

THE EFFECT OF 6-WEEK CONSUMPTION OF BAKERY PRODUCTS ON CHANGES IN SELECTED ANTHROPOMETRIC PARAMETERS IN WOMEN

Petra Lenártová¹, Martina Gažarová¹

¹Slovak University of Agriculture in Nitra, Faculty of Agrobiological and Food Resources, Institute of Nutrition and Genomics, Nitra, Slovakia

ABSTRACT

Background. The changes in consumers' eating habits are one of the most important factors affecting the consumption of selected foods. The most important factors for bakery products consumers are freshness, taste, price, durability, composition, and country of origin.

Objective. The aim of this study was to monitor changes in selected anthropometric characteristics in women from the general population in Slovakia, after 6 weeks of bakery products consumption.

Material and Methods. The study was conducted on healthy adult women from the general population (n=78), who were divided into four subgroups ("gluten" group, "gluten-free" group, "whole-grain" group and "control" group). The intervention dose consisted of a different combination of several types of bakery products (bread, pastries, soft pastries) within the individual weeks of consumption, while the intervention lasted 6 weeks, women consumed 150-200 grams per day. Anthropometric characteristics were measured by body composition analyser InBody 720 (Biospace Co. Ltd., Seoul, Republic of Korea), which works on the principle of bioelectrical impedance analysis. We also used the Lookin' Body 3.0 software to process the results. The collected data from these measurements were evaluated statistically and graphically in Microsoft Office Excel 2010 (Los Angeles, CA, USA). The levels of statistical significance were set at P<0.05 (*); P<0.01 (**); P<0.001 (***). Differences among subgroups of probands were tested with Pared Student t-test.

Results. The amount of fat mass (FM) in the participants changed along a similar curve as their body weight. After the 6-week intervention, there was a slight decrease in FM with statistical significance (P<0.01). In the third measurement, i.e., two months after the intervention, there was an increase in the amount of FM, but without statistical significance. Fat free mass (FFM) values in women increased very slightly after 6 weeks of consumption and remained approximately at the same level two months after the intervention (without statistical significance). We noted the most significant changes in the visceral fat area (VFA) parameter, which had a steadily increasing tendency. Changes in VFA values were statistically significant after the 6-week intervention (P<0.01) and also after two months of the intervention (P<0.05).

Conclusions. Changes in body composition in women were noted already after 6 weeks of bakery products consumption, especially in the FM, FFM, VFA and partially BMI parameters, but no changes were recorded in the WHR parameters during the entire duration of the study. One of the most significant results is the finding that there was an increase in VFA when consuming all types of bakery products (gluten, gluten-free and whole grain).

Key words: bakery products, anthropometric parameters, fat mass, FM, fat free mass, FFM, visceral fat area, VFA

INTRODUCTION

The challenge of provision of a much wider range of foods of relatively low glycaemic response than is currently available, especially in terms of cereal products, has been highlighted in recent years and this has relevance to bread consumption. Although there has been some transition to brown bread consumption, white bread remains a firm feature in the typical average western diet.

Food, which is the necessity of human life, is of critical importance in terms of community health. Bakery products and their derivatives have an important place in the food consumption all over the world [6, 9, 36]. Bread is, in fact, the only food known everywhere around the world, from the most developed to the most primitive cultures, and it is on the tables of everybody one way or another. Consumer perceptions and behaviour regarding this staple food are very important for the industrial bakery sector [29].

Corresponding author: Petra Lenártová, Slovak University of Agriculture in Nitra, Faculty of Agrobiological and Food Resources, Institute of Nutrition and Genomics, Tr. A. Hlinku 2, 94976 Nitra, Slovak Republic, phone: +421 37 641 4246, e-mail: petra.lenartova@uniag.sk

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Bread with its high nutritional value, a neutral aroma, availability, and cheapness is the basic food source in bakery products [9]. The quality and nutritional value of dietary proteins are determined by the quantity, digestibility, and bioavailability of essential amino acids (EAA), which play a critical role in human growth, longevity, and metabolic health. Plant-source protein is often deficient in one or more EAAs (e.g., branched-chain amino acids, lysine, methionine and/or tryptophan) and, in its natural form, is less digestible than animal-source protein. Nevertheless, dietary intake of plant-source protein has been promoted because of its potential health benefits, lower cost of production and lower environmental impact compared to animal-source protein. Implementation of dietary strategies that improve both human and planetary health are of critical importance and subject to growing interest from researchers and consumers [6]. The grain-based products have been and still are an important part of people's regular diets. Their nutritional properties recommend them as significant energy sources for the human body [3, 33].

