

REVIEW

Wheat-dependent exercise-induced anaphylaxis

K. A. Scherf¹, K. Brockow², T. Biedermann², P. Koehler¹ and H. Wieser¹

¹Deutsche Forschungsanstalt für Lebensmittelchemie, Leibniz Institut, Freising, Germany and ²Department of Dermatology and Allergy Biederstein, Technische Universität München, Munich, Germany

Clinical & Experimental Allergy

Summary

Wheat-dependent exercise-induced anaphylaxis (WDEIA) is a rare, but potentially severe food allergy exclusively occurring when wheat ingestion is accompanied by augmenting cofactors. It is clinically characterized by anaphylactic reactions ranging from urticaria and angioedema to dyspnoea, hypotension, collapse, and shock. WDEIA usually develops after ingestion of wheat products followed by physical exercise. Other cofactors are acetylsalicylic acid and other non-steroidal anti-inflammatory drugs, alcohol, and infections. The precise mechanisms of WDEIA remain unclear; exercise and other cofactors might increase gastrointestinal allergen permeability and osmolality, redistribute blood flow, or lower the threshold for IgE-mediated mast cell degranulation. Among wheat proteins, ω 5-gliadin and high-molecular-weight glutenin subunits have been reported to be the major allergens. In some patients, WDEIA has been discussed to be caused by epicutaneous sensitization with hydrolysed wheat gluten included in cosmetics. Diagnosis is made based on the patient's history in combination with allergy skin testing, determination of wheat-specific IgE serum antibodies, basophil activation test, histamine release test, and/or exercise challenge test. Acute treatment includes application of adrenaline or antihistamines. The most reliable prophylaxis of WDEIA is a gluten-free diet. In less severe cases, a strict limitation of wheat ingestion before exercise and avoidance of other cofactors may be sufficient.

Submitted 3 June 2015; revised 5 August 2015; accepted 13 September 2015

Correspondence:

K. A. Scherf, Deutsche Forschungsanstalt für Lebensmittelchemie, Leibniz Institut, D-85354 Freising, Germany.
E-mail: katharina.scherf@lrz.tum.de
Cite this as: K. A. Scherf, K. Brockow, T. Biedermann, P. Koehler, H. Wieser, *Clinical & Experimental Allergy*, 2016 (46) 10–20.

Introduction

Cereals are the most important staple foods world-wide with corn, rice, and wheat each yielding around 800 million metric tons annually [1]. Among cereals, wheat is unique due to the special bread-making properties of the flour. Numerous people cannot tolerate wheat consumption, because of harmful immune responses to specific wheat proteins. The most common wheat hypersensitivities are wheat allergy, baker's asthma [2], wheat-dependent exercise-induced anaphylaxis (WDEIA) [3, 4], coeliac disease (CD) [5, 6], and non-coeliac gluten sensitivity (NCGS) [7]. As an IgE-mediated allergic response, WDEIA is triggered by wheat ingestion only together with cofactors such as physical activity, but not by wheat ingestion alone. Wheat is the predominant allergen for food-dependent exercise-induced anaphylaxis (FDEIA), which includes reactions to many other foods [8]. Whereas wheat allergy and CD have been known for many decades, the first case of WDEIA was reported fairly recently in 1985 [9]. Since then, numerous studies

on WDEIA have been performed mostly in Japan and European countries. The present article reviews recent developments in diagnosis, treatment, clinical features, triggering wheat proteins and cofactors, and possible pathogenic mechanisms of WDEIA.

Food-dependent exercise-induced anaphylaxis

Anaphylaxis is a potentially life-threatening generalized or systemic hypersensitivity reaction involving several organs and systems, particularly skin, respiratory tract, gastrointestinal tract, and cardiovascular system [10]. Exercise-induced anaphylaxis (EIA) is thought to be associated with 5–15% of all cases of anaphylaxis [11]. It has been claimed that at least 33–50% of patients with EIA suffer from FDEIA. Two subtypes of FDEIA have been postulated: (i) non-specific FDEIA, where any meal followed by exercise induces EIA regardless of the food eaten, or (ii) a specific IgE-mediated FDEIA caused by the ingestion of a specific food prior to exercise [12]. In FDEIA, allergic reactions are elicited only

when a triggering cofactor, such as physical activity, is added after ingestion of the causative food. The time between meal and exercise is usually between 30 and 120 min, and the duration of exercise before the onset of symptoms lasts 10–50 min. Other cofactors in FDEIA are acetylsalicylic acid (ASS), other analgetics, alcohol, stress, or infections. Typical type I allergic symptoms such as pruritus, urticaria, and dyspnoea usually appear on the skin, mucous membranes, and the respiratory and gastrointestinal tracts. Acute myocardial infarction may be a rare, but severe complication of FDEIA [13]. FDEIA is potentially life-threatening, and cases of death have been ascribed to FDEIA [14, 15]. Despite being relatively rare, FDEIA presents an important and underdiagnosed entity for emergency physicians to consider [16].

The first case of FDEIA was reported in 1979 as a late allergic reaction to shellfish induced by strenuous exercise [17]. Kidd *et al.* [18] presented four such patients and designated the phenomena as FDEIA. Various kinds of causative foods have been identified including shellfish, wheat products, vegetables, fruits, nuts, eggs, mushrooms, corn, garlic, meat of pork and beef, or rice [8]. Allergenic proteins were identified only in wheat, soya bean (β -conglycinin) [19], and shrimp (70-kDa protein) [20]. In recent years, FDEIA has been increasingly reported. According to studies on Japanese children and students, 0.01–0.20% suffer from FDEIA [21, 22]. The disease appears to be more prevalent in Japanese children than in adults, and to affect men more frequently than women with a ratio of about 2 : 1.

Useful diagnostic tests for FDEIA are skin prick tests (SPT), oral food challenge combined with subsequent exercise and *in vitro* food-specific serum IgE assays. However, positive reactions in SPT or IgE assays do not necessarily indicate the triggering food [8]. If a causative food cannot be identified, a challenge test is necessary [23, 24]. Because FDEIA is still underdiagnosed, improved strategies to ensure accurate diagnosis are needed [25].

Serum histamine and tryptase levels are increased during symptomatic attacks indicating IgE-dependent degranulation of mast cells. However, the mechanism, by which exercise evokes clinical symptoms, is not fully understood. FDEIA may result from allergen leakage [26] by increased gastrointestinal permeability, blood flow redistribution, and increased osmolality triggered by physical effort after exposure to a sensitizing food allergen [27].

Wheat proteins

Wheat hypersensitivity is triggered by wheat proteins. The protein content of wheat grains covers a broad range (< 8% to > 16%) depending on the genotype

(species, variety) and the growing conditions (soil, climate, fertilization). Traditionally, this mixture of several hundred proteins has been classified into the four so-called Osborne fractions, the water-soluble albumins, salt-soluble globulins, alcohol-soluble gliadins, and insoluble glutenins, that are only alcohol-soluble in the presence of reducing (e.g. dithiothreitol) and disaggregating (e.g. urea) agents [28]. Albumins and globulins, predominantly present in the aleurone layer and the germ, are mainly metabolic proteins. Gliadins and glutenins are storage proteins located in the starchy endosperm as a supply of nitrogen and amino acids for the seedling. The contents of Osborne fractions also vary considerably depending on species, variety, and growing conditions. For example, quantitative studies on thirteen wheat flours resulted in the following ranges: albumins + globulins 16–34%, gliadins 48–62%, and glutenins 15–31% [29].

