



Wheat-dependent exercise-induced anaphylaxis in mice is caused by gliadin and glutenin treatments

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Abstract

Various foods may be associated with food-dependent exercise-induced anaphylaxis (FDEIA). However, although the most frequently reported cause of FDEIA has been wheat, the mechanism of FDEIA for wheat has remained largely uninvestigated. To investigate the effect of wheat-fractionated proteins on FDEIA, female B10.A mice (16–20 g) were divided into four groups; i.e. salt-soluble (S-group), gliadin-rich (GLI-group), and glutenin-rich (GLU-group)-sensitized mice, and unsensitized mice. The three sensitized groups were run on a treadmill after oral intake of each wheat-fractionated protein. The mice showed a significant increase in serum IgE, especially in the GLI- and GLU-group. After oral administration of each wheat-fractionated protein, the running time until exhaustion was remarkably shorter for the GLI- and GLU-group than for the S-group and unsensitized mice. The level of intestinal erosion was higher in all the sensitized mice than that in the unsensitized ones after exhaustive running. Furthermore, moderate exercise for 30 min after oral ingestion of each wheat-fractionated protein also induced intestinal erosion in the GLI- and GLU-group. In addition, we observed leaking of gliadin and glutenin proteins out of the intestine into the liver. These results indicated that the main factor involved in wheat-dependent exercise-induced anaphylaxis might be the gliadin and glutenin in wheat proteins.

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1. Introduction

Food-dependent exercise-induced anaphylaxis (FDEIA) has been shown by many clinical reports to be a distinct form of anaphylaxis occurring in association with exercise after ingestion of a specific food [1–3]. Previous studies have shown that various foods may be associated with FDEIA [4–6]. The most frequently reported cause of these reactions in FDEIA has been wheat [4,5,7,8]. Wheat protein is

composed of four classes of proteins: albumin and globulin, which are water/salt-soluble proteins, and gliadin and glutenin, which are insoluble proteins (gluten). Gluten, the chief component of wheat protein, is known to be a causative agent in several diseases [9,10]. Recently, Palosuo et al. [11] reported that a large skin prick test reaction was induced in wheat-dependent exercise-induced anaphylaxis (WDEIA) patients by gliadin treatment. However, the reason why gliadin is major allergen in WDEIA remains unclear. Previous studies of WDEIA were almost all clinical studies using the serum of patients. Although little has been published on allergen models and animal strains of FDEIA, recently we reported that the lysozyme-sensitized B10.A mouse is

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a useful model of FDEIAN [12]. A high concentration of plasma antigen-specific IgE was observed in these mice after sensitization and gastrointestinal mucosal lesions were noted after exercise. In this study, B10.A mice were sensitized with each wheat-fractionated protein and then were run on a treadmill, to determine the effect of WDEIAN on specific IgE, running performance, gastrointestinal mucosal lesions and allergen leakage.

2. Materials and methods

2.1. Fractionation of proteins from wheat flour

Wheat flour proteins were fractionated into three parts, albumin/globulin (water/salt extract), gliadin (extraction by 70% EtOH) and glutenin (extraction by alkali solution). Twenty grams of wheat flour (Nisshin Flour Milling) was treated with 200 ml of phosphate-buffered saline (PBS) overnight at 4 °C with stirring, was placed inside an ultrasonicator for 15 min in ice water, and centrifuged ($20,000 \times g$, 15 min). The supernatant, containing albumin and globulin proteins, was then extracted three times with PBS, and the supernatants of these extraction steps were discarded. The pellet was placed inside an ultrasonicator for 15 min in ice water and centrifuged ($20,000 \times g$, 15 min) with 70% ethanol. The supernatant, containing gliadin, was removed. To minimize cross-contamination between the gliadin and glutenin fractions, the ethanol extraction step was also repeated three times. The glutenin was extracted from the pellet with alkali solution (2% Na_2CO_3 /0.1N NaOH). After placement inside an ultrasonicator for 15 min in ice water and centrifugation the supernatant was stored. Gliadin- and glutenin-rich fractions were adjusted to pH 7.4, and dialysis was performed for three days in PBS with dialysis film (Sanko Junyaku). The protein concentrations of salt-soluble, gliadin- and glutenin-rich fractions were measured by the Lowry method [13].

Female ddY mice ($n=9$) were injected intraperitoneally under light ether anesthesia with 10 μg of extracted-protein (i.e. salt-soluble, gliadin- and glutenin-rich proteins) with 200 μl Freund's Complete Adjuvant (Rockland), with an interval of 14 days between the three injections. The collected sera were used as anti-wheat proteins.

