Monitoring of Daily Gliadin Intake in Patients on Gluten-free Diets

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Abstract: The aim of the study was to show patients suffering from the coeliac disease, their real gliadin daily intake, offer them very useful information concerning their diet and to find random possible mistakes. The monitoring was carried out within the context of their routine everyday diet regimen. The daily intake of gliadin in the diet was quantified on the basis of gliadin determination in their current daily food. The gluten-free diet was followed for 30 days. The patients were taking regular daily meals, drinks, and sometimes medicines or food supplements. The patients were provided with instructions, survey forms, digital scales, polyethylen bottles and sacks. The patients took out the stipulated amount, which served as a sample of each of their daily meals. The samples included both homemade meals as well as commercial products. The content of gliadin in daily meal was determined by the sandwich ELISA method. The daily gliadin intake was calculated on the base of the reported amount of meals ingested. 1,900 food samples were analyzed within the framework of this study. Several contaminated commercial foods were found; nevertheless this fact did not influence the otherwise satisfactory overall

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picture of the daily gliadin intake by the patients followed. The results in 14 patients revealed a satisfactory adherence to the gluten-free diet. It was proved that conscientiousness and awareness on the part of coeliac patients, or those taking care of them, is of paramount importance in determining the choice of foods comprising a gluten-free diet.

Introduction

Coeliac sprue (coeliac disease, gluten-sensitive enteropathy) is a chronic lifelong disease characterized by permanent intolerance to gluten and typical inflammatory changes of the mucosa of the small intestine. It is an autoimmune disorder associated with the presence of the genes HLA-DQ2/DQ8. The inflammatory changes of the intestinal mucosa lead to mucosal atrophy and consequently to the malabsorption of all nutrients, minerals and vitamins. The degree of this malfunction depends on the extent of the injury of the mucosa – this is why various clinical forms of this disorder occur, from the fully developed disease, through its incomplete forms, up to a complete lack of any symptoms. The disease is most frequently encountered in infancy, but it can manifest at any time in adults. It occurs all over the world, but shows pronounced regional differences in its incidence. In central Europe the prevalence is estimated at 1:100 in population, but only 10 percent of these are registered and subsequently treated (Farrell and Ciarán, 2002).

The mechanism of damage to the intestinal mucosa has not yet been fully elucidated. Obviously it is an abnormal immune response to gluten, or its prolamin (gliadin) fragments. Fundamental is the inadequate response of the T-cells of the immune system to the peptide grafts of the prolamin fraction, which trigger an inflammatory process leading to the atrophy of the intestinal mucosa (Catassi and Fasano, 2008; Troncone et al., 2008).

The evidence of genetic predisposition consists in both heritability (about 10% among first-degree relatives) and frequent laboratory or bioptic anomalies found in the symptom-free parents of the afflicted children.

Within the past 15 years sensitive and specific methods of antibody determination in blood serum have been used for screening and diagnosis of coeliac disease. Within the range of detectable antibodies, the determination of antibodies against gliadin of class IgA and IgG (AGA-A, AGA-G), and class IgA against endomysium (AMA) is used but of the utmost importance is the determination of antibodies against tissue transglutaminase of classes IgA a IgG (atTG-A and atTG-G) with up to 100 percent negative predictive value. The detection of these antibodies in patient's serum is an indication to perform enterobiopsy, which provides the definite proof of gluten enteropathy. Untreated patients show a typical picture of atrophic mucosa with the villi markedly flattened to completely atrophied, inflammatory alterations and lowered enzyme activity (Catassi and Fasano, 2008).

The only possible causal treatment of CD is a lifelong gluten-free diet. Gluten is in accordance with the Codex Alimentarius definition the principal constituent of wheat, rye, barley and oats (oats can be tolerated by most but not all people who are intolerant to gluten) grain. It is therefore necessary to avoid all foods containing flour made from these cereals, even in trace amounts. Maize, rice, soybean, buckwheat, millet, and possibly special gluten-free flour based on deproteinated starch and various mixtures of gluten-free cereals are allowed (Hischenhuber et al., 2006).

