UDC 612:616-092 doi: https://doi.org/10.15407/ubj90.05.071

EVALUATION OF METALLOTHIONEINS, OXIDATIVE STRESS AND SIGNS OF CYTOTOXICITY IN YOUNG OBESE WOMEN

H. I. FALFUSHYNSKA, O. I. HORYN, V. V. KHOMA, G. V. TERESHCHUK, D. V. OSADCHUK, N. I. RUSNAK, O. B. STOLIAR

Ternopil Volodymyr Hnatiuk National Pedagogical University, Ukraine; e-mail: falfushynska@tnpu.edu.ua

Obesity is rapidly increasing all over the world and pretends to be the global medical and social problem. Thus, the understanding of early signs of obesity and suitable biomarkers is urgently needed for developing an adequate strategy of the obesity prevention and a decrease in its growth rate. The parameters of the lipids' metabolism and oxidative stress, metallothioneins and signs of cytotoxicity have been investigated in blood samples of young obese women (O-group, 32 < Body Mass Index (BMI) < 37). With regard to persons of O-group they had higher catalase activity (by 435%), level of reactive oxygen species (by 129%), level of oxidised glutathione (by 55%), lipid peroxidation (by 26%) and protein carbonyls (by 345%) in the blood, when compared with control. The obesity was accompanied by an increase in concentration of metallothioneins which have a partial tread effect on radical processes and reduce manifestations of oxidative damage to biomolecules in obese patients. The obese women had the signs of cytotoxicity as higher lactate dehydrogenase activity (by 387%) and DNA fragmentation (by 42%). The principal component analysis revealed the set of biological traits which describes the obesity progress and it included metallothioneins, parameters of oxidative stress, cytotoxicity, BMI and a concentration of low density lipoproteins and total cholesterol. The BMI was in a good correlation with parameters of the lipid metabolism, oxidative injury and cytotoxicity ($r > |0.73|$, $P < 0.001$).

K e y w o r d s: obesity, oxidative stress, low density lipoproteins, high density lipoproteins, metallothioneins, cytotoxicity.

Obesity is a chronic disease that is accompanied by an enlargement of adipose tissue and represents a global social and medical problem [1]. Frequency of persons with overweight and obesity in the world has doubled since 1980, and by 2016, more than 1.9 billion adults with overweight and over 650 million with obesity, the main part of which belongs to young people [2]. According to WHO experts, 50% of women and 20% of men will be overweight, if obesity grows at the same pace by 2025. Ukraine ranks first in terms of childhood obesity (~28% of boys and girls) in Europe.

Reactive oxygen species (ROS) are physiologically necessary. They activate transcription factors and participate in gene expression, carry out transduction of hormonal and cellular signals, are involved in the oxidation of xenobiotics, have bactericidal action, as well as regulate the processes of cellular reproduction and control tissue homeostasis [3]. At the same time, the excessive formation of ROS and the excess of the tolerance limits of the organism's antioxidant system initiate free radical processes and the development of oxidative stress, which is one of the main determinants of a number of pathologies [4-6].

The adipose tissue is an endocrine and accumulation organ that consists of adipocytes and secretes hormones and adipokines [1]. In physiological and, especially, pathological conditions, adipokines produce ROS indirectly through NADPH-oxidase, which in turn initiates oxidative stress and causes imbalance of proliferation, differentiation and matu-

ration of adipose tissue cells. Peripheral deposition of fat in the abdominal cavity and in the area of internal organs determines the development of lipotoxicity under obesity, which becomes the basis for mitochondrial dysfunction and excessive production of ROS [5]. The latter, in combination with chronic systemic inflammation, become a trigger signal for the systemic acute phase response and determines the development of a number of pathological conditions, including concomitant diseases, in particular, atherosclerosis and other carrdiovascular diseases, metabolic syndrome and type II diabetes [7, 8].

Therefore, the purpose of the present work is to study the status of stress-responsive systems in combination with lipid metabolism and manifestations of oxidative damage and cytotoxicity in the blood of normal weight and obese women.

The parameters of the antioxidant system and metallothioneins, multifunctional metal-binding, stress-response proteins, which, depending on the conditions, can involve both the binding of metals and the sequestration of ROS [10], were included in the set of stress-responsive system's indices, according to the results we obtained earlier using ovarian cancerous tissue and human thyroid nodular goiter tissue [6, 9, 11].

Materials and Methods

We have screened the blood samples of 15 women with normal weight and 15 obese women aged 19-22 years who randomly sought regular health screening at the Ternopil hospital. Any person did not present any other pathologic condition that could interfere results and they did not take any vitamin–mineral supplementation and/or other medicines. BMI was calculated as weight (kg)/height (m^2) and persons who had BMI 19-24 kg/m^2 were determined as the control (C-group) and those who had BMI 32-37 kg/m^2, as the obese ones (O-group) as recommended by the WHO [2]. All studies and observations were conducted in accordance with the rules of the National Congress on Bioethics (Kyiv, 2000) and the protocol of estimation was approved by the Committee of Ethics in Volodymyr Hnatiuk National Pedagogical University of Ternopil (No 3, 2017).