SDS-PAGE and immunoblotting were used to evaluate each wheat-fractionated protein. The SDS-gels contained a 4.5% polyacrylamide stacking gel and a resolving gel with a linear gradient of 15% polyacrylamide. The stain solution was a 0.25% Brilliant blue R (Sigma), a decolorant solution containing methanol and acetic acid. Following electrophoresis, the gel was transferred to a cellulose nitrate membrane filter. Excessive proteins in the uncombined part of the membranes were saturated with 3% BSA/TBS (bovine serum albumine/tris-buffered saline, pH 7.5) overnight at 4 °C. Then the membranes were soaked in each mouse serum ($10^{-3} \times 1/2$) with 1% BSA/TBS solution for 1 h

at room temperature. After the membranes were washed, they were soaked in POD-linked goat IgG to mouse IgG ($10^{-3} \times 1/2$) with 1% BSA/TBS solution for 1 h at room temperature. After the membranes were washed again, they were stained with 6 μl 30% H_2O_2 /10 ml TBS including 6 mg 4-chloro-1-naphthol (Nakarai Tesuku)/2 ml ice-cold methanol with light shielding.

2.2. Animals and sensitization

Female B10.A mice (SLC) weighing 16–20 g were used in this experiment. The animals were kept on a standard diet and had access to tap water ad lib. The room temperature was maintained at 20 ± 2 °C, and a 12/12 h (8 a.m.–8 p.m.) light/dark cycle was used. The experiment procedures employed followed the guidelines set forth in the Care and Use of Animals in the Field of Physiological Sciences approved by the Council of the Physiological Society of Japan.

The mice were divided into four groups; salt-soluble (S-group), gliadin-rich (GLI-group), and glutenin-rich (GLU-group)-sensitized mice, and unsensitized mice. Each group of sensitized mice was injected intraperitoneally under light ether anesthesia with each wheat-fractionated protein (10 μg); salt-soluble, gliadin-rich, or glutenin-rich fractions in 0.5 ml of an aluminum hydroxide (Pierce Chem)/PBS suspension with an interval of 14 days between the four injections. Blood was obtained once every 2 weeks from the orbital veins of both sensitized and unsensitized mice under light ether anesthesia.

2.3. Enzyme-linked immunosorbent assay

The specific anti-wheat protein IgE in mouse serum was evaluated using the enzyme-linked immunosorbent assay (ELISA; [14]). First, 100 μl of a solution (10 $\mu\text{g}/\text{ml}$) of salt-soluble, gliadin-rich, or glutenin-rich fractions in wheat or whole wheat was added to each well of flat-bottomed microtiter plates, and they were incubated overnight at 4 °C. The wells were then washed with PBS containing 0.05% of Tween20 (PBS-T). Then 1% BSA/PBS-T solution was added to each well and they were incubated for 30 min at 37 °C. Then 100 μl of each mouse serum diluted 50 times with 1% BSA/PBS-T solution was applied to each well and they were incubated for 2 h at 37 °C. After each well was washed with PBS-T three times, 100 μl of peroxidase-conjugated goat anti-mouse IgG (Nordic Immunological Lab) in 1% BSA/PBS-T solution (10^{-3}) was added to each well and incubated for 2 h at 37 °C. Each well was washed with PBS-T three times, and then 100 μl of o-phenylenediamine (Wako) in citrate phosphate buffer (0.4 mg/ml) containing 0.03% H_2O_2 was added. The reaction was stopped with 50 μl of H_2SO_4 solution (2.5 mol/l) after 20 min at room temperature. The color development of the well was measured photometrically at 492 nm.

2.4. Estimation of small intestine damage

Damage to the small intestine was estimated by the method of Yano et al. [12]. Briefly, after all the animals were sacrificed under ether anesthesia, the small intestine was removed, spread open, and washed with PBS. The tissue was carefully cut into six parts and fixed with neutral-buffered 10% formalin. Each of the six parts was examined under a microscope. The presence of mucosal lesions on the surface was taken as a record of erosions. In this study, localization of the mucosal lesions was not limited, and differences in the size of each mucosal lesion were not considered. Accordingly, evaluation of the mucosal lesions in small intestine was shown by point scores. The cumulative number of all lesions in each part of the small intestine served as the measure of erosion damage; a very small lesion was scored as 0 (normal), a lesion of one point was scored as 1 (one), a lesion of two points was scored as 2 (two), and a lesion of more than three points was scored as 3 (three). The number of erosions in the small intestine was calculated by two independent observers, each of whom was blinded to the diagnosis and average value for each section.

2.5. Immunohistochemical estimation of wheat protein in liver

First, to prepare anti-wheat protein serum, Wister rats ($n = 3$) were injected intraperitoneally under light ether anesthesia with 10 μ g of extracted-protein (i.e. salt-soluble, gliadin- and glutenin-rich proteins, respectively) with 200 μ l Freund's Complete Adjuvant (Rockland).

