UDK 577.1 : 61 **ISSN 1452-8258**

J Med Biochem 36: 366–374, 2017

Original paper
Originalni naučni rad

THE MONITORING OF PROTEIN MARKERS OF INFLAMMATION AND SERUM LIPID CONCENTRATION IN OBESE SUBJECTS WITH METABOLIC SYNDROME

PRAĆENJE PROTEINSKIH MARKERA INFLAMACIJE I LIPIDNOG STATUSA U SERUMU GOJAZNIH I ISPITANIKA SA METABOLIČKIM SINDROMOM

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Summary

Background: Obesity is one of the most common modern health problems worldwide. Proinflammatory cells accumulate in the adipose tissue of the obese, and the presence of a low level chronic inflammation in obesity is associated with the emergence of a range of metabolic disorders including cardiovascular disease, insulin resistance, type-2 diabetes, fatty-liver disease, and others. Neutrophils are early participants in inflammatory processes. After the appropriate stimulation, these cells release reactive oxygen and nitrogen species, which leads to degranulation and secretion of myeloperoxidase and other enzymes. Myeloperoxidase and its reactive oxidants contribute to tissue damage during inflammatory processes in the human body.
Methods: The study included 175 subjects who were, in compliance with the International Diabetes Federation criteria, divided into 3 groups: normal weight subjects (N=106), subjects with abdominal obesity (N=37) and the third group consisted of subjects with the metabolic syndrome (N=32).
Results: By analyzing the myeloperoxidase enzyme activity (kU/L), and the levels of high-sensitivity C-reactive protein in the blood of all subjects, we detected their significantly higher activity and levels in subjects with the metabolic syndrome, as compared to normal weight subjects ($p<0.001$).
Conclusions: Based on our results, we can conclude that the MPO activity in the serum progressively increases with obesity and the metabolic syndrome, which indicates that this prooxidant enzyme may play a role in the pathophysiological mechanisms of the obesity and the metabolic syndrome related complications.

Keywords: obesity, metabolic syndrome, inflammation, myeloperoxidase, C-reactive protein

Kratak sadržaj

Uvod: Gojaznost je jedan od najčešćih savremenih zdravstvenih problema širom sveta. U masnom tkivu gojaznih akumuliraju se proinflamatorne ćelije, a prisustvo hronične inflamacije niskog intenziteta kod gojaznih udruženo je sa pojavom niza metaboličkih poremećaja uključujući kardiovaskularne bolesti, rezistenciju na insulin, dijabetes tip 2 i druge. Neutrofili su rani učesnici u inflamatornim procesima. Nakon odgovarajuće stimulacije, ove ćelije oslobađaju reaktivne vrste kiseonika i azota, dolazi do njihove degranulacije i izlučivanja mijeloperoksidaze i drugih enzima. Mijeloperoksidaza i njeni reaktivni oksidanti učestvuju u oštećenju tkiva tokom inflamatornih procesa u humanom organizmu.
Metode: Istraživanjem je obuhvaćeno 175 ispitanika koji su u skladu sa kriterijumima International Diabetes Federation podeljeni u 3 grupe: normalno uhranjeni (N=106), ispitanici sa abdominalnom gojaznošću (N=37) i treću grupu činili su ispitanici sa metaboličkim sindromom (N=32).
Rezultati: Analiziranjem aktivnosti enzima mijeloperoksidaze (kU/L) i koncentracije visokosenzitivnog C-reaktivnog proteina u krvi ispitanika utvrđena je njihova značajno viša aktivnost kod ispitanika sa metaboličkim sindromom, u odnosu na normalno uhranjene ($p<0,001$).
Zaključak: Na osnovu naših rezultata, može se zaključiti da aktivnost MPO u serumu progresivno raste sa gojaznošću i metaboličkim sindromom, što ukazuje da ovaj prooksidantni enzim može imati ulogu u patofiziološkim mehanizmima nastanka komplikacija gojaznosti i metaboličkog sindroma.