All the procedures were carried out at 4 °C. Plasma samples were obtained as the supernatants of anti-coagulated heparinized blood. All chemicals were purchased from Sigma Aldrich (St. Louis, USA) or Merck (Synbias, Kyiv, Ukraine), and were of the analytical grade or higher.

The glycated hemoglobin (HbA1c), total cholesterol and triglycerides were measured with diagnostic "LaChema" spectrometric kits and low density (LDL) and high density (HDL) lipoproteins were estimated with CormayLDL/HDL Direct spectrometric kits according to the manufacturer's instructions.

Catalase activity (EC 1.11.1.6) was measured in whole blood samples by Aebi method [12], which is based on the decomposition of hydrogen peroxide with the catalase derived from the sample. The test mixture contained 150 μg of protein in 50 mM K-phosphate buffer, pH 7.4 in the presence of 15 mM H_2O_2 in total volume of 3.0 ml. The reaction was initiated by adding the appropriate volume of blood sample, and then the absorbance at 240 nm within a 60-second interval was determined. Enzymatic activity was calculated by the millimolar coefficient of the hydrogen peroxide's absorbance ($\varepsilon = 0.04\ mM^{-1}\cdot cm^{-1}$) and expressed in $\mu mol\cdot min^{-1}\cdot mg^{-1}$ proteins.

The content of total glutathione was quantified in the blood plasma by the glutathione reductase recycling assay [13]. To estimate the oxidized glutathione (GSSG) level, the protein free sample was treated with 2-vinylpyridine 60 minutes prior to the assay at 2% final concentration [14]. The rate of 5-thionitrobenzoic acid formation was monitored spectrophotometrically at 412 nm. The concentration of reduced glutathione (GSH) was calculated as the difference of concentrations between total glutathione and its oxidized forms.

The content of ROS in the supernatant of the whole blood samples were determined using a ROS-sensitive fluorescent dye dihydrorhodamine which is converted by ROS to the fluorescent dye rhodamine-123 [15]. The supernatant was prepared using 20 mM HEPES-sucrose lysis buffer (pH 7.4) by centrifugation at 16,000×g for 45 min. Probe fluorescence signal was detected by using *f*-max fluorescence plate-reader [excitation (ex.) = 485 nm, emission (em.) = 538 nm] at time 0 and after 20 min, and the rates of ROS formation were calculated from these two values. The ROS level was expressed in relative fluorescence units (RFU) per 1 mg of protein.

Protein carbonyl (PC) concentration, as index of protein oxidation, was measured in the trichloroacetic acid-treated blood samples by the reaction with 2,4-dinitrophenylhydrazine (DNPH) [16]. Differences in the absorbance between the DNPH- and the HCl-treated samples were determined spectro-

photometrically at 370 nm, and the amount of carbonyls was determined by using molar extinction coefficient of $2.2 \cdot 10^4$ $M^{-1} \cdot cm^{-1}$. Data were expressed as nmol PC·mg-1 of soluble extracted protein.

Lipid peroxidation (LPO) was determined in the protein-free supernatant of trichloroacetic acid-treated blood samples by the production of thiobarbituric acid-reactive substances (TBARS) [16]. The formation of TBARS was calculated by the intensity of the absorption of a pink-colored complex at 532 nm by the molar extinction coefficient of the complex equal to $\varepsilon = 1.56 \cdot 10^5$ $M^{-1} \cdot cm^{-1}$ [16].

The activity of lactate dehydrogenase (LDH, EC 1.1.1.27) was determined using the UV assay with pyruvate and NADH [17] by determining the amount of NADH oxidation at 340 nm. Phosphate/pyruvate solution (3 ml) (50 mM phosphate, pH 7.5, 0.63 mM pyruvate) was pipetted into cuvettes and 50 µl NADH solution (11.3 mM β-NADH) added then 100 µl of the serum sample was spiked and mixed immediately and the extinction was read after every minute interval for a period of 4 min. A molar extinction coefficient of $6.22 \cdot 10^6$ $M^{-1} \cdot cm^{-1}$ was used. Glutathione-S-transferase (GST, EC 2.5.1.18) activity was measured according to [18] using CDNB as substrate. Enzyme activity was determined at 25 °C by monitoring changes in absorbance at 340 nm for 2 min at constant temperature. The GST activity was expressed as nmol $min^{-1} \cdot mg^{-1}$ protein.

Metallothioneins (MTs) were determined from thiols measure with 5,5′-dithio-bis-2-nitrobenzoic acid according to the method of Viarengo et al. [19] after the ethanol/chloroform extraction with slight modification. Briefly 200 µl fresh prepared blood plasma were mixed with a cold 20 mM Tris-sucrose buffer (1:3 v/v) with 0.05 ml of 0.01% β-mercaptoethanol and 0.5 mM PMSF solution and centrifuged at 16,000 g for 45 min. Then MTs were extracted with chloroform : absolute ethanol mixture (2:25 v/v).The levels of MTs were calculated assuming the relationship: 1 mol MTs = 20 mol GSH and were expressed as µg of MTs per ml of blood.

