DOI: 10.17816/KMJ2021-765

Indicators of lipid peroxidation and the activity of antioxidant system enzymes as predictors of the development of metabolic disorders in primary obesity

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Abstract

Aim. To assess the effectiveness of indicators of lipid peroxidation and the activity of antioxidant system enzymes in the early diagnosis of metabolic disorders.

Methods. The study included 269 women of fertile age with primary obesity. The control group consisted of 35 women. The clinical examination included identification of the type of obesity, whole-body fat percentage, the level of glycemia and the index of insulin resistance, biochemical markers of lipids, hormones (leptin and insulin), malondialdehyde and enzyme activity (peroxidase and catalase). The statistical significance of the differences was determined by using the inversion test. Spearman's rank correlation coefficient was used to assess the degree of relationship between quantitative characteristics, and scatter diagrams were used to compare two variables (Statistica software version 10.0).

Results. A statistically significant increase in basal and stimulated immunoreactive insulin was found in obese patients compared with the controls ($p < 0.01$). Stimulated immunoreactive insulin levels, insulin resistance score (HOMA-IR) and the level of leptin in the group of patients with android obesity was higher than in the group with gynoid obesity ($p < 0.01$). The relationship between the concentration of serum malondialdehyde and whole-body fat percentage was found to be more significant ($r=0.412$; $p < 0.001$) than the relationship with the type of obesity ($r=0.257$; $p < 0.01$). Positive correlations were found between serum malondialdehyde and insulin ($r=0.35$; $p < 0.001$) and leptin ($r=0.32$; $p < 0.001$) levels. The relationship between the concentration of serum malondialdehyde and the activity of enzyme systems was also noted. The activity of lipid peroxidation was higher in the group of patients with android obesity (malondialdehyde >3.3 μmol/L) compared with the group of patients with gynoid obesity. In the same group, a higher activity of enzyme systems was noted.

Conclusion. An increase in the concentration of serum malondialdehyde and the activity of enzyme systems should be considered as indicators of a high risk of developing metabolic syndrome.

Keywords: obesity, metabolic syndrome, lipid peroxidation, oxidative stress, malonic dialdehyde, peroxidase, catalase.

For citation: Nikishova T.V., Kurnikova I.A. Indicators of lipid peroxidation and the activity of antioxidant system enzymes as predictors of the development of metabolic disorders in primary obesity. *Kazan Medical Journal.* 2021; 102 (5): 765–772. DOI: 10.17816/KMJ2021-765.

Background. Obesity prevalence in the XXI century has acquired the scale of a global epidemic, with the rapidly growing number of people having this polyetiological disease [1–3]. A large proportion of cases of disability and mortality in the population of developed countries are associated with obesity and its complications [3, 4]. Primary alimentary-constitutive obesity is diagnosed in 80%–85% of cases [2]. Additionally, the risk of complications in the case of alimentary-constitutive obesity depends not only on the degree of obesity (by body mass index) but also on the characteristics of fat distribution in the body [3].

In 1947, J. Vague [5] described two types of obesity according to the adipose tissue topography, android (male) and gynoid (female). Wherein, android obesity is often combined with diabetes mellitus, cardiovascular diseases, gout, anovulatory infertility, and miscarriage [5–9]. It is the main link in the metabolic syndrome (MS) and is consi-

For correspondence: ntv116@mail.ru Received 29.04.2021; accepted 27.07.2021; published 15.10.2021.

dered a “metabolically malignant” or “metabolically unhealthy phenotype of obesity” [10–12].

Gynoid obesity is less hazardous for the health of women, especially in the initial stage, due to the absence of pronounced disorders of carbohydrate-fat metabolism. Adipose tissue, which determines the gynoid appearance in women, is believed to prevent the development of metabolic disorders and chronic noninfectious diseases and maintain reproductive health [11–13]. This type of obesity belongs to the “metabolically benign” or “metabolically healthy phenotype” (without pronounced MS) [11, 12, 14].

At the very core of the pathogenetic mechanisms of obesity, a special place is occupied by oxidative stress, which can be both a consequence and a trigger of obesity [15, 16]. With prolonged stress, the consumption of antioxidants exceeds their biosynthesis and causes biomembrane destructions, which consequently decreases the effect of the antioxidant system. This leads to the accumulation of under-oxidized products and decreased functional activity of biomembranes [15]. A high concentration of free radicals and oxidative stress contribute to the development of endothelial dysfunction in MS and, consequently, apoptosis of vascular endothelial cells [16, 17].

