

Rocz Panstw Zakl Hig 2024;75(1):67-73

https://doi.org/10.32394/rpzh.2024.0287

ORIGINAL ARTICLE

# EVALUATION OF LIPOPROTEINS AND HIGH SENSITIVITY CRP IN CONSUMERS OF BAKERY PRODUCTS

http://wydawnictwa.pzh.gov.pl/roczniki pzh/

Petra Lenártová<sup>1,</sup><sup>(b)</sup>, Martina Gažarová<sup>1,</sup><sup>(b)</sup>, Jana Kopčeková<sup>1,</sup><sup>(b)</sup>, Jana Mrázová<sup>1,</sup><sup>(b)</sup>

<sup>1</sup>Slovak University of Agriculture in Nitra, Faculty of Agrobiology and Food Resources, Institute of Nutrition and Genomics, Nitra, Slovakia

## ABSTRACT

**Background.** In recent years, a wider range of bakery products with a lower glycaemic response can be observed in the food industry. This contributes to the provision of a wider range of cereal bakery products. The gradual increase in the consumption of brown bread is significant, but despite this, white bread remains a part of the typical Western diet. Studies showed high intake of carbohydrates increase TG levels by enhancing hepatic synthesis of very low-density lipoprotein (VLDL) and decrease activity of lipoprotein lipase. White bread consumption has been therefore associated with an unhealthy lifestyle.

**Objective.** The aim of this study was to assess the influence of the consumption of gluten bakery products on lipids and inflammatory parameters of the probands.

**Material and Methods.** The monitored group consisted of 30 probands from the general population. The average age of the monitored group was 29.7 years. The intervention dose consisted of a different combination of several types of bakery products containing gluten (bread, pastries, soft pastries) within the individual weeks of consumption, while the intervention lasted 6 weeks. An intervention dose of 150 to 200 g per day was set for women and 200 to 250 g per day for men. Biochemical blood parameters were determined using a fully automatic Biolis 24i Premium blood serum biochemical analyzer, by end-point photometry method. We tested the differences between the biochemic parameters by one-factor analysis of variance (ANOVA) and compared them by Tuckey's Post Hoc Test.

**Results.** The measurement of the lipid profile showed that the average levels of total cholesterol (TC) were above the reference value (<5.00 mmol. l-1) in each of the three performed measurements (P<0.01). In the case of LDL, we found a similar trend in the development of lipoprotein values, while we positively evaluate a slight reduction of LDL in the measurement immediately after the intervention (P<0.001). Certain changes during the study were also noted in HDL parameters with high statistical significance (P<0.001). During the TG analysis, we found that probands have normal values (0.45-2.70 mmol. l-1). A reduction in average TG values was achieved in individual measurements, but without statistical significance (P>0.05). In high sensitivity CRP (hs-CRP) parameters was achieved a bell curve of the development of average values, with a maximum measured immediately after the intervention. Changes in hs-CRP during the study were without statistical significance (P>0.05)

**Conclusions**. The measurement of the lipid profile showed that the average levels of TC, LDL and HDL, there were above the reference value in each of the three measurements performed. Through the analysis of TG, we found normal values and during the study there was a slight decrease. Furthermore, we found that intervention with bakery products containing gluten was associated with an increase in hs-CRP levels in our probands.

Key words: gluten bakery products, lipoproteins, CRP, health, inflammatory reaction

# **INTRODUCTION**

Foods made from cereals are necessary to fulfil the need for nutrients. Therefore, assessing and managing grain quality is critical to our health and well-being. Grains have always been essential in the diet, as evidenced by the fact that growing grain was a reason for people to settle in one place, because that was the only way they could sow the seed and

harvest the harvest. The consequence of this act was the emergence of civilization [30].