Ključne reči: gojaznost, metabolički sindrom, inflamacija, mijeloperoksidaza, C-reaktivni protein

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Introduction

Obesity is a complex metabolic disorder, but also one of the most common modern health problems. Over the last thirty years, increased obesity prevalence has been noted worldwide. Obesity is characterized by a low-level chronic inflammation condition, which leads to an accelerated development of atherosclerosis, and is, thus, considered a risk factor for cardiovascular disease. The increase in the number of obese people in the world is followed by the increase of the most common modern human diseases, such as coronary heart disease, hypertension, insulin resistance and diabetes mellitus type 2 (1).

There is a need to explain the molecular link between obesity and chronic metabolic diseases. In this context, low-level chronic inflammation, mediated by the innate and adaptive immune cells, participates in creating this link between obesity and diseases (2). Numerous studies point to the positive association between persistent chronic low-level inflammation and obesity indices (3). In the obese, the adipose tissue is the primary site of inflammation as the proinflammatory cells (macrophages) of the visceral adipose tissue secrete different cytokines and have an important role in inflammation (4). The presence of a large number of macrophages in the adipose tissue of obese is probably caused by the reaction of adipose tissue to stress signals released by adipocytes (5), but the molecular events that initiate the recruitment and activation of immune system cells in adipose tissue of the obese are not yet fully known. Multiple inflammatory stimuli such as the increase in circulating cytokines, the reduction of protective factors (e.g. adiponectin) and the communication between inflammatory and metabolic cells, contribute to the onset of metabolic disorders in the body of the obese people. Myeloperoxidase that is present in neutrophils may also be involved in the initiation of an inflammatory response in the adipose tissue of the obese.

Myeloperoxidase (MPO; E.C. 1.11.1.2.) is one of the most prevalent proteins in human neutrophilic leukocytes but is less present in monocytes and tissue macrophages (6). The function of this enzyme is to catalyze a two-electron oxidation of chlorine ions in the presence of hydrogen peroxide. Myeloperoxidase together with H_2O_2 forms an enzyme-substrate complex that is able to oxidize halides (e.g. chloride, iodide), thus producing reactive products. The most important product in this reaction is hypochlorous acid (HOCl) due to the high concentration of chlorine ions (Cl^-) in biological systems (7).

Under *in vivo* conditions, the sources of hydrogen peroxide that serves as an MPO substrate are numerous, for example, leukocyte NADPH oxidase, xanthine oxidase, nitric oxide synthase and others (8). The production of reactive (chlorinating) species, such as HOCl, is important during the antimicrobial activity of MPO in the innate immune response. During the activation of leukocytes, MPO increases the oxidative potential of the respiratory burst by using hydrogen peroxide as a cosubstrate to form more reactive oxidant species (9). This can lead to the formation of numerous potent oxidant compounds capable of promoting oxidative modification of biomolecules. The generation of oxidized bioactive lipids is an important mechanism that connects MPO with inflammatory processes, while a heightened activity of MPO may be significant in inflammatory tissue injury (10). Many studies have shown that MPO and its reactive oxidants participate in the pathogenesis of many acute and chronic inflammatory conditions, particularly cardiovascular disease (6, 11).

Adipose tissue functions as an endocrine organ as well, due to the ability of adipocytes to produce numerous biologically active substances, adipocytokines. The increase in the number of adipocytes in the body of obese people leads to greater production of adipokines and contributes to the development of the metabolic syndrome (12). The presence of a number of related metabolic disorders in the human body, such as dysregulation of lipid metabolism, hyperglycemia, insulin insensitivity, increased blood pressure and abdominal obesity, is a characteristic of the metabolic syndrome. The mentioned metabolic disorders present significant risk factors for the occurrence of many complications.

The constant increase in the number of obese people imposes the need for finding reliable biomarkers in routine diagnosis and the assessment of the severity of metabolic disorders, as well as the possibility to predict the risks of any related complications.

The aim of the study was to monitor the concentrations of apolipoprotein A and B, their interrelation (apoB/apoA1 ratio) and myeloperoxidase enzyme activity, as early inflammatory markers in the serum of obese and the subjects with the metabolic syndrome.

Material and Methods

Samples

The study included 175 subjects from the student population who, after getting familiar with the goals of the study, voluntarily agreed to be involved in it. The study protocol was approved by the Ethics Committee of the Medical Faculty in accordance with the Helsinki Declaration. The criteria for the selection of patients were: that they were older than 18 years of age, did not have a diagnosed cardiovascular disease, as well as liver, kidney, central nervous system, endocrine and/or metabolic disorders; that they were not under a medical drug therapy that could affect the level of lipids and glycemic regulation and that their levels of the measured high-sensitivity C-reactive protein (hsCRP) were not higher than 10 mg/L.