Primary diagnostics of obesity at the stage of clinical manifestations is not particularly difficult. The degree of risk assessment of further disease development toward MS becomes a problem. MS-induced disorders are asymptomatic for a long time, therefore identifying the risk factors for the transition of obesity with less pronounced manifestations (metabolically healthy phenotype) to obesity with more pronounced manifestations and rapidly progressing course (metabolically unhealthy phenotype) and MS is advisable as soon as possible [9].

The aspects of the disease course in obesity include early manifestation of clinical symptoms (weight gain, waist circumference/hip circumference [WC/HC] ratio, etc.) and later laboratory parameters. Increased accuracy of primary diagnostics is achieved through examining the indicators of hormonal level (insulin, leptin, etc.). However, based on the assumption of oxidative stress as one of the etiological factors of MS, the search for early markers of oxidative stress in patients with obesity is a promising area of research.

The present study aimed to evaluate the efficiency of the use of indicators of lipid peroxidation (LPO) and activity of enzymes of the antioxidant system in the early diagnostics of metabolic disorders.

Materials and methods of research. The prospective cohort study included 269 women (mean age 37.5 ± 2.8 years) with exogenous constitutive obesity (ECO) (body mass index of 32.4 ± 0.9 kg/m^2), the type of which was determined based on the classification by localization adipose tissue in the body (the upper type is android, the lower type is gynoid or hypo-ovarian).

Taking into account the anthropometric diagnostics, two groups of patients were identified according to the ratio of “WC/HC,” namely the android type (ECO-AT) with a WC/HC ratio of >0.85 (n = 108, average age 36.5 ± 4.8 years) and gynoid type (ECO-GT) with the ratio of <0.85 (n = 161, average age 34.8 ± 4.3 years). The control group consisted of 35 women with normal body weight without signs of somatic diseases (mean age 33.9 ± 4.5 years). The groups were comparable by age.

The study was conducted in 2016–2019 at the Departmental Clinical Hospital of Kazan station of Russian Railways (Kazan) and the Central Clinical Hospital of Civil Aviation (Moscow). The study was approved by the ethical committee of the Kazan State Medical Academy, a branch of the Russian Medical Academy of Postgraduate Education of the Ministry of Health in Russia (protocol No. 2-09 of 09/08/2016).

The study excluded women with secondary obesity and complicated somatic diseases (pathology of the cardiovascular system with chronic heart failure and respiratory system with the development of respiratory failure and pulmonary heart disease, as well as the gastrointestinal tract with erosive and ulcerative processes).

In addition to history taking and physical examination, all patients underwent a complete blood test, urinalysis, and electrocardiography. The percentage of fat in the body was determined by the method of impedance monitoring on an Omron BF-302 apparatus (Japan). The biochemical analyzer Ciba Corning Express Plus (USA) studied the parameters of carbohydrate metabolism and lipid spectrum of the blood and determine the basal glycemia, triglycerides, total cholesterol, high-density lipoproteins (HDL), and low-density lipoproteins (LDL).

Immunoreactive insulin was determined on an empty stomach and 2 h after the intake of dextrose (glucose) using the enzyme immunoassay with reagents from Diagnostic System Laboratories (USA). The leptin level was determined using the enzyme immunoassay with Diagnostic System Laboratories and Phoenix Pharmaceuticals (USA) kits.

Insulin resistance was diagnosed using the levels of basal and postprandial insulinemia, the index of insulin resistance (Homeostasis Model Assessment of Insulin Resistance [HOMA-IR]), based on determining the concentration of serum glucose and insulin taken on an empty stomach.

Table 1. Main indicators of carbohydrate-fat metabolism (M ± SD) in patients of the compared groups

Indicators	ECO-AT group, n = 108	ECO-GT group, n = 161	Control group, n = 35	p_{1-2}	p_{1-3}	p_{2-3}
Adipose tissue content, %	42.21±6.55	36.25±3.18	27.75±2.51	0.0001	0.0001	0.0001
WC/HC	1.02±0.41	0.76±0.26	0.72±0.29	0.0001	0.0001	0.420
Glucose, mmol/l	5.23±0.77	4.87±0.39	4.70±0.46	0.0001	0.0001	0.025
Triglycerides, mmol/l	2.01±0.74	1.54±0.28	1.12±0.12	0.0001	0.0001	0.0001
Total cholesterol, mmol/l	5.65±0.71	5.43±0.49	4.95±0.38	0.003	0.0001	0.0001
HDL, mmol/l	1.15±0.18	1.44±0.14	1.67±0.21	0.0001	0.0001	0.0001
LDL, mmol/l	4.47±0.22	3.77±0.25	2.9±0.19	0.0001	0.0001	0.0001

Note: p_{1-2}: the significant differences in indicators between groups with exogenous constitutive obesity of the android type (ECO-AT) and gynoid type (ECO-GT); p_{1-3}: the significant differences between the ECO-AT and control groups; p_{2-3}: the significant differences between the ECO-GT and control groups; WC/HC: the WC/HC ratio; HDL: high-density lipoproteins; LDL: low-density lipoproteins.