The chemical composition of cereal grain varies according to the species, variety, soil and climatic conditions, agricultural techniques (fertilization, protection, etc.) and weather conditions in a particular year [18]. Cereals are mainly a source of carbohydrates (55-78%), mainly starch. The protein content is 7-19%. Cereal proteins are classified as incomplete,

**Corresponding author:** Petra Lenártová, Slovak University of Agriculture in Nitra, Faculty of Agrobiology 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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Publisher: National Institute of Public Health NIH - National Research Institute

the limiting amino acid is lysine [19]. They have a high content of proline and glutamine [9]. The fat content is in the range of 1-5%. Lipids should have a simple composition. Cereals are a source of vitamins of group B and vitamins of group E (tocopherols, tocotrienols), fiber, minerals, especially drugs, iron, magnesium, copper, manganese, zinc and phosphorus, antioxidants, and phytochemicals. Carotenoids (lutein), polyphenolic components (phenolic acids, alkylresorcinols, lignans), phytosterols and other biologically active substances (choline, betaine, etc.) play a significant role in nutrition and health [2, 6]. The content of individual nutrients in flour depends on the degree of milling [19].

The bread we eat today has changed little from the bread our ancestors ate a few millennia ago. There are hypotheses, partially supported by archaeological excavations, that mankind has known bread for about 6000 years. At the turn of the last century and the current one, there is also information that mankind consumed bread 15-20 thousand years ago. These opinions resulted from archaeological findings, allegedly also in the territory of Bohemia and Russia, where stones were found on which plant seeds were crushed and porridge was apparently made from this grain grinding [27].

Bread is an important stable food [8]. In recent years, a wider range of bakery products with a lower glycemic response can be observed in the food industry. This contributes to the provision of a wider range of cereal bakery products. The gradual increase in the consumption of brown bread is significant, but despite this, white bread remains a part of the typical Western diet [7].

In many European countries, the higher social classes more often prefer whole-grain and brown bread to white bread [5, 12, 17]. Total bread consumption is nevertheless often associated with low socioeconomic status [12, 23, 25]. Grains without bran have higher glycaemic load and glycaemic index than whole grains and have adverse effect on health. [29]. Studies showed high intake of carbohydrates increase TG levels by enhancing hepatic synthesis of very lowdensity lipoprotein (VLDL) and decrease activity of lipoprotein lipase [15]. White bread consumption has been therefore associated with an unhealthy lifestyle [20].

The aim of this study was to monitor the influence of the consumption of gluten bakery products on selected biochemical blood parameters of the probands.

#### **MATERIAL AND METHODS**

The monitored group consisted of 30 probands from the general Slovak population who were obtained by random selection, regardless of gender and age. All participants took part in the study expressly on a voluntary basis, without any coercion or financial or other remuneration for participating in the study. The research was approved by the Ethics Committee at the Specialized Hospital of St. Svorada Zobor, n.o., Nitra, Kláštorská 131, 94901 Nitra under number 012911/2016. This study was part of a larger study, partly already published [14].

For inclusion, or exclusion of probands, the following inclusion and exclusion criteria were used. The main inclusion criteria were good health, the absence of serious acute or chronic diseases, the absence of food allergies and intolerances, as well as the absence of drug treatment, and pregnancy in the case of women, as these factors could affect the results of the clinical study. Other conditions were not to change your eating habits, diet, physical activity, or lifestyle during the entire duration of the study. The condition for participation in the study was oral and written submission of information about the nature, course, and conditions of the study, which all enrolled probands confirmed by signing the informed consent.

The group consisted of 13 men (43.3%) and 17 women (56.7%). The average age of the monitored group was 29.7 years, while the age range varied from 21 to 53 years. The average height in the group was 174.53 cm (height range 154-196 cm) and the average body weight was 71.24 kg (weight range 42.8-104.1 kg). The intervention dose consisted of a different combination of several types of bakery products containing gluten (bread, pastries, soft pastries) within the individual weeks of consumption. Their amount considered the recommendations for the intake of bakery products regarding the gender of the probands. An intervention dose of 150 to 200 g per day was set for women and 200 to 250 g per day for men.