The health effects of cereal foods depend on the type of product. The intake of whole-grain cereal foods is consistently associated with health benefits at the population level, and increased consumption is therefore warranted and advocated in official dietary guidelines in many countries [22]. However, many observational studies have shown consistent inverse associations between high whole-grain intake and the risk of developing noncommunicable diseases such as type 2 diabetes, cardiovascular disease, colorectal cancer, and total and cause-specific mortality [19, 30]. In addition to observational studies, more than 200 dietary intervention studies have shown – depending on the cereal species – reduced body weight, decreased total cholesterol, improved systolic blood pressure [30] improved postprandial glucose and insulin homeostasis [21] decreased inflammatory markers, [10] and lowered total and low-density lipoprotein cholesterol [11, 13] when whole grains have been consumed, although it should be acknowledged that some results are conflicting. The evolution of wheat consumption has been of interest for a long time [1, 16]. However, concerns regarding the negative influence of its consumption through its derived products such as bread on health [5] or the environment [7] have increased. In view of the growing trend of obesity, some questions can be raised about the quality of nutritional information on food.

The changes in consumers' eating habits are one of the most important factors affecting the consumption of selected foods. The most important factors for bakery products consumers are freshness, taste, price, durability, composition, and country of origin. Rational aspects in the purchase of bakery products are factors

of composition and durability. The irrationality of consumers' purchasing behaviour can be accompanied by psychological factors, which include the perception of freshness and smell of bakery products, perception of the price, perception of the country of origin, as well as sensory aspects such as taste [18].

The most important factor influencing the purchase of bakery products is their price, but its importance decreased with the growth of consumer incomes. The quality of bakery products is more important to women, while special offers were more important to men. The frequency of buying bakery products is significantly influenced by age, with younger respondents buying more often than older categories of respondents. In addition, all age categories of consumers prefer supermarkets or hypermarkets to local stores [20].

Obesity is defined as an abnormal or excessive accumulation of fat that presents a risk to health [39]. Obesity, however, is also recognized as a heterogeneous disorder [14, 35]. Obese individuals are known to vary in their body fat distribution, and increasing evidence suggests that the regional distribution of adipose tissue might be more important than the total amount of body fat [15]. Body fat tissue has traditionally been thought to be distributed across two main compartments – subcutaneous fat, and visceral fat. These two different fat depots have been shown to have disparate functions, biochemical features, and metabolic characteristics [2].

The aim of the work was to evaluate the changes in the anthropometric characteristics of females from the general population after influence of 6 weeks of consumption of various bakery products.

MATERIALS AND METHOD

The study was conducted in healthy adult women from the general population ($n = 78$), who were divided into four subgroups (“gluten” group, “gluten-free” group, “whole-grain” group and “control” group). All participants signed written informed consent to participate in the study and were informed about the course and type of measurements, that will be used in the study. The research was approved by the Ethics Committee at the Specialized Hospital St. Zoerardus Zobor, n.o. (approval number 012911/2016).

Volunteers with the severe disease or with recommended special dietary regimen were excluded. Women of “gluten group” consumed gluten-containing bread and bakery products; women of “gluten-free group” consumed gluten-free bread and bakery products; women of “whole-grain” group consumed whole-grain containing bread and bakery products and women of “control group” not consumed bakery products. The amount of bread and bakery products consumed was determined according to

the recommended food consumption for the Slovak population, which means that women consumed 150-200 grams per day for 6 weeks. All participants were asked not to change their eating and physical activity habits.

Three measurements were taken during the study: (1) before the intervention; (2) after six-week intervention; (3) two months after intervention. Body height of probands was measured with device TANITA WB-380H, which measures body height and body weight at the same time. When measuring body height, the proband stands upright, his legs are close to him, his hands are next to his body. The head is in the orientation so-called Frankfurt location, i.e., his eyes are fixed on a point on the opposite side at the height of the person's eyes. The distance of the vertex from the plane on which the proband is standing is measured. The measured person stands without shoes, dressed only in underwear.