Table 2. The main indicators of the hormonal profile (M ± SD) in patients with exogenous constitutive obesity

Indicators	ECO-AT group, n = 108	ECO-GT group, n = 161	Control group, n = 35	p_{1-2}	p_{1-3}	p_{2-3}
Immunoreactive fasting insulin, μU/ml	25.2±9.8	27.3±8.4	8.4±1.5	0.061	0.0001	0.0001
Immunoreactive insulin after 2 hours, μU/ml	47.8±9.6	39.3±6.3	17.5±2.6	0.0001	0.0001	0.0001
HOMA-IR, c.u.	8.8±1.7	5.3±1.4	1.8±0.7	0.0001	0.0001	0.0001
Leptin, ng/ml	75.45±25.7	65.93±19.4	27.3±7.2	0.0001	0.0001	0.0001

Note: p_{1-2}: the significant differences in indicators between groups with exogenous constitutive obesity of the android type (ECO-AT) and gynoid type (ECO-GT); p_{1-3}: the significant differences between the ECO-AT and the control groups; p_{2-3}: the significant differences between the ECO-GT and control groups; WC/HC: the WC/HC ratio; HOMA-IR: insulin resistance index.

Special attention was paid to LPO parameters in blood serum, namely the primary products (diene conjugates) and secondary products (malondialdehyde [MDA]) [18, 19]. Catalase activity was assessed using the amount of hydrogen peroxide that decomposed under its influence [20], whereas peroxidase activity was determined using the method of photometric registration of a decreased concentration of indigo carmine (at a wavelength of 610 nm) [21].

Results were statistically processed using the Statistica 10.0 software package. The distribution of the attribute in the sample was performed according to the Shapiro–Wilk test. The data testing had the correct distribution in almost all cases, thus they were presented as mean ± standard deviation (M ± SD). The statistical significance of differences was established using the inversion test. Spearman's rank correlation coefficient was used to assess the degree of relationship between quantitative attributes. Differences were considered statistically significant at $p < 0.05$. Scatter plots were used to compare the two variables.

Results. Biochemical research demonstrated that the association of the type of obesity with some carbohydrate and fat metabolism parameters. Metabolic parameters were within the reference values in the ECO-AT of women; however, they exceeded similar indicators in the control group and higher values of glucose and triglycerides were recorded in reduced levels of HDLs and increased levels of LDLs in the ECO-GT group (Table 1).

Hormonal levels (fasting insulin and postprandial insulin) and HOMA-IR index are essential to assess the prognosis of the further development of metabolic disorders and the formation of insulin resistance. A statistically significant increase was found in basal and stimulated immunoreactive insulin patients with obesity compared to that of the control group ($p < 0.0001$). The level of stimulated immunoreactive insulin in the ECO-AT group was higher than in the ECO-GT group ($p < 0.0001$). The HOMA-IR index and the leptin levels were also statistically significantly higher in the android type of obesity (Table 2).

The study of LPO indices traced the relationship between the level of MDA and the type of obesity (Fig. 1), as well as the level of insulin in the blood plasma (Fig. 2). A positive correlation was established between increased insulin levels and in-

Table 3. Correlations between indicators of lipid peroxidation, enzyme activity, and type and severity of obesity in patients of the compared groups

Indicators	Insulin	Leptin	Catalase	Peroxidase	Malondialdehyde	Triglycerides	Cholesterol
WC/HC	0.369	0.335	0.110	0.104	0.257	0.243	0.214
Proportion of adipose tissue	0.689	0.667	0.147	0.328	0.412	0.397	0.412
Insulin	—	0.576	0.273	0.347	0.418	0.227	0.335
Leptin	0.576	—	0.289	0.147	0.324	0.240	0.318
Catalase	0.273	0.289	—	0.448	0.277	0.345	0.176
Peroxidase	0.347	0.147	0.448	—	0.366	0.153	0.149
Malondialdehyde	0.350	0.324	0.277	0.366	—	0.510	0.264
Triglycerides	0.227	0.240	0.345	0.153	0.510	—	0.145
Cholesterol	0.335	0.318	0.176	0.149	0.264	0.145	—

Note: Spearman's rank correlation ($p < 0.05$); WC/HC: the waist circumference/hip circumference ratio.