The intervention lasted 6 weeks. All measurements were taken before, immediately after the intervention and at an interval of 8 weeks after the end of the intervention. For these reasons, the total duration of the study was predetermined at 14 weeks. Monitored parameters (TC; DL; HDL; TAG, hs-CRP) were determined using a fully automatic Biolis 24i Premium blood serum biochemical analyzer (Tokyo Boeki Medical System Ltd., 569-6, Nobe, Akiruno, Tokyo 197-0823, Japan). It is an automatic biochemical analyzer for determination of substrates, enzymes, electrolytes, and specific proteins. The analyzer is compact, has the functions of larger analyzers. Highly efficient, noncontact (by air pressure) mixing of the reaction mixture in the cuvette is unique and ensures protection against any mutual contamination of the stirrer. An automatic washing system is used to wash the cuvettes. Reagents are identified based on the ID position or barcode according to the setting. The method of determination in this device is end-point photometry (turbidimetry), kinetics, homogeneous immunoanalysis. The device works at wavelengths: 40, 380, 405, 450, 505, 546, 570, 600, 660, 700, 750, 800 nm.

For statistical processing of the obtained data, we used the Microsoft Office Excel 2010 program (Los Angeles, CA, USA) in combination with XLSTAT (Version 2019.3.1). Statistical analyzes were also performed using the program Statistik Cz Version 10 (TIBCO Software Inc., Palo Alto, California, USA).

The resulting values of the measured parameters are presented as mean  $\pm$  standard deviation (SD). Statistical significance levels were set at P<0.05 (\*); P<0.01 (\*\*); P<0.001 (\*\*\*). We tested the differences between the anthropometric parameters by one-factor analysis of variance (ANOVA) and compared them by Tuckey's Post Hoc Test.

### **RESULTS AND DISCUSSION**

The lipid profile represents a group of lipid parameters - total cholesterol (TC), low density lipoproteins (LDL), high density lipoproteins (HDL) and triacylglycerols (TG), based on which it is possible to determine the risk of primarily cardiovascular and, in some cases, metabolic diseases. The lipid profile is considered a good indicator of the risk of developing cardiovascular complications of atherosclerosis, i.e., myocardial infarction and sudden stroke.

The average values of the levels of individual lipoproteins, evaluated within the lipid profile of the

probands during the study, are shown in Table 1. During a more detailed analysis of total cholesterol values, we found that before the intervention only 3 probands (1 woman and 2 men) had levels in the physiological range (<5.00 mmol. l<sup>-1</sup>), the remaining 27 probands had elevated values above this limit. After the intervention, we recorded the physiological values of cholesterol in 6 probands (3 women and 3 men) and the number of probands with hypercholesterolemia decreased to 24. At the same time, there was also a decrease in the average value of cholesterol in the group of women and in the entire group of probands, a slight increase in the average value was noted in men total cholesterol. Subsequently, in measurements with an interval of 2 months, we observed the physiological TC values in 5 probands (2 women and 3 men), 25 probands had increased TC values. At the same time, there was an increase in the average values of TC both in the whole group and also when differentiating by gender. The mentioned differences between the 2nd and 3rd measurements were statistically significant (P<0.01).

LDL particles should be in the range of 1.20-3.00 mmol. 1<sup>-1</sup> in the blood of a healthy person. In this case too, we found a similar trend in the development of lipoprotein values. In the first measurement, only 8 probands (5 women and 3 men) reached the physiological limit of LDL and the rest had elevated LDL values, in the second measurement there was a relatively significant improvement in LDL values,

	Before intervention $\overline{X} \pm SD$	After 6-weeks intervention $\overline{X} \pm SD$	$\begin{array}{c} 2 \text{ months after} \\ \text{intervention} \\ \overline{X} \pm \text{SD} \end{array}$	P value (significance) */**/***					
TC (mmol.l <sup>-1</sup> )									
All (n=30)	6.05±0.92	5.82±0.95	6.22±1.41	P<0.01(**)					
Women (n=17)	6.33±0.92	$5.84{\pm}0.97$	6.30±1.37	P<0.01(**)					
Men (n=13)	5.67±0.81	5.79±1.52	6.10±1.52	P>0.05					
LDL (mmol.l <sup>-1</sup> )									
All (n=30)	3.56±0.67	3.11±0.85	3.74±1.10	P<0.001(***)					
Women (n=17)	3.49±0.71	2.88±0.85	3.56±1.04	P<0.05(*)					
Men (n=13)	3.64±0.64	3.41±0.78	3.99±1.18	P<0.001(***)					
HDL (mmol.l <sup>-1</sup> )									
All (n=30)	1.75±0.85	1.75±0.52	1.93±0.54	P<0.001(***)					
Women (n=17)	2.04±0.58	1.99±0.52	2.20±0.49	P<0.001(***)					
Men (n=13)	1.36±0.29	1.42±0.32	1.56±0.35	P<0.05(*)					
TG (mmol.l <sup>-1</sup> )									
All (n=30)	1.22±0.73	1.07±0.44	1.07±0.46	P>0.05					
Women (n=17)	1.16±0.54	0.92±0.31	0.98±0.42	P<0.01(**)					
Men (n=13)	1.30±0.95	1.26±0.53	1.20±0.50 P>0.05						