Then, an immunohistochemical estimation of wheat protein in the liver was made using the method of Yano et al. [12] with some modifications. Briefly, the liver was perfused through the portal vein with 10 ml PBS under ether anesthesia, and then it was fixed in O.C.T. compound (Tissue-Tek, Sakura). Next, 6 μ m-thick cryostat sections were placed on acetone (4 °C) for 10 min, and then were washed twice with PBS-T (5 min each). The sections were treated with each sensitized rat serum ($10^{-2} \times 1/2$) overnight at 4 °C. After, washing twice with PBS-T (10 min each), the sections were treated with a fluorescein isothiocyanate-labelled secondary antibody, FITC-conjugated goat anti-rat IgG (10^{-2}) for 1 h at room temperature. After, washing twice with PBS-T (10 min each), the liver sections were washed in alcohol to remove water. The presence of wheat protein-stained liver was observed under a fluorescence microscope. Negative method controls in which the primary and/or secondary antibodies were omitted at the relevant stages were included.

2.6. Experiment 1: effect of each wheat-fractionated protein sensitization on exhaustive running performance after wheat protein ingestion

After no feeding for 24 h, the sensitized mice were challenged orally with 20 mg/0.5 ml PBS with a concentration of each wheat-fractionated protein. The unsensitized group

received PBS alone or wheat protein. Then they were run on a treadmill from an initial speed of 20 m/min, on a 5% gradient for 30 min. The speed was increased every 30 min by 3 m/min until exhaustion. Exhaustion was defined as the point at which a mouse refused to run despite being given mild physical prodding. Then the mice were sacrificed, and the small intestine was removed to examine damage to it.

2.7. Experiment 2: immunohistochemical estimation of wheat protein in liver and damage to the small intestine in wheat protein-ingested mice after moderate exercise

The sensitized mice were challenged orally with a 20 mg/0.5 ml PBS concentration of each wheat-fractionated protein. The unsensitized group received PBS alone. Then groups of exercising mice in each of sensitized and unsensitized groups were run at 15 m/min for 30 min on a 5% gradient on a treadmill. Resting mice in each group remained in the cage for 30 min after oral ingestion. After exercise or rest, each mouse was sacrificed, and the liver and small intestine were removed to estimate wheat protein in the liver and to examine damage to the small intestine.

2.8. Statistical analysis

Data were expressed as the means $\pm$ standard deviation (S.D.). Within-group analysis was performed first with a one or two-way analysis of variance (ANOVA), and then post hoc analysis was performed with the Fisher PLSD test. We used these procedures, a data base, and a statistical package (Stat View J-4.5, Abacus Concepts) in which $p < 0.05$ was considered significant.

3. Results

3.1. Extraction of wheat protein

The proteins of three different solubility fractions (salt-soluble, and gliadin- and glutenin-rich) in wheat were resolved by SDS-PAGE. A comparison of these solubility fractions showed that the salt-soluble protein was distributed in a range from 15 to 100 kDa, whereas the gliadin- and glutenin-rich proteins were restricted to specific regions of the SDS-gel. Gliadin-rich protein was between 30 and 70 kDa, and glutenin-rich protein varied from 35–45 kDa (LMW subunits) to 80–110 kDa (HMW subunits). In addition to the HMW and LMW glutenin subunits, some other polypeptide bands below 30 kDa and between 45 and 60 kDa were also visible in the glutenin fraction. These polypeptides were most likely salt-soluble protein impurities which had not been completely removed by the preceding extraction steps with PBS and 70% ethanol (Fig. 1A). However, each POD-conjugated mouse anti-wheat protein serum reacted, respectively, with salt-soluble, and gliadin- and glutenin-rich proteins