DNA damage was evaluated by the levels of protein-free DNA strand breaks in the 1:10 (v/v) blood serum in 50 mM Tris-EDTA buffer, pH 8.0 which contains 0.5% sodium dodecyl sulphate (SDS) by the alkaline DNA precipitation assay [20] using Hoescht 33342 dye. To reduce the possible interference with traces of SDS, the assay was carried out in the presence of 0.4 M NaCl, 4 mM sodium cholate, and 0.1 M Tris (pH 9). Probe fluorescence signal was detected by using *f*-max fluorescence plate-reader (excitation = 360 nm, emission = 450 nm). The content of fragmented DNA was expressed as a mass fraction of DNA strand break in a sample to the total DNA.

Data were tested for normality and homogeneity of variance by using Kolmogorov-Smirnoff and Levene's tests, respectively. Whenever possible, data were normalized by Box-Cox common transforming method. For the data that were not normally distributed even after the transformation, non-parametric tests (Kruskall–Wallis ANOVA and Mann–Whitney U-test) were performed. Pearson's correlation test was used to assess correlations between the studied traits. Normalized, Box-Cox transformed data were subjected to the principal component analysis (PCA) to differentiate the individual specimens by the set of their indices. For evaluation of the antioxidative-prooxidative equilibrium and generalization of organism's response we used the self-proposed integral index of oxidative stress as the ratio of antioxidant defence parameters (catalase, reduced glutathione, glutathione transferase, MTs) and oxidative injury manifestations (ROS, oxidized glutathione, TBARS, protein carbonyls) after the standardization of data (http://uapatents.com/5-45298). All statistical calculations were performed with Statistica v. 12.0 and Excel for Windows-2013. Differences were considered significant, if the probability of type I error was less than 0.05. For all biological traits and all studied groups, the sample size was 15. The data are presented as means ± standard deviation (SD).

Results and Discussion

Determination of lipid metabolism indices in the examined persons of the O-groups indicates that in obese patients, the concentration of total cholesterol (by 29%) and lower density lipoproteins (LDL, by 59%) is higher, while the high-density lipoproteins (HDL, by 19%) is lower than in the control group (Table 1). The ratio of HDL/LDL in the control group is 0.82 and decreases for obesity to 0.44. Parameters of lipid metabolism correlate with BMI ($r > |0.73|$, $P < 0.001$). The concentration of triglycerides and glycosylated hemoglobin is similar in both groups of patients.

The analysis of the indices of the antioxidant system shows (Table 2) that in patients with obesity, catalase activity is higher (by 435%), and glutathione-S-transferase activity (by 27%) and the content of reduced glutathione (by 36%) are lower

Table 1. Parameters of lipid metabolism and concentration of glycated haemoglobin in the blood samples of normal weight and obese women, M ± SD, n = 15

Parameters	Group	
	Control	Obesity
Glycated haemoglobin, %	4.9 ± 0.1	4.9 ± 0.2
Total cholesterol, mM	4.3 ± 0.3	5.5 ± 0.4*
Triglycerides, mM	0.9 ± 0.3	1.1 ± 0.3
High density lipoproteins, mM	1.8 ± 0.2	1.5 ± 0.2*
Low density lipoproteins, mM	2.2 ± 0.4	3.4 ± 0.3*

Here and in Table 2: * statistically significant differences compared to correspondent control, $P < 0.05$

than in the control. Also, the content of metallothionein is higher (by 66.7%) in patients with obesity. At the same time, the patients had higher levels of ROS (by 129%), concentration of oxidized glutathione (by 55%), TBARS (by 26%) and protein carbonyl (by 345%) in comparison with the control group. The integral index of oxidative stress is -0.43 and indicates a shift in redox-balance in the cell and prooxidant changes.

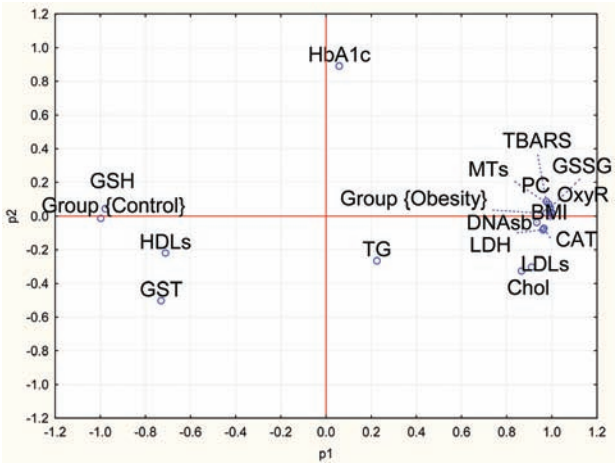
There are signs of cytotoxicity in patients with obesity, among them elevated, compared to control, the level of DNA fragmentation (by 42%) and higher lactate dehydrogenase activity (by 387%).

The use of the principal component analysis with the NIPALS algorithm (type of multi-factor analysis of data) allowed us to find correlations between investigated parameters of the examined normal-weight and obese individuals. About 85% of the absolute values of the investigated biological traits belong to Factors 1 and 2 (Figure). Metallothioneins form a joint cluster with parameters of oxidative stress, cytotoxicity, index of body mass, total cholesterol, and low density lipoprotein. These indices are also crucial in the development of obesity, as they correlate with O-group with a high significance. The control group is located in opposition to the O-group and includes indices of reduced glutathione and high density lipoprotein. This arrangement proves the relation of the patterns within the cluster and their opposite nature between the two clusters.