Table 1. Lipoprotein levels of probands

TC – total cholesterol; LDL – low density lipoprotein; HDL – high density lipoproteins; TG – triacylglycerols;  $\overline{X}$  – average; SD – standard deviation

as 15 probands (50%) had physiological values (12 women and 3 men) (P<0.001). We negatively evaluate the repeated decrease in the number of probands with physiological LDL values in the third measurement, only 8 probands (6 women and 2 men) reached them (P<0.001).

Certain changes during the study were also noted in the HDL parameter (P<0.001). The physiological range (women 1.20-2.70 mmol.  $1^{-1}$ ; men 1.00-2.10 mmol.  $1^{-1}$ ) was reached in the first measurement by 15 women (1 had reduced and 1 increased HDL values) and 12 men (1 man had reduced HDL values). In the second measurement, the situation in women did not change, while in men, 1 proband normalized HDL from a low value and the other proband reached values of 2.16 mmol.  $1^{-1}$ , which is already a value slightly above the norm. The situation in the third measurement in men did not change, in women compared to the second measurement we recorded an increase in HDL in one subject to a value of 2.73 mmol.  $1^{-1}$ , which is above the reference value.

By evaluating TG, we found that up to 29 probands had normal values (0.45-2.70 mmol. 1-1) in the 1st measurement, which is quite positive information from the point of view of evaluating their health and nutritional status. One proband (a man) had an initial TG value of 4.04 mmol. l-1, which, among other things, may reflect not only errors in the diet, but also an incorrect lifestyle (irregular regime, little sleep, low level of physical activity, etc.). However, in the measurement after the intervention, his TG values decreased, so all probands reached optimal levels of these lipoproteins in the blood. In the third measurement, all probands again had normal TG values. Average TG values had a decreasing trend during the study (from 1.22±0.73 mmol.  $1^{-1}$  in the first measurement to  $1.07\pm0.46$  mmol. 1<sup>-1</sup> in the third measurement), but without statistical significance (P > 0.05).

In a study by Sawicki et al. [24], a higher intake of whole grains was associated with a greater increase in HDL cholesterol and a decrease in triglyceride concentrations, and conversely, a higher intake of refined cereal products was associated with a smaller decrease in triglyceride concentration (P<0.001). Evidence from observational studies found that greater consumption of whole grain bakery products is also associated with a lower risk of cardiovascular disease (CVD) [3], as well as obesity, type 2 diabetes, hypertension [1] and all-cause mortality [11, 31]. Nevertheless, intake of refined grains remains high. Some, but not all, studies suggest that a higher intake of refined grains is associated with a higher risk of CVD [13]. Higher intake of whole grains was associated with a smaller increase in waist circumference (about 1.4±0.2 cm compared to refined grains, where the increase in waist circumference was 3.0±0.1 cm (P<0.001). The

changes were also in fasting glucose concentrations (an increase of  $0.7\pm0.4$  mg.dl<sup>-1</sup> compared to  $2.6\pm0.2$  mg.dl<sup>-1</sup> (P < 0.001)) and systolic blood pressure (by  $0.2\pm0.5$  mmHg compared to  $1.4\pm0.3$  mmHg (P<0.001). When stratified by sex, a stronger association of consumption of individual types of cereals with waist circumference was observed in women than in men [24]. Therefore, replacing refined grains with a higher intake of whole grains and their products is a potential dietary strategy to reduce CVD risk.

