

Exercise-Independent Wheat-Induced Anaphylaxis Caused by ω -5 Gliadin in Mice

Mamoru Tanaka^a Takao Nagano^a Hiromi Yano^b Ken Haruma^c Yasuko Kato^a

Departments of ^aClinical Nutrition and ^bHealth and Sports Science, Kawasaki University of Medical Welfare, and ^cDivision of Gastroenterology, Department of Internal Medicine, Kawasaki Medical School, Kurashiki, Japan

Key Words

Wheat-dependent exercise-induced anaphylaxis · B10.A mouse · ω -5 Gliadin · Food allergy

Abstract

Background: ω -5 Gliadin is known as a major allergen in wheat-dependent exercise-induced anaphylaxis. The characteristics that make ω -5 gliadin an allergen remain unclear. **Methods:** Mice were sensitized by means of intraperitoneal injection of gliadin fractions together with the adjuvant alum. Anaphylactic responses were assessed by measuring body temperature and voluntary physical activity. Specific IgE levels in mouse serum were evaluated by ELISA. After oral administration of proteins to mice, concentrations of administered proteins in their portal blood were also analyzed by competitive inhibition ELISA. **Results:** Oral administration of ω -5 gliadin evoked significant decreases in body temperature and physical activity of sensitized mice, whereas the gliadin fraction did not induce these effects at the same dose. These responses were exercise independent. ELISA analysis revealed that IgE antibodies from sensitized mice react to ω -5 gliadin with great efficacy. After oral administration of either the gliadin fraction or ω -5 gliadin, blood levels of ω -5 gliadin were much higher than those of the gliadin fraction.

Conclusions: ω -5 Gliadin caused anaphylaxis in sensitized mice, whereas the gliadin fraction did not at the same dose. The anaphylactic response was exercise independent. It is likely that IgE of sensitized mice reacts strongly to ω -5 gliadin and that ω -5 gliadin is better absorbed from the gastrointestinal tract than other components of the gliadin fraction. These results indicated that ω -5 gliadin has prominent characteristics as an allergen and that exercise might be an indirect factor in anaphylaxis induction.

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Introduction

FDEIA (food-dependent exercise-induced anaphylaxis) is a severe form of allergy which is caused by physical exercise after ingestion of a specific food [1]. Although several foods, including shellfish, fruits and nuts, are reportedly associated with FDEIA, the most frequently reported cause of FDEIA is wheat [2–9]. Our group has previously reported a murine model of FDEIA used to assess whether exercise is an essential driving force in FDEIA induction. Studies using this model showed that physical exercise and sensitization affected the absorption of allergens from the gastrointestinal tract because increased

damage to the villi in the small intestine occurred following exercise in sensitized mice [10]. These results raise the possibility that exercise is an indirect factor acting to stimulate anaphylactic responses.

Wheat endosperm proteins consist of water-soluble albumins, salt-soluble globulins and water-insoluble gliadins and glutenins. Gliadins and glutenins are the two main protein groups of gluten and account for up to 80% of total wheat protein [11]. Gliadins are heterogeneous and subdivided into α , β , γ and ω fractions according to their mobility on polyacrylamide gel electrophoresis at acidic pH [12–14]. The ω -gliadins are characterized by a high content of glutamine, proline and phenylalanine, which account for approximately 80% of the proteins, and by being sulfur-poor proteins with no cysteine residues [15]. Based on the analysis of N-terminal or complete amino acid sequences, amino acid compositions and molecular weight, they can be grouped into 2 types: ω -5 and ω -1,2 gliadins [16]. Although the distribution of total gliadins among the different types depends strongly on the wheat variety and growing conditions, it can be generalized that the α/β - and γ -gliadins are major components, whereas the ω -gliadins occur in much lower proportions. The amount of ω -5 gliadin in total gluten proteins has been estimated to be 3–6% [16]. Denery-Papini et al. [17] determined the amount of ω -5 gliadin for 11 wheat cultivars and reported this protein to account for between 1.2 and 7.8% of total gliadin.