Metallothioneins are low molecular weight, stress-response proteins with high content of sulfur (up to 30% of the amino acid composition falls on cysteine), and d-metals deposited with zinc and copper are involved in the cadmium detoxification in cells and, due to the presence of three elements in the promoter of the gene, metal-response (MRE), antioxidant-response (ARE) and glucocorticoid-response (GRE), are induced by metals, numerous stressors and prooxidants and, *in vitro*, inhibit radical processes and apoptosis [11, 21, 22]. Meanwhile, the properties of metallothioneins of adipose tissue and their role in the development of obesity in mammals, and especially humans, are not well-estimated [23]. Existing data are mostly sporadic. In particular, it has been shown that over obesity, metallothioneins expression in hypodermic adipose tissue cells, adipocytes and SVF cells of mammals and humans is higher than normal [21, 23]. At the same time, a

Table 2. Blood biochemical parameters of normal weight and obese women, M ± SD, n = 15

Parameters	Group	
	Control	Obesity
Metallothioneins concentration, μg·ml^{-1} blood	11.7 ± 1.4	19.5 ± 1.9*
Catalase activity, μmol·min^{-}1·mg^{-1} proteins	13.6 ± 2.8	72.9 ± 13.5*
TBARS, nmol·ml^{-1} blood	2.3 ± 0.3	2.9 ± 0.3*
Protein carbonyls, nmol·mg^{-1} proteins	1.1 ± 0.1	4.9 ± 0.6*
Glutathione oxidized, μmol·ml^{-1} blood	0.20 ± 0.02	0.31 ± 0.03*
Glutathione reduced, μmol·ml^{-1} blood	2.2 ± 0.3	1.4 ± 0.2*
Reactive oxygen species concentration, RFU·mg^{-1} proteins	5.4 ± 0.3	12.2 ± 0.6*
Glutathione transferase, nmol·min^{-1}·mg^{-1} proteins	100.7 ± 15.5	73.8 ± 9.8*
DNA strand break, %	6.3 ± 0.7	8.9 ± 0.3*
Lactate dehydrogenase activity, μmol·min-1·mg-1proteins	0.42 ± 0.08	2.1 ± 0.4*



Integral analysis of indices of lipid metabolism, stress-response systems and molecular injury of normal weight and obese women

two-week diet with an energy value of 800 calories and a prolonged fasting does not affect the level of expression of MT2 [23, 24]. The protective role of metallothioneins for the development of obesity by regulating adipocyte proliferation, signaling leptin and synthesis of ATP is proved on the example of MT-I- and MT-II-zero ($MT^{-/-}$) and wild type ($MT^{+/+}$) lines of female mice supporting a high calorie diet [21, 22]. It has also been shown that the level of metallothioneins in the blood of obese women is higher when compared to the control, moreover, the metallothioneins mRNA level is 5-15 times higher than that of ZnT-1 and Zip-1 zinc transporter mRNA, however, similar parameters for the control group have not been studied [25].

The examined obese women have a higher concentration of metallothioneins than those in the control group. In combination with oxidative injury, inhibition of the antioxidant system parameters, metallothioneins perform a protective role and, apparently, function primarily not as metal binding proteins, but as radical scavenger using free thiol groups. The inclusion of MT into a joint cluster with oxidative stress indices (Fig.) and the existence of a correlation between the concentration of metallothioneins and oxidation damage of proteins and lipids in a regression analysis model demonstrates the benefit of the proposed hypothesis: Metalothioneins = -10.65 + 0.005×BMI – 0.024×Cholesterol + 0.004×LDL + 9.39×TBARS* + 0.503×PC* 0.026×ROS + 0.0005×Catalase, $R^2 = 0.99$; $F(7.8) = 5053.8$, $P < 0.001$; * – parameter inputs the significant value in the statistical model. Involvement of metallothioneins in the elimination of ROS has been proved using human nodular goiter and ovary cancer models [6, 9, 10]. Therefore, the further study of the possibility of using metallothioneins as nutritional supplements to reduce the effects of oxidative damage to biomolecules in people with overweight is urgently needed. Certain attempts in the use of compounds enriched in cysteine (among them N-acetylcysteine) or substances that modulate the synthesis of metallothioneins as a means of anti-obesity are known in the literature [26, 27].

Oxidative modifications of lipids associated with low density lipoproteins play an important role in endothelial dysfunction and inflammatory processes associated with atherosclerosis [4,5]. Partly protective, antioxidant role in the oxidation of low density lipoproteins is performed by high density lipoproteins that has been demonstrated *in vitro* by the example of copper-induced oxidation of lipoproteins in women with type 2 diabetes [28] and postmenopausal women [29]. In the present study, BMI is negatively correlated with the ratio of HDL/LDL. Thus, in obese women the portion of high density lipoproteins is reduced in agreement with the increase of protein carbonyls and DNA fragmentation: DNAsb = 8.51 – 1.52×TBARS + 0.95×PC* + 0.23×HDL/LDL; $R^2 = 0.85$, $F(3.12)=30.45$, $P < 0.001$. These factors can be considered as determinants of the pathogenesis of concomitant metabolic diseases, which is consistent with the literature [30].