Decree No. 54/2004 Coll., dealing with foods for special dietary purposes and the methods of their use, treats gluten-free foods in the Czech Republic in part 7. This decree proceeds from the descriptions and statements of Codex Alimentarius document (ALINORM 06/29/23). In this decree the limits of gluten content are expressed in mg per kg of food ready for consumption. Gluten-free foods must not contain more than 100 mg gluten per 1 kg of food ready for consumption. Foods labelled as "naturally gluten-free" must not contain any ingredients from wheat and other wheat species, such as spelt, kamut or durum wheat, barley, rye and oats and their crossbred varieties. Gluten levels must not exceed 20 mg per 1 kg of food ready for consumption. It should be mentioned, that normal population not taking gluten-free diet consumes tens of grams of gluten in its daily meals. The final changes of Codex Standard for foods for special dietary use for persons intolerant to gluten (Codex Standard 118-1979) were adopted during 2008 and hence are not included in Czech food legislation. European legislation concerning gluten-free foods (Commission Regulation No. 41/2009) was adopted in the year 2009 and may be already used in the Czech Republic too. The question of oat acceptability in gluten-free diet has not yet been solved. Avenin is not consider harmful, but there is a significant possibility of contamination by wheat, so that some physicians or dietitians advice to avoid also oat (Lundin et al., 2003; Peräaho et al., 2004).

Based on the fact that a gluten free diet is the only causal therapy of coeliac disease, the present study was aimed at monitoring of the adherence to a gluten-free diet of patients suffering from coeliac disease within the context of their routine daily diet regimens. The level of antibodies against tTG and gliadin was determined at the beginning and at the end of the study period. Daily intake of gliadin by determination of gliadin content in patients' food was quantified.

Methods

Patients

The patients monitored were both adults and children chosen with the cooperation of the Coeliac Association of the Czech Republic (CACR; www.celiac.cz). A short consultation was carried out with the parents and adult participants to explain the monitoring process and answer all questions. All adult participants and the parents were members of CACR. According to their information, they use mainly food labelling for food choice, recommendation and discussion among the members

of the association. The participants receive only general information from their gastroenterologist after coeliac disease diagnosis. The participants did not get any support from dieticians for this study.

Diet monitoring

The monitoring period of daily food intake was 30 days. Blood assay for antibodies determination was carried out before and after this period. Prior to the monitoring the patients were given PE bottles and grip-seal PE bags for sampling their food, beverages and also their potential medicine and food supplements ingested each day. Individual components of the daily menu, the producers or distributors of respective commercial foods (not strictly required in samples not suspected to contain gluten), and weight or volume of all consumed components of the menu were recorded using a standardized form. The bottles and bags were marked 1A–1X (day 1) to 30A–30X (day 30). The patients took out the stipulated amount, which served as a sample of each of their daily meals. The samples included both homemade meals as well as commercial products (foods considered as gluten-free, foods labelled as gluten-free and naturally gluten-free). Those foods which were consumed repeatedly were sampled only once prior to their first intake, and all the subsequent consumption was only recorded without repeated sampling. The daily gliadin intake was calculated on the basis of the reported amount of meals ingested. Simple digital scales were made available for weighing the meals.

Gliadin content determination in foods

The gliadin ELISA kit (IM3717), Immunotech and Beckman Coulter Company, was used to determine the content of gliadin and other prolamins.

The kit was developed and validated under a research project and further verified by two interlaboratory ring trials with international participation (Gabrovská et al., 2004, 2006).

Principle of determination

The kit for gliadin determination in foods by means of specific monoclonal and polyclonal antibodies is suitable for quantitative determination of prolamins originating from wheat and rye, and for detecting the presence of barley prolamins (Sánchez et al., 2007).

The ELISA procedure for gliadin determination is a two-step sandwich-type immunochemical assay on a microtitration plate. The standard solutions or diluted extracts of the samples are incubated in the plate wells coated with two monoclonal antibodies against gliadin. During the incubation stage gliadin is captured by these antibodies. After incubation and washing, the conjugate of polyclonal antibody with horse radish peroxidase is added to the respective wells, where it is bound to the solid-phase antibody-antigen complex. After additional incubation, the wells are washed and the antigen-antibody complex bound to the

wells is detected by the addition of a chromogenic substrate. The colour intensity is proportional to the gliadin concentration in standard solutions or diluted extracts of the samples. The preparation of the sample is very simple, consisting in the extraction of 1 g (1 ml) of the homogenized sample in 10 ml of 40% ethanol, agitated for 1 minute. The sample is then centrifuged and the resulting supernatant is properly diluted and then used for ELISA determination.