Gliadins and glutenins have extremely imbalanced amino acid compositions with high proportions of glutamine (32–53 mol-%) and proline (11–29 mol-%) and low contents (< 2 mol-%) of lysine, methionine, and tryptophan. Gliadins are mainly monomeric proteins (molecular weights: 30 000–50 000) and glutenins are polymeric proteins linked by interchain disulphide bonds (molecular weights: 600 000 to more than 10 million). The chemical reduction of disulphide bonds results in glutenin subunits (molecular weights: 30 000 to 80 000).

Based on similar amino acid sequences and molecular weights, gluten proteins can be classified into the following protein types [30]: α/β -, γ -, ω 1,2-, and ω 5-gliadins, low-molecular-weight (LMW-GS), and high-molecular-weight glutenin subunits (HMW-GS). α/β -Gliadins, γ -gliadins, and LMW-GS belong to the major and ω 5-gliadins, ω 1,2-gliadins, and HMW-GS to the minor components (Table 1) [31].

The amino acid sequences of all protein types consist of two or three strongly different structural domains [6]. Unique to each type are distinctive domains that contain repetitive sequences rich in glutamine and proline. α/β -Gliadins are characterized by repetitive motifs such as QPQPFPQQPYP, γ - and ω 1,2-gliadins by QPQPFP, ω 5-gliadins by QQQFP, LMW-GS by QQQPPFS, and HMW-GS by QQPQGQ interspersed by YYPTSP and QQP. These motifs are frequently modified by exchange of single amino acid residues and the number of repeats. These repetitive sequences are responsible for the development of CD [6] and WDEIA.

Wheat-dependent exercise-induced anaphylaxis

WDEIA, a special form of FDEIA, exhibits allergic symptoms after a combination of wheat intake and cofactors such as exercise. The frequency of WDEIA in

Table 1. Proportions of storage protein types from 14 wheat cultivars, rye (cultivar Halo), and barley (cultivar Golden Promise)*

Wheat	Rye	Barley
α/β -Gliadins (m) 27–39%	–	–
γ -Gliadins (m) 21–30%	γ -40k-Secalins (m) 25%	γ -Hordeins (m) 32%
ω 1,2-Gliadins (m) 4–7%	ω -Secalins (m) 18%	C-Hordeins (m) 36%
ω 5-Gliadins (m) 3–7%	–	–
LMW-GS (p) 16–23%	γ -75k-Secalins (p) 48%	B-Hordeins (p) 27%
HMW-GS (p) 7–12%	HMW-Secalins (p) 9%	D-Hordeins (p) 5%

*From [31, 99, 100].

All percentages based on total gluten contents.

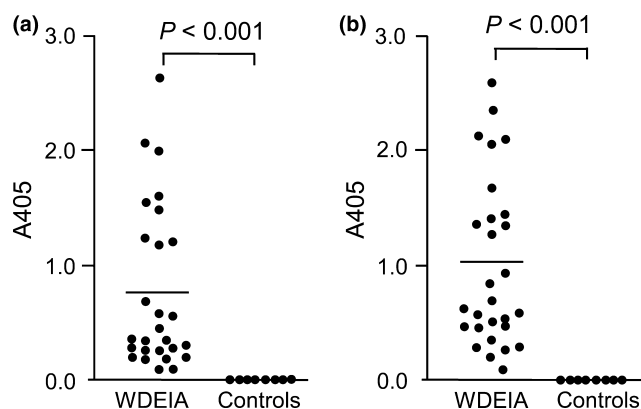
m, monomeric; p, polymeric.

Japanese patients with FDEIA was found to be 56% [32], but data on the prevalence of WDEIA in different countries is scarce. In contrast to reports on FDEIA, WDEIA occurs more frequently in adults than in children.

Clinical features

Symptoms of WDEIA are usually elicited by physical activity one to four hours after wheat intake, but a delayed onset may occur in rare cases [33]. In contrast, symptoms may also occur if food is ingested soon after exercise [34]. The frequency of WDEIA events varies from patient to patient and ranges from a singular episode to multiple episodes [35]. The clinical manifestations and severity of allergic reactions from urticaria to anaphylaxis also vary.

WDEIA patients typically present pruritus, local and generalized urticaria, angioedema, dyspnoea, upper respiratory tract obstruction, gastrointestinal symptoms, hypotension, and if extensive, bronchospasm, vascular collapse, and anaphylactic shock. High concentrations of IgE antibodies specific to wheat protein fractions are observed in sera of WDEIA patients [23, 36, 37]. In particular, IgE antibody levels to a crude gliadin extract and to ω 5-gliadin were shown to be significantly higher in WDEIA patients as compared to controls ($P < 0.001$) (Fig. 1). Moreover, levels of IgA antibodies to total gliadin and ω 5-gliadin were distinctly increased. Both allergens released high levels of histamine from basophils of WDEIA patients, but the structures of the digested gluten allergens are still unknown. Mast cell degranulation due to higher osmolality from dehydration during exercise has also been reported [27, 38]. Beside histamine, elevated plasma noradrenaline, and increased tryptase and leukotriene levels, consistent

**Fig. 1.** IgE antibody levels to (a) crude gliadin extract and (b) ω 5-gliadin in ELISA (absorbance units (AU) at 405 nm) in 27 patients with WDEIA and 21 healthy controls ($P < 0.001$) (adapted from [36]).

with mast cell activation, have been found [15, 23, 39, 40].

Causative proteins

The first allergens to be identified in WDEIA were wheat gliadin and the corresponding ethanol-soluble proteins from rye (secalins), barley (hordeins), and oats (avenins) [41]. Initially, α/β - and γ -gliadins were found to be the presumptive allergens [42–44]. Subsequently, ω 5-gliadin (called Tri a 19) was described as the major allergen of immediate wheat allergy and WDEIA [45, 46]. Of the seven identified epitopes within the amino acid sequence of ω 5-gliadin, four were found to be dominant (QQIPQQQ, QQFPQQQ, QQSPEQQ, and QQSPQ QQ) [47]. Later on, four additional IgE-binding epitopes (QQFHQQQ, QSPEQQQ, QQPPQQ, and YQQYPQQ) were identified [48, 49].

Synthetic overlapping dodecapeptides spanning the complete sequences (Pepscan) were used to identify the major immunogenic epitopes from α/β -gliadin, γ -gliadin, ω 1,2-gliadin, and ω 5-gliadin [50]. All epitopes were localized in the glutamine- and proline-rich repetitive domains of the proteins. Cross-reactivity of allergens from barley, rye, and oats was studied, and among 23 patients with WDEIA, 21 showed IgE-binding to γ -75k-secalin, 19 to γ -40k-secalin, and 21 to γ 3-hordein; oat proteins did not cross-react [51]. To examine whether intestinal tissue transglutaminase (TG2) could be involved in the IgE-binding ability, ω 5-gliadin was enzymatically digested and treated with TG2 [52]. The binding ability of ω 5-gliadin was retained after digestion, and TG2 treatment formed peptide/TG2 conjugates with molecular weights ranging from 40 000 to > 200 000. These conjugates bound IgE antibodies more intensely than untreated or digested ω 5-gliadin and caused larger mean SPT weals.

