EFFECT OF GLUTEN-FREE BAKERY PRODUCTS ON SERUM LEVELS OF MINERAL ELEMENTS IN HEALTHY CONSUMERS*

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Healthy nutrition requires the intake of all macronutrients and micronutrients in optimal ratio and quantity. Nowadays, many people tend to gluten-free food without health and medical reasons. Many gluten-free products are nutritionally poorer than gluten-containing analogues. Although in many cases gluten-free products are nutritionally rich (either naturally or as a result of fortification), the bioavailability of nutrients is limited. The study focused on the influence of a six-week consumption of gluten-free bread and bakery products on mineral status in thirty healthy people without coeliac disease and the impacts eight weeks after the end of the consumption. Since the recommended daily intake for phosphorus, calcium, magnesium and iron was satisfied at only 84–88% for our volunteers, we consider the increase in the average serum levels of the monitored parameters to be positive (except for calcium). The six-week consumption of gluten-free bakery products had a positive effect on the mineral status of the monitored group of healthy consumers. We found a significant increase in phosphorus ($P < 0.001$) and magnesium ($P < 0.01$) levels, a certain increase in iron ($P > 0.05$), whereas the level of calcium insignificantly lowered ($P > 0.05$).

gluten-free products, nutrition, phosphorus, calcium, magnesium, iron

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INTRODUCTION

The nutritional value of bread, not only gluten-free, is very important because bread is the basis of nutrition for most of the world's population. Gluten-free eating patterns are frequently perceived to be healthier than the gluten-containing ones and good health is the primary reason given for consuming a gluten-free diet in healthy population. Lucisano et al. (2012) reported that most gluten-free products have poor cooking quality compared with their wheat counterparts and there is a need to improve the nutritional quality of these products. People on gluten-free diet tend to consume refined gluten-free cereal products, which do not have the same nutritional composition as their gluten-containing analogues (Thompson et al., 2005) and gluten-free cereal products often do not contain the same levels of micronutrients, such as thiamine, riboflavin, niacin, folate, vitamin D, calcium and iron (Saturni et al., 2010). Most of the gluten-free products are produced from corn and rice which are not good sources of micronutrients. Several gluten-free foodstuffs contain more fat including saturated and salt but fewer minerals and vitamins than their

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equivalents with gluten (Pellegrini, Agostoni, 2015). Gluten-free diet must be nutritionally balanced and cover nutrient requirements. The content of potassium, phosphorus, calcium, iron, zinc and group B vitamins of almost all types of gluten-free bread and bakery products considered is lower than that in the conventional products (European Institute of Oncology, 2008; Wild et al., 2010). The micronutrient comparison of a gluten-free diet against a gluten-containing diet released by Larretxi et al. (2019) indicated higher levels of vitamin E, zinc, potassium and calcium intake but lower of provitamins A, thiamine, riboflavin, niacin, vitamin B6, vitamin B12, folate, biotin, vitamin D, pantothenate, magnesium, sodium, iron, copper, iodine, chlorine, manganese and selenium intake.

In coeliacs, the ingestion of gluten leads to inflammation and mucosal damage in the small intestine and results in malabsorption of micronutrients such as iron, folic acid, calcium and fat-soluble vitamins (Gujral, Rosell, 2004; Brites et al., 2018). The nutritional status of coeliacs at diagnosis depends on many factors (duration of the disease, extent of inflammation, dietary intake) (Theethira et al., 2014).

Many gluten-free products are nutritionally rich (Sharoba et al., 2014), but the bioavailability of nutrients from these products is subject to many factors and impacts. The bioavailability of mineral ingredients from gluten-free products is still a serious problem among coeliacs leading to many nutritional and health deficiencies (do Nascimento et al., 2014).