Antitransglutaminase and antigliadin antibody determination

The antigliadin antibodies (IgA and IgG isotype) in the patients' sera were determined by the ELISA method as described in the previous publications (Tučková et al., 1997; Vančíková et al., 2002). Peripheral blood of patients was collected, centrifuged and sera were stored at –80 °C until analysis. In short, gliadin (Crude Gliadin, Sigma; concentration 100 µg/ml; volume 50 µl/well) was bound to the walls of the wells of a microtitre plate (Gama České Budějovice). After fixation with 0.25% (v/v) glutaraldehyde (Sigma) solution and blocking with 1% (w/v) bovine serum albumin (BSA; Sigma) solution, the patients' sera diluted in 1% BSA (1:20, 1:100, 1:500) were added. After incubation and washing the secondary antibodies aHu IgA Px (Binding Site) diluted 1:500 in 1% BSA and aHu IgG Px (Binding Site) diluted 1:1000 in 1% BSA were applied. The detection was done by the addition of TMB (Sigma) solution and H_2O_2. The reaction was then stopped by adding 2 M H_2SO_4 and the optical density was measured at 450 µm using ELISA Reader (Multiskan Ascent, Labsystems, Dynex Prague). Antibodies of IgA isotype against tTG were determined by BioSystems Antitransglutaminase Antibodies (Anti tTG) kit according to the manufacturer's instructions.

Ethical approval

According to national legislation an ethical approval (informed consent) was not necessary for this study. All study was carried out in cooperation and in agreement with the Coeliac Association of the Czech Republic.

Results

From the huge amount of data obtained we chose a single example of the forms completed by the participating patients, examples of the forms after daily gliadin intake calculation, the summary of daily gliadin intake by the 14 patients and the values of antibodies observed in the patients before and after diet monitoring.

Table 1 is an example of a form with daily gliadin intake calculated without any unintentional gliadin ingestion.

Table 2 is an example of an unintended conflict with dietary recommendations. Home-made black cake with icing was baked from an allegedly gluten-free mixture, which was later found to contain more than 10 mg gliadin/100 g. Another batch of this gluten-free mixture was therefore analyzed, but its gliadin content was determined as satisfactory.

Table 3 includes coconut biscuits, which were declared as gluten-free. However, the content of gliadin was found to exceed 10 mg/100 g. This product did not meet the requirements of Czech food legislation and could not be labelled as gluten-free. In this case unintentional gliadin ingestion was found as well.

Table 4 shows the final results of average daily gliadin intake at 14 patients. The maximum of the average gliadin daily intake determined during the study was 5.5 mg (with the highest SD 5.5). Some authors consider the daily intake of 10 mg

Table 1 – Example of a filled-in form with calculated daily gliadin intake

Meal	Code	Description	Quantity (g or ml)	Gliadin (mg/100 g)	Total gliadin (mg)
Breakfast	1a	bread	91	0.00	0.00
	1b	home-made jam	20	0.30	0.06
	2a	veal liver sausage	18	0.42	0.08
	2b	Camembert	33	0.00	0.00
Snack	2c	coffee with milk	250	0.29	0.73
Lunch	2d	meat loaf	84	0.00	0.00
	2e	peas with carrots	80	0.00	0.00
Snack	2c	coffee with milk	250	0.29	0.73
Evening meal	2f	pink grape juice	250	0.32	0.80
	1a	bread	100	0.00	0.00
	2d	meat loaf	50	0.00	0.00
	2a	veal liver sausage	20	0.42	0.08
	2b	Camembert	40	0.00	0.00
	2g	yoghurt "Jogobella Fit"	175	0.28	0.49
Other	2f	pink grape juice with water	125	0.32	0.40

Daily gliadin intake 3.36 mg

Table 2 – Example of a filled-in form with calculated daily gliadin intake, showing unintentional gliadin ingestion

Meal	Code	Description	Quantity (g or ml)	Gliadin (mg/100 g)	Total gliadin (mg)
Breakfast	19a	black cake with icing	25	11.90	2.98
Snack	19a	black cake with icing	34	11.90	4.05
Lunch	19b	roasted cod fillets	43	0.00	0.00
Snack	19a	black cake with icing	100	11.90	11.90
Evening meal	19b	roasted cod fillets	20	0.00	0.00
Other	20a	yoghurt "South Bohemian Blackberries"	160	0.00	0.00
	5h	candy with menthol	3.5	0.19	0.01
	20b	coloured cake icing	ingredient	7.05	