Anthropometric characteristics were measured by body composition analyser InBody 720 (Biospace Co. Ltd., Seoul, Republic of Korea), which works on the principle of bioelectrical impedance analysis. Proband was informed about measurements procedure, explained the possible risks of measuring in the case of pregnancy or having an artificial pacemaker at the heart. The measurement must be performed before a meal or 2 hours after a meal and after taking it toilets. It is not recommended to drink large amounts of fluids before the examination. There should be no major physical activity before the examination, as temporary changes in body composition may occur. When measuring during menstruation, there will be an increase in body water, so it is better to perform measurement only after its completion. It is also not advisable to perform the test after or after showering sauna, because sweating causes temporary changes in body composition. During testing it is suitable for a temperature of 20-25 °C. If a repeat test is performed, it is necessary him under the same conditions. We also used the Lookin' Body 3.0 software to process the results.

The collected data from these measurements were evaluated statistically and graphically in Microsoft Office Excel 2010 (Los Angeles, CA, USA). The levels of statistical significance were set at $P < 0.05$ (*); $P < 0.01$ (**); $P < 0.001$ (***). Differences among subgroups of probands were tested with Pared *Student t*-test.

RESULTS AND DISCUSSION

In the study, we monitored the influence of bakery product consumption on women's body composition. Anthropometric measurements were performed in 78 women with an average age of 31.64 ± 9.42 years (in "gluten" group it was 31.76 years; in "gluten-free"

group 29.11 years; in "whole-grain" group 44.08 years and in "control" group it was 27.86 years). To determine the body composition the bioelectrical impedance analysis was performed.

Single-frequency bioelectrical impedance analysis is recognized as an appropriate measurement of body composition [27, 34]. BIA is a method for estimating body composition, in particular body fat and muscle mass, where a weak electric current flows through the body and the voltage is measured in order to calculate impedance (resistance) of the body. Most body water is stored in muscle. Therefore, if a person is more muscular there is a high chance that the person will also have more body water, which leads to lower impedance. BIA determines the electrical impedance, or opposition to the flow of an electric current through body tissues which can then be used to estimate total body water (TBW), which can be used to estimate fat-free body mass and, by difference with body weight, body fat [31].

Table 1 shows the changes in anthropometric parameters of all study women ($n=78$). Average body weight ranged around 63.43 kg (maximum 101.53 kg; minimum 43.17 kg). By evaluating the changes in the body composition of the entire group of women during the study, we found that the body weight after the 6-week intervention decreased slightly with statistical significance ($P < 0.001$). However, with an interval of two months, an increase in body weight was recorded in the probands to a higher average value of body weight than before the intervention with a statistically significant difference ($P < 0.05$).

Body mass can be divided into two major components: body fat (energy stores) and lean mass (including muscle, organs, and bone), each of which has distinct biological significance and was likely subject to different selective pressures during human evolution. Humans have a high proportion of body fat compared to other primates, and to mammals more widely [28, 37, 41]. In contrast, skeletal muscle mass (a major constituent of lean mass) is low compared with our closest relatives [41], other primates [24].

When evaluating the changes due to the six-week consumption of various types of bakery products, we found that there were partial changes not only in body weight, but also in its individual components.

The amount of fat mass (FM) in the participants changed along a similar curve as their body weight. After the 6-week intervention, there was a slight decrease in FM with statistical significance ($P < 0.01$). In the third measurement, i.e., two months after the intervention, there was an increase in the amount of FM, but without statistical significance. The average values of FM during the study were 18.66 kg. The mean of the maximum during study was 51.33 kg; the mean of the minimum was 8.63 kg.

Table 1. Changes in anthropometric parameters of all study women (n=78)

Parameters	Before intervention	After 6-weeks intervention	Two months after intervention
Weight (kg)			
mean±SD	63.34±10.83	63.16±10.75	63.79±10.93
P value, Significance (*/**/****)	0.0873	0.0005***	0.0393*
FM (kg)			
mean±SD	18.64±7.83	18.43±7.74	18.91±7.91
P value, Significance (*/**/****)	0.0558	0.0011**	0.3006
FFM (kg)			
mean±SD	44.70±4.79	44.72±4.77	44.88±4.69
P value, Significance (*/**/****)	0.8180	0.4011	0.3177
VFA (cm²)			
mean±SD	75.38±27.96	75.02±28.29	76.81±28.20
P value, Significance (*/**/****)	0.4262	0.0027**	0.0426*
BMI (kg.m⁻²)			
mean±SD	22.81±3.68	22.74±3.63	22.95±3.75
P value, Significance (*/**/****)	0.0750	0.0004***	0.0330*
WHR			
mean±SD	0.87±0,06	0,88±0.05	0.88±0.06
P value, Significance (*/**/****)	0.2517	0.2418	0.0482*