It is believed that hydrogen peroxide is one of the key molecules that cause oxidative damage to biological systems. The mechanisms of cytotoxic action of hydrogen peroxide include its conversion to the highly reactive hydroxyl radical that damages proteins, lipids and DNA. Glutathione peroxidase, peroxyredoxin and catalase are the enzymes that are involved in the neutralization of H_2O_2 and catalase has the least affinity for the hydrogen peroxide [31]. In our case study, catalase activity in obese women exceeded the basal activity of the control group by 435%, consistent with a threefold increase in protein carbonyls ($r = 0.96$, $P < 0.001$). Similar results were obtained using mice that were kept on a fat-enriched diet [32]. Thus, catalase reacts sensitively to dyslipidemia and can be considered as a sensitive marker for exceeding the threshold concentration of hydrogen peroxide, while glutathione peroxidase and peroxyredoxin are manipulated with nanomolar peroxide amounts necessary for signal transduction [33]. Activation of catalase and its up-regulation on the

principle of sharp-phase response is noted in animals under a number of model acute toxic conditions, for example, after copper treatment [34].

Glutathione transferase belongs to the key enzymes of Phase II detoxification and protects cells from oxidative stress and numbers of organic and inorganic toxic compounds and their metabolites. Our results show that in patients with obesity GST activity is lower than in the comparison group. Similar data were obtained in mice with induced and genetic obesity (ob/ob) [35, 36]. Lower relative expression of GSTP1/2 in the liver of obese mice which corresponds to a smaller number of GSTP class proteins and may result in decreasing the enzyme catalytic activity was demonstrated using transcriptional analysis. It has recently been proven that the human GSTP1 gene polymorphism is associated with increased susceptibility to diabetes and abdominal obesity [37].

There is a number of evidence that the increase of proliferation and hypertrophy of adipocytes in animals and humans is accompanied by the development of hypoxia and LDH activation [7, 38]. LDH of blood is considered a marker of cytotoxicity and tissue damage, as well as malignancy of pathomorphological changes in a number of diseases, including cancer, myocardial infarction, liver disease, sepsis, and so on [39]. The results obtained by us testify that LDH activity in young women correlates with the body mass index ($r = 0.9$, $P < 0.001$) on the one hand and manifestations of oxidative damage on the other ($r = 0.95$, $P < 0.001$ for protein carbonyls and $r = 0.94$, $P < 0.001$ for lipid peroxidation), which may indicate an unfavorable clinical pattern of the disease and development, over a period of time, in the examined concomitant metabolic diseases. In obese patients, who concomitantly had the colorectal cancer, high LDH activity and IL-8 levels determined five times higher mortality than in those with normal body weight [40].

A series of publications are devoted to the search for early markers for the prognosis of the appearance and progression of obesity in young people. However, the main focus is on anthropometric measurements, markers of inflammatory processes (e.g., IL-6, C-reactive protein) and lipidogram parameters [41-43]. However, their separate evaluation could not reflect a coherent picture of existing pathological changes, and complicates the prognosis of the course of the disease, including the possibility development of concomitant metabolic diseases. Up-to-date, more and more works have appeared, especially in the field of oncology and cardiovascular pathologies, which argue that only the definition of a set of indicators can provide an early detection and adequate prediction of the course of a process and improve understanding of the mechanisms of its occurrence [44, 45]. According to the analysis of all our data by the principal component analysis, the development of obesity is determined by a set of indices, which include metallothioneins, oxidative stress, cytotoxicity, body mass index, low density lipoprotein and total cholesterol concentrations. Among them, the highest index of variability ($2<IV<5$), and thus the greatest sensitivity to obesity, is shown by catalase, products of oxidative destruction of proteins, LDH, the ROS level and the ratio of high-density lipoprotein to low-density lipoproteins. Therefore, this panel of blood parameters, as covering various functional systems of the body, can be used to assess the degree of pathomorphological changes in obesity.

To sum up, the integrated analysis of the selected parameters of lipids' metabolism and oxidative stress, metallothioneins and signs of cytotoxicity in obese young women allowed us to determine the amount of features that deepens pathological changes. It is the discrepancy in oxidative-reductive status related to simultaneous activation of catalase and manifestation of oxidative injury, the redox-equilibrium shift in the direction of the prooxidant processes and the disturbance of the balance of anaerobic / aerobic glycolysis and NAD^+/NADH, reducing the portion of high-density lipoproteins to low-density lipoproteins, as well as increasing the DNA fragmentation. Metallothioneins have a partial tread effect on radical processes and reduce manifestations of oxidative damage to biomolecules in obese patients.

This work was supported by the Ministry of Education and Science of Ukraine (N133B) and by Alexander von Humboldt Scholarship to H.F.