Daily gliadin intake 18.9 mg

Table 3 – Another example of a completed form with calculated daily gliadin intake, a real technological mistake by the producer

Meal	Code	Description	Quantity (g or ml)	Gliadin (mg/100 g)	Total gliadin (mg)
Breakfast	22a	coconut biscuits	57	12.36	7.05
Snack	4d	Panini	53	0.00	0.00
	23a	Brussels paté	17	0.00	0.00
	23b	eucalyptus candy	9	0.72	0.06
	23c	fruit tea "Wild Fruits"	1000	0.00	0.00
Lunch	23d	chicken curry with rice	300	0.42	1.26
	23e	chocolate milk	200	0.00	0.00
	23f	fizzy caramels "Zozole"	14	0.00	0.00
Snack	23g	sesame peanuts "Twiny"	22	0.00	0.00
Evening meal	23h	instant rice pudding	300	0.00	0.00
	23i	orange juice "Jupí"	200	0.00	0.00
	23j	candy "Sour Fish"	21	0.00	0.00
Other	1n	multivitamin "Small Martians – Strawberry"	1	0.35	0.00
	1p	food supplement Preventan	0.19	1.06	0.00
	3h	orange beverage "Juwík"	10	0.00	0.00

Daily gliadin intake 8.31 mg

Table 4 – Summary table of average daily intake of gliadin

	Daily intake of gliadin (mg/day)	SD	Corrected daily intake of gliadin (mg/day)*
Monitored in 2005			
Boy, age 4.5, weight 25 kg	1.5	0.7	1.1
Boy, age 7, weight 20 kg	1.7	0.7	1.1
Woman, age 32, weight 55 kg	3.7	0.8	3.4
Monitored in 2006			
Girl, age 10, weight 35 kg	2.4	1.2	2.6
Woman, age 21, weight 58 kg	1.8	1.0	–
Boy, age 16, weight 44 kg	5.5	5.5	3.1
Pregnant woman, age 29, weight 65 kg	2.0	1.4	–
Woman, age 45, weight 58 kg	2.8	1.9	–
Monitored in 2007			
Boy, age 12, weight 37 kg	2.8	2.1	2.2
Girl, age 7.5, weight 16 kg	2.3	4.2	0.7
Woman, age 42, weight 58 kg	4.7	2.4	4.2
Monitored in 2008			
Boy, age 10, weight 23.5 kg	1.3	0.7	–
Girl, age 4, weight 14 kg	0.8	0.7	–
Woman, age 36, weight 92 kg	2.2	1.7	–

*Days with unintentional gliadin ingestion or with missing samples (e.g. beverages) were excluded from the data for calculating the average daily gliadin intake

as a safe level (Collin et al., 2004; Catassi and Fasano, 2007; Catassi et al., 2007). The results of the study indicate that the level of 10 mg gliadin per day was not exceeded in any case. Table 5 shows the results of antigliadin antibodies (IgA and IgG isotype) and antibodies against tTG (IgA isotype) determination in patients' sera.

Table 5 – Results of antitransglutaminase and antigliadin antibody determination (percent of internal standard)*

Patient	Antibody	1st blood draw	2nd blood draw
Boy, age 4.5, weight 25 kg	IgA tTG	13.4	13.7
	IgA gliadin	14.4	13.2
	IgG gliadin	62.4	45.3
Boy, age 7, weight 20 kg	IgA tTG	18.7	15.8
	IgA gliadin	20.7	23.4
	IgG gliadin	47.2	42.1
Woman, age 32, weight 55 kg	IgA tTG	19.8	31.9
	IgA gliadin	31.8	24.8
	IgG gliadin	35.1	20.5
Girl, age 10, weight 35 kg	IgA tTG	44.1	36.9
	IgA gliadin	16.5	17.6
	IgG gliadin	102.7	106.7
Woman, age 21, weight 58 kg	IgA tTG	21.9	19.1
	IgA gliadin	36.9	27.9
	IgG gliadin	57.3	50.1
Boy, age 16, weight 44 kg	IgA tTG	20.2	23.4
	IgA gliadin	20.6	24.1
	IgG gliadin	40.3	38.5
Pregnant woman, age 29, weight 65 kg	IgA tTG	18.7	23.7
	IgA gliadin	22.5	25.0
	IgG gliadin	45.7	46.4
Woman, age 45, weight 58 kg	IgA tTG	13.0	14.9
	IgA gliadin	22.3	34.4
	IgG gliadin	32.0	34.9
Boy, age 12, weight 37 kg	IgA tTG	28.4	27.1
	IgA gliadin	25.7	24.2
	IgG gliadin	19.4	8.7
Girl, age 7.5, weight 16 kg	IgA tTG	21.9	13.9
	IgA gliadin	41.1	24.7
	IgG gliadin	32.3	12.9
Woman, age 42, weight 58 kg	IgA tTG	96.6	71.0
	IgA gliadin	76.9	34.2
	IgG gliadin	80.5	78.6

