an exercise and aspirin challenge combined with wheat ingestion in both patients with WDEIA and healthy subjects. These research results provide circumstantial support for the hypothesis that exercise and aspirin facilitate the absorption of causative allergens from the intestine into blood circulation and elicit the allergic symptoms in FDEIA.

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