In addition to ω 5-gliadin, HMW-GS were shown to be the most potent allergens [53] with three predominant epitopes (QQPGQ, QQPGQGQQ, and QQSGQGQ) [49]. Two recombinant LMW-GS also reacted with IgE antibodies [54] and a subsequent study confirmed the WDEIA-specific antigenicity of LMW-GS [55]. A comprehensive analysis of IgE reactivity to different wheat proteins was performed by ImmunoCAP[®] (ThermoFisher, Uppsala, Sweden) and a microarray [56]. By ImmunoCAP[®], IgE antibodies to ω 5-gliadin were detectable in 82%, to $\alpha/\beta/\gamma$ -gliadin in 82%, and to HMW-GS in 59% of patients. The microarray revealed specifically γ -gliadin as important allergen beside ω 5-gliadin. In ω 5-gliadin-negative patients, IgE to $\alpha/\beta/\gamma$ -gliadins and HMW-GS are of additional diagnostic value.

An anaphylactic response to deamidated wheat proteins is a separate entity of WDEIA. These WDEIA patients did not respond to native wheat proteins, but to a deamidated wheat protein isolate [57]. In ELISA, all sera displayed IgE-binding to deamidated total gliadin as well as to deamidated γ - and ω 5-gliadins. Epitope mapping revealed that the consensus sequence QPEEPFPE, derived from the native sequence QPQQPF PQ, had the highest IgE-binding intensity.

In conclusion, ω 5-gliadin and HMW-GS appear to be the major allergens in WDEIA. However, other types of gluten proteins such as α/β - and γ -gliadins and LMW-GS also can sensitize WDEIA patients.

The dependence of WDEIA induction on the amount of allergenic proteins has not been well characterized. In any case, the amount of allergenic proteins in WDEIA is much higher than in CD (20 mg daily gluten intake). Challenge tests showed that 64 g, but not 45 g, of bread induced generalized urticaria in one case. Further food challenges with udon (a Japanese noodle made from wheat) also triggered allergic reactions in a dose-dependent manner: 200 g, but not 100 g or 150 g, of udon combined with exercise elicited weals and erythema [58].

Cofactors

The most important and prevalent cofactor is physical activity. In many patients, strenuous exercise such as jogging, dancing, or cycling triggered anaphylaxis after food ingestion, but milder exercise, for example walking or gardening, may be sufficient. The characteristics of the exercise such as intensity, duration, heart rate, and maximal oxygen uptake that are necessary to induce anaphylaxis are not clear. It is also unknown whether this dose may vary at different times for the same individual. Exercise may act through two different pathways [32]: (i) Exercise up-regulates intestinal absorption resulting in increased amounts of food anti-

gens in the circulating blood, and (ii) exercise enhances the degranulation of mast cells in combination with IgE cross-linking through an unknown mechanism. However, only limited data are available on the precise mechanisms of exercise-induced enhancement of gastrointestinal absorption of allergens into the circulation [8]. In addition, infectious diseases, especially in early phases or clinically mild forms, may serve as cofactors to augment anaphylaxis. In contrast to other cofactors, infections are particularly dangerous, because they cannot be avoided or prevented [3].

ASS (e.g. Aspirin[®]) and other NSAIDs including indomethacin, ibuprofen, and diclofenac sodium have also been described as cofactors of WDEIA [32, 59, 60]. They induce symptoms in combination with food ingestion even without exercise. In some cases, WDEIA was not induced by the combination of wheat and exercise, but only by the combination of wheat and ASS [61]. ASS is widely used and it has been reported that patients with ASS-provoked WDEIA took ordinary doses of ASS (500–1000 mg per day). Even low doses of ASS (81 mg per day) taken regularly may be sufficient to contribute to WDEIA [62].

The exact mechanism by which NSAIDs act as cofactors for WDEIA remains to be elucidated. ASS is known to cause damage to the tight junctions in the gastrointestinal mucosa, which could lead to increased permeability [63, 64], and facilitate allergen absorption. Serum gliadin can be detected in WDEIA patients and healthy subjects, who consumed ASS before wheat ingestion [65]. ASS inhibits cyclooxygenase function, which results in decreased prostaglandin and increased leukotriene synthesis. Misoprostol, a synthetic analogue of prostaglandin E1 (PGE1), compensates the ASS-induced inhibition of prostaglandin synthesis. PGE1 may suppress allergen absorption and the outbreak of allergic symptoms induced by ASS in patients with WDEIA [66].

ASS has been reported to directly augment the degranulation of mast cells and accelerate histamine release via increased Syk kinase activation [22, 60]. This increased histamine release by NSAIDs may be one possible cause of aggravated symptoms in patients with chronic urticaria and FDEIA. However, another study suggested that this worsening of symptoms is not mediated by direct effects of ASS on mast cell and basophil activation [67]. Thus, further studies are required to clarify these mechanisms. Ethanol may also act as cofactor for inducing WDEIA [68] and it has been reported to increase gastric allergen absorption and augment histamine release [12].

Pathomechanism

The precise mechanism underlying allergic sensitization in WDEIA is crucial, but it remains poorly understood.

The route of allergen sensitization to wheat proteins is thought to be via either the gastrointestinal tract or the skin. There is increasing evidence that an epidermal barrier defect may play an important role. Loss-of-function mutations in the gene encoding filaggrin, a key epidermal protein in skin barrier function, are known to be significant predisposing factors for the development of atopic diseases such as eczema, asthma, and rhinitis [69] and might also be involved in the pathogenesis of WDEIA [70].

Recent investigations indicate that major events occur in the intestine, and various activating factors have to be taken into consideration. There is a general agreement that IgE-dependent mast cell degranulation and histamine release are the key features of WDEIA. Several theories have been proposed to explain details of the mechanism [3].

(i) TG2 may become activated during exercise, which results in conjugates between wheat peptides and TG2 followed by IgE binding [52], but the presence of such conjugates in the circulation is still unconfirmed. (ii) Exercise mobilizes and activates immune cells from gut-associated depots stimulating pro-inflammatory responses that are normally countered by anti-inflammatory responses [71]. (iii) Exercise and ASS increase gastrointestinal permeability and facilitate allergen absorption into the circulating blood [65]. (iv) Exercise induces locally increased osmolality in the intestine, enhances mast cell degranulation, and thus, increases gut permeability [38, 72]. (v) Blood flow redistribution during exercise may carry allergens to tissues containing mast cells that are not tolerant to these allergens. This may result in an allergic reaction during exercise and tolerance at rest [73]. (vi) Plasma noradrenaline levels are significantly increased, when anaphylactoid symptoms have been induced [40]. Noradrenaline may be involved in the onset of WDEIA, because it selectively inhibits T helper 1 (Th1) functions while favouring Th2 and humoral responses, or acts directly on both cellular and humoral immunity [74].

Studies on lysozyme-sensitized mice demonstrated that allergen leakage from the intestinal tract into the circulation was strongly induced by exercise and that damage of the villi also occurred [26]. The authors hypothesized that the same mechanism might underlie WDEIA. Gliadin- and glutenin-sensitized mice had a greater elevation of serum IgE and higher intestinal erosion than albumin/globulin-sensitized mice or control animals [75]. Bodinier *et al.* [76] established an IgE Fc receptor-humanized rat cell line (RBL-2H3) and demonstrated a correlation between mast cell degranulation and IgE levels induced by sera from WDEIA patients. IgE antibodies of gliadin-sensitized mice reacted strongly with ω 5-gliadin, which is consistent with findings that ω 5-gliadin is a primary cause of WDEIA [77].