FDEIA caused by wheat is referred to as wheat-dependent exercise-induced anaphylaxis (WDEIA). Among the wheat proteins, gliadins were found to be the major allergen in patients exhibiting WDEIA using skin tests and immunoblotting studies [18–20]. In addition, ω -5 gliadin was detected as a major allergen among the entire gliadin fractions [21–24]. Palosuo et al. [21] first demonstrated that a purified protein with the N-terminal sequence SRLL reacted strongly with IgE from WDEIA patients. Matsuo et al. [25] reported that IgE antibodies from 80% of patients with WDEIA reacted strongly to ω -5 gliadin, and IgE antibodies from the remaining patients reacted to high-molecular-mass glutenin subunits. Although we already reported that whole-gliadin-sensitized mice had a greater elevation in serum IgE, a remarkably shorter running time and a higher level of intestinal erosion when compared with control animals [26], it remains unclear whether ω -5 gliadin alone causes anaphylaxis symptoms, such as a decrease in body temperature and physical activity, after oral administration of ω -5 gliadin in sensitized animals or humans. In addition, very recently we observed that ω -5 gliadin also caused WDEIA in

a similar manner to the whole gliadin fraction [27]. However, the mechanism by which ω -5 gliadin causes WDEIA is currently poorly understood.

The objectives of the present study were to elucidate the characteristics of ω -5 gliadin as an allergen and examine the impact of exercise on anaphylaxis responses. The effects of ω -5 gliadin on anaphylaxis were investigated in comparison with those of the whole gliadin fraction. Our findings indicated that ω -5 gliadin induced anaphylaxis in sensitized mice, but the gliadin fraction did not at the same dose. Interestingly, this anaphylaxis response was exercise independent. The reactivity of IgE from sensitized mice and the intestinal absorption of ω -5 gliadin and the gliadin fraction were studied to reveal the characteristics of ω -5 gliadin as an allergen.

Materials and Methods

Materials

Soft wheat flour was kindly provided by Nisshin Flour Milling Inc. (Tokyo, Japan). Female B10.A mice (5 weeks old, weighing 15–20 g) and female ICR mice (6 weeks old, weighing 25–27 g) were purchased from Japan SLC (Shizuoka, Japan). Aluminum hydroxide (Imject Alum) was obtained from Pierce Biotechnology (Rockford, Ill., USA), horseradish peroxidase (HRP)-conjugated goat IgG fraction to mouse IgE and Fc specific from Nordic Immunological Laboratories (Tilburg, The Netherlands) and HRP-conjugated goat anti-rabbit IgG from Millipore (Bedford, Mass., USA). Tween 20 was purchased from National Diagnostics (Atlanta, Ga., USA) and bovine serum albumin (BSA) from Wako Pure Chemical Industries (Osaka, Japan). All other chemicals used in this study were of reagent grade and were used without further purification.

Gliadin Fraction and Purified ω -5 Gliadin

The fractionation of gliadin and purification of ω -5 gliadin were performed as described previously [27]. All fractionation procedures were performed at room temperature. Wheat flour (50 g) was stirred for 2 h in 0.5 M NaCl (500 ml) and centrifuged at 1,700 g for 15 min. The supernatant was discarded and the resulting pellet was resuspended in 500 ml of distilled water before being stirred for another 2 h and again centrifuged (1,700 g for 15 min). This procedure with distilled water was repeated once more (twice in total). The pellet was collected, resuspended in ethanol (500 ml, 70% v/v) and stirred for 2 h before being centrifuged at 1,700 g for 15 min. The supernatant (the gliadin fraction) was dialyzed against 1% acetic acid for 60 h and then freeze dried. The freeze-dried gliadin fraction was dissolved in 10 mM glycine-acetate buffer (pH 4.6) containing 3 M urea. The gliadin fraction solution was separated by strong cation exchange chromatography using a Macro-Prep High S column (5 × 20 cm; Bio-Rad, Hercules, Calif., USA) and eluted with a linear gradient of 0–0.25 M NaCl. The peak containing ω -5 gliadin was concentrated to 10 ml using centrifugal concentrators (Vivaspin 20; Sartorius Stedim Japan, Tokyo, Japan) and separated by size exclusion chro-

matography using a Sephacryl S-300 HR column (2.5 × 75 cm; GE Healthcare Japan, Tokyo, Japan) in 6 M urea and 1% acetic acid. The eluted peak of ω -5 gliadin was dialyzed against 1% (v/v) acetic acid for 60 h and freeze dried. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was carried out on 10% polyacrylamide gels according to the method of Laemmli [28]. The amount of ω -5 gliadin in the prepared gliadin fraction was measured by densitometry.

Animals

B10.A mice (n = 34) and ICR mice (n = 122) were housed in a room with a 12-hour light/dark cycle maintained at 20 ± 2°C. Mice had free access to standard chow and tap water. The experimental design was in accordance with the guidelines for animal experimentation and was approved by the Animal Experimental Committee of Kawasaki, University of Medical Welfare (authorization number: 09-004).