Table 4. The main indicators of lipid peroxidation (M ± SD) in the patient groups

Indicators	ECO-AT group, *n* = 108	ECO-GT group, *n* = 161	Control group, *n* = 35	$p_{1–2}$	$p_{1–3}$	$p_{2–3}$
Diene conjugates, r.u./ml	7.94±1.25	6.88±0.69	3.73±0.44	0.0001	0.0001	0.0001
Malondialdehyde, μmol/l	3.86±0.71	3.31±0.38	1.49±0.26	0.0001	0.0001	0.0001

Note: $p_{1–2}$: the significant differences in indicators between groups with exogenous constitutive obesity of the android type (ECO-AT) and gynoid type (ECO-GT); $p_{1–3}$: the significant differences between the ECO-AT and the control groups; $p_{2–3}$: the significant differences between the ECO-GT and control groups.

creased MDA levels, where the mutual influence of these indicators on each other is revealed (Fig. 2).

An increased MDA level above the reference values (1.15–1.85 μmol/L) was registered in 68.5% of patients with ECO-AT (n = 74) and 61.5% of women with ECO-GT (n = 99).

Concurrently, activity indicators of the enzymes catalase and peroxidase, as well as their correlation with the level of hormones and LPO were studied (Table 3). The MDA values correlated not only with the level of triglycerides but with the average strength with the levels of hormones (insulin and leptin), as well as with the activity of enzyme systems (Table 3). The relationship between the MDA and percentage of adipose tissue in the body was found to be more significant ($r = 0.412$; $p < 0.001$) than the relationship with the type of obesity ($r = 0.264$; $p < 0.01$). Particularly, the diagnostic value of MDA identified disorders even at the stage with no pronounced clinical manifestations of MS and android type redistribution of adipose tissue.

A statistically significantly increased concentration of diene conjugates and MDA in the blood serum was revealed in patients of the studied groups compared with healthy women ($p < 0.01$). Concurrently, these indicators were higher in the ECO-AT group than in the ECO-GT group (Table 4).

Discussion. Determination of the LPO process activities in patients with various diseases, including obesity, is quite rarely performed, although the influence of oxidative stress on the development of obesity is known [16, 17, 22]. Obesity is a special disease since the process of metabolic disorder development is compensated for a long time and undergoes a reverse development in the case of effective treatment. Imaginary well-being creates in the patient the illusion of "safety" of obesity for his life and health, and the process of treatment and rehabilitation starts, as a rule, with the development of MS manifestations, such as dyslipidemia, impaired carbohydrate tolerance, type 2 diabetes mellitus, arterial hypertension, hyperuricemia, etc.

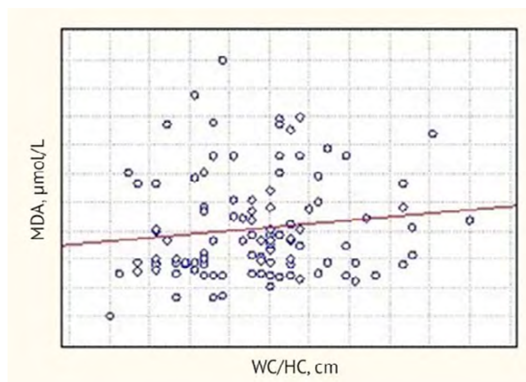

Fig. 1. Correlation of malondialdehyde (MDA) index and waist circumference/hip circumference (WC/HC) ratio ($r = 0.257$) in patients with exogenous constitutive obesity

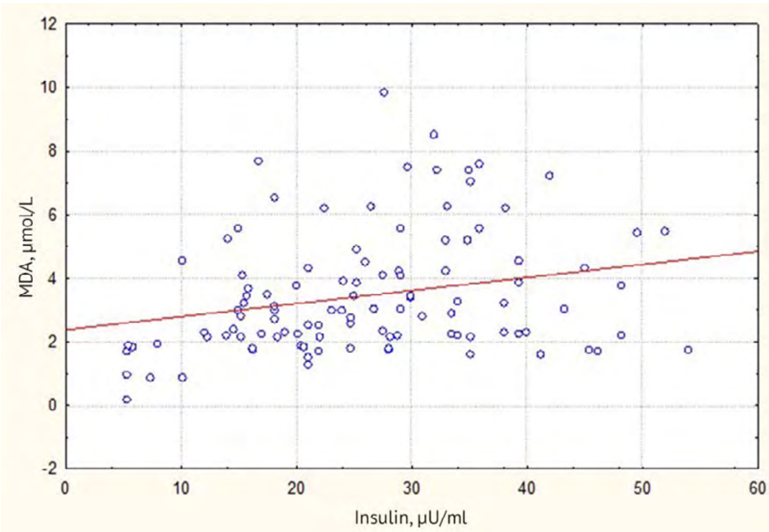

Fig. 2. Correlation of the level of malondialdehyde (MDA) and insulin (r = 0.350) in patients with exogenous constitutive obesity

Therefore, the search for markers that allow early identification and quantitative assessment of the risk of MS in patients with obesity is an important task of modern medicine.