Diagnosis

The precise diagnosis of WDEIA is extremely important, but there is a lack of awareness of WDEIA among patients as well as physicians. A prolonged time lag (32–62 months) to diagnosis is not uncommon [78]. WDEIA may be mistaken for other diseases such as acute urticaria, exercise-induced anaphylaxis, or idiopathic anaphylaxis, and a high index of clinical suspicion is required. At the beginning of the diagnostic procedure, allergy history data of the patient are recorded together with a detailed medical examination. If the cause of an anaphylaxis is unknown, patients need to be taught how to carefully chart all factors relating to any subsequent episodes to facilitate diagnosis and minimize excessive reliance on laboratory tests. The diagnostic tools for WDEIA can be divided into *in vivo* tests such as SPT and provocation tests as well as *in vitro* tests including basophil activation test (BAT), histamine release test (HRT), and wheat-specific IgE testing.

The skin prick test (SPT) is performed with a small amount of wheat extract, gluten, or gliadin using prick lancets. Recent studies on challenge-confirmed WDEIA patients showed that SPT using gluten had a sensitivity of 100% and a specificity of 96% [79].

Figure 2 presents a proposed scheme of the diagnostic procedure for WDEIA [80]. Placebo-controlled wheat/exercise challenge is the gold standard investigation. The test includes wheat allergen ingestion, subsequent exercise, and/or pre-treatment with ASS. Thirty minutes after wheat intake (100 g of wheat flour or 20 g of gluten at a minimum), exercise was performed for 15–20 min using a treadmill while monitoring heart rate, blood pressure, and electrocardiogram. Patients, who were evaluated as having a positive result (weals, anaphylaxis) in the provocation test, were included in the group of definitive WDEIA (group I) [80]. Patients with negative results were divided into either undetectable group II (serum gliadin level < 15 pg/mL) or detectable group III (gliadin level > 15 pg/mL). Group II patients were considered insufficiently challenged and required to undergo retesting including ASS pre-treatment [22, 61]. Serum gliadin monitoring during challenge testing may be useful to check challenge efficacy [65, 80]. Recent studies showed that high doses of gluten (up to 80 g) were able to overcome non-responsiveness of patients despite a clear history [79] (Fig. 3).

Wheat/exercise challenge is recommended for a complete diagnostic assessment in patients with suspected WDEIA [3], may help understand the pathomechanism, and is especially important in cases where diagnosis is unclear. However, it can only confirm the diagnosis of about 70% of patients [22, 67] and entails inevitable risks for the patient. Therefore, it might not need to be

performed as clinical routine when serum IgE antibody test results are positive and the clinical history is suggestive of WDEIA.

Among *in vitro* tests for the diagnosis of WDEIA [35], the BAT is useful in identifying patients and predicting the causative allergen. Moreover, it can distinguish two phenotypes of WDEIA, conventional WDEIA (CO-WDEIA) and WDEIA induced by hydrolysed wheat proteins (HWP) [81]. For the HRT [60], a commercial kit (Allerport® HRT, Shionogi & Co., Osaka, Japan), covering five different allergens (egg white, cow's milk, wheat, soya bean, and rice), is available for routine examination [82]. A disadvantage of both cellular tests is that basophils of some patients do not react with the relevant allergen (non-responders), and sensitivity and specificity of the test have not fully been investigated [35].

As alternative, serum IgE antibodies to wheat proteins can be determined by fluorescence enzyme immunoassays (FEIA) [83]. Because crude extracts from foodstuffs are usually employed as allergens, the sensitivities and specificities of these immunoassays are not always satisfactory. Recombinant allergens of reproducible quality allow a standardization of the method with improved diagnostic results. In the case of WDEIA, the ImmunoCAP® including wheat extract, gluten, and recombinant ω 5-gliadin as allergens has been used most frequently. For example, the sensitivity of the ω 5-gliadin-specific CAP was 80%, but the sensitivities of the wheat extract and gluten CAPs were only 48% and 56%, respectively [84]. The log-transformed specific IgE ratio of ω 5-gliadin to wheat responses, determined by the corresponding ImmunoCAP®, may be useful for

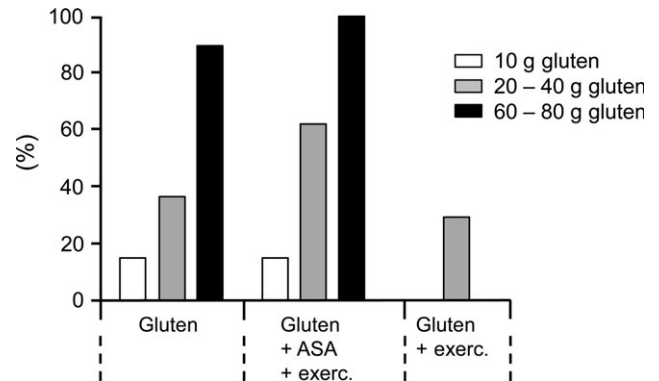


Fig. 3. Cumulative percentage of positive challenge results with gluten alone, gluten + aspirin + alcohol (ASA), and gluten + exercise depending on different amounts (10 g; 20–40 g; 60–80 g) of gluten used for challenge (adapted from [79]).

the diagnosis of WDEIA patients [85]. An improved microarray for the detection of serum IgE against ω 5-gliadin showed promising results adequate to FEIA [86]. However, IgE antibodies specific to ω 5-gliadin were detected not only in WDEIA patients, but also in patients with immediate wheat allergy [45, 87]. Therefore, a CAP-FEIA with recombinant HMW-GS (subunit 1Ax1) was constructed [88]. Sera from 48 patients with WDEIA, 16 patients with atopic dermatitis (who had never experienced wheat allergy), and 12 healthy controls were analysed with commercial wheat, gluten, and ω 5-gliadin CAPs and the customized HMW-GS CAP: Positive rates, sensitivities, and specificities of the tests are shown in Table 2. The healthy group sera did not react with any of the allergens. The specificities of the tests were 50% (wheat), 89.3% (gluten), 100% (ω 5-

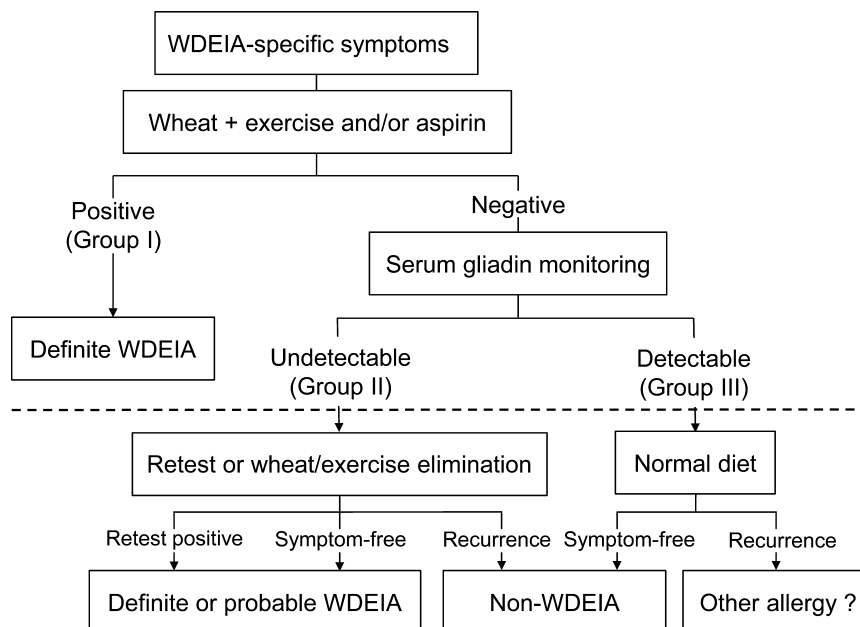


Fig. 2. Proposed diagnostic procedure for WDEIA including serum gliadin determination (adapted from [80]).