Sensitization

B10.A mice were randomly divided into sensitized (n = 26) and unsensitized (n = 8) groups. Sensitized mice were initially injected intraperitoneally with 100 μ g of the gliadin fraction and 1 mg of aluminum hydroxide in 0.2 ml of phosphate-buffered saline (PBS; pH 7.0). The gliadin fraction was then reduced to 50 μ g for the remainder of the injections. Each animal was administered one injection every week for 6 weeks. Unsensitized animals received vehicle alone. To assess sensitization, blood was obtained from the orbital veins of mice under light anesthesia 37 days after the initial injection and subjected to enzyme-linked immunosorbent assay (ELISA) specific for the IgE to gliadin.

Animal Protocol

Before any experimental treatment, all mice were exercised during a 3-day acclimation period. The sensitized mice were orally administered 0.1 mg of ω -5 gliadin (n = 8), low-dose gliadin (0.1 mg of the gliadin fraction; n = 9) or high-dose gliadin (0.8 mg of the gliadin fraction; n = 9) in 0.5 ml of 1 mM acetic acid solution. Unsensitized mice (n = 8) were orally administered 0.5 ml of 1 mM acetic acid alone, serving as a control. Following this administration, nonexercised mice were put in a cage for 30 min and exercised mice were forced to run on a treadmill set to 15 m/min and 20% gradient for 30 min. To evaluate body temperature, rectal temperature was measured 30 min after oral administration of ω -5 gliadin with a digital thermocouple thermometer (SK-1250MC; Sato Keiryoki Mfg., Tokyo, Japan). To determine voluntary physical activity, the mice were housed in cages with a running wheel, and the number of revolutions per hour was monitored for 15 h from 20.00 to 11.00 h using a counting and accumulating printer (Natsume Seisakusho, Tokyo, Japan).

ELISA for Specific IgE Levels

Specific IgE levels in mouse serum were evaluated by ELISA as described previously [26]. Flat-bottomed microtiter plates were precoated with 100 μ l of either the gliadin fraction or ω -5 gliadin (10 μ g/ml) in 0.1 M carbonate buffer (pH 9.6) containing 3 M urea and incubated overnight at 4°C. After the wells were washed with PBS containing 0.05% Tween 20 (PBST), a 1% BSA/PBST solution was added to each well and incubated for 1 h at 37°C. Then, 100 μ l of each serum sample diluted 50-fold with 1% BSA/PBST was applied to each well and incubated for 1 h at 37°C. After each well

was washed 5 times with PBST, 100 μ l of HRP-conjugated goat anti-mouse IgE (diluted 1:1,000 in 1% BSA/PBST) was added to each well and incubated for 1 h at 37°C. Each well was washed with PBST 5 times, and 100 μ l of *o*-phenylenediamine (0.4 mg/ml) in citrate-phosphate buffer (pH 5.0) containing 0.006% H₂O₂ was added. The reaction was quenched with 50 μ l of 2.5 M H₂SO₄ (aqueous solution) after 5 min at room temperature, and color development was measured by colorimetric photometry at 492 nm.

Quantification of Gliadin and ω -5 Gliadin in Mouse

Portal Blood

After no feeding for 24 h, ICR mice were orally administered 0.1, 1 or 10 mg of either the gliadin fraction or ω -5 gliadin in 0.5 ml of 1 mM acetic acid. Then, the nonexercised mice were put in a cage and exercised mice were forced to run on a treadmill set to 15 m/min and 20% gradient for 30 min. Portal blood samples were collected 1 h after oral administration of the protein samples. The portal blood samples obtained were freeze dried and then stored at -20°C until use. Concentrations of gliadin and ω -5 gliadin in mouse blood were determined by competitive inhibition ELISA. Flat-bottomed microtiter plates were precoated with 100 μ l of either gliadin or ω -5 gliadin solution (100 μ g/ml) in 0.1 M carbonate buffer (pH 9.6) containing 3 M urea and incubated for 1 h at 37°C. After the wells were washed with PBST, a 1% BSA/PBST solution was added to each well, and the wells were incubated for 1 h at 37°C and then washed 3 times with PBST. The freeze-dried blood samples were dissolved in 100 μ l of 1% BSA/PBST containing 3 M urea and mixed with the same amount of rabbit anti-gliadin IgG solutions in 1% BSA/PBST containing 3 M urea. The mixture solutions were incubated for 1 h at 37°C for an antigen-antibody reaction and added to either gliadin- or ω -5-gliadin-solution-coated plates. Each well was treated with HRP-conjugated goat anti-rabbit IgG, as described above.