Authors of several studies have suggested that the bioavailability of minerals may be higher in enriched than non-enriched products. Fibre content and pH also affect the bioavailability of minerals (Regula, Gramza-Michalowska, 2010). Regula et al. (2018) monitored the *in vitro* bioavailability of calcium, magnesium, iron, zinc and copper from gluten-free bread enriched with natural ingredients in the form of powdered milk and seeds. The addition of milk and seeds significantly increased the energy value of both types of tested rice and buckwheat breads. The gluten-free rice and gluten-free buckwheat breads without added ingredients had a similar calcium content. The addition of milk increased the calcium content significantly to 2330 $mg.kg^{-1}$ and the addition of milk with seeds increased the calcium content to approximately 2207-2225 $mg.kg^{-1}$. The magnesium content was significantly higher in buckwheat bread (866 $mg.kg^{-1}$) compared to rice bread (189 $mg.kg^{-1}$), but the addition of milk and milk with seeds increased the amount of magnesium in rice bread to 606 $mg.kg^{-1}$ and 696 $mg.kg^{-1}$, respectively. In the case of enriched buckwheat bread, the magnesium content was increased in both cases to 1170 $mg.kg^{-1}$. In the case of iron, each addition to rice and buckwheat bread has led to a significant increase in this mineral amount. Despite the high calcium content, its relatively low bioavailability has been demonstrated in rice and buckwheat bread with the addition of milk. The highest bioavailability was observed in rice bread without added ingredients. Buckwheat breads without or with ingredients demonstrated a generally lower bioavailability of calcium compared to rice bread with the same additives (Regula et al., 2018). The bioavailability of calcium in gluten-free bread is low. Research suggests that the type of added ingredients used to increase nutrient absorption is very important. Despite the high content of nutrients in the initial product, bioavailability may be low, which is often the case for products with a high nutritional value and nutrient density (Krupa-Kozak, 2015). In the case of magnesium, rice and buckwheat types of bread showed an increase in potential bioavailability with an increasing amount of ingredients. The potential bioavailability of this mineral was influenced not only by the additive used but also by the type of bread. Higher values were observed in rice breads. Iron also showed higher bioavailability in rice bread. In the case of buckwheat bread, the highest bioavailability was observed in bread with the addition of dried milk and seeds with all ingredients. The worst bioavailability of iron was found in rice bread made with milk (Regula et al., 2018).

Pseudocereals are very good sources of iron and magnesium, but their aroma and especially flavour limit their use in significant quantities in foods. In the case of iron, the highest bioavailability was achieved after enrichment of gluten-free bread with iron pyrophosphate and iron glycinate (Kiskini et al., 2007). This depends, of course, also on the content of the ingredient in the product and the presence of other minerals that can improve or worsen the absorption and utilisation of this nutrient (Zielinska-Dawidziak et al., 2016).

Due to the increased popularity of gluten-free diet, the influence of this diet was evaluated in healthy subjects. Following gluten-free diet did not significantly affect metabolic syndrome prevalence and cardiovascular risk in healthy subjects (Kim et al., 2017).

This study was aimed to find the effect of a six-week consumption of gluten-free bread and bakery products on mineral elements (P, Ca, Mg, Fe) profile in non-coeliac consumers determined as the serum levels at the start and at the end of the experiment as well as two months after the end.

MATERIAL AND METHODS

Characteristics of the participants

The trial was approved by the Ethic Committee at the Specialized St. Svorad Hospital Zobor, Nitra, Slovakia (protocol No. 012911/2016). The requirement for participation in the research was an informed

Table 1. Recommended and actual dietary allowance of selected nutrients of the study participants