*Data are expressed as average ± standard deviation (SD); n – number of participants; FM – fat mass; FFM – fat free mass; VFA – visceral fat area; BMI – body mass index; WHR – waist to hip ratio

Fat free mass (FFM) values in women (average value 44.70 kg; maximum 57.60 kg; minimum 34,20 kg) increased very slightly after 6 weeks of consumption (average value 44.72 kg; maximum 57.70 kg; minimum 34.20 kg and remained approximately at the same level two months after the intervention (without statistical significance). The mean of the maximum FFM was 57.77 kg; the mean of the minimum FFM was 34.53 kg.

Visceral fat area is important factor used in the assessment of cardiometabolic risk and is correlated with the metabolic syndrome even at the normal body mass index indicating the absence of obesity [4, 17]. In this study, we noted the most significant changes in the visceral fat area (VFA) parameter, which had a steadily increasing tendency. Changes in VFA values were statistically significant after the 6-week intervention (P<0.01) and also after two months of the intervention (P<0.05). VFA values higher than 100 cm² indicate abdominal obesity. In the set of monitored women, we recorded VFA values above 100 cm² in 16 women, of which 11 women during all three measurements; in 3 women, there was an increase in the values above the 100 cm² limit until two months after the intervention; 1 woman had an increased VFA only before the intervention, and 1 woman had the VFA decreased up to two months after the intervention. 31 women had borderline VFA values (71-100 cm²), and

31 women had borderline VFA values (71-100 cm²). 30 women had normal values (41-70 cm²).

In this study, average values of VFA were achieved to 75.74 cm² (the mean of the maximum was 159.67 cm²; the mean of the minimum was 33.71 cm²). From the point of view of assessing cardio-metabolic risk, VFA values are very important, as the accumulation of fat in the abdominal cavity and waist area is a risk factor for the development of typical metabolic (insulin resistance, type 2 diabetes mellitus...) and many cardiovascular diseases. However, the fact is that even low VFA values (<40 cm²) are endocrinologically undesirable. We found them in 7 women.

WHO compiled uniform categories of BMI. Four categories were established: underweight, normal, overweight, and obese. An individual would be considered underweight if their BMI was between 15 and 19.9 kg.m⁻², normal weight if their BMI was 20 to 24.9 kg.m⁻², overweight if their BMI was 25 to 29.9 kg.m⁻², and for obesity, if it were 30 to 35 kg.m⁻² or bigger. Using linear regression, a BMI of 16.9 kg.m⁻² in men and 13.7 kg.m⁻² in women represents a complete absence of body fat stores [8, 25, 39].

Body mass index (BMI) is a metric currently used to define anthropometric height/weight characteristics in adults and to classify (categorize) them into groups. A common interpretation is that it represents an individual's fatness index. It is also widely used

as a risk factor for the development or prevalence of several health problems. BMI has been useful in population-based studies based on its wide acceptance in defining specific categories of body weight as a health problem. But it's becoming increasingly clear that BMI is a poor indicator of body fat percentage. Importantly, BMI also does not capture information about the amount of fat in different parts of the body [25], which also confirm our findings. Based on BMI, there were 15 women with underweight 44 women with normal weight, 13 women with overweight and 6 women with obesity. The average values of BMI during the study were 22.83 kg.m⁻². The mean of the maximum during study was 35.96 kg.m⁻²; the mean of the minimum was 17.37 kg.m⁻².

The average values of WHR of the entire group of women did not change during the study, BMI increased slightly after the 6-week intervention, then decreased only minimally two months after the intervention. We did not observe statistically significant differences in both indexed parameters (WHR and BMI). WHR has been used as an indicator or measure of health, and as a risk factor for developing serious health conditions. WHR is used as a measurement of obesity, which in turn is a possible indicator of other more serious health conditions. The WHO states that abdominal obesity is defined as a waist–hip ratio above 0.90 for males and above 0.85 for females, or a body mass index (BMI) above 30.0 [38]. If obesity is redefined using WHR