The anthropometric indicators were measured for all of the subjects: body weight (kg), body height (cm), waist and hip circumferences (cm), and systolic and diastolic blood pressure (mmHg). Body Mass Index (BMI) was determined based on these results, as well as the ratio between waist (cm) and hips (cm) circumference (WC/HC). Body mass index was calculated as weight in kilograms (kg) divided by height in meters squared (m^2).

Venous blood was obtained from each of the subjects in the study via peripheral venipuncture on an empty stomach (after at least 12–14 hours of night fasting) for laboratory tests to determine: erythrocytes count, leukocytes count, platelet count, erythrocyte sedimentation rate (SE), and the levels of fibrinogen (g/L) and high-sensitivity C-reactive protein (hsCRP) (mg/L), glycosylated hemoglobin (HbA1c, %), total cholesterol (mmol/L), triacylglycerol (mmol/L), HDL (mmol/L), LDL (mmol/L), VLDL-cholesterol (mmol/L), apolipoprotein A1 (ApoA1) and apolipoprotein B (ApoB), ApoB/ApoA1 ratio, the levels of glucose (mmol/L), urea (mmol/L), creatinine (μmol/L), and uric acid (μmol/L), and the myeloperoxidase enzyme (MPO) activity (kU/L).

Methods. The methods used for laboratory tests were as follows: erythrocyte sedimentation rate (SE) was determined according to the Westergren method, leukocytes count, platelet count as well as hemoglobin and hematocrit levels were determined using an automated blood cell counter (Sysmex K 21). By means of automatic spectrophotometric methods, the biochemical analyzer Architect 4000c (Abbott, USA), and commercial test reagents, we determined the levels of total cholesterol, triacylglycerol, lipoprotein cholesterol (HDL, VLDL), glucose, urea, creatinine and uric acid. The LDL-cholesterol levels were determined using Friedewald's formula (13). Levels of hsCRP were measured by a high-sensitivity automated immunoturbidimetric method with a measuring range from 0.1 to 160 mg/L on an Architect c4000 analyzer (Abbott, USA) using fully prepared test reagents made by the same company. To quantitatively determine the glycosylated hemoglobin A1c levels (HbA1c) in blood, we used an Architect System analyzer (Abbott, USA) for a chemiluminescent immunoassay test with fully prepared test reagents. Apoprotein B and A1 (Apo B and apoA1) levels were determined by an immunoturbidimetric test, based on the degree of turbidity due to the formation of insoluble immune complexes, after adding ApoB/ApoA1 antibodies to the sample. Atherogenic index was calculated as the ratio between the individual lipid fractions: total cholesterol – HDL/HDL. The reference value for this index is 2.3. We calculated the apoB/apoA1 ratio, whose value greater than 0.60 indicates an increased atherogenic risk.

Chlorinating myeloperoxidase activity was spectrophotometrically determined in a system consisting of hydrogen peroxide, taurine, and TNB (2-nitro-5-thiobenzoyl acid) at 25 °C and pH 7.4 (14). Hypochlorous acid is generated as a result of MPO activity, which then reacts with taurine to form taurine chloramine that oxidizes TNB into DTNB (5,5'-dithiobis-[2-nitrobenzoic acid]), detected by measuring the absorbance at 412 nm. One unit of MPO activity is defined as the amount of enzyme that catalyzes the production of HOCl to the extent that is sufficient to lead to the formation of 1.0 nmol of taurine chloramine at 25 °C and pH 7.4 per 30 min in the presence of 100 nmol of chloride and 100 mmol H_2O_2. Chlorinating MPO activity was expressed as kU/L.

Statistics

With the data obtained in the study and using the IBM SPSS 20 Statistics program, we created a data file which helped with analyzing the gathered data. The descriptive methods we used in statistical analysis were frequencies, percentages, mean values and the variability range while the analytical methods used were chi-square test, Kruskal-Wallis test, Mann-Whitney U test, linear correlation, One-factor Analysis of Variance and Sidak's multiple comparisons test in cases when the analysis of variance showed statistically significant differences between groups.