	Energy			Proteins			Carbohydrates			Lipids		
	RDA (kJ)	ADA (kJ)	+/– (kJ)	RDA (g)	ADA (g)	+/– (g)	RDA (g)	ADA (g)	+/– (g)	RDA (g)	ADA (g)	+/– (g)
Mean	9600	7840.40	–1759.60	53.13	69.01	16.20	372.83	230.51	–142.32	66	80.67	14.78
Max	11500	17290.35	8290.35	66	161.33	110.33	442	643.82	291.82	80	208.42	143.42
Min	9000	129.96	–9370.04	51	14.58	–51.42	352	66.75	–302.25	60	25.11	–39.89
Med	9500	7346.65	–2551.66	52	66.97	14.88	369	200.1	–172.585	65	71.885	4.85
	Phosphorus			Calcium			Magnesium			Iron		
	RDA (mg)	ADA (mg)	+/– (mg)	RDA (mg)	ADA (mg)	+/– (mg)	RDA (mg)	ADA (mg)	+/– (mg)	RDA (mg)	ADA (mg)	+/– (mg)
Mean	1200	1040.59	–159.41	1000	773.58	–226.42	345.00	338.89	–6.11	15.47	13.17	–2.30
Max	1200	2923.66	1723.66	1000	2111.8	1111.8	400	5004.57	4654.57	16	36.44	21.44
Min	1200	162.22	–1037.78	1000	185.87	–814.13	300	78.94	–271.06	12	3.33	–12.67
Med	1200	957.12	–242.88	1000	744.49	–255.51	350	269.215	–78.995	16	11.55	–4.38

RDA = Recommended Dietary Allowance, ADA = Actual Dietary Allowance, +/– = differences between recommended and actual dietary allowance (+ = excessive intake, – = insufficient intake), Max = maximum value, Min = minimum value, Med = median value of a range of values

consent of volunteers with all the study and measurement conditions which they would have to complete during the research. All participants signed the written informed consent to participate in the study. The participant group was composed of volunteers from the general population and consisted of 30 healthy adults (3 men and 27 women), who during a six-week period consumed gluten-free bread and gluten-free bakery products, however the participants of the study were not allowed a total gluten-free diet. Participants with an acute severe disease or with a recommended special dietary regimen were excluded from the study group. The amount of bread and bakery products was determined according to the recommended consumption of food for the Slovak population as follows: women consumed 150–200 g per day; men 200–250 g per day. All participants were asked not to change their eating habits and also not to change their habits related to the physical activity. Volunteers were subjected to totally 3 measurements (the first measurement before the consumption of gluten-free bakery products, the second after the six-week consumption of gluten-free bread and bakery products, and the last one 2 months after the end of consuming gluten-free bakery products) (Kolesarova et al., 2018).

Dietary assessment

For study purposes, we monitored the nutritional intake of study participants in order to evaluate the recommended nutritional doses and to better assess the potential impact of consumption of gluten-free bakery products on the blood concentrations of the monitored minerals. Dietary intake was assessed using three 24-hour food recalls, two on weekdays and one at the weekend. We used the nutritional software program Mountberry – Nutrition & Fitness Software (Version 1.1, 2011). Mountberry provides a complete analysis of food, meals, recipes based on an updated food database and nutritional recommendations for nutrient intake, health insights, dietary guidelines, and individual user needs. The average nutrient intake for participants was assessed according to the Recommended Dietary Allowance (RDA) in Slovakia updated in 2015 (Kajaba et al., 2015) (Table 1). During the trial we focused on basic parameters such as energy, proteins, carbohydrates, fats and also minerals (phosphorus, calcium, magnesium and iron).

Blood sampling and serum analyses

In our study, we focused on changes in phosphorus, calcium, magnesium and iron concentrations in serum during intervention, after gluten-free consumption, and also after further two months. Participants were divided into groups according to the respective concentration values of the individual parameters either in the reference range, below or above the reference standard boundary. In the case of phosphorus and calcium concentrations, they did not drop below the reference standard lower limit during the study. Most volunteers had the values of monitored parameters within the range of the reference standard. Blood samples were obtained before the start of consumption, after 6 weeks of consumption, and 8 weeks after the end of consumption. Venous blood was collected in the morning after 8 h of fasting using 1 × 2.5 ml EDTA tube and 2 × 7.5 ml serum gel tubes. After the

Table 2. Representation of participants in categories according to reference values during study