C-reactive protein (CRP) is considered the most sensitive reactant of the acute phase of inflammation, CRP concentrations rise very quickly during inflammatory processes to many times the norm. CRP activates the complement system, triggers opsonization and phagocytosis of damaged cells, but its main function is to bind and detoxify endogenous toxic substances arising as a product of tissue damage. About two decades ago, the importance of small, chronic elevations of CRP as an indicator of cardiovascular risk in healthy people was identified. A very sensitive method has been developed for measurement, therefore CRP measured by this method is called highly sensitive C-reactive protein (hs-CRP).

Several studies have concluded that the hs-CRP value can be used as an indicator of cardiovascular risk, i.e., a marker for predicting the risk of developing coronary heart disease in healthy people, as well as an indicator of the prognosis of cardiovascular events with its very close relationship to the "inflammatory" theory of the pathogenesis of atherosclerosis [26]. Both methods (CRP and hs-CRP) measure the same protein in the blood. "Classic" CRP is normal if the CRP concentration is less than 5 mg. 1<sup>-1</sup>. Accurate determination of CRP concentrations in the range of 0-5 mg. 1<sup>-1</sup> can only be realized using a special highly sensitive hs-CRP test. In healthy adults, the average value of CRP is 0.8 mg. 1<sup>-1</sup>, with a distribution of 90% of the population below 3 mg. l<sup>-1</sup>. The concentration tends to be slightly higher in women compared to men. This difference is due to the effect of estrogen. CRP values increase with age, but only minimally. The basal CRP level can be influenced by several factors, mostly influenceable. CRP increases e.g., inappropriate lifestyle (smoking, sedentary lifestyle, higher BMI, overweight), metabolic disorders (obesity, low HDL level, high LDL level, high TG level, hypertension, metabolic syndrome, diabetes mellitus), chronic infections and inflammatory processes, use of estrogens and progesterone. Conversely, factors that reduce CRP levels are increased physical activity, weight loss, quitting smoking, moderate alcohol consumption, some medications - statins, etc. [4]. For these reasons, we decided to use hs-CRP to evaluate the inflammatory response and to estimate the risk of cardiovascular diseases. Before the intervention, hsCRP was present in the probands at an average value of  $1.80\pm2.25$  mg.  $1^{-1}$  (maximum 9.48 mg.  $1^{-1}$ ; minimum 0.06 mg.  $1^{-1}$ , median 0.75 mg.  $1^{-1}$ ). Immediately after the intervention, the average value increased to  $3.25\pm5.66$  mg.  $1^{-1}$  (maximum 26.59 mg.  $1^{-1}$ ; minimum 0.12 mg.  $1^{-1}$ , median 0.56 mg.  $1^{-1}$ ). 2 months after the end of the intervention, hs-CRP levels decreased to  $1.75\pm2.76$  mg.  $1^{-1}$  (maximum 12.39 mg.  $1^{-1}$ ; minimum 0.07 mg.  $1^{-1}$ , median 0, 66 mg.  $1^{-1}$ ). Changes in hs-CRP during the study were without statistical significance (P>0.05) (Table 2).

women. They found that increasing the intervention dose by every 50 g.day-1 was associated with an increase in hs-CRP concentration by 0.23 mg. 1<sup>-1</sup>. They found no statistically significant interactions by gender or BMI.

Evidence from studies regarding the health consequences of consuming refined grains is inconsistent, with most studies reporting negative or neutral effects on the development of low-grade inflammation that accompanies an increase in hs-CRP [16, 22] and to the development of cardiovascular and