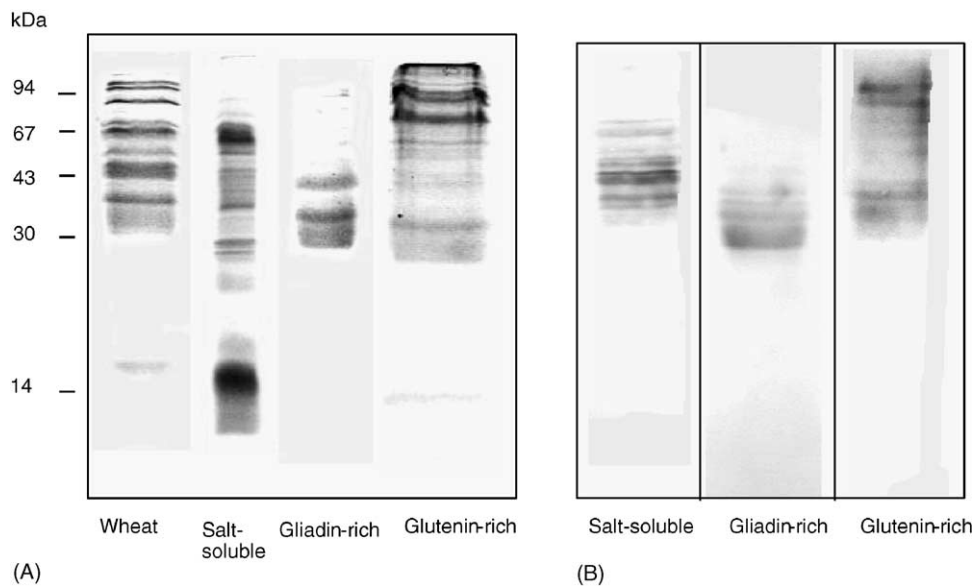


Fig. 1. SDS-PAGE (A) and immunoblotting (B) of each wheat-fractionated protein (Wheat: wheat protein, Salt-soluble: salt-soluble fractionated wheat protein, Gliadin-rich: gliadin-rich fractionated wheat protein, and Glutenin-rich: glutenin-rich fractionated wheat protein).

(Fig. 1B), indicating that the extraction of these proteins was exact.

3.2. Sensitization by wheat proteins

B10.A mice were injected intraperitoneally with each wheat-fractionated protein. Blood was obtained, and specific anti-wheat protein IgE in mouse serum was evaluated using the ELISA (Fig. 2). The specific IgE titer in the GLI-group

was higher than that in the other groups ($p < 0.01$), indicating that gliadin in wheat protein causes a marked increase in sensitization. The glutenin-rich protein induced a slight increase in the specific IgE titer ($p < 0.05$). In contrast, the antibody value in the S-group did not increase easily, despite intraperitoneal administration of the same dose of protein.

3.3. Exercise performance of sensitized mice

The sensitized mice were run on a treadmill until exhaustion after oral ingestion of each wheat-fractionated protein. The unsensitized mice were run on a treadmill until exhaustion after oral ingestion of PBS alone (UP-group) or wheat protein (UW-group). The running times to exhaustion are shown in Fig. 3 (data of the UW-group not shown). The exhaustion time in the UP-group was 199 ± 29 min and that in the UW-group was 179 ± 29 min. In contrast, the exhaustion times in the GLI-group (16% versus the UP-group, $p < 0.01$) and GLU-group (27% versus the UP-group, $p < 0.01$) were significantly lower than that in the UP-group, although initially all the mice were running well. No significant difference, however, was observed between the exhaustion times in the S- and UP-group (74% versus UP-group). In addition, the reduction of exhaustion times in the GLI- and GLU-group was significantly lower than that in the S-group ($p < 0.01$ and 0.05 , respectively).

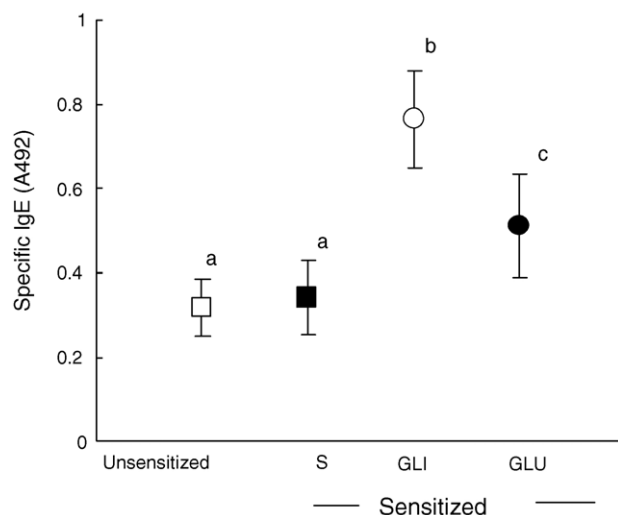


Fig. 2. Mean (S.D.) effect of sensitization of each wheat-fractionated protein on serum antigen-specific IgE (S: salt-soluble fractionated wheat protein-sensitized mice, GLI: gliadin-rich fractionated wheat protein-sensitized mice, and GLU: glutenin-rich fractionated wheat protein-sensitized mice). IgE levels were expressed as absorbance (A492 nm). The differences between the values not associated with a common superscript letter were significant ($p < 0.05$).

3.4. Damage to the small intestine in sensitized mice after exhaustive exercise

To investigate the effect of exhaustive exercise after oral ingestion of allergen on gastrointestinal damage in each group

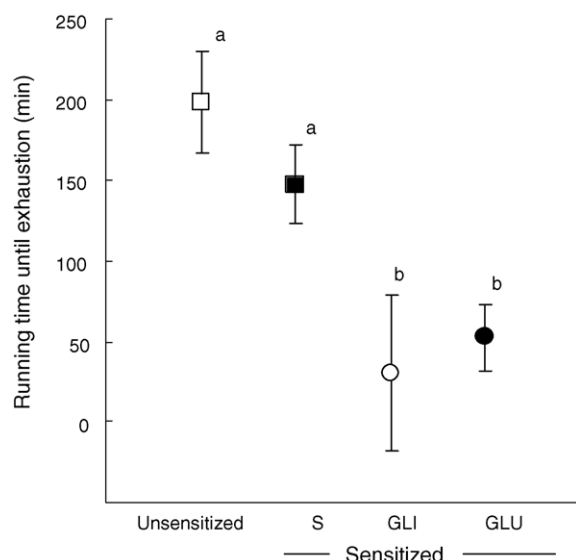


Fig. 3. Mean effect of oral wheat protein ingestion on exercise performance in each wheat-fractionated protein-sensitized group of mice and the unsensitized mice. Exercise performance was estimated from the time to run until exhaustion on a treadmill.

