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Specific IgG levels to wheat in wheat tolerant professional cyclists may depend on a homeostatic immune response to a high consumption of wheat

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Key words

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Summary

Background. *Implication of IgG antibodies to wheat has been alleged in gastrointestinal symptoms. Precise data on the specific IgG levels in healthy subjects are lacking. Our objectives are to compare levels of IgG antibodies to wheat protein fractions in healthy non atopic or atopic subjects, and in healthy professional cyclist subjects, taking into account the quantitative consumption of wheat.* ***Methods***. *24 control subjects and 26 professional cyclist subjects were selected. ELISA was performed to 2 wheat commercial solutions and to 3 wheat protein fractions.* ***Results***. *No significant difference was observed between non atopic and atopic subjects. For wheat flour extract, physiological norm determined was 3.27 mg/L sIgG concentration ± 1.25 CI (95% confidence intervals) for the professional cyclists (vs 1.56 mg/L ± 0,91 CI in control subjects, p-value: 0.040). For gluten solution, physiological norm was 1.42 mg/L ± 0.60 CI (vs 0.50 ± 0,24 CI in control subjects, p-value: 0.010).* ***Conclusion***. *Atopic and non atopic healthy adults have a similar level of sIgG to wheat. Increased levels of sIgG are observed correlatively with an excessive consumption, and could contribute to homeostasis of tolerance. Studies searching for a pathogenic role of sIgG in certain pathologies should take into account the quantitative consumption.*

Abbreviations
IBS: Irritable bowel syndrome
AD: Atopic dermatis
SPT: Skin prick test
SD: Standard deviation
UL: Upper limit
WA: Wheat allergic

Introduction

Wheat is a staple food worldwide. It can be responsible of food hypersensitivity in individuals at all ages, as coeliac disease, IgE-mediated food allergy (multiple food allergy syndrome in infants, exercise-induced anaphylaxis, anaphylactic shock, atopic dermatitis (AD), chronic urticaria, recurring angioedema) (1-5) or gluten sensitivity (6). Gastrointestinal

symptoms can be induced by wheat allergy (4, 7). Rare cases of ulcerous colitis and of Crohn disease have been described (8). More recently wheat has been incriminated in eosinophilic esophagitis, and eosinophilic gastroenteritis and colitis (9, 10). Wheat allergy has been incriminated in the pathogenesis of irritable bowel syndrome (IBS). The implication of IgG-dependent mechanism has been alleged (11-13). In IgE dependent wheat allergy, increased levels of sIgG have been observed (14, 15). Antigenic profiles of IgE and IgG antibodies against prolamins have been shown to be quite similar (16).

However sIgG antibodies are found in healthy subjects, reflecting the physiological immune response of tolerance to dietary proteins (17, 18). Thus, the meaning of wheat sIgG in gastrointestinal allergy should be revisited in the light of precise data of the sIgG levels of healthy subjects who tolerate wheat flour, in order to take into account a possible relation between this level and the quantities regularly consumed.

The aims of this study were then to compare the levels of IgG antibodies to different fractions of wheat in healthy non-atopic and atopic subjects, and to examine the relationship of sIgG with the consumption of wheat products, comparing these healthy subjects to healthy professional cyclist subjects who are characterized by an important daily consumption of breakfast cereals, pasta, and bread.

Materials and methods

Population selected for the biological study

The study was carried out with 2 populations of healthy subjects, tolerant to wheat. A written consent was obtained.

The group 1 included subgroup 1A: 12 non atopic subjects (11 females and 1 male) with an age range of 24 to 62 years (means: 44 years). The subgroup 1B included 12 atopic subjects (8 females and 4 males) with an age range of 7 to 40 years (means: 24 years).

The group 2 included 26 professional cyclists males aged between 19 to 35 years (means: 26 years). Of them, subgroup 2A included 12 atopic cyclists and subgroup 2B included 14 non atopic. They were training regularly and intensively several hours per day all the year.

Criteria of selection

Atopy was based on a past history of atopic dermatitis or infantile asthma joined to positive prick test to one or more common allergens. Besides, subgroup 2A was selected on the basis of allergy to grass pollen in order to take into account a possible cross reactivity of IgG antibodies between wheat flour proteins and grass pollen proteins (19).