Results

In accordance with the criteria of the International Diabetes Federation (IDF) (15) and based on the measured anthropometric parameters and biochemical indicators, the subjects were divided into 3 groups. The first group consisted of normal weight respondents with a BMI 18.5–24.9 kg/m^2, waist circumference <94 cm in men, and <80 cm in women. For this group of subjects, the measured level of triacylglycerol was up to 1.70 mmol/L, HDL cholesterol above 1.03 mmol/L in men, and above 1.29 mmol/L in women, blood glucose was below 5.6 mmol/L, systolic blood pressure was lower than 130 mmHg and diastolic lower than 85 mmHg. The second group consisted of subjects with abdominal obesity whose measured waist circumference was over 94 cm in men, and over 80 cm in women. The blood analysis of this group of subjects showed levels of triacylglycerol up to 1.70 mmol/L, HDL cholesterol over 1.03 mmol/L in men, and over 1.29 mmol/L in women, blood glucose below 5.6 mmol/L, systolic blood pressure lower than 130 mmHg and diastolic pressure lower than 85 mmHg. The third group consisted of subjects with the metabolic syndrome in which the measured waist circumference was higher than 94 cm in men, or higher than 80 cm in women. More specifically, the respondents in this group had abdominal obesity and two or more parameters including triglycerides levels above 1.70 mmol/L, HDL cholesterol levels lower than 1.03 mmol/L in men, and lower than 1.29 mmol/L in women, glucose levels above 5.6 mmol/L, systolic blood pressure higher

Table I Descriptive characteristics of subjects.

	Normal weight (NW)	Overweight (OW)	Metabolic syndrome (MS)	p*		
				NW-OW	NW-MS	MS-OW
Number of subjects	106	37	32			
Gender, n (%)						
male	32 (30.2%)	4 (10.8%)	17 (53.1%)	<0.001	<0.001	0.001
female	74 (69.8%)	33 (89.2%)	15 (46.9%)	<0.001	<0.001	0.001
BMI (kg/m^2)	21.2 ± 2.2	25.2 ± 5.5	26.6 ± 3.5	<0.001	<0.001	0.293
WC (male) (cm)	79.9 ± 5.8	101.7 ± 4.9	96.9 ± 7.2	<0.001	<0.001	0.372
WC (female) (cm)	68.8 ± 5.2	87.5 ± 8.7	89.7 ± 6.6	<0.001	<0.001	0.621
HC (male) (cm)	101.3 ± 5.0	114.4 ± 3.8	109.9 ± 6.7	<0.001	<0.001	0.468
HC (female) (cm)	95.6 ± 5.5	105.5 ± 11.3	103.5 ± 5.6	0.000	0.016	0.919
WC/HC (male)	0.8 ± 0.07	0.9 ± 0.03	0.9 ± 0.04	0.024	<0.001	0.993
WC/HC (female)	0.7 ± 0.05	0.8 ± 0.04	0.9 ± 0.05	<0.001	<0.001	0.042
SBP (mmHg)	112.6 ± 8.6	109.9 ± 10.5	120.3 ± 11.8	0.364	<0.001	<0.001
DBP (mmHg)	74.5 ± 6.7	72.8 ± 7.5	80.6 ± 9.1	0.567	<0.001	<0.001

Data are presented as mean ± SD, or n (%). Body mass index (BMI), waist circumference (cm) (WC), hip circumference (cm) (HC), waist to hip ratio (WC/HC), systolic blood pressure (SBP), diastolic blood pressure (DBP). p value (p*) refers to the statistical significance of the differences between: normal weight and obese subjects (NW-OW) and normal weight subjects with metabolic syndrome (NW-MS), and obese subjects with metabolic syndrome (MS-OW).

than 130 mmHg and/or diastolic blood pressure higher than 85 mmHg.