of wheat-fractionated sensitized mice, the small intestine was removed from all mice and was observed microscopically. Since the localization of mucosal lesions in the small intestine was not limited in Fig. 4, we have shown the total mucosal lesion score in the small intestine. The mucosal lesion scores of the small intestine in the S-group (2.3 ± 0.2 , $p < 0.01$), GLI-group (2.1 ± 0.6 , $p < 0.01$), and GLU-group (2.3 ± 0.2 , $p < 0.01$) were significantly higher than that in the UP-group (1.3 ± 0.4).

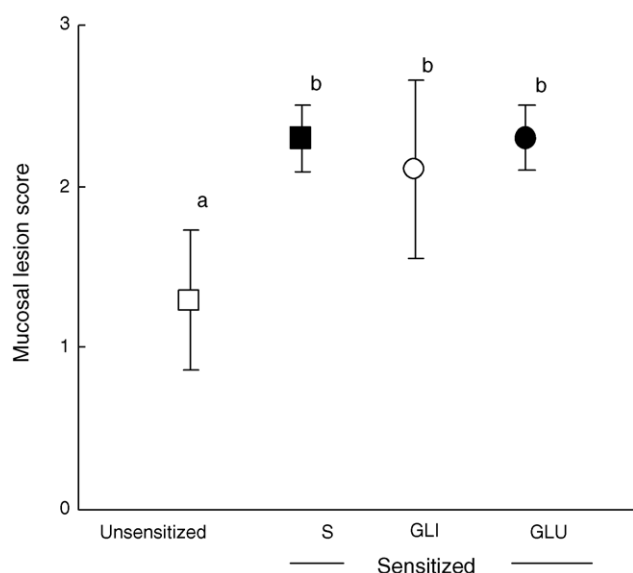


Fig. 4. Mean effect of exhaustive exercise after oral ingestion of wheat protein on the mucosal lesion score in each wheat-fractionated protein-sensitized group of mice and in the unsensitized mice.

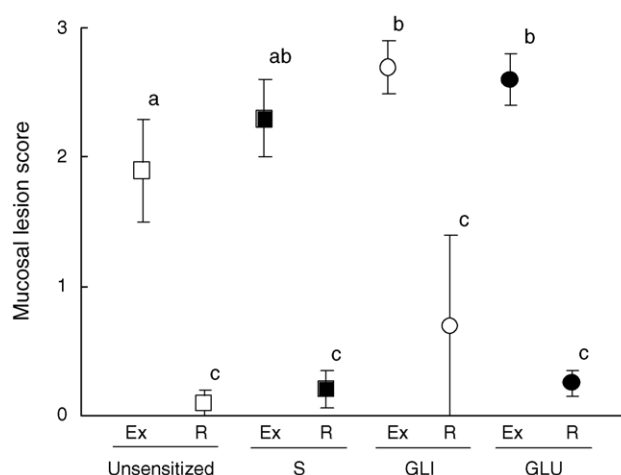


Fig. 5. Mean effect of moderate exercise or rest after oral ingestion of wheat protein on the mucosal lesion score in each wheat-fractionated protein-sensitized group of mice and in the unsensitized mice (Ex: the group with exercise after oral ingestion of wheat protein and R: the group with rest after oral ingestion of wheat protein).

3.5. Damage to the small intestine in sensitized mice after moderate exercise

To clarify the effect of moderate exercise on food-dependent anaphylaxis, the number of mucosal lesions in the small intestine of the sensitized and unsensitized mice was determined after oral ingestion of each wheat-fractionated protein and running on a treadmill for 30 min (Fig. 5). As a reference, the number of mucosal lesions in the small intestine of sensitized and unsensitized mice after oral ingestion of each wheat-fractionated protein and rest in a cage for 30 min was determined. The mucosal lesion scores of the small intestine in the unsensitized mice with rest, the S-group with rest, the GLI-group with rest, and GLU-group with rest were 0.1 ± 0.1 , 0.2 ± 0.2 , 0.7 ± 0.7 , and 0.3 ± 0.1 , respectively. The mucosal lesion score of the small intestine in the GLI-group with exercise (2.7 ± 0.2 , $p < 0.05$) and GLU-group with exercise (2.6 ± 0.2 , $p < 0.05$) were significantly higher than that in the unsensitized mice with exercise (1.9 ± 0.4). In the S-group with exercise, however, the mucosal lesions in the small intestine were similar to those in the unsensitized mice with exercise (2.3 ± 0.3). Moreover, the mucosal lesion score of the small intestine in each exercise-group was significantly higher than that in each rest-group ($p < 0.01$, respectively).