Non atopic subjects were characterized on the basis of no previous atopic diseases (AD, asthma or allergic rhinitis) and on the negativity of skin prick tests (SPTs) to 12 common aeroallergens including grass pollen.

Population selected for evaluation of wheat protein consumption

This evaluation was carried out on 26 professional cyclists (group 2) and 10 healthy male subjects with similar ages.

The estimation was based on a questionnaire with medical interview to estimate the frequency of the consumptions of food with wheat flour. The subjects had photos of known quantitative portions. We made sure of stable consumer habits and the information made reference to the two weeks before the questionnaire.

The quantitative estimation was based on the totality of the wheat proteins ingested weekly. The protein quantities were determined on the following base: 100 g of bread = 7.5 g of proteins (250 g of French stick = 18.5 g of proteins), 100 g of muesli represent 8.8 g of proteins including 66% of cereals = 5.8 g of cereal proteins. A large plate (180 g of cooked pasta) corresponds to 90 g of dried pasta = 11.25 g of wheat proteins.

Skin prick tests

Skin prick tests were performed with 12 aeroallergens: *Dermatophagoides pteronyssinus* and *D. farinae*, cockroaches, Alternaria, cat and dog epithelia, latex and grass, tree, Artemisia, plantain and ash pollens. SPT were carried out with two commercial wheat extracts: water/salt soluble fraction containing albumin/globulin proteins (wheat flour extract) and ethanol soluble fraction containing gluten proteins (gluten extract) (ALK-Abelló, France). The negative and positive controls SPT were physiological saline serum, control-gluten buffer (negative controls), and 9% codeine phosphate (positive control). A SPT was considered positive if the wheal diameter was at least 3 mm larger than the diameter of the negative control and/or >50% of the diameter of the positive control .

Biological study

Wheat protein fractions

Albumin/globulin and gliadin fractions were obtained as

previously described (16, 20). Protein concentration was determined by Bradford method (Interchim) with bovine serum albumin as a standard. Wheat flour and gluten SPT solutions were used as allergen reference in specific IgG detection test.

Specific IgG levels

Enzyme-linked immunosorbent assay (ELISA) were performed using albumin/globulin, gliadin and glutenin stock fractions, and wheat SPT solutions (wheat flour and gluten). ELISA were performed in wells of microtiter plates (Immulon-2HB, Thermo Lab systems, Franklin, MA, USA) coated with 500 ng of allergen extracts in 0.05 M carbonate buffer pH 9.6, overnight at 4°C. After this and each subsequent step, the wells were washed three times with PBS buffer containing 0.05% (v/v) Tween 20 (PBS/T). Unoccupied binding sites were blocked with PBS/T containing 0.5% (w/v) gelatin (SERVA Electrophoresis, GmbH, Heidelberg) (PBS/T/G) during 1 h at 37°C. The plates were incubated 2 h at 37°C with sera from the subjects diluted 1:50 in PBS/T/G.

One serum was used as calibrator, selected on the basis of its high levels of sIgG antibodies to wheat proteins (gluten [f79] and wheat flour [f4]), as previously determined by UniCAP-100 system (Pharmacia Diagnosis, Uppsala, Sweden). This reference serum contained 12 mg/L of specific IgG to wheat flour and 7 mg/L to gluten. After dilution 1:10 of reference serum in PBS/T/G, 100 µL of twofold serial dilution were added in wells coated by wheat SPT solutions (wheat flour extract and gluten extract).

Peroxidase-labelled goat anti-human IgG (γ-chain specific, A-2290, Sigma) diluted 1:10000 in PBS/T/G were added 2 h at 37°C (100 µL/well). The peroxidase activity was detected by adding the peroxidase substrate (TMB/H2O2 substrate, KPL, Maryland, USA), and the plates were incubated 1 h at room temperature. The absorbance was read at 595 nm with an automated ELISA reader (OpsysMR, Dynex, Thermo Life Sciences). All sera tested were performed in duplicates. For each serum, the absorbance values of wells containing no antigen were subtracts from absorbance values of wheat proteins tested.

Tests for specific IgG detection were assessed in all non atopic, atopic individuals, and cyclist subjects for three wheat fractions and the two wheat SPT solutions. Using serial dilution of reference serum, a linear standard curve was expressed between specific IgG levels to wheat flour extract or gluten extract and absorbance values of each serum. The standard curve of wheat flour extract was used to obtain sIgG levels to wheat flour extract and albumins/globulins fraction. The standard curve of gluten extract was used to obtain sIgG levels to gluten extract, gliadins and glutenins fractions. Levels were expressed in mg/L of specific IgG.