Of the 175 subjects involved in the study, 30% (N=53) were men, while 70% (N=122) were women. Women were predominant in the group of subjects with normal weight, and the one with abdominal obesity, while there was a higher number of men in the group with the metabolic syndrome, which was confirmed by a chi-square test (chi-square=14.533; p=0.001) (*Table I*). The study found that the measurements for body weight, body mass index (BMI), waist circumference and hip circumference were significantly lower in the group with normal weight subjects compared to obese and the subjects with the metabolic syndrome (p<0.001), whereas among obese and subjects with the metabolic syndrome no significant differences were established for the above mentioned parameters (*Table I*). By analyzing the ratio between the waist and hips circumferences (WC/HC), we established a significant difference between obese and subjects with the metabolic syndrome compared to normal weight subjects (p<0.001) (*Table I*). The measured values of systolic and diastolic blood pressure were significantly higher in subjects with the metabolic syndrome compared to normal weight and obese subjects (p<0.001) (*Table I*).

We measured significantly higher levels of total cholesterol, LDL-cholesterol, VLDL-cholesterol (mmol/L) and triacylglycerol (mmol/L) in the serum of subjects with the metabolic syndrome compared to normal weight and obese subjects. Also, the atherogenic index values were the highest in the group of subjects with the metabolic syndrome (p<0.001) (*Table II*). Through the analysis of the measured levels of HDL-cholesterol in serum, we determined significantly lower levels in patients with the metabolic syndrome compared to normal weight and obese subjects (p<0.001), whereas we could not establish a significant difference between normal weight and obese subjects (*Table II*). The levels of glycosylated hemoglobin (HbA1c) were significantly lower in the blood of normal weight subjects compared to obese and the subjects with the metabolic syndrome (p<0.001), whereas there were no significant differences found between obese and the subjects with the metabolic syndrome (*Table II*). By analyzing the measured levels of blood glucose (mmol/L), urea (mmol/L), creatinine (mmol/L) in the serum of subjects, as well as the erythrocyte count (x10^{12}/L), leukocyte count (x10^9/L), platelet count (x10^9/L) and hematocrit levels, we found no significant differences between the two groups (*Table II*).

We determined no significant differences between the measured values of erythrocyte sedi-

Table II Biochemical characteristics of subjects.

	Normal weight (NW)	Overweight (OW)	Metabolic syndrome (MS)	p*		
				NW-OW	NW-MS	MS-OW
Fasting glucose (mmol/L)	4.3±0.5	4.3±0.4	4.4±0.6	0.365	0.256	0.289
HbA1c (%)	5.3±0.2	5.6±0.2	5.6±0.3	<0.001	<0.001	0.710
Total cholesterol (mmol/L)	4.5±0.6	4.7±0.8	5.1±1.1	0.289	<0.001	0.115
Triglyceride (mmol/L)	0.9±0.3	1±0.3	2±1.2	0.991	<0.001	<0.001
HDL (mmol/L)	1.5±0.2	1.5±0.3	1.1±0.2	0.499	<0.001	<0.001
LDL (mmol/L)	2.8±0.5	3.1±0.8	3.6±0.9	0.098	<0.001	0.005
VLDL (mmol/L)	0.2±0.07	0.2±0.07	0.4±0.2	0.989	<0.001	<0.001
Atherogenic index	1.9±0.3	2.3±0.7	3.8±1.1	0.001	<0.001	<0.001
ApoA1 (g/L)	1.8±0.1	1.3±0.09	1.1±0.09	<0.001	<0.001	<0.001
ApoB (g/L)	0.7±0.1	1.5±0.1	1.8±0.08	<0.001	<0.001	<0.001
The ApoB/ApoA1 ratio	0.4±0.07	1.2±0.1	1.6±0.2	<0.001	<0.001	<0.001
Erythrocytes (x 10^{12}/L)	4.9±0.4	4.7±0.4	4.9±0.4	0.200	0.548	0.047
Hemoglobin (g/L)	137.9±15.5	133.3±10.5	143.4±13.1	0.441	0.075	0.011
Leukocytes (x 10^9 /L)	5.8±1.4	6.6±1.7	6.6±2.2	0.254	0.059	0.012
Hematocrit (%)	0.4±0.03	0.4±0.03	0.4±0.03	0.019	0.019	0.018
Platelets (x 10^9/L)	238.9±53.2	243.5±56.6	249.4±51.1	0.032	0.035	0.028
Urea (mmol/L)	4.2±1.1	3.9±1.0	4.0±0.8	0.156	0.186	0.187
Creatinine (mmol/L)	78.9±11.9	75.6±8.5	79.7±12.0	0.034	0.039	0.046

Data are presented as mean±SD. p value (p*) refers to the statistical significance of the differences between: normal weight and obese subjects (NW-OW) and normal weight subjects with metabolic syndrome (NW-MS), and obese subjects with metabolic syndrome (MS-OW). The apolipoprotein B/apolipoprotein A1 ratio (the ApoB/apoA-I ratio), low-density lipoprotein cholesterol (LDL-c), high-density lipoprotein cholesterol (HDL-c).