	Reference values		Baseline		After 6 weeks of consumption		Two months after end of consumption	
			n	mean	*n*	mean	*n*	mean
Phosphorus (mmol.l^{-1})	low	< 0.81	0	–	0	–	0	–
	normal	0.81–1.45	29	1.14	10	1.28	17	1.24
	high	> 1.45	1	2.36	20	1.7	13	1.6
Calcium (mmol.l^{-1})	low	< 2	0	–	0	–	0	–
	normal	2–2.75	23	2.56	25	2.48	29	2.52
	high	> 2.75	7	2.87	5	2.96	1	2.82
Magnesium, women (mmol.l^{-1})	low	< 0.77	22	0.49	3	0.52	4	0.69
	normal	0.77–1.03	4	0.87	24	0.87	23	0.86
	high	> 1.03	1	1.8	0	–	0	–
Iron, women (μmol.l^{-1})	low	< 10.7	6	7.1	7	8.87	8	6.78
	normal	10.7–32.2	21	18.27	18	16.76	18	17.24
	high	> 32.2	0	–	2	46.14	1	39.9

Table 3. Changes of phosphorus, calcium, magnesium and iron levels during the trial

	Phosphorus (mmol.l^{-1})			Calcium (mmol.l^{-1})		
	baseline	after 6 weeks of consumption	two months after end of consumption	baseline	after 6 weeks of consumption	two months after end of consumption
Mean	1.18	1.56	1.39	2.63	2.56	2.53
± SD	0.29	0.24	0.22	0.18	0.24	0.11
Max	2.36	1.95	1.78	3.20	3.16	2.82
Min	0.84	1.11	0.98	2.30	2.22	2.32
Mod	0.98	1.33	1.78	2.54	2.63	2.57
Med	1.15	1.60	1.43	2.60	2.60	2.53
P	< 0.001***^{a}	0.0019**^{b}	< 0.001***^{c}	0.1999	0.5309	0.0112*^{c}
	Magnesium (mmol.l^{-1})			Iron (μmol.l^{-1})		
	baseline	after 6 weeks of consumption	two months after end of consumption	baseline	after 6 weeks of consumption	two months after end of consumption
Mean	0.62	0.91	0.84	16.41	16.75	17.09
± SD	0.33	0.25	0.10	7.12	9.38	11.20
Max	1.80	2.15	1.03	30.54	54.42	55.00
Min	0.11	0.69	0.57	5.60	7.43	2.38
Mod	0.88	0.91	0.78	ND	ND	14.45
Med	0.57	0.87	0.83	15.91	15.02	14.45
P	0.0025**^{a}	0.1835	< 0.001***^{c}	0.8210	0.8820	0.7321

SD = standard deviation, Max = maximum value, Min = minimum value, Mod = the value that appears most often, Med = median value of a range of values, the levels of statistical significance chosen for the comparisons were $P < 0.05$ (*), $P < 0.01$ (**), $P < 0.001$ (***), ND = not detectable, a = intra-group differences after 6 weeks of consumption of gluten-free bakery products, b = differences between data after 6 weeks of consumption and post-intervention data, c = differences between baseline data and post-intervention data

separation of blood serum, the phosphorus, calcium, magnesium and iron levels were measured with an automated biochemical analyser Biolis 24i Premium (Tokyo Boeki Machinery Ltd., Japan) using direct

Table 4. The study participants' compliance with the standard for nutrient intake

	Energy (%)	Proteins (%)	Carbohydrates (%)	Lipids (%)	Phosphorus (%)	Calcium (%)	Magnesium (%)	Iron (%)
Mean	88	133	62	124	88	84	85	85
Max	294	302	151	297	214	369	200	224
Min	32	49	22	44	29	20	27	22

ion selective electrodes methods in the laboratories of the Department of Clinical Biochemistry of the Specialized St. Svorad Hospital Zobor.

Statistical analysis

We evaluated the data collected from the measurements both statistically and graphically in MS Excel 2010. The changes in different groups were analysed using paired Student's *t*-test and the data were presented as the means ± standard deviation (SD). The levels of statistical significance were set at $P < 0.05$ (*), $P < 0.01$ (**), $P < 0.001$ (***).