Table 2. Positive rate of allergen-specific IgE tests (CAP-FEIA) with wheat proteins*

CAP-FEIA	WDEIA (<i>n</i> = 48)	AD (<i>n</i> = 16)	Sensitivity	Specificity
	Positive (%)	Positive (%)	(%)	(%)
Wheat	17 (35.4)	14 (87.5)	35.4	50.0
Gluten	18 (37.5)	3 (18.8)	37.5	89.3
ω5-Gliadin	39 (81.3)	0 (0.0)	81.3	100.0
HMW-GS	8 (16.7)	2 (12.5)	16.7	92.9
ω5-Gliadin + HMW-GS	45 (93.8)	2 (12.5)	93.8	92.9

*All healthy controls (*n* = 12) negative.

Adapted from [88].

AD, atopic dermatitis.

gliadin), and 92.9% (HMW-GS and combined ω5-gliadin + HMW-GS). The combination of both ω5-gliadin and HMW-GS antibodies is additionally useful, because patients, who do not have antibodies specific to ω5-gliadin (around 20%), have IgE antibodies specific to HMW-GS [53]. More recently, the sensitivity of IgE to wheat, gluten, and ω5-gliadin was found to be 81%, 100%, and 100%, with specificities of 87%, 95%, and 100%, respectively [79].

Treatment

Acute treatment includes immediate termination of physical effort at the earliest warning signs to prevent potentially life-threatening cardiovascular symptoms. Pharmaceuticals such as adrenaline, antihistamines, and systemic corticosteroids may be applied and patients should be instructed on how to use adrenaline autoinjectors.

To prevent further episodes of WDEIA, the education of patients is crucial and they should receive a written anaphylaxis management plan as well as dietary counselling. The classical recommendation of refraining from exercise for 4–6 h after wheat intake may be insufficient, because unexpected exercise, NSAID administration, or infective illness may occur. Currently, there is no prospective data on the optimal management plan for WDEIA, so that risks and recommendations need to be considered on a case-by-case basis. The level of exertion required to provoke reactions, severity of the reaction, and social factors have to be taken into account. In cases of low-threshold provocation, severe reactions, and highly demanding professions (military personnel, pilots, police, etc.), a gluten-free diet is recommended. In less severe cases, the avoidance of wheat ingestion combined with cofactors may be sufficient. Furthermore, due to cross-reactivity of wheat proteins with corresponding proteins from rye, barley, and oats, it is not entirely clear whether patients need to avoid wheat only or also other gluten-containing cereals.

Some pharmacologic protection has also been reported. A case report demonstrated that olopatadine hydrochloride and ketotifen completely inhibited anaphylaxis [40, 89]. Oral sodium chromoglycate (SCG) prevented WDEIA in two children even if they exercised after lunch, but they developed WDEIA, when they forgot to take SCG [90].

WDEIA sensitized with hydrolysed wheat proteins

Wheat gluten, a by-product of wheat starch production, is widely used as additive for numerous kinds of products. For example, gluten in its native form ('vital gluten') is applied as improver for bakery products. One of the modified forms is 'hydrolysed wheat gluten protein' (HWP), which is the water-soluble, emulsifying, and foaming product obtained after different procedures of gluten hydrolysis with acid, alkaline, or enzymes. HWP is used as additive for soaps, shampoos, creams, etc. instead of chemical foaming agents. In 2000 and 2003, the first cases of contact urticaria triggered by epicutaneous sensitization with HWP were reported [91, 92]. In 2013, there were 2026 cases with immediate wheat allergy and WDEIA due to HWP according to the Japanese Society of Allergy. Most patients were likely to have been sensitized to HWP through the use of HWP-supplemented soap (Glupearl 19S).

The predominant symptom of HWP-WDEIA was angioedema on the eyelids [93] and anaphylactic shock occasionally developed. The measurement of serum IgE antibodies by means of CAP-FEIA revealed remarkable differences (Table 3). As expected, IgE antibodies specific to the combination of ω5-gliadins and HMW-GS were useful in diagnosing CO-WDEIA (94%), but not for HWP-WDEIA (16.6%). Gluten- and wheat-specific IgE tests positively recognized 70% and 77% of HWP-WDEIA patients, respectively. The soap Glupearl 19S enhanced CD203c expression in basophils in all HWP-WDEIA patients [81]. In another study, HWP-WDEIA patients developed facial symptoms [94], but they suffered less frequently from hypotension than patients with CO-WDEIA. All patients were positive against

Table 3. Positive rate of ω5-gliadin- and HMW-GS-specific IgE measurement in CO-WDEIA and HWP-WDEIA patients*

CAP-FEIA	CO-WDEIA (<i>n</i> = 54)	HWP-WDEIA (<i>n</i> = 30)
	Positive (%)	Positive (%)
Wheat	17 (31.4)	21 (70.0)
Gluten	20 (37.0)	23 (76.6)
ω5-Gliadin	43 (79.6)	2 (6.6)
HMW-GS	10 (18.5)	5 (16.6)
ω5-Gliadin + HMW-GS	51 (94.4)	5 (16.6)

*Adapted from [93].

HWP in HRT, and among wheat antigens, glutenins induced the highest histamine release.

The specificity of IgE serum antibodies of one CO-WDEIA and one HWP-WDEIA patient, sensitized with HWP via the gastrointestinal tract or via the skin, was compared with different wheat, barley, and rye proteins [95]. The antibodies from both sera strongly reacted with γ 3-hordein, but differences were observed with ω -gliadin, γ -40k- and γ -75k-secalins, LMW-GS, and C-hordein. HWP-WDEIA IgE antibodies bound to α/β -, γ -, and ω 1,2-gliadins and an epitope from γ -gliadin (QPQQPFQ) was identified. The deamidated peptide (PEEPFP) bound to IgE more strongly compared to the native peptide [96].

Regarding the diagnosis of HWP-WDEIA, SPT using Glupers 19S is considered a fast and sensitive method. In addition, other immunological methods such as ELISA [97], Dot Blotting, Western Blotting, or BAT with Glupers 19S as antigen were recommended.

Conclusion

WDEIA is a relatively rare, but potentially severe form of food allergy. Previously uncommon, it is now recognized with increasing frequency, particularly in the Far East and Europe. The prevalence is unknown, mostly due to lack of awareness. The major allergens in WDEIA appear to be ω 5-gliadins and HMW-GS, but other gluten proteins are also involved. It is important to use the most appropriate well-characterized allergen for diagnostic tests. Wheat extracts are unsuitable, because they are poor in gluten, whereas gliadin prepa-

rations lack glutenins. Recombinant proteins are not representative for the whole spectrum of gluten proteins. Only gluten isolated from wheat dough contains all proteins known for their potential allergenicity and constitutes a suitable base material to isolate well-defined ω 5-gliadins and HMW-GS for sensitive testing in the case of WDEIA. Because gluten is hardly soluble in aqueous systems, using an enzymatic gluten digest may be an option [98]. Data on tolerable quantities of wheat products are urgently needed. Correlations between the amount of wheat and the intensity of exercise with the severity of the hypersensitivity reaction should be clarified while taking other cofactors such as ASS, other NSAIDs, or alcohol into account. To better understand the immunological pathways of WDEIA, further research towards appropriate animal models has to be carried out. More research on all aspects of WDEIA will enable better diagnostics, increase awareness, and improve treatment options.