ОЦІНКА ВМІСТУ МЕТАЛОТІОНЕЇНІВ, ПОКАЗНИКІВ ОКИСНОГО СТРЕСУ ТА ЦИТОТОКСИЧНОСТІ В МОЛОДИХ ЖІНОК З ОЖИРІННЯМ

Г. І. Фальфушинська, О. І. Горин, В. В. Хома, Г. В. Терещук, Д. В. Осадчук, Н. І. Руснак, О. Б. Столяр

Тернопільський національний педагогічний університет імені В. Гнатюка, Україна;
e-mail: falfushynska@tnpu.edu.ua

Ожиріння набуває загрозливих масштабів та являє глобальну медико-соціальну проблему. Відтак, існує необхідність вивчення причин виникнення та механізмів його прогресування для вироблення єдиної стратегії профілактики ожиріння та зменшення темпів його приросту. Досліджували показники ліпідограми та окисного стресу, вміст металотіонеїнів та ознаки цитотоксичності в крові молодих жінок, хворих на ожиріння (О-група, 32<індекс маси тіла (ІМТ) < 37). В обстежених жінок О-групи встановлено значно вищу активність каталази (на 435%), підвищені рівні активних форм оксигену (на 129%), концентрація окисленого глутатіону (на 55%), вміст ТБК-АП (на 26%) і карбонільних похідних протеїнів (на 345%) порівняно з обстеженими в контрольній групі. За ожиріння збільшувався вміст металотіонеїнів, які відіграють частково протекторну роль щодо радикальних процесів у клітинах та зменшують прояви окисного ушкодження біомолекул в жінок з ожирінням. У хворих на ожиріння проявлялися ознаки цитотоксичності зі збільшенням лактатдегідрогеназної активності (на 387%) та рівня фрагментації ДНК (на 42%). Згідно з методом головних компонентів розвиток ожиріння визначається сукупністю ознак, до яких належать металотіонеїни, показники окисного стресу, цитотоксичності, ІМТ, концентрація ліпопротеїнів низької щільності та холестеролу. ІМТ корелює з показниками ліпідного обміну, окисного ушкодження та цитотоксичності (r > |0,73|, P < 0,001).

Ключові слова: ожиріння, окисний стрес, ліпопротеїни низької щільності, ліпопротеїни високої щільності, металотіонеїни, цитотоксичність.

ОЦЕНКА СОДЕРЖАНИЯ МЕТАЛЛОТИОНЕИНОВ, ПОКАЗАТЕЛЕЙ ОКИСЛИТЕЛЬНОГО СТРЕССА И ЦИТОТОКСИЧНОСТИ У МОЛОДЫХ ЖЕНЩИН С ОЖИРЕНИЕМ

Г. И. Фальфушинская, О. И. Горын, В. В. Хома, Г. В. Терещук, Д. В. Осадчук, Н. И. Руснак, О. Б. Столяр

Тернопольский национальный педагогический университет имени В. Гнатюка, Украина;
e-mail: falfushynska@tnpu.edu.ua

Ожирение приобретает угрожающие масштабы и представляет глобальную медико-социальную проблему. Следовательно, существует необходимость изучения причин возникновения и механизмов его прогрессирования для выработки единой стратегии профилактики ожирения и уменьшения темпов его прироста. Исследовали показатели липидограммы и окислительного стресса, содержание металлотионеинов и признаки цитотоксичности в крови молодых женщин, больных ожирением (О-группа, 32<индекс массы тела (ИМТ) < 37). У обследованных женщин О-группы установлена значительно более высокая активность каталазы (на 435%), повышенные уровень активных форм кислорода (на 129%), концентрация окисленного глутатиона (на 55%), содержание ТБК-АП (на 26%) и карбонильных производных протеинов (на 345%) по сравнению с обследованными в контрольной группе. При ожирении увеличивалось содержание металлотионеинов, которые исполняют частично протекторную роль относительно радикальных процессов в клетках и уменьшают проявления окислительного повреждения биомолекул у женщин с ожирением. У больных с ожирением проявлялись признаки цитотоксичности с увеличением лактатдегидрогеназной активности (на 387%) и уровня фрагментации ДНК (на 42%). Согласно методу главных компонентов развитие ожирения определяется совокупностью признаков, к которым относятся металлотионеины, показатели окислительного стресса, цитотоксичности, ИМТ, концентрация липопротеинов низкой плотности и холестерина. ИМТ коррелирует с показателями липидного обмена, окислительного повреждения и цитотоксичности (r > |0,73|, P < 0,001).

Ключевые слова: ожирение, окислительный стресс, липопротеины низкой плотности, липопротеины высокой плотности, металлотионеины, цитотоксичность.