Table III The indicators of inflammation in the blood of subjects.

	Normal weight (NW)	Overweight (OW)	Metabolic syndrome (MS)	p*		
				NW-OW	NW-MS	MS-OW
Myeloperoxidase (kU/L)	9.5±1.4	18.2±2.4	26.5±2.8	<0.001	<0.001	<0.001
Fibrinogen (g/L)	3.2±0.5	3.9±1.1	3.6±0.7	0.001	0.117	0.518
Uric acid (mmol/L)	238.4±72.7	239.4±83.3	387.6±79.4	0.512	<0.001	0.000
SE (mm/h) (median)	8 (1–36)	13 (2–48)	9 (1–42)	0.007	0.338	0.559
hsCRP (mg/L) (median)	0.2 (0.1–0.8)	1.0 (0.1–4.3)	1.2 (0.2–3.4)	<0.001	<0.001	0.486

Data are presented as mean±SD. p value (p*) refers to the statistical significance of the differences between: normal weight and obese subjects (NW-OW) and normal weight subjects with metabolic syndrome (NW-MS), and obese subjects with metabolic syndrome (MS-OW). The erythrocyte sedimentation rate (SE).

mentation rate (mm/h), uric acid (mmol/L) and fibrinogen (g/L) within the tested groups (*Table III*). By analyzing the myeloperoxidase enzyme activity (kU/L) and high-sensitivity C-reactive protein (hsCRP) levels in the blood of the subjects, we determined differences between the three groups of subjects, namely significantly higher MPO activity and hsCRP values measured in subjects with the metabolic syndrome as compared to normal weight subjects ($p<0.001$) (*Table III*).

Discussion

Obesity is associated with a low-level chronic inflammation condition, caused by progressive infiltration of macrophages in adipose tissue. The increasing volume of adipose tissue and the accumulation of macrophages in it increase the production of proinflammatory cytokines and acute-phase molecules that contribute to pathological changes in obesity. Cytokines produced by macrophages and adipokines produced by the adipose cells cause inflammation (16). The increased levels of high-sensitivity C-reactive protein (hsCRP), fibrinogen, amyloid-A, monocyte-chemoattractant protein-1 (MCP-1), plasminogen inhibitors (PAI-1), cytokines (TNF-α, IL-6, etc.) and other biological markers of inflammation in the blood of obese subjects confirmed the presence of chronic inflammation (17). The action of these inflammatory molecules may represent the molecular link between adipose tissue and the metabolic and cardiovascular complications in obese.

During our study, we measured significantly higher values of systolic and diastolic blood pressure ($p<0.001$) in subjects with the metabolic syndrome ($p<0.001$) compared to obese and normal weight subjects (*Table I*). It is possible that the inflammatory condition that occurs in obesity may contribute to an increase in blood pressure. High blood pressure may increase cardiovascular risk by causing chronic endothelial damage leading to structural and functional vascular changes, especially in the microvascular (capillary) network.

During our tests, we measured higher levels of hsCRP in the blood of obese and the subjects with the metabolic syndrome compared to those with optimal body weight ($p<0.001$) (*Table III*). C-reactive protein is an acute phase protein produced by hepatocytes stimulated by cytokines (IL-1, IL-6). Interleukin-6 (IL-6) is expressed in adipose tissue in inflammatory conditions and it regulates CRP expression in hepatocytes at the transcriptional level.

Several studies have pointed to obesity as the main predictor of increased hsCRP blood levels. Also, the results of the Ebrahimi M. et al. (18) study showed that increased hsCRP levels represent a predictive factor for the development of the metabolic syndrome, while the Maffeis et al. (19) study confirmed that high hsCRP levels in the blood of young people point to greater future risks of coronary artery disease.