Statistical Analysis

Data are expressed as means ± SEM. All statistical analysis was performed using SPSS 14.0J for Windows (SPSS Japan, Tokyo, Japan). Between-group analysis was performed using a Student *t* test. Within-group analysis was conducted by one-way analysis of variance (ANOVA), with data concerning hourly changes in the number of revolutions run by mice analyzed by a two-way ANOVA with repeated measures. Tukey's tests were also performed post hoc. Data were considered to be significantly different when *p* values were less than 0.05.

Results

Specific IgE in Sera of Sensitized Mice

Specific IgE to the gliadin fraction in mouse sera was determined by ELISA (fig. 1a). The amount of IgE specific for gliadin produced by mice injected with gliadin was significantly greater than that produced by unsensitized mice, indicating that sensitization to the gliadin fraction had occurred following administration. The reactivity of IgE to ω -5 gliadin in sera from sensitized mice was also measured (fig. 1b). IgE of sensitized mice reacted

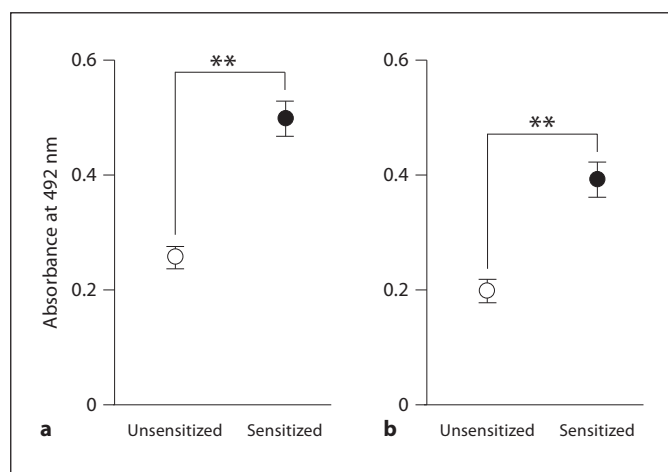


Fig. 1. Levels of specific IgE to the gliadin fraction (a) and purified ω-5 gliadin (b) in sera from unsensitized mice (n = 8) or mice sensitized with the gliadin fraction (n = 26). B10.A mice were sensitized by intraperitoneal injection of the gliadin fraction with an alum adjuvant. Each animal was given one injection every week for 6 weeks. The unsensitized animals received the vehicle alone. Blood was obtained from the orbital veins of mice 37 days after the initial injection and subjected to ELISA specific for IgE to the gliadin fraction and ω-5 gliadin. Data are expressed as means ± SEM. ** p < 0.01.

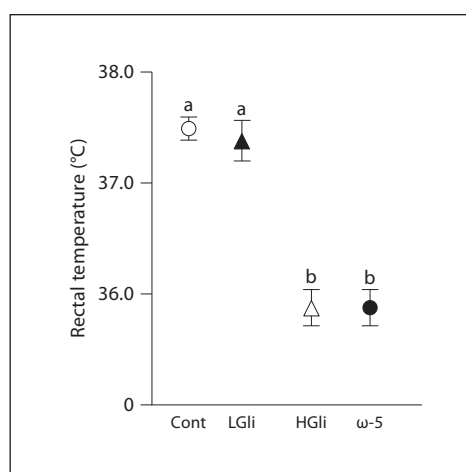


Fig. 2. Effect of the gliadin fraction and purified ω-5 gliadin on the rectal temperature of sensitized mice. Sensitized mice were orally administered low-dose gliadin (LGli; n = 9), high-dose gliadin (HGli; n = 9) or ω-5 gliadin (ω-5; n = 8) in 1 mM acetic acid. The unsensitized mice (n = 8) were orally administered 1 mM acetic acid alone, serving as the control. The rectal temperature was measured 30 min after oral administration. Data are expressed as means ± SEM. Values marked with different letters are significantly different from each other: p < 0.01.

to ω-5 gliadin with greater efficacy compared with IgE specific to the gliadin fraction, indicating that the sensitized mice reacted strongly to ω-5 gliadin.

Assessment of Exercise-Independent Wheat-Induced Anaphylaxis Using a Mouse Model

During systemic anaphylaxis, decreases in both whole body temperature and rates of voluntary physical activity are commonly observed [29–31]. An oral dose of 0.1 mg of ω-5 gliadin was administered in this experiment. It was estimated that 0.8 mg of the gliadin fraction contained 0.1 mg of ω-5 gliadin, as the amount of ω-5 gliadin in the gliadin fraction was 14%. Thus, 0.1- and 0.8-mg doses of the gliadin fraction were chosen and referred to as low dose and high dose, respectively.