Statistical analyses

The clinical diagnosis of non atopic or atopic subjects was considered as the reference. Data were represented as mean values and ± 95% confidence intervals (CI). Statistical analyses were performed using the Fisher test and the Student t test to compare arithmetic mean values performed. Two populations were significantly identical is rejected if p-value is less than 0.05 at the 95% confidence level.

Results

All the subjects had negative SPT to the two commercial wheat extracts (wheat flour and gluten).

The weekly consumption of wheat proteins was evaluated semi-quantitatively to be 70.91 g ± 44.65 g for 10 healthy young male subjects. The consumption of wheat proteins was 133.25 g ± 43.85 g for 26 professional cyclist subjects ($P<0.001$).

Specific IgG from non atopic and atopic subjects

The reactivity of sIgG, from 12 non atopic and 12 atopic subject sera, to wheat SPT solutions (wheat flour extract and gluten extract) and to wheat protein fractions (albumin/globulin, gliadin and glutenin fractions) determined by ELISA showed that 83% of non atopic sera and 92% of atopic sera contain sIgG antibodies to at least one wheat protein fraction. The reactivity of sIgG antibodies was similar on frequencies and levels between wheat flour extract and albumin/globulin fraction, and between gluten extract and gliadin fraction (Table 1). In contrast, sIgG levels to glutenin fraction were very weak. No significant difference was observed between non atopic and atopic subjects for sIgG level mean values and standard deviations (SD) with wheat flour and gluten extract (Table 2) and wheat protein fractions (Fig. 1A).

Therefore, physiological norms (mean value), SD and physiological upper limit (UL) (physiological norms adding to 2 SD) were determined for these 24 subjects. For wheat flour, the physiological norm of sIgG concen-

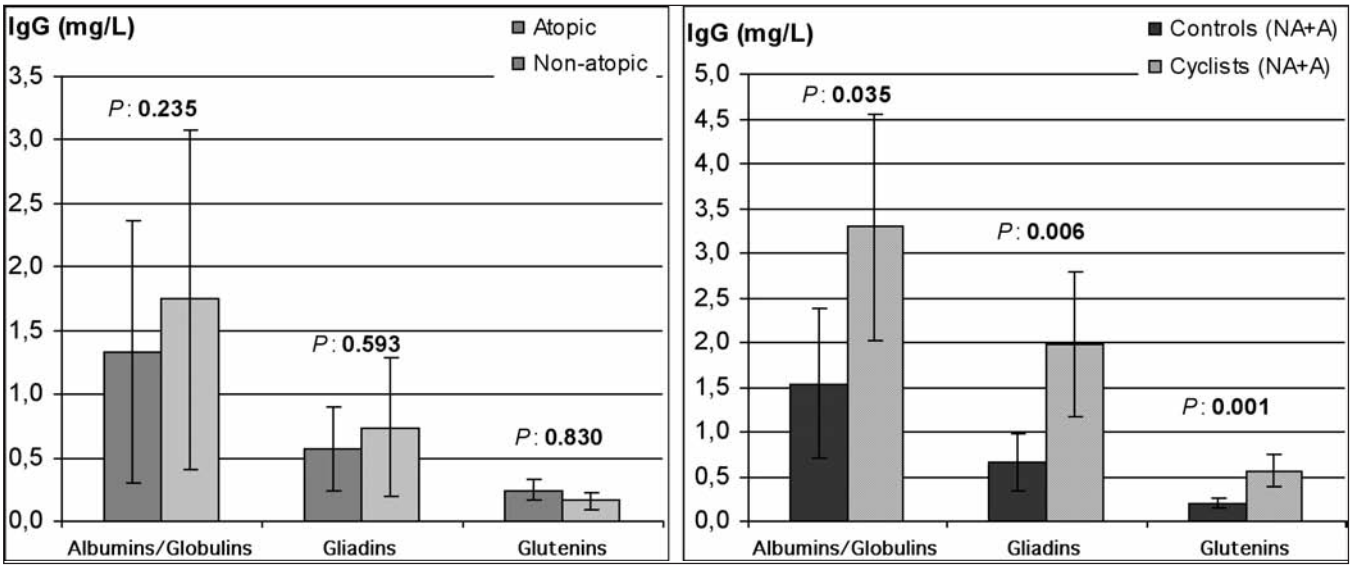
Table 1 - Specific IgG concentrations (mg/L) to SPT solutions and three wheat fractions for non-atopic and atopic subjects and professional cyclists obtained by ELISA. Specific IgG responses reported were sIgG concentrations in mg/L obtained by the background-corrected absorbance of samples where serum was incubated in wells without antigen and then calculated using reference serum which IgG concentration was known