*Results for only 11 patients. Another kit for determination of antibodies was used for the remainder of the patients. There was correlation of the results concerning reliability and sensitivity of the antibody levels when comparing both kits

Discussion

Coeliac disease is common but often underdiagnosed, resulting in major medical complications. The prevalence of coeliac disease is approximately 1% worldwide. The disease is caused by gluten intolerance based on immune-mediated mechanisms with autoimmune features. Recently, screening for coeliac disease has become easier with autoantibodies against transglutaminase and deamidated gliadin. Diagnosis is confirmed by a small intestinal biopsy with characteristic histological changes. Beside gut mucosa, coeliac disease affects a number of other organs: extra-intestinal symptoms are pronounced mainly in adult coeliac patients. The results of the studies suggest that activation of innate immunity cells by some food proteins, e.g. gliadin, could lead to mucosal inflammation, participate in the impairment of the intestinal mucosal barrier and consequently lead to the development of inflammatory and autoimmune diseases (Tlaskalová-Hogenová et al., 2005). Various immunologically mediated chronic diseases were demonstrated to be associated with coeliac disease. Interestingly, in a subset of non-coeliac patients suffering from various chronic diseases introduction of a gluten free diet was shown to improve their clinical symptoms (Tlaskalová-Hogenová et al., 2007).

A so-called "recommended daily intake" of gliadin has been sought in connection with a gluten-free diet. Several clinical tests were carried out in Finland and Italy. Coeliac patients were administered various doses of gluten and subsequently their levels of antibodies were measured and biopsy was carried out. Very different values (for example 30 mg of gluten/day vs. 10 mg of gluten/day) were found as acceptable amounts of daily gliadin intake and no definite conclusion has been reached thus far (Collin et al., 2004; Hischenhuber et al., 2006; Catassi and Fasano, 2007; Catassi et al., 2007; Kinsey et al., 2008).

Elimination of gluten from the diet has been the standard of care for coeliac patients during the last half century. For coeliac patients, particularly young people, adherence to a gluten-free diet may be difficult to achieve and gluten restriction may lead to insufficient nutrient intake and an unbalanced food intake resulting in overweight (Hopman et al., 2006). Gluten-free food products can be contaminated by traces of gluten and the absence of symptoms and increase in antibody levels may be due to these difficulties (Mothes and Stern, 2003). It is therefore of great importance to simultaneously monitor daily gluten intake and changes in serological markers of coeliac disease in patients.

The results in 14 patients revealed a satisfactory adherence to their gluten-free diets. Several raw food materials or final products with an elevated or "above the limit" gliadin content were found during monitoring, nevertheless this fact did not influence the otherwise satisfactory overall picture of the daily gliadin intake by the patients followed. 1,900 food samples (common foods, gluten-free and naturally gluten-free foods) were analyzed within the framework of this study (160 bakery products, 23 potato products, 398 dairy products, 319 meat products, 13 fish,

185 cereal products, 427 confectionary products, 39 vegetable or fruit products, 23 soy products, 124 drinks, 123 spices and flavour-enhancers, 66 food supplements and medicines).

In 2005, the diet was monitored in two children (boys 4.5 and 7 years old) and a woman (32 years old). Daily intake amounted to 1.5 and 1.7 mg respectively in the children, 3.7 mg in the woman. It became apparent, though, that the intake of gliadin in the form of drinks and soups constituted a problem. These foods, containing very little gliadin but consumed in large quantities, seemed to substantially increase the daily sum of gliadin ingested. For example, if the drinks were omitted from calculating the total consumption, the values of the daily intake dropped to 1.1 mg (boy, 7) and 3.4 mg (woman). The corrected daily intake of gliadin in the other boy was obtained by the omission of the consumption of the yogurt drink Activia, which increased the daily intake of gliadin by either 0.93 mg or 1.83 mg, depending on the ingested quantity. No unintentional gliadin ingestion was recorded in these patients.