RESULTS AND DISCUSSION

Phosphorus

The phosphorus intake of our study participants is shown in Table 1. None of them had serum values below the lower limit of the reference values during the study (Table 2). However, individual values of participants varied radically between measurements. At the beginning of the study the average phosphorus level of the group was 1.18 ± 0.29 mmol.l^{-1}. Most participants (97%) had the phosphorus level within the range of the reference values. During the consumption of gluten-free bread and bakery products we found a significant increase in phosphorus to 1.56 ± 0.24 mmol.l^{-1} ($P < 0.001$) (Table 3) and only 10 participants (33%) were found within the reference range. The remaining 2/3 of participants had the phosphorus level above the upper reference limit. After the third blood sampling the mean phosphorus level decreased ($P < 0.01$) but compared to the baseline, the mean phosphorus value of the group was significantly higher (1.39 ± 0.22 mmol.l^{-1}, $P < 0.001$). At the conclusion of the study we found that, compared to the initial values, the phosphorus level was reduced only in 5 probands and increased in 25 subjects (83%). Regarding the phosphorus intake, participants were below recommendations because they reached, on average, only 87% of requirements.

Phosphorus accounts for approximately 1% of the total body weight. It is present in all body cells but is most commonly found in bones and teeth. The main sources of phosphorus are protein-based food groups such as meat and milk, and processed foods containing phosphate-based additives, dried brewer's yeast and yeast extract, dried skim milk, yoghurt, wheat germ, soy flour and nuts (Takeda et al., 2012). Low calcium intake with excessive protein and phosphorus intake, as well as inappropriate calcium/phosphorus and calcium/protein ratios, may have an adverse effect on bone tissue mineralization at the time of reaching peak bone mass (Czeczelewski et al., 2011). Although the typical adult diet contains enough phosphorus, 10–15% of older women receive less than 70% of the recommended daily dose (Heaney et al., 2010). A study focused on dietary data of Spanish children taken before and after one year on a gluten-free diet found that patients increased their phosphorus intake (Salazar Quero et al., 2015). The content of mineral elements in bread and bakery products depends on the recipe's composition. Winiarska-Mieczan, Kwiecien (2011) confirmed that whole-wheat bread is richer in mineral elements in comparison with white bread. In the case of phosphorus, the highest mean amount was found in crisp bread (5765 mg.kg^{-1}) and pumpkin bread (2975 mg.kg^{-1}). According to the authors, a statistical daily intake of 166 g of bread means consumption of 512 mg of phosphorus with bread (73% of RDA in Poland).

Calcium

The recommendation for calcium intake of our study group was fulfilled at 84% (Tables 1, 4). The average calcium levels varied during the study with a decreasing trend. The initial value 2.63 ± 0.18 mmol.l^{-1} dropped to 2.56 ± 0.24 mmol.l^{-1} without a significant change, but at the third sampling the value dropped compared to the baseline significantly to 2.53 ± 0.11 mmol.l^{-1} ($P < 0.05$). Serum calcium levels of most volunteers were within the reference range of 2–2.75 mmol.l^{-1} during the study period (Table 2). The consumption of gluten-free bread and bakery products resulted in a decrease of calcium levels in 18 subjects (60%) and an increase in values of 11 subjects.

Suliburska et al. (2013) found in their study 446.2 mg.kg^{-1} dry weight (d.w.) of calcium in the gluten-free bread but potential *in vitro* bioavailability of this mineral element from this product was only 8.48%. A similar amount of calcium was found in peas puff (458 mg.kg^{-1} d.w.), with bioavailability of 21.86%.

The highest bioavailability of calcium was reported by the authors in the case of corn porridge (~68%) with the lowest amount of calcium (34.3 mg.kg^{-1} d.w). The lowest potential bioavailability was found in the gluten-free pasta (7.72%).