Figure 2 displays the rectal temperature of sensitized mice after challenge with either the gliadin fraction or ω-5 gliadin. The ω-5 gliadin and high-dose gliadin challenges evoked significant decreases in body temperature but low-dose gliadin challenge did not. Figure 3 shows the number of revolutions achieved hourly or in 15 h by each treatment group following challenge with either the gliadin fraction or ω-5 gliadin. The sensitized mice administered with ω-5 gliadin and high-dose gliadin ran sig-

nificantly less during the first 7 h than the sensitized mice administered low-dose gliadin and the control group (fig. 3a), suggesting that the allergen reduced the physical activity of the sensitized mice for an acute time period. The sensitized animals subjected to ω-5 gliadin and high-dose gliadin displayed a significantly reduced number of revolutions compared with the control group, but the sensitized mice administered low-dose gliadin did not (fig. 3b). These results indicated that ω-5 gliadin and high-dose gliadin challenges caused decreases in voluntary physical activity while low-dose gliadin challenge did not. Taken together, the results of body temperature and voluntary physical activity indicated that the oral challenges with ω-5 gliadin and high-dose gliadin caused anaphylaxis while low-dose gliadin did not. These anaphylactic responses were exercise independent.

Assessment of WDEIA Caused by the Gliadin Fraction Using a Mouse Model

The low-dose gliadin challenge did not cause anaphylaxis. Thus, WDEIA was assessed by measuring body temperature and voluntary physical activity in sensitized mice after a low-dose gliadin challenge and enforced exercise for 30 min immediately after gliadin administra-

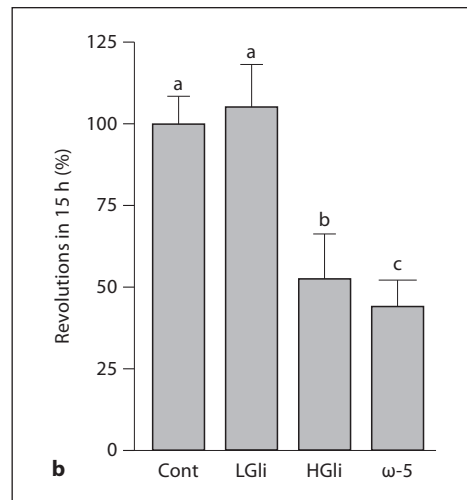
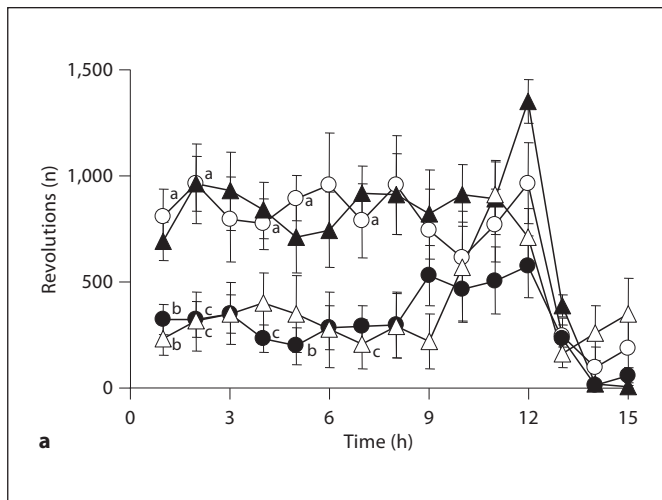


Fig. 3. Effect of the gliadin fraction and purified ω -5 gliadin on the number of revolutions achieved hourly (a) or in 15 h (b) by the sensitized mice. The sensitized and unsensitized mice were orally administered as described in figure 2. Each mouse was housed in a cage with a running wheel, and the number of revolutions per hour was monitored for 15 h from 20.00 to 11.00 h. a $\circ$ = Control group (n = 8); $\blacktriangle$ = low-dose-gliadin-challenged group (n = 9);

$\triangle$ = high-dose-gliadin-challenged group (n = 9); $\bullet$ = ω -5-gliadin-challenged group (n = 8). b Cont = Control group; LGli = low-dose-gliadin-challenged group; HGli = high-dose-gliadin-challenged group; ω -5 = ω -5-gliadin-challenged group. Data are expressed as means $\pm$ SEM. Values marked with different letters are significantly different from each other: p < 0.05 for a versus b; p < 0.01 for a versus c.