In 2006, diet was monitored in five coeliacs. Unintentional gliadin ingestion was recorded in a patient who was suffering from both coeliac disease and diabetes. This situation complicated his food choices considerably. The unintentional gliadin ingestion lay in the use of a commercial mix for buckwheat pancakes containing more than 9 mg gliadin/100 g. A different problem was with a chocolate bar. Chocolate bars recommended for diabetics contained wheat germ as one of the ingredients. This participant did not check the ingredients properly. It is possible to consider this case as a dietary mistake. The samples contained 18 mg/100 g and 45 mg/100 g respectively. The increased daily intake also resulted from the consumption of 400 g potato soup, which contained 0.5 mg gliadin/100 g. The days without the complete set of samples available were excluded from the calculation of the corrected daily gliadin intake in a girl (age 10), with the resulting value of 2.6 mg/day. The overall results of monitoring indicated strict compliance to a gluten-free diet except for one patient, where one mistakes was found. However, despite the various unintentional gliadin ingestion, daily gliadin intake fell below the lower limit of the theoretical tolerated dose, which is supposed to range between 5 and 15 mg gliadin per day (Collin et al., 2004; Catassi and Fasano, 2007; Catassi et al., 2007).

During 2007 four coeliacs were monitored for daily intake of gliadin. The unintentional gliadin ingestion was found in the cases of three coeliacs and four food samples with increased gliadin content were recognized. Initial detection of excessive gliadin content in coconut biscuits was not confirmed by repeated analysis of the same batch; it was not clear whether it was a production fault or a subsequent accidental contamination in the patient's household. A gluten-free flour mix was used for baking a homemade black cake with icing and yeast pancakes and originally was not available for analysis. It was analysed after detecting an above-limit gliadin level in the aforementioned black cake. This analysis confirmed some

contamination of the mix (gliadin content 12 mg/100 g). Another batch of the same gluten-free flour mix was therefore obtained; it contained 0.6 mg gliadin/100 g. The coloured cake icing was analyzed twice; the second batch was also high in gliadin. The basic component of this icing is wheat starch and starch syrup. Increased gliadin content was also found in coconut biscuits.

Three patients on gluten-free diets were followed in 2008. Homemade foods prepared from three kinds of flours (maize, chickpea and millet – considered as naturally gluten-free, but not labelled as naturally gluten-free) were included in the diet of a four years old girl. These flours were found to be above limit. Daily gliadin intake was thus increased on several occasions. The analyses found chickpea flour to contain 22.2 mg gliadin/100 g, which is a quantity markedly above limit, whereas maize and millet flours contained only 4.7 mg gliadin/100 g. Chickpea and millet flours were used for making raw potato pancakes, which were revealed to contain 4.2 mg gliadin/100 g. This value complies with the aforementioned Decree, but a higher and more frequent consumption of such homemade meals from these flours could increase daily gliadin intake. Maize flour was used as an ingredient of cheese soufflé. The gliadin content of the soufflé amounted to only 0.24 mg/100 g, which was ascribed to the simultaneous use of other, low-gliadin ingredients for its preparation. In spite of using contaminated flours, the total daily gliadin intake (0.8 mg/day) was very low because these flours were not used for more than two of the 29 days of the monitoring period.

Determination of antibodies was carried out in 14 patients. Table 5 shows the results for 11 patients. Another kit for determination of antibodies was used for the remainder of the patients and the results correlated well with the results of the other 11 patients. Good correlation of the results concerning reliability and sensitivity of the antibody levels when comparing both kits was found in our previous studies. The final conclusion is that no high values of antibody activity and no changes in the antibody activity between the first and last day of monitoring were found for all 14 patients evaluated. Based on these and previous findings it could be suggested that measuring the levels of anti-gliadin and anti-transglutaminase antibodies is not sufficiently sensitive to reflect potential mild changes of immune reactivity which could occur as a consequence of ingestion of scarce amount of gliadin (Jelínková et al., 2000).

Conclusion

It was proved that conscientiousness and awareness on the part of coeliac patients, or those taking care of them, is of paramount importance in determining the choice of foods comprising a gluten-free diet. In some cases the gluten-free diet was followed almost too zealously, in other cases better attention should have been paid to food choices. In any case, the fare of the persons monitored reflects their individual preferences and also their ages.

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