instead of BMI, the proportion of people categorized as at risk of heart attack worldwide increases threefold [40]. WHR may be less accurate in individuals with a BMI of 35 kg.m⁻² or higher, and more complex to interpret since an increased WHR may result from increased abdominal fat or decreased lean muscle mass around the hips [12]. The body fat percentage is considered to be an even more accurate measure of relative weight. Of these three measurements, only the waist–hip ratio takes account of the differences in body structure. Hence, it is possible for two people of the same sex to have different body mass indices but the same waist–hip ratio, or to have the same body mass index but different waist–hip ratios. WHR has been shown to be a better predictor of cardiovascular disease than simple waist circumference and body-mass index [23]. The average values of WHR during the study were 0.88. The mean of the maximum during study was 1.01; the mean of the minimum was 0.78. Women with a WHR value higher than 0.8 and men with a value higher than 1.0 have a higher risk of health complications due to inappropriate distribution of fat in the body. In our study, there were only 7 women with a WHR value below 0.8, and therefore up to 71 women had an increased risk of health complications related to fat distribution.

When dividing the probands in more detail based on the bakery products type consumed (i.e., with gluten content; gluten-free; whole grain and without

Table 2. Changes in anthropometric parameters of “gluten” group (n=17)

Parameters	Before intervention	After 6-weeks intervention	Two months after intervention
Weight (kg)			
mean±SD	61.39±11.64	61.54±11.27	61.45±10.67
P value, Significance (*/**/****)	0.6249	0.7917	0.8843
FM (kg)			
mean±SD	15.48±6.92	15.59±6.62	15.52±6.57
P value, Significance (*/**/****)	0.6156	0.7999	0.9221
FFM (kg)			
mean±SD	45.91±5.96	45.95±6.06	45.94±5.54
P value, Significance (*/**/****)	0.8680	0.9643	0.9369
VFA (cm²)			
mean±SD	63.26±28.51	63.86±26.64	64.26±26.92
P value, Significance (*/**/****)	0.5653	0.7324	0.5447
BMI (kg.m⁻²)			
mean±SD	21.58±3.19	21.63±2.98	21.61±2.79
P value, Significance (*/**/****)	0.6708	0.8956	0.8481
WHR			
mean±SD	0.86±0.06	0.86±0.05	0.86±0.06
P value, Significance (*/**/****)	0.4477	0.3109	0.1635

*Data are expressed as average ± standard deviation (SD); n – number of participants; FM – fat mass; FFM – fat free mass; VFA – visceral fat area; BMI – body mass index; WHR – waist to hip ratio

Table 3. Changes in anthropometric parameters of “gluten-free” group (n=27)

Parameters	Before intervention	After 6-weeks intervention	Two months after intervention
Weight (kg)			
mean±SD	63.51±11.93	63.29±12.09	64.04±12.09
P value, Significance (**/**/***)	0.1550	0.0041**	0.093
FM (kg)			
mean±SD	18.60±9.44	18.66±9.27	19.36±9.44
P value, Significance (**/**/***)	0.8080	0.0038**	0.0171*
FFM (kg)			
mean±SD	44.91±4.08	44.63±4.05	44.68±4.19
P value, Significance (**/**/***)	0.2456	0.8033	0.5308
VFA (cm²)			
mean±SD	74.10±30.16	74.77±31.37	76.77±29.99
P value, Significance (**/**/***)	0.3732	0.0668	0.0231*
BMI (kg.m⁻²)			
mean±SD	22.75±4.12	22.67±4.13	22.94±4.19
P value, Significance (**/**/***)	0.1797	0.0043.94**	0.0900
WHR			
mean±SD	0.87±0.06	0.87±0.06	0.87±0.06
P value, Significance (**/**/***)	0.1726	0.7326	0.1109

*Data are expressed as average ± standard deviation (SD); n – number of participants; FM – fat mass; FFM – fat free mass; VFA – visceral fat area; BMI – body mass index; WHR – waist to hip ratio

Table 4. Changes in anthropometric parameters of “whole grain” group (n=12)

Parameters	Before intervention	After 6-weeks intervention	Two months after intervention
Weight (kg)			
mean±SD	64.19±9.66	63.86±9.74	64.78±10.21
P value, Significance (**/**/***)	0.0487*	0.0061**	0.1152
FM (kg)			
mean±SD	20.19±6.46	19.68±6.72	20.28±6.91
P value, Significance (**/**/***)	0.0061**	0.0161*	0.9898
FFM (kg)			
mean±SD	44.00±4.68	44.19±4.61	44.51±4.69
P value, Significance (**/**/***)	0.2515	0.2298	0.0390*
VFA (cm²)			
mean±SD	82.45±24.19	80.81±25.47	83.31±25.88
P value, Significance (**/**/***)	0.0095**	0.0097**	0.6121
BMI (kg.m⁻²)			
mean±SD	23.48±3.47	23.36±3.46	23.65±3.71
P value, Significance (**/**/***)	0.0426*	0.0064**	0.1066
WHR			
mean±SD	0.89±0.05	0.89±0.05	0.89±0.05
P value, Significance (**/**/***)	0.8224	0.4661	0.5818