Nutrition plays an important role in bone homeostasis, providing the necessary substrates for the metabolic functions of bone tissue. Reduced calcium intake or malabsorption lead to increased parathyroid hormone secretion which promotes bone turnover and cortical bone loss (Holick, 2007). The main sources of calcium in the diet are milk and dairy products, while non-dairy sources are green leafy vegetables (cabbage, celery, Chinese cabbage) that provide about 7% of dietary calcium (Buchowski, 2015). Other excellent sources are fish, eggs and nuts. The group of people at risk of calcium deficiency is created by persons with chronic intestinal disease, including coeliac disease, which is an immune-mediated systemic disorder caused by ingestion of gluten or related prolamines in genetically susceptible individuals (Olivares et al., 2013). Calcium deficiency and metabolic bone disease are frequent comorbidities in patients with this disease; approximately 75% of newly diagnosed patients with coeliac disease have decreased bone mass (Pantaleoni et al., 2014). Villous atrophy developing in the proximal portion of the small bowel under the influence of the disease affects the absorption of active calcium. Some studies have shown that people suffering from this disease may have nutritional disorders resulting not only from intestinal abnormalities but also from an inadequate nutrient intake in the gluten-free diet (Saturni et al., 2010). Several studies (Caruso et al., 2013; Shepherd, Gibson, 2013; Orecchio et al., 2015) have shown that compared to wheat products, many commercially available gluten-free products provide low levels of vitamins and minerals, including calcium. Coeliacs reduce the dietary calcium intake due to the reduced intake of milk and dairy products in order to avoid lactose because secondary lactose intolerance is a consequence of reduced production of lactase in damaged small intestine mucosa. Kinsey et al. (2008) described a mean daily calcium intake below the recommended 1500 mg per day in 92% coeliac patients on a gluten-free diet. Larretxi et al. (2019) found that over a half of coeliac participants did not reach recommendations for vitamin D and did not fulfil the recommendations for folic acid and calcium. Dietetic studies have revealed that patients with coeliac disease who are on a strict gluten-free diet often consume less than the recommended amounts of calcium and vitamin D. An insufficient calcium intake in children and adolescents on a gluten-free diet was also observed in a study of Blazina et al. (2010). However, the state of nutrition of coeliac disease patients depends on the length of active disease persistence, the extent of intestinal injury, the degree of malabsorption as well as on dietary habits and food choices. In children and adolescents, gluten-free diet can help recover any disrupted bone density (Kavak et al., 2003), but rarely normalises bone density in adulthood (Capriles et al., 2009). The enrichment of wheat and other flours with calcium is mandatory or recommended in many countries and is a relatively inexpensive and effective method for increasing calcium intake by food, taking into account the fact that the consumption of flour and bread and bakery products is high. In Denmark and Great Britain, flour is enriched with $CaCO_3$, where the amount of calcium in the form of $CaCO_3$ ranges from 900 to 1500 mg per 1 kg of flour (Reinwald et al., 2008; Sudha, Leelavathi, 2008). Allen, Orfila (2018) found that only 5% of gluten-free breads were fortified with mandatory nutrients such as calcium, iron, nicotinic acid or nicotinamide and thiamine and only 28% were fortified with calcium and iron only. Fortification of gluten-free flour is less common and a very limited number of studies have analysed the effect of calcium addition on the technological and sensory properties of gluten-free bakery products. Generally, it is expected that the enrichment of gluten-free bakery products with calcium improves its content in the final product without negatively affecting its overall quality.

Magnesium

The magnesium intake and fulfillment of recommendations of our study group are shown in Tables 1, 4. The reference values of magnesium and iron are determined by gender. Given that 90% of our study participants were women, we focused on evaluating just the females and the values of men were excluded. For females, the reference range was 0.77–1.03 mmol.l^{-1}. The initial mean magnesium level of the female group was below the lower reference limit (0.62 ± 0.33 mmol.l^{-1}). At baseline, the majority of women (81%) had a magnesium level below the reference limit (Table 2). During the consumption of gluten-free bakery products, there was a positive increase in magnesium levels in up to 23 female volunteers (77%), which was also reflected on the average magnesium level in the group of women as the value increased significantly to 0.91 ± 0.25 mmol.l^{-1} ($P < 0.01$). Between the second and third sampling, the mean magnesium level dropped, however insignificantly (0.84 ± 0.1 mmol.l^{-1}). However, compared to the initial mean magnesium level, the value was significantly higher ($P < 0.001$).