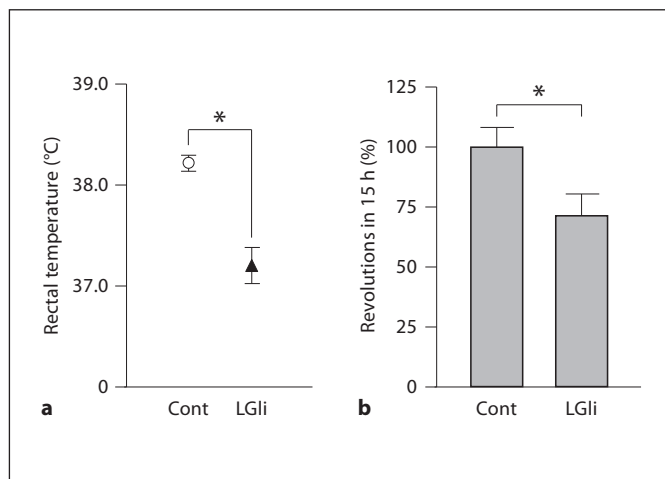


Fig. 4. Effect of the gliadin fraction challenge and exercise on the rectal temperature of sensitized mice (a) and the number of revolutions achieved in 15 h by the sensitized mice (b). The sensitized and unsensitized mice were orally administered as described in figure 2. The mice were then forced to run on a treadmill set to 15 m/min and a 20% gradient for 30 min. The rectal temperature was measured as soon as the mice had exercised, and the number of revolutions was monitored over 15 h as described in figure 3. Cont = Control group (n = 8); LGli = low-dose-gliadin-challenged group (n = 8). Data are expressed as means $\pm$ SEM. * p < 0.05.

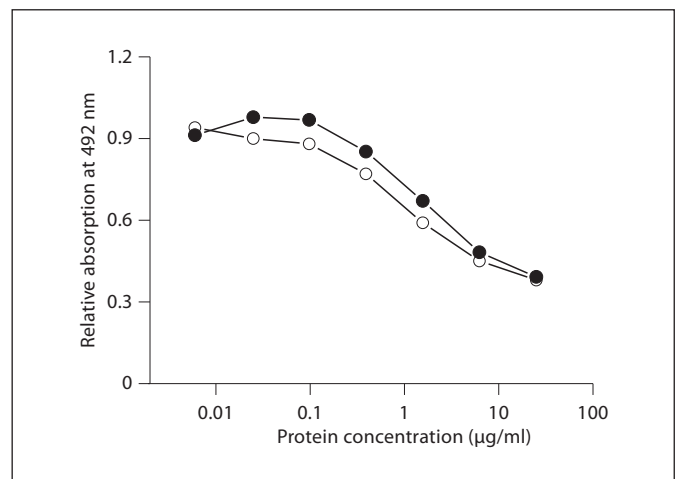


Fig. 5. Standard curves obtained by competitive inhibition ELISA for the gliadin fraction ($\circ$) and ω -5 gliadin ($\bullet$) in mouse blood. Competitive inhibition ELISA was conducted as described in Materials and Methods.

Table 1. Concentrations of gliadin and ω -5 gliadin in mouse portal blood in nonexercised and exercised mice as determined by competitive inhibition ELISA

Dose, mg		Gliadin, $\mu\text{g/ml}$	ω -5 Gliadin, $\mu\text{g/ml}$
0.1	nonexercised mice	ND	ND
0.1	exercised mice	ND	0.22 ± 0.07 (n = 10)
1.0	nonexercised mice	ND	1.42 ± 0.26 (n = 10)
1.0	exercised mice	ND	1.61 ± 0.22 (n = 10)
10.0	nonexercised mice	0.18 ± 0.03 (n = 15) ^a	1.53 ± 0.26 (n = 10) ^c
10.0	exercised mice	0.37 ± 0.04 (n = 26) ^b	1.57 ± 0.22 (n = 16) ^c

Portal blood samples were collected 1 h after ICR mice were orally administered 0.1, 1 or 10 mg of either the gliadin fraction or ω -5 gliadin. Exercised mice were forced to run on a treadmill set to 15 m/min and 20% gradient for 30 min after oral administration of the protein samples. ND = Not detected. Values marked with different superscripts are significantly different from each other: $p < 0.01$.

tion. The low-dose gliadin challenge and exercise evoked a significant drop in body temperature (fig. 4a). Animals subjected to a low-dose gliadin challenge and exercise achieved a significantly lower number of revolutions than the control group (fig. 4b), indicating that low-dose gliadin challenge and exercise caused a decrease in voluntary physical activity. These results of body temperature and voluntary physical activity indicated that low-dose gliadin challenge and exercise caused WDEIA in sensitized mice, although anaphylaxis was not induced by low-dose gliadin challenge alone.