Group	Controls	Wheat flour extract	Albumins/ globulins	Gluten extract	Gliadins	Glutenins	Group	Cyclists	Wheat flour extract	Albumins/ globulins	Gluten extract	Gliadins	Glutenins
1A	99	0.14	0.00	0.00	0.00	0.07	2A	C-1	7.27	7.35	3.88	4.90	0.43
	86	3.95	4.28	1.08	1.66	0.16		C-2	0.72	0.63	0.00	0.10	0.43
	87	5.50	5.24	2.18	2.88	0.33		C-3	0.80	0.62	0.11	0.19	0.58
	88	0.26	0.23	0.14	0.20	0.36		C-4	5.13	4.04	2.48	4.25	0.22
	188	4.07	3.14	0.83	0.86	0.37		C-5	1.37	1.48	1.26	1.16	0.50
	187	0.83	0.84	0.32	0.28	0.12		C-6	0.30	0.30	0.39	0.44	0.00
	193	7.69	6.84	1.59	2.35	0.10		C-7	7.72	8.08	3.98	5.36	2.37
	192	0.00	0.00	0.00	0.10	0.18		C-8	2.43	2.33	0.70	1.09	0.71
	191	0.14	0.18	0.32	0.42	0.15		C-9	5.06	5.43	2.70	4.15	0.55
	186	0.00	0.00	0.00	0.00	0.00		C-10	1.70	1.68	0.35	0.38	0.87
	185	0.00	0.00	0.00	0.00	0.00		C-11	2.94	2.47	0.87	1.02	0.50
	190	0.23	0.17	0.00	0.08	0.08		C-12	0.80	0.79	0.33	0.42	0.36
1B	90	0.00	0.00	0.13	0.16	0.29		C-13	0.00	0.00	0.45	0.37	0.15
	91	7.10	7.14	1.44	1.55	0.49		C-14	0.30	0.56	0.42	0.98	0.40
	94	0.00	0.00	0.41	0.31	0.17	2B	C-15	7.68	9.13	1.22	1.28	0.36
	176	0.24	0.28	0.16	0.32	0.37		C-16	1.23	0.86	0.60	0.88	0.51
	177	1.73	1.87	0.95	1.41	0.17		C-17	3.38	4.21	1.41	1.70	0.48
	178	0.14	0.28	0.09	0.10	0.12		C-18	0.62	0.59	0.10	0.11	0.16
	180	2.10	2.09	1.24	1.69	0.31		C-19	4.11	3.54	0.69	0.87	0.19
	181	1.34	1.53	0.33	0.34	0.21		C-20	1.18	1.20	0.68	0.92	0.99
	163	0.59	0.45	0.07	0.08	0.30		C-21	9.73	9.82	4.01	5.42	0.96
	189	1.12	1.06	0.00	0.10	0.00		C-22	3.51	3.10	1.17	1.63	0.43
	184	0.00	0.00	0.00	0.00	0.00		C-23	12.21	11.88	6.33	8.36	1.36
	183	0.32	1.28	0.82	0.80	0.45		C-24	3.00	3.37	1.76	3.52	0.41
	Mean	1.56	1.54	0.50	0.65	0.20		C-25	1.63	1.75	0.62	1.39	0.10
	Median	0.29	0.37	0.24	0.30	0.17		C-26	0.17	0.26	0.36	0.73	0.47
								Mean	3.27	3.29	1.42	1.99	0.56
								Median	2.07	2.04	0.70	1.06	0.45

Table 2 - sIgG concentration mean values to wheat flour and gluten extract between non-atopic and atopic subjects and between controls and professional cyclists. CI: 95% confidence intervals; P: p-values calculated by student t tests; SD: standard deviation. Fisher and Student tests were used for each analysis but only p-values obtained with Student test were showed.