Acknowledgment

The H. + W. Winkler Stiftung Hilfe für den Menschen kindly supports research on and management of patients with wheat-dependent exercise-induced anaphylaxis.

Conflicts of interest

The authors declare that they have no conflict of interests.

References

- Food and Agriculture Organization of the United Nations. Available from: <http://faostat3.fao.org> (Last accessed 21 March 2015).
- Sicherer SH, Sampson HA. Food allergy: epidemiology, pathogenesis, diagnosis, and treatment. *J Allergy Clin Immunol* 2014; 133:291–307.
- Wölbing F, Fischer J, Köberle M, Kaesler S, Biedermann T. About the role and underlying mechanisms of cofactors in anaphylaxis. *Allergy* 2013; 68:1085–92.
- Tatham AS, Shewry PR. Allergens in wheat and related cereals. *Clin Exp Allergy* 2008; 38:1712–26.
- Ludvigsson JF, Green PH. Clinical management of coeliac disease. *J Intern Med* 2011; 269:560–71.
- Wieser H, Koehler P, Konitzer K. Celiac disease – multidisciplinary challenges and opportunities. 1st edn. London, Waltham, San Diego: Elsevier Ltd., Academic Press, 2014.
- Catassi C, Elli L, Bonaz B *et al.* Diagnosis of non-celiac gluten sensitivity (NCGS): the Salerno experts' criteria. *Nutrients* 2015; 7:4966–77.
- Morita E, Kohno K, Matsuo H. Food-dependent exercise-induced anaphylaxis. *J Dermatol Sci* 2007; 47:109–17.
- Kushimoto H, Aoki T. Masked type I wheat allergy. Relation to exercise-induced anaphylaxis. *Arch Dermatol* 1985; 121:355–60.
- Johansson SG, Hourihane JO, Bousquet J *et al.* A revised nomenclature for allergy. *Allergy* 2001; 56:813–24.
- Du Toit G. Food-dependent exercise-induced anaphylaxis in childhood. *Pediatr Allergy Immunol* 2007; 18:455–63.
- Chong S-U, Worm M, Zuberbier T. Role of adverse reactions to food in urticaria and exercise-induced anaphylaxis. *Int Arch Allergy Immunol* 2002; 129:19–26.
- Yaegashi T, Nakamura Y, Sakagami S, Saeki T, Omi W, Ikeda K. Acute myocardial infarction following food-dependent exercise-induced anaphylaxis. *Intern Med* 2011; 50:451–4.
- Noma T, Yoshizawa I, Ogawa N, Ito M, Aoki K, Kawano Y. Fatal buckwheat dependent exercise-induced anaphylaxis. *Asian Pac J Allergy Immunol* 2001; 19:283–6.
- Flannagan LM, Wolf BC. Sudden death associated with food and exercise. *J Forensic Sci* 2004; 49:543–5.
- Goldflam K, Silvers CT. Exercise-induced anaphylaxis as a cause of syncope. *J Emerg Med* 2012; 43:651–4.
- Maulitz RM, Pratt DS, Schocket AL. Exercise-induced anaphylactic reaction to shellfish. *J Allergy Clin Immunol* 1979; 63:433–4.

- 18 Kidd JM, Cohen SH, Sosman AJ, Fink JN. Food-dependent exercise-induced anaphylaxis. *J Allergy Clin Immunol* 1983; **71**:407–11.
- 19 Adachi A, Horikawa T, Shimizu H *et al.* Soybean beta-conglycinin as the main allergen in a patient with food-dependent exercise-induced anaphylaxis by tofu: food processing alters pepsin resistance. *Clin Exp Allergy* 2009; **39**:167–73.
- 20 Adachi A, Tanaka A, Chinuki Y, Morita E. Identification of 70 kDa shrimp protein as a positive new allergen for shrimp allergy. *Arerugi* 2013; **62**:960–7.
- 21 Tanaka S. An epidemiological survey on food-dependent exercise-induced anaphylaxis in kindergartners, schoolchildren and junior high school students. *Asia Pac J Public Health* 1994; **7**:26–30.
- 22 Aihara Y, Takahashi Y, Kotoyori T *et al.* Frequency of food-dependent, exercise-induced anaphylaxis in Japanese junior-high-school students. *J Allergy Clin Immunol* 2001; **108**:1035–9.
- 23 Dohi M, Suko M, Sugiyama H *et al.* Food-dependent exercise-induced anaphylaxis: a study on 11 Japanese cases. *J Allergy Clin Immunol* 1991; **87**:34–40.
- 24 Romano A, Di Fonso M, Giuffreda F *et al.* Diagnostic work-up for food-dependent, exercise-induced anaphylaxis. *Allergy* 1995; **50**:817–24.
- 25 Teo S-L, Gerez IFA, Ang EY, Shek LP. Food-dependent exercise-induced anaphylaxis – a review of 5 cases. *Ann Acad Med Singapore* 2009; **38**:905–9.
- 26 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.
- 27 Barg W, Medrala W, Wolanczyk-Medrala A. Exercise-induced anaphylaxis: an update on diagnosis and treatment. *Curr Allergy Asthma Rep* 2011; **11**:45–51.
- 28 Koehler P, Wieser H. Chemistry of cereal grains. In: Gobetti M, Gaenzle M, eds. *Handbook of sourdough biotechnology*. New York: Springer, 2013:11–45.
- 29 Koenig A, Konitzer K, Wieser H, Koehler P. Classification of spelt cultivars based on differences in storage protein compositions from wheat. *Food Chem* 2015; **168**:176–82.
- 30 Wieser H. Chemistry of gluten proteins. *Food Microbiol* 2007; **24**:115–9.
- 31 Wieser H, Kieffer R. Correlations of the amount of gluten protein types to the technological properties of wheat flours. *J Cereal Sci* 2001; **34**:19–27.
- 32 Harada S, Horikawa T, Ashida M, Kamo T, Nishioka E, Ichihashi M. Aspirin enhances the induction of type 1 allergic symptoms when combined with food and exercise in patients with food-dependent exercise-induced anaphylaxis. *Br J Dermatol* 2001; **145**:336–9.
- 33 Rongfei Z, Wenjing L, Nan H, Guanghui L. Wheat-dependent exercise-induced anaphylaxis occurred with a delayed onset of 10 to 24 hours after wheat ingestion: a case report. *Allergy Asthma Immunol Res* 2014; **6**:370–2.
- 34 Oyefara BI, Bahna SL. Delayed food-dependent, exercise-induced anaphylaxis. *Allergy Asthma Proc* 2007; **28**:64–6.
- 35 Morita E, Chinuki Y, Takahashi H. Recent advances of in vitro tests for the diagnosis of food-dependent exercise-induced anaphylaxis. *J Dermatol Sci* 2013; **71**:155–9.
- 36 Lehto M, Palosuo K, Varjonen E *et al.* Humoral and cellular responses to gliadin in wheat-dependent, exercise-induced anaphylaxis. *Clin Exp Allergy* 2003; **33**:90–5.
- 37 Lee J-Y, Yoon S, Ye Y-M, Hur GY, Kim S, Park H-S. Gliadin-specific IgE in wheat-dependent exercise-induced anaphylaxis. *Allergy Asthma Proc* 2008; **29**:614–21.
- 38 Wolanczyk-Medrala A, Barg W, Gogolewski G *et al.* Influence of hyperosmotic conditions on basophil CD203c upregulation in patients with food-dependent exercise-induced anaphylaxis. *Ann Agric Environ Med* 2009; **16**:301–4.
- 39 Hosey RG, Carek PJ, Goo A. Exercise-induced anaphylaxis and urticaria. *Am Fam Physician* 2001; **64**:1367–72.
- 40 Kato Y, Nagai A, Saito M, Ito T, Koga M, Tsuboi R. Food-dependent exercise-induced anaphylaxis with a high level of plasma noradrenaline. *J Dermatol* 2007; **34**:110–3.
- 41 Varjonen E, Vainio E, Kalimo K. Life-threatening, recurrent anaphylaxis caused by allergy to gliadin and exercise. *Clin Exp Allergy* 1997; **27**:162–6.
- 42 Palosuo K, Alenius H, Varjonen E *et al.* A novel wheat gliadin as a cause of exercise-induced anaphylaxis. *J Allergy Clin Immunol* 1999; **103**:912–7.
- 43 Morita E, Yamamura Y, Mihara S, Kameyoshi Y, Yamamoto S. Food-dependent exercise-induced anaphylaxis: a report of two cases and determination of wheat gamma-gliadin as the presumptive allergen. *Br J Dermatol* 2000; **143**:1059–63.
- 44 Morita E, Kameyoshi Y, Mihara S, Hiragun T, Yamamoto S. Gamma-gliadin: a presumptive allergen causing wheat-dependent exercise-induced anaphylaxis. *Br J Dermatol* 2001; **145**:182–4.
- 45 Palosuo K, Varjonen E, Kekki OM *et al.* Wheat omega-5 gliadin is a major allergen in children with immediate allergy in ingested wheat. *J Allergy Clin Immunol* 2001; **108**:634–8.
- 46 Morita E, Matsuo H, Mihara S, Morimoto K, Savage AWJ, Tatham AS. Fast omega-gliadin is a major allergen in wheat-dependent exercise-induced anaphylaxis. *J Dermatol Sci* 2003; **33**:99–104.
- 47 Matsuo H, Morita E, Tatham AS *et al.* Identification of the IgE-binding epitope in ω -5 gliadin, a major allergen in wheat-dependent exercise-induced anaphylaxis. *J Biol Chem* 2004; **279**:12135–40.
- 48 Matsuo H, Kohno K, Morita E. Molecular cloning, recombinant expression and IgE-binding epitope of omega-5 gliadin, a major allergen in wheat-dependent exercise-induced anaphylaxis. *FEBS J* 2005; **272**:4431–8.
- 49 Matsuo H, Kohno K, Niihara H, Morita E. Specific IgE determination to epitope peptides of omega-5 and high molecular weight glutenin subunit is a useful tool for diagnosis of wheat-dependent exercise-induced anaphylaxis. *J Immunol* 2005; **175**:8116–22.
- 50 Battais F, Mothes T, Moneret-Vautrin DA *et al.* Identification of IgE-binding epitopes on gliadins for patients with food allergy to wheat. *Allergy* 2005; **60**:815–21.
- 51 Palosuo K, Alenius H, Varjonen E, Kalkkinen N, Reunala T. Rye γ -70 and γ -35 secalins and barley γ -3 hordein cross-react with ω -5 gliadin, a major allergen in wheat-dependent, exer-