3.6. Leakage of wheat in the liver from the gastrointestinal tract

In Fig. 6, leakage of each wheat-fractionated protein in the liver from the gastrointestinal tract is shown. After oral intake of each wheat-fractionated protein and rest for 30 min, the liver in unsensitized mice (Fig. 6A), the S-group (Fig. 6C), the GLI-group (Fig. 6E), and the GLU-group (Fig. 6G) was

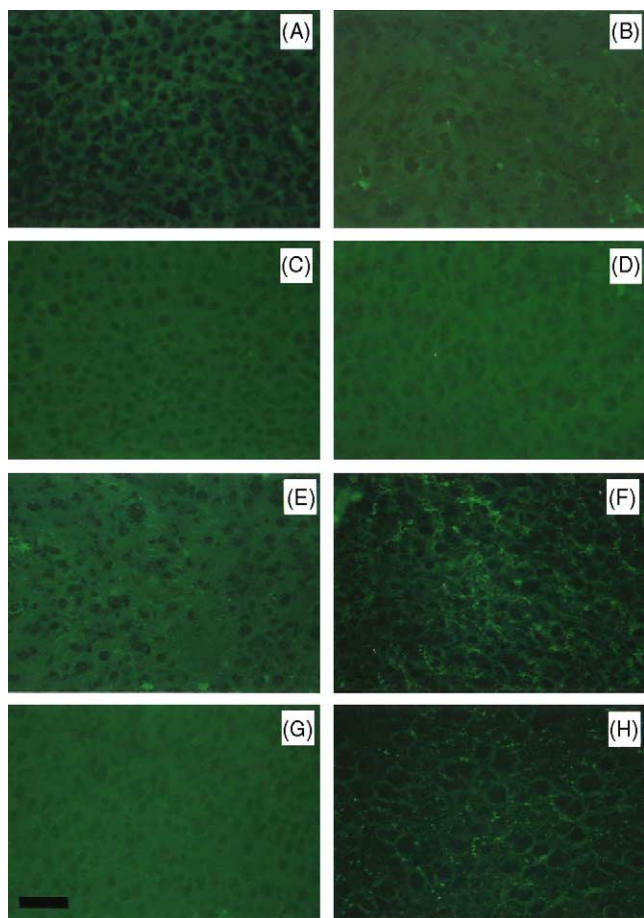


Fig. 6. Photographs of negatively stained liver in unsensitized mice with rest (A) or exercise (B), and of positively stained liver in the S-group with rest (C) or exercise (D), GLI-group with rest (E) or exercise (F), and GLU-group with rest (G) or exercise (H). All of the sensitized mice ingested wheat-fractionated proteins, while the unsensitized mice ingested only PBS before moderate exercise (bar = 50 μ m and 100 $\times$ magnification).

not stained. After oral intake of each wheat-fractionated protein and exercise for 30 min, the liver in the GLI-group with exercise was stained (Fig. 6F), and that in the GLU-group with exercise was slightly stained (Fig. 6H), but no staining was observed in the S-group with exercise (Fig. 6D). The liver in unsensitized mice was not stained, even when we used all of the rat serum (Fig. 6B).

4. Discussion

In this study, we found that the allergen activities of WDEIAn differed with each extraction protein of wheat. WDEIAn was induced in the GLI-group by oral challenge and then acute exercise. Specifically, the GLI-group exhibited a high IgE antibody titer in plasma, reduction of exercise performance and high leakage of allergen to the liver. These changes have been reported to be representative of anaphylactic reactions [12]. Our results are

supported by studies showing that the major allergen of WDEIAn is gliadin [11,15]. A slight increase in IgE and reduction of exercise performance until exhaustion also occurred in the GLU-group. These results suggest that glutenin might also induce WDEIAn. In fact, Lehto et al. [15] reported that in three of every nine patients with WDEIAn, an increase in histamine release was induced by gliadin. Therefore, there is a possibility that glutenin is also one of the allergens in WDEIAn. Thus, wheat insoluble proteins might cause WDEIAn. In contrast, the S-group in our study did not show any increase in plasma IgE. Although it has been reported that non-IgE immunoglobulins mediate antigen-specific systemic anaphylactic reactions in IgE-deficient mice [16], the S-group did not exhibit any reduction in exercise performance after the oral challenge. At least in the present study, WDEIAn might not be induced by salt-soluble protein in wheat. It is known that IgE antibody, which binds to high-affinity Fc ϵ RI receptors on mast cells, macrophages and dendritic cells, plays an important role in mediating type I hypersensitivity responses. A previous study showed that the rectal temperature of sensitized mice is regulated by the activity of histamine, which can be released from mast cells following the binding of antigen by IgE antibodies [17]. Although we did not measure plasma histamine in this study, we observed decreases in body temperature as one of the symptoms of anaphylactic shock [18,19]. (GLI-group: $\sim$ 36.3 and $\sim$ 34.9 $^{\circ}$ C, before and after moderate exercise, respectively).