		Wheat flour extract			Gluten extract		
		Mean (mg/L) ± CI	SD	P	Mean (mg/L) ± CI	SD	P
Controls	Non-atopic	1.90 ± 1.52	2.68	0.489	0.54 ± 0.41	0.73	0.793
	Atopic	1.22 ± 1.08	1.99		0.47 ± 0.28	0.51	
Cyclists	Non-atopic	2.61 ± 1.38	2.64	0.275	1.28 ± 0.73	1.39	0.638
	Atopic	4.04 ± 2.18	3.85		1.58 ± 1.02	1.81	
Controls (NA+A)		1.56 ± 0.91	2.33	0.040	0.50 ± 0.24	0.62	0.010
Cyclists (NA+A)		3.27 ± 1.25	3.26		1.42 ± 0.60	1.57	

Figure 1 - Comparison of sIgG concentration (mg/L) mean values and 95% confidence intervals to wheat protein fractions between atopic and non-atopic subjects (1A) and between controls (atopic and non-atopic) and professional cyclists (1B), obtained by ELISA. P: p-values calculated by student t tests to analyse relationships between atopic and non-atopic subjects and then controls (atopic and non-atopic) and professional cyclists.



tration was 1.56 mg/L with 2.33 SD and the physiological UL (adding to 2 SD, 1.56 + 2 X 2.33) was 6.22 mg/L. For gluten, the physiological norm was 0.50 mg/L with 0.62 SD and the physiological UL was 1.74 mg/L. For gliadins, the same values were 0.65 mg/L (SD: 0.82) and 2.29 mg/L respectively.

Specific IgG from professional cyclists compared to controls

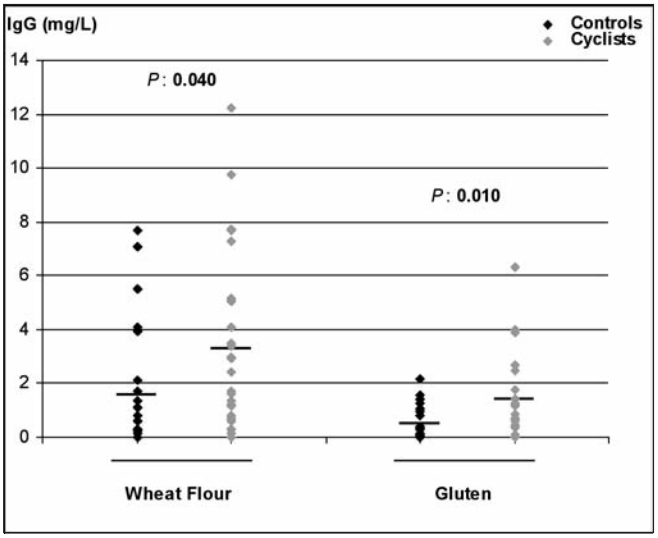
100% of the cyclist sera (vs. 88% of the control subjects) had specific IgG directed to at least one wheat protein fraction (Table 1). No significant difference was observed between non atopic and atopic cyclists for sIgG level mean values and SD with wheat flour and gluten extract (Table 2) and wheat protein fractions (data not shown). Statistical analyses of sIgG level mean values to wheat flour (P:0.040) and gluten (P:0.010) SPT solutions revealed a significant difference between control and professional cyclist subject sera (Table 2 and Fig. 2). Specific IgG levels obtained to albumin/globulin, gliadin and glutenin fractions were also

statistically higher in cyclists sera than in controls sera (Fig. 1B). In addition, the mean value and the UL (mean + 2 SD) were higher (3.27 and 9.79 mg/L) for wheat flour, as it was for gluten (1.42 and 4.56 mg/L) (Table 2). However, as observed for cyclists, some individuals in the control group have also high sIgG levels sera principally against the wheat flour extract (Fig. 2).
Seven cyclists out of 26 exceed the physiological UL (27%).