*Data are expressed as average ± standard deviation (SD); n – number of participants; FM – fat mass; FFM – fat free mass; VFA – visceral fat area; BMI – body mass index; WHR – waist to hip ratio

Table 5. Changes in anthropometric parameters of “control” group (n=22)

Parameters	Before intervention	After 6-weeks intervention	Two months after intervention
Weight (kg)			
mean±SD	62.95±9.44	62.45±9.36	62.95±9.86
P value, Significance (*/**/****)	0.0358*	0.0997	1.0000
FM (kg)			
mean±SD	19.14±5.91	18.54±6.15	19.05±6.38
P value, Significance (*/**/****)	0.0165*	0.0713	0.7755
FFM (kg)			
mean±SD	43.81±4.64	43.91±4.58	43.90±4.55
P value, Significance (*/**/****)	0.6561	0.9392	0.7147
VFA (cm²)			
mean±SD	77.98±21.93	75.85±23.01	77.60±23.04
P value, Significance (*/**/****)	0.0069**	0.1054	0.7511
BMI (kg.m⁻²)			
mean±SD	22.80±3.52	22.61±3.44	22.80±3.68
P value, Significance (*/**/****)	0.0337*	0.0953	0.953
WHR			
mean±SD	0.88±0.04	0.87±0.04	0.87±0.04
P value, Significance (*/**/****)	0.3386	1.0000	0.5229

*Data are expressed as average ± standard deviation (SD); n – number of participants; FM – fat mass; FFM – fat free mass; VFA – visceral fat area; BMI – body mass index; WHR – waist to hip ratio

consumption of bakery products) we found certain differences. Tables 2, 3, 4 and 5 show the changes in anthropometric parameters of “gluten” group; “gluten-free” group; “whole-grain” group and “control” group.

After six weeks of intervention with different bakery products, we found that women in the “whole grain” group achieved a slight increase in fat-free mass (FFM) and this increase was maintained two months after the intervention ($P < 0.05$), similarly, the value of FFM increased at the same level in the other monitored groups. Only in the gluten-free group did the FFM values decrease (without statistical significance).

Furthermore, there was an increase visceral fat area (VFA) in the “gluten” and “gluten-free” groups, and a decrease VFA in the “control” and “whole grain” groups. The values of fat mass (FM) increased slightly in the “gluten” and “gluten-free” groups and decreased in the “whole-grain” and “control” groups.

The consumption of different types of bakery products thus caused changes in the body structure of women after only 6 weeks of intervention.

Some studies have suggested that promoting the Mediterranean diet as a healthy eating model may help prevent weight gain and the development of overweight/obesity. The consumption of bread, which was part of the traditional Mediterranean diet, has been declining because the general public believes that bread is fattening. A reduction in consumption of white bread, but not whole grain bread, within

a Mediterranean-style diet is associated with lower weight gain and abdominal fat. The difference in composition between whole grain bread and white bread appears to make a difference in its effect on body weight and abdominal fat [32].

Whole grains have received increased attention for their potential role in weight regulation. A high intake has been associated with smaller weight gain in prospective cohort studies, whereas the evidence from randomized controlled studies has been less consistent [26].

CONCLUSION

Changes in body composition were noted already after 6 weeks of consumption, especially in the FM, FFM, VFA and partially BMI parameters, but no changes were recorded in the WHR parameters during the entire duration of the study. One of the most significant results is the finding, that there was an increase in VFA when consuming all types of bakery products (gluten, gluten-free and whole grain), which represents a serious cardio-metabolic risk. Therefore, we consider it necessary to improve lifestyle factors in order to improve this unflattering situation of women from the general Slovak population. Further research is needed to monitor the effect of different types of bakery products on body composition, especially fat mass, including visceral fat, in a larger number of

volunteers with using daily records of physical activity during the entire study period.

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Conflict of interest

The authors declare no conflict of interest.

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