References

1. Sikaris KA. The clinical biochemistry of obesity. *Clin Biochem Rev.* 2004; 25(3): 165-181.
2. Obesity and overweight. Key facts issued by World Health Organisation. 2017. Electronic resource. Available from http:. www.who.int. en. news-room. fact-sheets. detail. obesity-and-overweight (assessed 19.06.2018).
3. Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M. Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chem Biol Interact*. 2006; 160(1): 1-40.
4. Chisolm GM, Steinberg D. The oxidative modification hypothesis of atherogenesis: an overview. *Free Radic Biol Med*. 2000; 28(12): 1815-1826.
5. Schrauwen P, Schrauwen-Hinderling V, Hoeks J, Hesselink MK. Mitochondrial dysfunction and lipotoxicity. *Biochim Biophys Acta*. 2010; 1801(3): 266-271.
6. Falfushynska HI, Gnatyshyna LL, Deneha HV, Osadchuk OY, Stoliar OB. Manifestations of oxidative stress and molecular damages in ovarian cancer tissue. *Ukr Biochem J.* 2015; 87(5): 93-102.
7. Hensley K, Robinson KA, Gabbita SP, Salsman S, Floyd RA. Reactive oxygen species, cell signaling, and cell injury. *Free Radic Biol Med*. 2000; 28(10): 1456-1462.
8. Fernández-Sánchez A, Madrigal-Santillán E, Bautista M, Esquivel-Soto J, Morales-González A, Esquivel-Chirino C, Durante-Montiel I, Sánchez-Rivera G, Valadez-Vega C, Morales-González JA. Inflammation, oxidative stress, and obesity. *Int J Mol Sci*. 2011; 12(5): 3117-3132.
9. Falfushynska HI, Gnatyshyna LL, Osadchuk OY, Shidlovski VO, Stoliar OB. Metal-binding functions and antioxidant properties in human thyroid gland under iodine deficient nodular colloidal goiter. *Ukr Biokhim Zhurn*. 2011; 83(6):92-97. (In Ukrainian).
10. Maret W. Redox biochemistry of mammalian metallothioneins. *J Biol Inorg Chem*. 2011; 16(7): 1079-1086.
11. Falfushynska HI, Gnatyshyna LL, Osadchuk OY, Shidlovski VO, Stoliar OB. Trace elements storage peculiarities and metallothionein content in human thyroid gland under iodine deficiency euthyroid nodular goiter. *Ukr Biochem J.* 2014; 86(3): 107-113. (In Ukrainian).
12. Aebi H. Catalase. *Methods Enzym Anal*. 1974; 2: 673-684.
13. Anderson ME. Determination of glutathione and glutathione disulfide in biological samples. *Methods Enzymol*. 1985; 113: 548-555.
14. Griffith OW. Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine. *Anal Biochem*. 1980; 106(1): 207-212.
15. Viarengo A, Burlando B, Cavaletto M, Marchi B, Ponzano E, Blasco J. Role of metallothionein against oxidative stress in the mussel Mytilus galloprovincialis. *Am J Physiol*. 1999; 277(6 Pt 2): R1612-R1619.
16. Lushchak VI, Bahniukova TV, Lushchak OV. Indices of oxidative stress. 1. TBA-reactive substances and carbonylproteins. *Ukr Biokhim Zhurn*. 2004; 76(3): 136-141. (In Ukrainian).
17. Bergmeyer HU, Bernt E. UV-Assay with Pyruvate and NADH. *Methods Enzym Anal*. 1974; 2: 574-579.
18. Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. *J Biol Chem*. 1974; 249(22): 7130-7139.
19. Viarengo A, Ponzano E, Dondero F, Fabbri R. A simple spectrophotometric method for metallothionein evaluation in marine organisms: an application to Mediterranean and Antarctic molluscs. *Mar Environ Res*. 1997; 44(1): 69-84.
20. Olive PL. DNA precipitation assay: a rapid and simple method for detecting DNA damage in mammalian cells. *Environ Mol Mutagen*. 1988; 11(4): 487-495.
21. Miles AT, Hawksworth GM, Beattie JH, Rodilla V. Induction, regulation, degradation, and biological significance of mammalian metallothioneins. *Crit Rev Biochem Mol Biol*. 2000; 35(1): 35-70.
22. Sato M, Kawakami T, Kondoh M, Takiguchi M, Kadota Y, Himeno S, Suzuki S. Development of high-fat-diet-induced obesity in female metallothionein-null mice. *FASEB J.* 2010; 24(7): 2375-2384.