- cise-induced anaphylaxis. *Clin Exp Allergy* 2001; 31:466–73.
- 52 Palosuo K, Varjonen E, Nurkkala J *et al.* 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.
 - 53 Morita E, Matsuo H, Chinuki Y, Takahashi H, Dahlström J, Tanaka A. Food-dependent exercise-induced anaphylaxis – importance of omega-5 gliadin and HMW-glutenin as causative antigens for wheat-dependent exercise-induced anaphylaxis. *Allergol Int* 2009; 58:493–8.
 - 54 Snegaroff J, Branlard G, Bouchez-Mahiout I *et al.* Recombinant proteins and peptides as tools for studying IgE reactivity with low-molecular-weight glutenin subunits in some wheat allergies. *J Agric Food Chem* 2007; 55:9837–45.
 - 55 Bouchez-Mahiout I, Snegaroff J, Tylichova M, Pecquet C, Branlard G, Lauriere M. Low molecular weight glutenins in wheat-dependent exercise-induced anaphylaxis: allergenicity and antigenic relationships with omega 5-gliadins. *Int Arch Allergy Immunol* 2010; 153:35–45.
 - 56 Hofmann SC, Fischer J, Eriksson C, Bengtsson Gref O, Biedermann T, Jakob T. IgE detection to $\alpha/\beta/\gamma$ -gliadin and its clinical relevance in wheat-dependent exercise-induced anaphylaxis. *Allergy* 2012; 67:1457–60.
 - 57 Denery-Papini S, Bodinier M, Larre C *et al.* Allergy to deamidated gluten in patients tolerant to wheat: specific epitopes linked to deamidation. *Allergy* 2012; 67:1023–32.
 - 58 Hanakawa Y, Tohyama M, Shirakata Y, Murakami S, Hashimoto K. Food-dependent exercise-induced anaphylaxis: a case related to the amount of food allergen ingested. *Br J Dermatol* 1998; 138:898–900.
 - 59 Izumi K, Aihara M, Ikezawa Z. Effects of nonsteroidal antiinflammatory drugs (NSAIDs) on immediate-type food allergy analysis of Japanese cases from 1998 to 2009. *Arerugi* 2009; 58:1629–39.
 - 60 Matsuo H, Yokooji T, Morita E *et al.* Aspirin augments IgE-mediated histamine release from human peripheral basophils via Syk kinase activation. *Allergol Int* 2013; 62:503–11.
 - 61 Matsukura S, Aihara M, Sugawara M *et al.* Two cases of wheat-dependent anaphylaxis induced by aspirin administration but not by exercise. *Clin Exp Dermatol* 2009; 35:233–7.
 - 62 Fujii H, Kambe N, Fujisawa A, Kohno K, Morita E, Miyachi Y. Food-dependent exercise-induced anaphylaxis induced by low dose aspirin therapy. *Allergol Int* 2008; 57:97–8.
 - 63 Asero R, Mistrello G, Roncarolo D, Antoniotto P, Falagiani P. Exercise-induced egg anaphylaxis. *Allergy* 1997; 52:687–9.
 - 64 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.
 - 65 Matsuo H, Morimoto K, Akaki T *et al.* Exercise and aspirin increase levels of circulating gliadin peptides in patients with wheat-dependent exercise-induced anaphylaxis. *Clin Exp Allergy* 2005; 35:461–6.
 - 66 Inoue Y, Adachi A, Ueno M *et al.* The inhibition effect of a synthetic analogue of prostaglandin E1 to the provocation by aspirin in the patients of WDEIA. *Arerugi* 2009; 58:1418–25.
 - 67 Fukunaga A, Shimizu H, Tanaka M *et al.* Limited influence of aspirin intake on mast cell activation in patients with food-dependent exercise-induced anaphylaxis: comparison using skin prick and histamine release tests. *Acta Derm Venereol* 2012; 92:480–3.
 - 68 Fiedler E-M, Zuberbier T, Worm M. A combination of wheat flour, ethanol and food additives inducing FDEIA. *Allergy* 2002; 57:1090–1.
 - 69 Irvine AD, McLean WH, Leung DYM. Filaggrin mutations associated with skin and allergic diseases. *N Engl J Med* 2011; 365:1315–27.
 - 70 Mizuno O, Nomura T, Ohguchi Y *et al.* Loss-of function mutations in the gene encoding filaggrin underlie a Japanese family with food-dependent exercise-induced anaphylaxis. *JEADV* 2015; 29:805–8.
 - 71 Cooper DM, Radom-Aizik S, Schwindt C, Zaldivar F. Dangerous exercise: lessons learned from dysregulated inflammatory responses to physical activity. *J Appl Physiol* 2007; 103:700–9.
 - 72 Barg W, Wolanczyk-Medrała A, Obojski A, Wytrychowski K, Panaszek B, Medrała W. Food-dependent exercise-induced anaphylaxis: possible impact of increased basophil histamine releasability in hyperosmolar conditions. *J Invest Allergol Clin Immunol* 2008; 18:312–5.
 - 73 Robson-Ansley P, Du Toit G. Pathophysiology, diagnosis and management of exercise-induced anaphylaxis. *Curr Opin Allergy Clin Immunol* 2010; 10:312–7.
 - 74 Elenkov IJ, Wilder RL, Chrousos GP, Vizi ES. The sympathetic nerve – an integrative interface between two supersystems: the brain and the immune systems. *Pharmacol Rev* 2000; 52:595–638.
 - 75 Kozai H, Yano H, Matsuda T, Kato Y. Wheat-dependent exercise-induced anaphylaxis in mice is caused by gliadin and glutenin treatments. *Immunol Lett* 2006; 102:83–90.
 - 76 Bodinier M, Brossard C, Triballeau S *et al.* Evaluation of an in vitro mast cell degranulation test in the context of food allergy to wheat. *Int Arch Allergy Immunol* 2008; 146:307–20.
 - 77 Tanaka M, Nagano T, Yano H *et al.* Impact of ω -5 gliadin on wheat-dependent exercise-induced anaphylaxis in mice. *Biosci Biotechnol Biochem* 2011; 75:313–7.
 - 78 Wong GKY, Huissoon AP, Goddard S, Collins DM, Krishna MT. Wheat dependent exercise induced anaphylaxis: is this an appropriate terminology? *J Clin Pathol* 2010; 63:814–7.
 - 79 Brockow K, Kneissl D, Valentini L *et al.* Using a gluten oral food challenge protocol to improve diagnosis of wheat-dependent exercise-induced anaphylaxis. *J Allergy Clin Immunol* 2015; 135:977–84.
 - 80 Kohno K, Matsuo H, Takahashi H *et al.* Serum gliadin monitoring extracts patients with false negative results in challenge tests for the diagnosis of wheat-dependent exercise-induced anaphylaxis. *Allergol Int* 2013; 62:229–38.
 - 81 Chinuki Y, Kaneko S, Dekio I *et al.* CD203c expression-based basophil activation test for diagnosis of wheat-dependent exercise-induced anaphylaxis. *J Allergy Clin Immunol* 2012; 129:1404–6.