A comprehensive assessment of oxidative stress provides determines the level of MDA as a mandatory marker, as well as some other substances along with it (antioxidant defense enzymes, markers of cytolysis, hormones, etc.) [22, 23]. The opinion of the majority of researchers is unanimous that the MDA level is increased in patients with MS. Opinions on the other criteria listed are ambiguous, and correlations between disorders at the cellular and subcellular levels and the regulatory level (hormonal regulation) are under study [23]. The data on studies of oxidative stress in patients with complicated forms of obesity are most frequently published. Direct correlations of MDA levels and inverse correlations of superoxide dismutase and peroxidase with markers of cytolysis were found in patients with MS and non-alcoholic fatty liver disease [22].

Quite a lot questions are still raised related to the assessment of the activity of various antioxidant defense enzymes under different conditions and diseases, the identification of the most significant criteria in practical work, and early diagnostics and assessment of the risk of disease progression.

This study answered some of the questions.

Oxidative stress, in which the balance between the activity of oxidative processes and the antioxidant defense system (the balance of prooxidant and antioxidant components) is disturbed, is one of the key links in the development of several diseases and pathological conditions. The study showed that LPO processes are closely related to metabolic disorders both at the level of regulation (connection with hormones) and at the cellular level (enzymes). The activation of LPO processes and increased level of activation of enzymes of the antioxidant system were most pronounced in patients with ECO-AT, which evaluated these processes as a risk factor for the formation of an android type of obesity. LPO activation causes more pronounced metabolic disorders in the group of patients with ECO-AT, such as decreased HDL levels and increased LDL and triglyceride indicators. Lipids in obesity represent a source of oxidative processes [24].

This study revealed the additional mechanisms of the influence of the type of adipose tissue distribution on the increased risk of MS formation. Undoubtedly, the proportion of adipose tissue in the body is of great importance, and our study demonstrated that the body fat content in patients with ECO-AT was higher (> 42%) than in the group of patients with ECO-GT (> 36%) using impedance measurement. In addition, these 6% differences in the content of visceral fat led to significantly ($p < 0.0001$) higher indicators of glycemia, triglycerides, HDLs, and LDLs in the ECO-AT group than that in the ECO-GT group. Moreover, the revealed a higher correlation with the percentage of body fat (in comparison with the type of obesity) confirms the value of LPO indicators as early markers of the formation of MS. Several studies revealed that the visceral adipose tissue triggers pro-inflammatory and prooxidant processes in obesity [24, 25].

The basal insulin level in patients in the compared groups did not have statistically significant differences, but the level of stimulated insulin confirmed a high probability of insulin resistance formation in the ECO-AT group. Further, one of the significant mechanisms in the development of this process is the LPO impairment. The accumulation of glycosylation products is known to increase the activity of oxidases, and the products of glucose oxidation become sources of the hydroxyl radical and superoxide anion radicals [26].

A direct correlation was revealed between MDA and immunoreactive insulin ($r = 0.42$, $p < 0.001$) and triglyceride levels ($r = 0.51$, $p < 0.001$). These associations with peroxidase activity were confirmed but to a lesser extent, which indicates that LPO disorders not only start early (in patients at the stage of obesity only without other MS components) but also have a relationship with the level of immunoreactive insulin that increases progressively along with the percentage of body fat and dyslipidemia.

CONCLUSION

The study results consider the LPO indicators, such as the level of MDA and the activity of enzymes of the antioxidant system (peroxidase and catalase), for early diagnostics of the occurrence of MS in female patients with alimentary-constitutive obesity.

Author contributions. T.V.N. conducted the research, was responsible for collecting and analyzing data, wrote the article, approved the final version, and agreed to be responsible for all aspects of the work; I.A.K. created the concept and design of the study, performed scientific guidance, wrote the article, approved the final version and agreed to be responsible for all aspects of the work.

Funding. The study had no external funding.

Conflict of interest. The authors declare no conflict of interest.

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