A disorder of blood lipid levels is usually present in the blood of obese people, and it is manifested as greater levels of triglycerides and LDL cholesterol, as well as reduced levels of HDL-cholesterol (20). During our study, dyslipidemia was also observed in the form of elevated levels of triacylglycerol, LDL, VLDL and total cholesterol in the blood of obese and subjects with the metabolic syndrome ($p<0.001$) (*Table II*). Also, the obese and the subjects with the metabolic syndrome had higher levels of atherogenic index and lower levels of HDL-cholesterol in blood ($p<0.001$) (*Table II*). We detected significantly lower levels of apoA1 (g/L) ($p<0.001$), while the apoB levels (g/L), and apoB/apoA1 ratio were significantly higher ($p<0.001$) in obese and subjects with the metabolic syndrome ($p<0.001$) (*Table II*). It is likely that HDL modification and/or the suppression of its synthesis in the liver is mediated by MPO generated oxidants, which results in reduced levels of HDL particles in blood. The reduced level of HDL-cholesterol in blood is seen as a risk factor for the development of pathological changes in the cardiovascular system due to its important role in the metabolism of cholesterol, and its antiinflammatory and atheroprotective effects (21). The elevated levels of LDL-cholesterol and apo B, the increased apoB/apoA1 ratio and/or reduced levels of HDL-cholesterol and apoA1 indicate a higher risk of a cardiovascular disease. Numerous studies have shown that the apoB/apoA1 ratio is a stronger risk predictor for development of a cardiovascular disease compared to levels of other lipids and lipoproteins. As a component of atherogenic lipoproteins, apolipoprotein B directly reflects elevated levels of atherogenic lipoproteins in plasma. Apolipoprotein A is the main protein component of high-density lipoprotein, and reflects the level of antiatherogenic lipoproteins in plasma, while the

Table IV Correlation between ApoB/ApoA1 ratio and different variables.

ApoB/ApoA1 Ratio	TC mmol/L	HDL mmol/L	LDL mmol/L	TG mmol/L	hs-CRP mmol/L	MPO kU/L	Atherogenic index
r	0.261**	-0.478**	0.365**	0.465**	0.612**	0.921**	0.600**
p	0.001	0.001	0.001	0.001	0.001	0.001	0.001

Table V Correlation between myeloperoxidase and different variables.

MPO kU/L	BW (kg)	BMI kg/m^2	WC cm	SBP mm/Hg	HT mmol/L	LDL mmol/L	Atherogenic index	hsCRP mmol/L	UA µmol/L
r	0.496**	0.556**	0.719**	0.212**	0.283**	0.397**	0.636**	0.563**	0.312**
p	0.001	0.001	0.001	0.005	0.001	0.001	0.001	0.001	0.001

Body weight (BW), uric acid (UA).

ApoB/ApoA1 ratio reflects the balance of potentially atherogenic and antiatherogenic lipoprotein particles. It is believed that apoB levels and the apoB/apoA1 ratio may be useful predictors of cardiovascular events and that elevated levels of plasma apoB and/or apoB/apoA1 ratio may be the reason for the increased hsCRP levels and the onset of inflammation in the vasculature (22). Data obtained in the Lu M. et al. (23) study show that the subjects with higher BMI, waist/hips circumference ratio, SBP and DBP mean values and triglycerides and total cholesterol levels in the blood, have a higher risk of cardiovascular events (24, 25).

The study in our patients revealed a significant negative correlation between the apoB/apoA1 ratio and HDL ($r=-0.478$, $p<0.001$) and a positive correlation between apoB/apoA1 ratio and the levels of LDL ($r=0.365$, $p<0.001$), total cholesterol ($r=0.261$, $p<0.001$) and triglycerides ($r=0.465$, $p<0.001$) (*Table IV*). Also, a significant positive correlation was established between the apoB/apoA1 ratio and hsCRP levels ($r=0.612$, $p<0.001$), MPO activity ($r=0.921$, $p<0.001$) and the atherogenic index ($r=0.600$, $p<0.001$) (*Table IV*), which indicates that apoB/apoA1 ratio is related not only to the lipids level, but also to chronic inflammation caused by obesity.