- 82 Urisu A, Ebisawa M, Ito K *et al.* Japanese guideline for food allergy 2014. *Allergol Int* 2014; **63**:399–419.
- 83 Hamilton RG, Adkinson NF Jr. In vitro assays for the diagnosis of IgE-mediated disorders. *J Allergy Clin Immunol* 2004; **114**:213–25.
- 84 Matsuo H, Dahlström J, Tanaka A *et al.* Sensitivity and specificity of recombinant omega-5 gliadin-specific IgE measurement for the diagnosis of wheat-dependent exercise-induced anaphylaxis. *Allergy* 2008; **63**:233–6.
- 85 Park H-J, Kim J-H, Kim J-E *et al.* Diagnostic value of the serum-specific IgE ratio of ω -5 gliadin to wheat in adult patients with wheat-induced anaphylaxis. *Int Arch Allergy Immunol* 2012; **157**:147–50.
- 86 Brans R, Sauer I, Czaja K, Pfuetschner W, Merk HF. Microarray-based detection of specific IgE against recombinant ω -5-gliadin in suspected wheat-dependent exercise-induced anaphylaxis. *Eur J Dermatol* 2012; **22**:358–62.
- 87 Jacquenet S, Morisset M, Battais F *et al.* Interest of ImmunoCap system to recombinant ω -5 gliadin for the diagnosis of exercise-induced wheat allergy. *Int Arch Allergy Immunol* 2009; **149**:74–80.
- 88 Takahashi H, Matsuo H, Chinuki Y *et al.* Recombinant high molecular weight-glutenin subunit-specific IgE detection is useful in identifying wheat-dependent exercise-induced anaphylaxis complementary to recombinant omega-5 gliadin-specific IgE test. *Clin Exp Allergy* 2012; **42**:1293–8.
- 89 Choi JH, Lee HB, Ahn IS, Park CW, Lee CH. Wheat-dependent, exercise-induced anaphylaxis: a successful case of prevention with ketotifen. *Ann Dermatol* 2009; **21**:203–5.
- 90 Sugimura T, Tananari Y, Ozaki Y *et al.* Effect of oral sodium cromoglycate in 2 children with food-dependent exercise-induced anaphylaxis (FDEIA). *Clin Pediatr (Phila)* 2009; **48**:945–50.
- 91 Varjonen E, Petman L, Makinen-Kiljunen S. Immediate contact allergy from hydrolyzed wheat in a cosmetic cream. *Allergy* 2000; **55**:294–6.
- 92 Pecquet C, Lauriere M. New allergens in hydrolysates of wheat proteins. *Rev Fr Allergol Immunol Clin* 2003; **43**:21–3.
- 93 Chinuki Y, Morita E. Wheat-dependent exercise-induced anaphylaxis sensitized with hydrolyzed wheat protein in soap. *Allergol Int* 2012; **61**:529–37.
- 94 Hiragun M, Ishii K, Hiragun T *et al.* The sensitivity and clinical course of patients with wheat-dependent exercise-induced anaphylaxis sensitized to hydrolyzed wheat protein in facial soap – secondary publication. *Allergol Int* 2013; **62**:351–8.
- 95 Snegaroff J, Bouchez-Mahiout I, Pecquet C, Branlard G, Lauriere M. Study of IgE antigenic relationships in hypersensitivity to hydrolyzed wheat proteins and wheat-dependent exercise-induced anaphylaxis. *Int Arch Allergy Immunol* 2006; **139**:201–8.
- 96 Yokooji T, Kurihara S, Murakami T *et al.* Characterization of causative allergens for wheat-dependent exercise-induced anaphylaxis sensitized with hydrolyzed wheat proteins in facial soap. *Allergol Int* 2013; **62**:435–45.
- 97 Nakamura M, Yagami A, Hara K *et al.* A new reliable method for detecting specific IgE antibodies in the patients with immediate type wheat allergy due to hydrolyzed wheat protein: correlation of its titer and clinical severity. *Allergol Int* 2014; **63**:243–9.
- 98 Ellis HJ, Pollock EL, Engel W *et al.* Investigation of the putative immunodominant T cell epitopes in coeliac disease. *Gut* 2003; **52**:212–7.
- 99 Gellrich C, Schieberle P, Wieser H. Biochemical characterization and quantification of the storage protein (secalin) types in rye flour. *Cereal Chem* 2003; **80**:102–9.
- 100 Lange M, Vincze E, Wieser H, Schjoerring JK, Holm PB. Suppression of C-hordein synthesis in barley by antisense constructs results in a more balanced amino acid composition. *J Agric Food Chem* 2007; **55**:6074–81.