A common diet meets the recommended daily needs for magnesium for a healthy population, but not for people with congenital genetic disorders. The nutritional requirements are best satisfied through a balanced diet (Breitschaedel et al., 2005). According to Winiarska-Mieczan, Kwiecien (2011), in Poland, a daily portion of bread contains 93 mg of magnesium (i.e. approximately 22–30% of RDA). The re commended daily intake of magnesium is 320–350 mg. A study by Suliburska et al. (2013) revealed the highest magnesium content in corn porridge and gluten-free bread (331 mg.kg^{-1} and 314 mg.kg^{-1} d.w., respectively) with potential bioavailability of 44.26% and 20.98%, respectively. The highest bioavailability was found in peas puff (54.25%) with the lowest magnesium content from the all selected gluten-free products.

People living in areas with soft water or using diuretics or predisposed for increased magnesium loss or ectopic heart attacks may have increased demands on their intake (Klevay, Milne, 2002). Taetzsch et al. (2018) found that gluten-free eating patterns do not have healthier micronutrient profile and based on the results of the study these patterns are less optimal for dietary fibre, folate, total protein, vitamin E, magnesium and potassium. Gonzalez et al. (2018) found out in their study that coeliac men consumed lower amounts of vitamin E, niacin and magnesium than the control group. Coeliac women fulfilled magnesium requirements better than coeliac men (Churruca et al., 2015; Gonzalez et al., 2018) but Wild et al. (2010) showed that female patients adhering to a gluten-free diet had lower intakes of magnesium, iron, zinc, manganese, selenium and folate. In male patients, intakes of magnesium and selenium were particularly low. Moderate magnesium deficiency through the exacerbation of chronic inflammatory stress can significantly contribute to the occurrence of chronic diseases such as atherosclerosis, hypertension, osteoporosis, diabetes mellitus and cancer (Nielsen, 2010). Subjects with magnesium intake lower than the recommended are more likely to have elevated CRP which may contribute to the development of cardiovascular disease (King et al., 2005).

Iron

Even in the case of iron, the reference standard is influenced by gender, so we have evaluated this parameter only in women, like in the previous case. The optimal serum level of iron is between 10.7 and 32.2 μmol.l^{-1}. The mean iron levels during the study are shown in Table 3. The iron level gradually increased from initial 16.41 ± 7.12 μmol.l^{-1} to 16.75 ± 9.38 μmol.l^{-1} after consumption and final 17.09 ± 11.2 μmol.l^{-1} without significant changes and kept approximately the same level. Especially in women this parameter is influenced by many factors, so its levels change significantly over a short period of time. Even for this parameter, the recommended daily intake of the study group was fulfilled at only 85%. Based on the distribution of participants into groups according to iron levels, most of them were within the range of reference values (Table 2). After the 1st blood sampling, 21 women had iron levels in the range of reference values (78%, 18.27 μmol.l^{-1} on average) and 18 females at the 2nd and 3rd sampling (67%, 16.76 μmol.l^{-1} and 17.24 μmol.l^{-1}, respectively). An individual assessment of changes in all volunteers shows that during the six-week consumption of gluten-free bread and bakery products the iron level was reduced in a half of them (53%). When we assessed the iron initial and final values of individuals, there was a decrease in 12 individuals, while for the rest we observed an increase in iron level.