Quantification of the Gliadin Fraction and ω -5 Gliadin in Mouse Portal Blood

To determine intestinal absorption of the gliadin fraction and purified ω -5 gliadin, concentrations of orally administered proteins in mouse portal blood were measured by competitive inhibition ELISA. First, the experiments were conducted under the same conditions for anaphylaxis assessments, with the same amount of ω -5 gliadin and low-dose and high-dose gliadin given orally to sensitized B10.A mice. However, our measuring system could not detect the administered proteins in mice portal blood. Thus, we conducted further studies using unsensitized ICR mice. No differences were observed between sensitized B10.A mice and ICR mice in the experiments we conducted.

Figure 5 displays standard curves obtained by competitive inhibition ELISA for the gliadin fraction and ω -5 gliadin dissolved in mouse blood. The standard curves revealed that concentrations of both the gliadin fraction and ω -5 gliadin in blood can be determined in the range

0.10–6.3 $\mu\text{g/ml}$ by the competitive inhibition ELISA system.

The effects of dose and exercise on concentrations of orally administered proteins in mouse portal blood were also investigated (table 1). The gliadin fraction in mouse blood could be detected neither in nonexercised nor exercised mice when 0.1 or 1 mg of the gliadin fraction was given to mice. When 10 mg of the gliadin fraction was given, the concentrations of the gliadin fraction in mouse blood were 0.18 ± 0.03 $\mu\text{g/ml}$ for nonexercised mice and 0.37 ± 0.04 $\mu\text{g/ml}$ for exercised mice. The concentration of the gliadin fraction in the blood of exercised mice was significantly higher than that in nonexercised mice, indicating an increase in the absorption of the gliadin fraction following exercise. In contrast, ω -5 gliadin in blood could not be detected in nonexercised mice, whereas the concentration of ω -5 gliadin in blood was 0.22 ± 0.07 $\mu\text{g/ml}$ for exercised mice when 0.1 mg of ω -5 gliadin was given, indicating an increase in the absorption of ω -5 gliadin following exercise. The concentrations of ω -5 gliadin in blood were 1.42 ± 0.26 $\mu\text{g/ml}$ for nonexercised mice and 1.61 ± 0.22 $\mu\text{g/ml}$ for exercised mice when 1 mg of ω -5 gliadin was given. The concentrations of ω -5 gliadin in blood were 1.53 ± 0.26 $\mu\text{g/ml}$ for nonexercised mice and 1.57 ± 0.22 $\mu\text{g/ml}$ for exercised mice when 10 mg of ω -5 gliadin was given. There were no differences in concentrations of ω -5 gliadin in blood between dose or exercise regimens when either 1 or 10 mg of ω -5 gliadin was given. The concentrations of ω -5 gliadin in mouse blood were 8.5 and 4.2 times higher than those of the gliadin fraction for nonexercised and exercised mice, respectively, when 10 mg of protein was given orally. These

results indicate that the intestinal absorption of the gliadin fraction and ω -5 gliadin was increased by exercise at certain doses and that ω -5 gliadin was absorbed from the gastrointestinal tract more effectively than other gliadin components.

Discussion

A minor component of wheat gliadin, ω -5 gliadin, is known as a major allergen in WDEIA [21–24]. The effects of ω -5 gliadin on WDEIA were investigated in this study using a mouse model. Anaphylactic responses were assessed by measuring body temperature and voluntary physical activity. Anaphylaxis was induced by challenge with 0.1 mg of ω -5 gliadin in sensitized mice but not by low-dose gliadin (0.1 mg of the gliadin fraction) challenge. The low-dose gliadin challenge and exercise caused WDEIA in sensitized mice, whereas the high-dose gliadin (which contained an estimated 0.1 mg of ω -5 gliadin) challenge caused anaphylaxis in sensitized mice, but this anaphylaxis response was exercise independent. These results suggest that ω -5 gliadin functions as an allergen and that exercise is an indirect factor in inducing anaphylaxis.