In this study, intestinal damage was observed in the wheat protein-sensitized mice, and particularly in the GLI- and GLU-group. Antigen analysis by Kushimoto and Aoki [7] disclosed that a pepsin-digested gluten fraction with the same or greater allergenicity than the gluten allergen itself, but additional trypsin-digestion greatly diminished or completely abolished the allergenicity of the pepsin-digested gluten allergen in their patients. Sturgess et al. [20] also reported that gliadin peptides damage the gut mucosa in celiac disease. Li et al. [21] reported that increased intestinal permeability in mouse occurred as a result of food allergies. Ogawa et al. [22] also observed that significant villus atrophy and increased crypt depth in the small intestine occurred in OVA-sensitized rats given OVA. Furthermore, we observed intestinal damage in the S-group after exhaustive exercise. Exercise has also been previously reported to increase gastrointestinal permeability [23]. In addition, we have already reported on gastrointestinal damage in LYZ-sensitized mice of B10.A when these mice showed a symptom of FDEIAn after LYZ intake and running exercise [12]. The intestinal damage in the S-group of the present study might have been dependent on exercise intensity because that group showed little damage to the small intestine with moderate exercise. Therefore, exercise might strongly induce gastrointestinal permeability when sensitized mice are fed a food allergen.

The results of immunohistochemistry of the liver might correlate with leakage from the gastrointestinal tract, because

in the GLI- and GLU-group, the hepatic sinusoid was stained, and the villi in the small intestine were remarkably damaged compared with those in the unsensitized mice. These results suggest that insoluble proteins were absorbed and/or leaked from the intestine and reached the liver through the portal vein. Mucosal lesions of the small intestine seem to be related to the gastrointestinal leakage of gliadin and glutenin as antigens, because alterations caused by damage to the intestinal wall could allow the escape of gliadin and glutenin from the gastrointestinal tract into the portal circulation.

Our results suggested that specific food allergens might induce gastrointestinal damage. Furthermore, they suggested that exercise after the intake of allergens causes significant development of mucosal lesions in the small intestine. The damage to the small intestine may allow antigens to pass more easily in significant amounts into the systemic circulation than they would in an undamaged small intestine. Yano et al. [12] reported that small intestine mucosal lesions in unsensitized mice were induced by acute exercise. It is known that reduction in mesenteric blood flow depends on exercise intensity [24,25], so exercise could induce an increase in gastrointestinal permeability [23] and gastrointestinal damage [12,26]. A previous study [12] also reported damage to villi of the small intestine in LYZ-sensitized mice that were not exercised which was then markedly accelerated when they were exercised. Also, in this study, erosion of the small intestine in sensitized non-exercise mice was rarely observed. It seems that exercise might be related to the cause of erosions of the small intestine. Although we could not clarify the mechanism of exercise-induced small intestine erosions, it is interesting that the difference in wheat protein had an effect on these erosions.

In summary, leakage from the gastrointestinal tract into the circulation was strongly induced by acute exercise in the mice sensitized with gliadin and glutenin as allergens. This finding suggests that WDEIA might be related to the titer of specific IgE and that the gastrointestinal leakage of allergen was caused by exercise-induced gastrointestinal mucosal lesions. If the wheat insoluble proteins, gliadin and glutenin, are found to be allergens in WDEIA, WDEIA could be investigated using this mouse model system.