Winiarska-Mieczan, Kwiecien (2011) found the highest content of iron in crisp bread and the lowest in graham bread. According to the authors, a daily portion of bread contains 4.5 mg of iron (ca. 25–45% of RDA). However, iron present in grain products is characterised by low assimilability (Hotz et al., 2003). A relatively low potential iron bioavailability was observed from gluten-free products in the study by Suliburska et al. (2013). The highest *in vitro* bioavailability for iron was found in gluten-free biscuits followed by gluten-free bread, peas puff, gluten-free pasta and corn porridge (~58%, ~38%, ~36%, ~28% and ~28%, respectively). Anaemia is the most frequently seen disorder in the haematologic system that often develops due to iron deficiency (Catal et al., 2015) and iron deficiency anaemia is one of the most common nutritional disorders in the world (Ghasemi et al., 2010). This may be due to a low iron intake, lack of absorption in the intestine, excessive blood loss and/or increased need (Akhter et al., 2005). The pathogenesis of anaemia is usually dependent on malabsorption of iron or vitamins (Halfdanarson et al., 2007), although occult blood loss from the gastrointestinal tract may occur (Fine, 1996). Health benefits of iron include mainly transferring of oxygen to human blood cells. About two-thirds of bodily iron is found in haemoglobin. Iron deficiency can often cause severe fatigue, physical weakness, and related health problems (https://www.organicfacts.net/health-benefits/minerals/health-benefits-of-iron.html). Although the aetiology of iron deficiency anaemia is multifactorial, it usually results from the fact that the iron requirements of the organism are not satisfied by its adequate absorption regardless of the cause (Clark, 2008). The recommended iron values usually are based on the genetic and diet iron-bioavailability, which can be considered as the principal factor that differs among the cultures and influences the distinct levels of recommendation among countries (Diego Quintaes et al., 2017). Anaemia cannot only be explained by nutritional deficiencies. Not all dietary ingested iron, heme or nonheme, will be available to absorption. In coeliac patients suppression of intestinal inflammatory changes as a result of a gluten-free diet improves anaemia by correcting iron malabsorption as well as mechanisms contributing to anaemia of chronic disease (Bergamaschi et al., 2008). Wierdsma et al. (2013) assessed coeliac patients before starting a gluten-free diet measuring their serum levels of folic acid, vitamins A, B6 and B12, vitamin D, iron and zinc and found out that 87% of patients had deficient values for at least one serum vitamin or mineral, especially zinc and iron. Larretxi et al. (2019) found in their study that about 1/4 of coeliac participants did not reach recommendations for iron. According to Gonzalez et al. (2018) the iron, iodine, potassium and selenium dietary reference intakes were better complied by coeliac men than women. Harper et al. (2007) found iron deficiency in 33% of males and 19% of females and anaemia in 20% of coeliac patients. The iron food fortification is considered more cost effective and economically more attractive than the iron supplementation. Thompson (2000) reported on the iron fortification in 23% of gluten-free breads and no fortification in US gluten-free pasta products. There are many iron compounds available to be used in iron fortification and cereals represent a target food group to iron fortification programs due to high consumption (Diego Quintaes et al., 2017). Suliburska et al. (2011) found that most of the analysed food products fortified with iron had significantly higher contents and total and relative bioavailability of this element in comparison with the non-fortified analogues and concluded that the fortified with iron food products appear to be better sources of potentially bioavailable iron in comparison with the non-fortified analogues.

CONCLUSION

A long-term unbalanced diet can cause serious chronic and civilization diseases. Gluten-free diet is considered to be a healthier alternative to diet, but gluten-free products are also considered nutritionally deficient as compared to their gluten-containing counterparts. Without supplementation of the nutrients, the main source of which is cereal-based food, the diet may become deficient. As shown by the analysis of dietary habits and daily nutrient intake, the gluten-containing diet of our healthy volunteers did not satisfy the recommended daily intake of investigated micronutrients. However, the results of our study show that replacement of the gluten-free bread and bakery products has no negative effect on the mineral profile of healthy consumers. Apart from calcium, the levels of the monitored parameters (phosphorus, magnesium and iron) have increased. We cannot claim unambiguously that gluten-free food is inappropriate for healthy consumers. Many people with normal dietary habits eat poorly under the influence of various lifestyle factors. In many cases, the reason for the preference of alternative forms of diet is to improve nutritional intake and nutrient balance in the diet. If the consumer has sufficient knowledge about nutrition, food composition, nutritional needs and metabolism, the substitution of any gluten-containing product by a gluten-free product, whether for a shorter or longer period, should not adversely affect the consumer's health.

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