hs-CRP (mg. l <sup>-1</sup> )									
	Before intervention $\overline{X} \pm SD$	After 6-weeks intervention $\overline{X} \pm SD$		$\begin{array}{c} 2 \text{ months after} \\ \text{intervention} \\ \overline{X} \pm \text{SD} \end{array}$		P value (significance) */**/***			
All (n=30)	1.80±2.25	3.25±5.66		1.75±2.76		P>0.05			
Women (n=17)	$1.69{\pm}2.61$	4.21±7.07		1.79±3.09		P>0.05			
Men (n=13)	1.94±1.77	1.99±2.80		1.69±2.35		P>0.05			
Cardiovascular risk of probands									
	Low risk (hs-CRP<1 mg.l <sup>-1</sup> )		Medium risk (hs-CRP 1-3 mg.l <sup>-1</sup> )		High risk (hs-CRP>3,0 mg.l <sup>-1</sup> )				
Before intervention	17 (12 women;5 men)		7 (2 women;5 men)		6 (3 women;3 men)				
After 6-weeks intervention	18 (9 women;9 men)		3 (2 women;1 men)		9 (6 women;3 men)				
2 months after intervention	18 (11women;7men)		8 (4 women;4 men)		4 (2 women;2 men)				

Table 2. Cardiovascular risk of probands by using the hs-CRP

hs-CRP - high sensitivity C-reactive protein;  $\overline{X}$  – average; SD - standard deviation.

As can be seen from the data in Table 2, based on the average levels of hs-CRP (> 3.0 mg.  $l^{-1}$ ), were immediately after the intervention 9 probands (6 women and 3 men) at high cardiovascular risk. In the measurement with an interval of 2 months from intervention, we found that only 4 probands (2 women and 2 men) had a high cardiovascular risk. From which we could conclude that an inflammatory response occurred during the study, but we also need to think about other factors that affect the hs-CRP level. These results show that intervention with bakery products containing gluten was associated with an increase in hs-CRP levels. When assessing hs-CRP levels according to gender, we found a more massive increase in hs-CRP in women, which could have been contributed to by the presence of female sex hormones, as well as a higher proportion of adipose tissue in their body (part of CRP is also formed in adipose tissue). Similar results were reached by Taskinen et al. [28], who observed the effect of consumption of whole grain and refined (white) bakery products on hs-CRP levels. At the intervention dose of 92 g.day<sup>-1</sup> in men and 75 g.day<sup>-1</sup> in women, the average serum concentration of hs-CRP was 1.77 mg. 1-1 in men and 1.96 mg. 1-1 in

metabolic diseases [1]. Data on the association between grain consumption and low-grade inflammation in intervention studies are somewhat conflicting. A recent meta-analysis of 14 randomized controlled trials found no significant effect of whole grain intake on serum concentrations of CRP, interleukin 6 (IL-6), tumor necrosis factor alpha (TNF- $\alpha$ ), or plasminogen activator inhibitor type 1 (PAI-1) [21], while in another meta-analysis of 17 randomized controlled trials, whole grain intake resulted in significantly lower serum hs-CRP and IL-6, but not TNF-alpha, compared to consumption of refined (white types) bakery products [10].

#### **CONCLUSION**

Measurement of the lipid profile showed that mean TC and LDL levels were above reference values throughout the duration of the study. The slight reduction of LDL immediately after the 6-week intervention was positively evaluate. Certain changes during the study were also noted in HDL parameters. It was found that the average TG values had a decreasing tendency, which is quite positive from the point of view of evaluating the health and nutritional status.

Furthermore, it was found that intervention with bakery products containing gluten was associated with an increase in hs-CRP levels. When evaluating hs-CRP levels by gender it was found a more massive increase in hs-CRP in women, which could have been contributed to by the presence of female sex hormones, as well as a higher proportion of adipose tissue in the body. Therefore, we consider it is necessary to improve the overall lifestyle of the general Slovak population as soon as possible. Further research is needed with a larger number of probands and the use of other methods of monitoring the nutritional and health status of the population.

#### Acknowledgments

This research was funded within the project of Grant Agency of Faculty of Agrobiology and Food Resources, SUA in Nitra ,,04/2023 – Blood biochemical parameters in the evaluation of the health and nutritional status of selected population groups".

Publication was also created thanks to the support of the KEGA 003SPU-4/2022 Integration of university education with practice through the implementation of practical training in the new subject Nutritional counselling.

#### **Conflict of interest**

The authors declare no conflict of interest.

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Received: 07.09.2023 Revised: 27.01.2024 Accepted: 30.01.2024

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