The results of our study show an increase in myeloperoxidase activity in the serum of obese and the subjects with the metabolic syndrome ($p<0.001$) (*Table III*). These results are consistent with the results of Zur B. et al. (26) that showed a significantly higher myeloperoxidase activity in obese compared with the subjects with optimal body weight ($p<0.001$), as with the results of the Andrade VL et al. (27) study, the research that involved female subjects and where a significant increase in myeloperoxidase activity was measured in obese compared to normal weight women ($p<0.05$). Olza J. et al. (26) study that examined overweight and normal weight children showed that myeloperoxidase may be an early marker associated with the risk of developing cardiovascular disease in obese children in the period before puberty. The increased MPO activity in obese subjects indicates that the activation of neutrophils likely leads to inflammatory conditions (low-level inflammation) associated with obesity.

The role of MPO and its reactive oxidant species in the promotion of pathological events involved in the development of atherosclerotic changes and coronary artery disease was confirmed by several research studies (29–31). By analyzing the interdependent relationship that exists between MPO activity and lipid profile in the blood of the subjects, the total cholesterol levels ($r=0.283$, $p<0.001$), LDL ($r=0.397$, $p<0.001$) and the atherogenic index ($r=0.636$, $p<0.001$) (*Table V*), we determined a significant positive correlation. This suggests a possible role of the MPO and its reactive oxidant species in the development of endothelial dysfunction through the modification of lipoproteins.

Through the study of the links between MPO activity (kU/L) and the indicators of nutritional status of the organism, BMI ($r=0.556$, $p<0.001$), body weight ($r=0.496$, $p<0.001$) and waist circumference ($r=0.719$, $p<0.001$), we determined a significant positive correlation (*Table V*). This correlation confirmed that, with increasing amounts of adipose tissue in the body of obese, there is an increased accumulation of macrophages in adipose tissue and the MPO production. We established a significant positive correlation between the indicators of inflammation such as erythrocyte sedimentation rate ($r=0.147$, $p<0.001$), fibrinogen ($r=0.204$, $p<0.001$) and hsCRP ($r=0.563$, $p<0.001$) and the MPO activity (*Table V*), which indicates that with increasing amounts of adipose tissue and the accumulation of macrophages in it, there is an increased production of acute phase proteins in obese subjects. Also, we revealed a significant positive correlation between MPO activity and uric acid levels in our subjects ($r=0.312$, $p<0.001$) (*Table V*). We believe that this association indicates a probable role of MPO in the pathophysiological mechanism of endothelial dysfunction, more so since the uric acid levels in the serum were higher in obese with the metabolic syndrome. Hyperuricemia is a known risk factor for atherosclerotic events and is associated with other cardiovascular risk factors such as dyslipidemia and hypertension.

Determining the inflammatory markers such as MPO and hsCRP levels in the blood of obese can be important in deciding on the antiinflammatory therapy and the prevention of the development of CVD events in obese subjects. Monitored MPO activity in the blood of obese subjects may be a significant marker indicating cardiovascular risks, a mediator of atherogenesis and a potential factor for the preven-

tion of a cardiovascular disease. Namely, it is confirmed that the application of statins causes the repression of MPO gene expression and reduces systemic levels of protein modification by MPO-catalyzed pathways (32).

The importance of MPO was also confirmed by detecting myeloperoxidase inhibitors that can prevent myeloperoxidase dependent inflammation and oxidative stress and possibly serve as a new therapeutic method in delaying the development of cardiovascular and other complications in obese patients (33). Observations of the association of MPO activity in circulation and future cardiovascular risks indicate that monitoring MPO may prove significant in predicting clinical risks. Also, the MPO is a potential factor in the development of new therapeutic methods which would delay the development of cardiovascular complications in obese patients.

Conclusion

Based on our results, we can conclude that MPO activity in serum increases progressively with the development of obesity and the metabolic syndrome, suggesting that this prooxidant enzyme may have a role in the pathophysiological mechanisms of the obesity and metabolic syndrome related complications. Our results suggest examining parameters that are indicators of atherosclerotic changes in obese patients which can lead to earlier application of treatment in patients with a high risk of developing a cardiovascular disease.

Conflict of interest statement

The authors stated that they have no conflicts of interest regarding the publication of this article.

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Received: February 7, 2017

Accepted: February 16, 2017