ω -5 Gliadin has previously been proposed to be a major allergen in WDEIA following the observation that IgE of patients with WDEIA reacts strongly to ω -5 gliadin [21–25]. Elevated titers of antigen-specific IgE are found in allergic patients, and antigen-specific IgE is essential for type I allergic reactions. The disease is preceded by a sensitization phase without symptoms, in which allergen-specific T and B cells are primed and IgE is produced. Recurrent allergen challenge of sensitized individuals results in IgE cross-linking on tissue mast cells that subsequently release their proinflammatory mediators [32]. In this study, the reactivity of IgE in sera of sensitized mice to ω -5 gliadin was measured using ELISA. As compared with specific IgE to the gliadin fraction, the IgE of sensitized mice reacted strongly to ω -5 gliadin. In a previous report [25], IgE-binding epitopes in ω -5 gliadin from patients with WDEIA were analyzed, and 4 predominant epitopes, QQIPQQQ, QQFPQQQ, QQSPEQQ and QQSPQQQ, were found within the primary sequence of ω -5 gliadin. These IgE-binding epitopes were found to appear repetitively in the primary sequence of ω -5 gliadin. These results highlight the relevance of ω -5 gliadin as a food allergen, as it possesses the capacity to provoke IgE antibody production.

The amount of allergen that can be absorbed from the gastrointestinal tract is a key predictor of anaphylaxis

causation [10, 26]. We hypothesized that ω -5 gliadin might induce anaphylaxis at a lower dose than the gliadin fraction because ω -5 gliadin is better absorbed from the gastrointestinal tract than other gliadin components. In this study, dose effects on concentrations of orally administered proteins in mouse portal blood were investigated in nonexercised mice to determine the intestinal absorption of the gliadin fraction and ω -5 gliadin. The gliadin fraction was not detected in mouse blood when 0.1 or 1 mg of the gliadin fraction was given orally to mice. In contrast, ω -5 gliadin was determined in mouse blood when 1 mg of ω -5 gliadin was administered orally. The concentration of ω -5 gliadin in mouse blood was 8.5 times higher than that of the gliadin fraction when 10 mg of either protein was given orally. These results indicate that ω -5 gliadin is absorbed from the gastrointestinal tract more effectively than any other gliadin component, and they help to explain why ω -5 gliadin induces anaphylaxis in nonexercised mice. The decrease in proline concentration in the proteins renders them more susceptible to enzymatic hydrolysis in the gut [33], and the murine stomach also has higher pH than the human stomach [34]. Thus, it might be feasible that ω -5-gliadin-derived peptides are absorbed from the gut more effectively than other gliadin-derived peptides.

FDEIA is caused by physical exercise after ingestion of a specific food [1]. Our group previously reported the development of a murine model for FDEIA [10]. Investigations using our animal model found that physical exercise in sensitized mice affected the absorption of allergens due to increased damage to the villi within the small intestine. Moreover, we investigated the effect of each specific wheat protein (i.e. the salt-soluble, gliadin and glutenin fractions) on WDEIA and found that gliadin- and glutenin-sensitized mice displayed WDEIA that was accompanied by a higher level of intestinal erosion than that observed in salt-soluble-fraction-sensitized mice or control animals [26]. In this study, we show that low-dose gliadin challenge alone did not cause anaphylaxis in sensitized mice, whereas low-dose gliadin challenge and exercise induced WDEIA. Exercise increased the concentrations of both the gliadin fraction and ω -5 gliadin in mouse blood at certain doses. These results are consistent with the hypothesis that WDEIA is stimulated by exercise because physical exercise affects the absorption of allergens due to increased damage to the villi within the small intestine [10].

Perhaps the most contentious issue in this field is why ω -5 gliadin has been reported as a major allergen in WDEIA despite the fact that ω -5 gliadin has prominent

characteristics as an allergen to induce anaphylaxis. It is possible that this is due to the low levels of ω -5 gliadin in wheat products. As described in the Introduction, although the distribution of total gliadins among the different types depends strongly on wheat variety and growing conditions, it can be generalized that the ω -gliadins are minor components. Food-allergic individuals can react adversely to exposure to small quantities of the offending food, but it is now well documented that there are threshold levels below which adverse reactions will not be experienced in these individuals [35]. It is likely that wheat-allergic individuals consume ω -5 gliadin from wheat products below these threshold doses, explaining why they do not experience adverse reactions in their daily lives. However, WDEIA is caused in certain wheat-allergic individuals as exercise increases the absorption of ω -5 gliadin from wheat products. Further studies concerning threshold doses for ω -5 gliadin are necessary to further elucidate this hypothesis.

In conclusion, our study has shown that ω -5 gliadin induces anaphylaxis in sensitized mice at a lower dose than the whole gliadin fraction and that these anaphylactic responses are exercise independent. Two features of ω -5 gliadin as an allergen were revealed by our study. One is that IgE antibodies of sensitized mice strongly react to ω -5 gliadin and the other is that ω -5 gliadin is absorbed from the gastrointestinal tract more effectively than other components of the gliadin fraction. These two prominent characteristics of ω -5 gliadin as a food allergen provide key insights into a better understanding of the mechanism of WDEIA.

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