23. Do MS, Nam SY, Hong SE, Kim KW, Duncan JS, Beattie JH, Trayhurn P. Metallothionein gene expression in human adipose tissue from lean and obese subjects. *Horm Metab Res.* 2002; 34(6): 348-351.
24. Cancello R, Zulian A, Gentilini D, Mencarelli M, Della Barba A, Maffei M, Vitti P, Invitti C, Liuzzi A, Di Blasio AM. Permanence of molecular features of obesity in subcutaneous adipose tissue of ex-obese subjects. *Int J Obes (Lond).* 2013; 37(6): 867-873.
25. dos Santos Rocha PB, de Castro Amorim A, de Sousa AF, do Monte SJ, da Mata Sousa LC, do Nascimento Nogueira N, Neto JM, do Nascimento Marreiro D. Expression of the zinc transporters genes and metallothionein in obese women. *Biol Trace Elem Res.* 2011; 143(2): 603-611.
26. Kim JR, Ryu HH, Chung HJ, Lee JH, Kim SW, Kwun WH, Baek SH, Kim JH. Association of anti-obesity activity of N-acetylcysteine with metallothionein-II down-regulation. *Exp Mol Med*. 2006; 38(2): 162-172.
27. Wang S, Gu J, Xu Z, Zhang Z, Bai T, Xu J, Cai J, Barnes G, Liu QJ, Freedman JH, Wang Y, Liu Q, Zheng Y, Cai L. Zinc rescues obesity-induced cardiac hypertrophy via stimulating metallothionein to suppress oxidative stress-activated BCL10/CARD9/p38 MAPK pathway. *J Cell Mol Med.* 2017; 21(6): 1182-1192.
28. Sanguinetti SM, Brites FD, Fasulo V, Verona J, Elbert A, Wikinski RL, Schreier LE. HDL oxidability and its protective effect against LDL oxidation in Type 2 diabetic patients. *Diabetes Nutr Metab.* 2001; 14(1): 27-36.
29. Brites F, Zago V, Verona J, Muzzio ML, Wikinski R, Schreier L. HDL capacity to inhibit LDL oxidation in well-trained triathletes. *Life Sci*. 2006; 78(26): 3074-3081.
30. Brennan ML, Hazen SL. Amino acid and protein oxidation in cardiovascular disease. *Amino Acids.* 2003; 25(3-4): 365-374.
31. Gaetani GF, Ferraris AM, Rolfo M, Mangerini R, Arena S, Kirkman HN. Predominant role of catalase in the disposal of hydrogen peroxide within human erythrocytes. *Blood*. 1996; 87(4): 1595-1599.
32. Rindler PM, Plafker SM, Szweda LI, Kinter M. High dietary fat selectively increases catalase expression within cardiac mitochondria. *J Biol Chem*. 2013; 288(3): 1979-1990.
33. Manta B, Hugo M, Ortiz C, Ferrer-Sueta G, Trujillo M, Denicola A. The peroxidase and peroxynitrite reductase activity of human erythrocyte peroxiredoxin 2. *Arch Biochem Biophys.* 2009; 484(2): 146-154.
34. Craig PM, Wood CM, McClelland GB. Oxidative stress response and gene expression with acute copper exposure in zebrafish (Danio rerio). *Am J Physiol Regul Integr Comp Physiol*. 2007; 293(5): R1882-R1892.
35. Koide CL, Collier AC, Berry MJ, Panee J. The effect of bamboo extract on hepatic biotransforming enzymes–findings from an obese-diabetic mouse model. *J Ethnopharmacol.* 2011; 133(1): 37-45.
36. Roe AL, Howard G, Blouin R, Snawder JE. Characterization of cytochrome P450 and glutathione S-transferase activity and expression in male and female ob/ob mice. *Int J Obes Relat Metab Disord.* 1999; 23(1): 48-53.
37. Amer MA, Ghattas MH, Abo-Elmatty DM, Abou-El-Ela SH. Evaluation of glutathione S-transferase P1 genetic variants affecting type-2 diabetes susceptibility and glycemic control. *Arch Med Sci*. 2012; 8(4): 631-636.
38. Azuma M, Shi M, Danenberg KD, Gardner H, Barrett C, Jacques CJ, Sherod A, Iqbal S, El-Khoueiry A, Yang D, Zhang W, Danenberg PV, Lenz HJ. Serum lactate dehydrogenase levels and glycolysis significantly correlate with tumor VEGFA and VEGFR expression in metastatic CRC patients. *Pharmacogenomics.* 2007; 8(12): 1705-1713.
39. Adeva-Andany M, López-Ojén M, Funcasta-Calderón R, Ameneiros-Rodríguez E, Donapetry-García C, Vila-Altesor M, Rodríguez-Seijas J. Comprehensive review on lactate metabolism in human health. *Mitochondrion*. 2014; 17: 76-100.
40. Shah MS, Fogelman DR, Raghav KP, Heymach JV, Tran HT, Jiang ZQ, Kopetz S, Daniel CR. Joint prognostic effect of obesity and chronic systemic inflammation in patients with metastatic colorectal cancer. *Cancer.* 2015; 121(17): 2968-2975.
41. Brisbois TD, Farmer AP, McCargar LJ. Early markers of adult obesity: a review. *Obes Rev.* 2012; 13(4): 347-367.
42. Romero A, Rezende L, Romero S, Villar B. Relationship between obesity and biochemical markers in Brazilian adolescents. *Rev Bras Cineantropom Desempenho Hum*. 2014; 16(3): 268-276.

43. Laimer M, Ebenbichler CF, Kaser S, Sandhofer A, Weiss H, Nehoda H, Aigner F, Patsch JR. Markers of chronic inflammation and obesity: a prospective study on the reversibility of this association in middle-aged women undergoing weight loss by surgical intervention. I*nt J Obes Relat Metab Disord*. 2002; 26(5): 659-662.
44. de Albuquerque A, Kaul S, Breier G, Krabisch P, Fersis N. Multimarker Analysis of Circulating Tumor Cells in Peripheral Blood of Metastatic Breast Cancer Patients: A Step Forward in Personalized Medicine. *Breast Care (Basel)*. 2012; 7(1): 7-12.
45. O'Donoghue ML, Morrow DA, Cannon CP, Jarolim P, Desai NR, Sherwood MW, Murphy SA, Gerszten RE, Sabatine MS. Multimarker Risk Stratification in Patients With Acute Myocardial Infarction. *J Am Heart Assoc*. 2016; 5(5): e002586.

Received 14.05.2018