Discussion

The weekly consumption of wheat proteins in 10 healthy young male subjects was correlated with the general data from INSEE for the total French population (data from the French National Institute of Statistics (INSEE)). The consumption for professional cyclists subjects was significantly higher ($P < 0.001$).
Specific IgG have been studied in celiac disease, wheat allergy and in IBS (11, 21-23). In the celiac disease, the diagnosis depends on anti-transglutaminase IgG. Specific IgG to gliadins are most often associated. Specific IgG to gliadin deamidated peptides could have a certain interest for the diagnosis (21, 22). In food allergy, the presence of sIgG is commonly associated with the specific IgE (23). It should be emphasized that specific IgG to food are not reliable tools of diagnosis (24, 25). In IBS, specific IgG have been shown and a single randomized study has indicated an improvement of IBS by an avoidance diet based on these specific IgG (11). However, their implication in the pathogeny has been debated (12).
Noteworthy, food sIgG antibodies are a part of a normal immune response and depend on exposure to foods. Data upon the levels of anti-gluten and anti-gliadin IgG levels in healthy individuals are scarce (26-28).
This series characterizes specific IgG by ELISA to salt soluble albumins/globulins, ethanol soluble gliadins and glutenins fractions of wheat. Data show that the water/salt soluble fraction is much more immunogenic than gliadins, glutenins being less immunogenic (Fig 1A and 1B). The higher immunogenicity of albumins/globulins by the oral route compared to gluten proteins which are yet the major flour proteins, may be due to their solubility and better ability to be absorbed in the gut. Wheat water/salt soluble proteins have been shown for a long time to play an important role as inhalant and food allergens. 81% of wheat allergic (WA) children with AD and 92% of WA children with AD and asthma, have IgE antibodies to albumins/globulins (29). Non soluble gluten proteins may rather consider 82% of WA patients with urticaria. 100% of WA patients with anaphylaxis have specific IgE to prolamins (7, 29).
In this study, atopic and non atopic subjects were for the greater part women while the cyclists are all men. However the sex has never been incriminated as a possible difference of level of anti-food antibodies. Specific IgG to wheat flour proteins were analyzed from 12 non atopic, 12 atopic and 26 professional cyclist subject sera. IgG levels to any fraction of wheat are similar in atopic and non atopic adults, though elevated IgG to foods (including wheat) are correlated with atopy in young children with atopic dermatitis (30). The explanation could be linked to a gut hyperpermeability since it is frequently associated with eczema (31, 32). Thus, we considered physiological mean value and UL for the 24 control subjects. The physiological upper limits of sIgG antibodies were determined at 6.22 mg/L and 1.74 mg/L for wheat flour and gluten, respectively.
The upper limits of sIgG antibodies in sera from cyclists were 9.79 mg/L and 4.56 mg/L for wheat flour and gluten proteins, respectively, about two times higher than those observed from subjects of same sex and age with a

Figure 2 - ELISA performed on 24 control subjects and 26 professional cyclists sera to analyse specific reactivity against wheat flour and gluten extracts (SPT solutions). Each point represent the sIgG concentration (mg/L) obtained for each serum and the bars show the mean values of each group. P: p-values calculated by student t tests to analyse relationships between controls (atopic and non-atopic) and professional cyclists.



common consumption of wheat. Professional cyclists consumed a high quantity of wheat foods: 133.25 g of proteins ± 43.85 g, vs 70.91 g ± 44.65 g in control subjects ($P < 0.001$). The ratio of the mean sIgG of Cyclist/Controls is 2.10 for the wheat flour and 2.84 for gluten.

It is known that the exercise is a triggering factor for the IgE dependent wheat food allergy (7). Among the studied mechanisms, the accent is put on the intestinal hyperpermeability due to effort (33, 34). This one is secondary to the splanchnic hypoperfusion and to the ischaemia (35). It was shown that the intestinal hyperpermeability provokes a greater passage of the food antigens, which provoke a greater synthesis of the specific IgGs (36).

However, this increase of the specific IgGs can be also due to a higher consumption of wheat flour. This increase of the IgGs can be considered as a physiological immune response, aiming to control the potential pathological risk of a strong antigenic stimulation. Indeed, the cyclists are asymptomatic and even the atopic cyclists are not sensitized to wheat.

A complementary study concerning sedentary subjects having a high consumption would make clear, by comparison with these data, the relative implication of these factors.

In conclusion, this study provides a physiological norm of specific IgG to wheat antigenic main fractions, in atopic and non atopic subjects, with the prerequisite of a usual consumption of wheat. The fact that the level is significantly increased for both soluble and insoluble fractions of wheat in subjects with a raised consumption indicates that studies searching for a pathogenic role of specific IgG to wheat in every kind of diseases with a suspected intestinal hyperpermeability should take into account the consumption of wheat products.

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