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References

- [1] Kemp SF, Lockey RF, Wolf BL, Lieberman P. Anaphylaxis: a review of 266 cases. *Arch Intern Med* 1995;155:1749–54.
- [2] Castells MC, Horan RF, Sheffer AL. Exercise-induced anaphylaxis (EIA). *Clin Rev Allergy Immunol* 1999;17:413–24.
- [3] Garcia-Ara MC, Sanchez AV, Martinez MT, Diaz Pena JM. Cow's milk-dependent exercise-induced anaphylaxis: case report of a patient with previous allergy to cow's milk. *J Allergy Clin Immunol* 2003;111:647–8.
- [4] Romano A, Di Fonso M, Giuffreda F, Papa G, Artesani MC, Viola M, Venuti A, Palmieri V, Zeppelli P. Food-dependent exercise-induced anaphylaxis: clinical and laboratory findings in 54 subjects. *Int Arch Allergy Immunol* 2001;125:264–72.
- [5] Guinnee MT, Eloit C, Raffard M, Brunet-Moret MJ, Rasse-mont R, Laurent J. Exercise-induced anaphylaxis: useful screening of food sensitization. *Ann Allergy Asthma Immunol* 1996;77:491–6.
- [6] Shadick NA, Liang MH, Partridge AJ, Bingham C, Wright E, Fossel AH, Sheffer AL. The natural history of exercise-induced anaphylaxis: survey results from a 10-year follow-up study. *J Allergy Clin Immunol* 1999;104:123–7.
- [7] Kushimoto H, Aoki T. Masked Type I Wheat Allergy. *Arch Dermatol* 1985;121:355–60.
- [8] Varjonen E, Vainio E, Kalimo K. Life-threatening, recurrent anaphylaxis caused by allergy to gliadin and exercise. *Clin Exp Allergy* 1997;27:162–6.
- [9] Kendall MJ, Schneider R, Cox PS, Hawkins CF. Gluten subfractions in celiac disease. *Lancet* 1972;2:1065–7.
- [10] Kieffer M, Barnetson RSC. Increased gliadin antibodies in dermatitis herpetiformis and pemphigoid. *Br J Dermatol* 1983;108:673–8.
- [11] Palosuo K, Varjonen E, Nurkkala J, Kalkkinen N, Harvima R, Reunala T, Alenius H. Transglutaminase-mediated cross-linking of a peptic fraction of omega-5 gliadin enhances IgE reactivity in wheat-dependent, exercise-induced anaphylaxis. *J Allergy Clin Immunol* 2003;111:1386–92.
- [12] Yano H, Kato Y, Matsuda T. Acute exercise induces gastrointestinal leakage of allergen in lysozyme sensitized mice. *Eur J Appl Physiol* 2002;87:358–64.
- [13] Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951;193:265–75.
- [14] Engvall E, Perlmann P. Enzyme-linked immunosorbent assay (ELISA). Quantitative assay of immunoglobulin G. *Immunochemistry* 1971;8:871–4.
- [15] Lehto M, Palosuo K, Varjonen E, Majuri ML, Andersson U, Reunala T, Alenius H. Humoral and cellular responses to gliadin in wheat-dependent, exercise-induced anaphylaxis. *Clin Exp Allergy* 2003;33:90–5.
- [16] Oettgen HC, Martin TR, Wynshaw-Boris A, Deng C, Drazen JM, Leder P. Active anaphylaxis in IgE-deficient mice. *Nature* 1994;370:367–70.
- [17] Makabe-Kobayashi Y, Hori Y, Adachi T, Ishigaki-Suzuki S, Kikuchi Y, Kagaya Y, Shirato K, Nagy A, Ujike A, Takai T, Watanabe T, Ohtsu H. The control effect of histamine on body temperature and respiratory function in IgE-dependent systemic anaphylaxis. *J Allergy Clin Immunol* 2002;110:298–303.
- [18] Djuric VJ, Markovic BM, Lazarevic M, Jankovic BD. Anaphylactic shock-induced conditioned taste aversion. Part II. Correlation between taste aversion and indicators of anaphylactic shock. *Brain Behav Immun* 1988;2:24–31.
- [19] Okunuki H, Teshima R, Sakushima J, Akiyama H, Goda Y, Toyoda M, Sawada J. Induction of active systemic anaphylaxis by oral sensitization with ovalbumin in mast-cell-deficient mice. *Immunol Lett* 2000;74:233–7.
- [20] Sturgess R, Day P, Ellis HJ, Lundin KE, Gjertsen HA, Kontakou M, Ciclitira PJ. Wheat peptide challenge in coeliac disease. *Lancet* 1994;343:758–61.
- [21] Li XM, Schofield BH, Huang CK, Kleiner GI, Sampson HA. A murine model of IgE mediated cow's milk hypersensitivity. *J Allergy Clin Immunol* 1999;103:206–14.

- [22] Ogawa T, Miura S, Tsuzuki Y, Ogino T, Teramoto K, Inamura T, Watanabe C, Hokari R, Nagata H, Ishii H. Chronic allergy to dietary ovalbumin induces lymphocyte migration to rat small intestinal mucosa that is inhibited by MAdCAM-1. *Am J Physiol Gastrointest Liver Physiol* 2004;286:G702–10.
- [23] Lambert GP, Broussard LJ, Mason BL, Mauermann WJ, Gisolfi CV. Gastrointestinal permeability during exercise: effects of aspirin and energy-containing beverages. *J Appl Physiol* 2001;90:2075–80.
- [24] Bortoff A. Influence of exercise on gastrointestinal function. In: Horton ES, Terjung RL, editors. *Exercise, nutrition, and energy metabolism*. New York: McMillan; 1988. p. 159–71.
- [25] Yano L, Yano H, Taketa K. Portal blood flow during and after high-intensity physical exercise in rats: response of plasma endothelin-1 and catecholamine. *Hepatol Res* 1998;11:1–11.
- [26] Yano H, Kinoshita S, Kira S. The effects of acute moderate exercise on the phagocytosis of Kupffer cells in rats. *Acta Physiol Scand* 2004;182:151–60.