Crosstalk between Oxidative Stress and Inflammation in Obesity

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Obesity is a multifactorial disease in which oxidative stress and inflammation play important roles. The aim of our study was to evaluate oxidative stress levels and inflammation markers in obese subjects vs. controls and to investigate the relationship between these values. We found increased levels of reactive oxygen species and inflammation markers (fibrinogen, ferritin, CRP, NLR) and decreased levels of antioxidants in obese subjects vs. controls.

Keywords: obesity, inflammation, antioxidants, reactive oxygen species

Obesity is a multifactorial disease, characterized by the accumulation of abnormal or excessive fat. Considered a global health problem, obesity is involved in the development of a myriad of health issues, such as diabetes mellitus, cardiovascular disease, cancer, infertility, asthma, sleep disorders, irritable bowel syndrome, hepatic and renal dysfunctions. According to the World Health Organization (WHO) definition of overweight and obesity, an overweight subject has a body mass index (BMI) of 25 to 29.9 kg/m^2, whereas the BMI of an obese subject exceeds 30 kg/m^2. Recent studies have underlined that, in obese subjects, the adipose tissue dysfunction derives also from the interplay between reactive oxygen species (ROS) signalling and inflammatory response pathways [1-9].

Oxidative stress, defined as the imbalance between the production of ROS and the levels of antioxidants in the body, is a key element in the development of obesity and many obesity-related comorbidities. The main ROS involved in this process are the superoxide anion $^{\bullet}O_2^-$, the hydrogen peroxide H_2O_2 and the hydroxyl radical $^{\bullet}OH$, whereas antioxidant enzymes, i.e. superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), uric acid, cofactors (e.g. NADPH), vitamin E, vitamin C, thioredoxin and glutathione (GSH) and trace metals (e.g. selenium) counteract pro-oxidant effects of ROS. Oxidative stress can also be induced by obesity *per se* by several mechanisms such as oxidative phosphorylation in the mitochondria, oxidation of fatty acids, over-consumption of oxygen, protein kinase C activation, hyperleptinemia, postprandial ROS generation due to lipid-rich and carbohydrate-rich diets, low antioxidant defenses, endothelial dysfunction due to reduced bioavailability of nitric oxide and increased levels of endothelium-derived contractile factors, and chronic inflammation [10-18].

In the adipose tissue, local hypoxia and the hypertrophy of adipocytes resulted during the overgrowth of fat cells can act as inflammation triggers. Hypoxia is correlated with the macrophage infiltration into the fat tissue and with an excess of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6). Thus, in the adipose tissue, low-grade localized inflammation can develop and then lead to systemic inflammation which acts as an essential element in the development of many obesity-associated comorbidities [9]. TNF-α is a pro-inflammatory cytokine which binds to specific receptors and is involved in immunity, adipose cell apoptosis, lipid metabolism, insulin signalling and ROS production by enhancing NF-κB signalling [1]. As a response to tissue injury, infection or as part of the immune response, monocytes release IL-1β, a pyrogenic cytokine. By the release of IL-6, IL-1β and TNF-α enhance the systemic acute-phase response and decrease the levels of anti-inflammatory factors like adiponectin. Apart from its role in the acute phase reaction, IL-6 is a pro-inflammatory cytokine also involved in osseous metabolism, hematopoiesis and cancer progression, playing an important role in transitioning from a state of acute inflammation to a chronic inflammatory disorder in conditions such as insulin resistance, obesity and inflammatory diseases [17].

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The aim of the study was to evaluate the levels of oxidative stress and inflammation markers in a group of patients stratified in different classes of obesity and to investigate the relationship between these variables.

Experimental part

We evaluated oxidative stress levels and inflammation markers in 85 obese patients vs. 30 non-obese controls. Patients were divided by sex, age and obesity class. The diagnosis of obesity was based on body mass index (BMI) values according to the WHO criteria: first class obesity – BMI of 30.0 to 34.9 kg/m^2, second class obesity – BMI of 35.0 to 39.9 kg/m^2, and third class obesity – BMI > 40kg/m^2. To assess BMI, height and weight were measured with a standard height-weight scale, and BMI was calculated as weight (kg) divided by height (m) squared. The complete blood count was determined using an automated analyzer BM-800. Serum glucose levels, uric acid and serum iron were measured by spectrophotometry using a KONELAB601 analyzer. Oxidative stress levels were evaluated using a CR3000 analyzer. Reactive oxygen species levels were measured using the FORT (Free Oxygen Radicals Testing) test and the antioxidant capacity using the FORD (Free Oxygen Radical Defence). Inflammatory status was evaluated by acute phase proteins (fibrinogen, C-reactive protein, serum ferritin) and the neutrophil-to-lymphocyte ratio (NLR). Statistical analysis of data was performed using Microsoft Excel (Microsoft Office Professional Plus 2013) and GraphPad QuickCalcs (https://www.graphpad.com). Informed consent was obtained from all subjects involved. The study had the approval of the Ethics Committee of the University of Medicine and Pharmacy of Craiova (approval number: 40/27.03.2018).

Results and discussions

The study group involved 85 obese patients (mean age 61.85 ± 10.60 years, age range 24-82): 17 males (20.00%, mean age 62.65 ± 8.90 years, age range 43-77) and 68 females (80.00%, mean age 61.65 ± 10.97 years, age range 24-82). Most patients lived in rural areas (46 patients, 54.12%) as compared to urban areas (39 patients, 45.88%). The control group included 30 non-obese subjects (mean age 44.60 ± 18.45 years, age range 18-85). The mean BMI was 36.44 ± 3.63 kg/m^2 in obese subjects (men: 35.35 ± 3.41 kg/m^2, women: 36.71 ± 3.63 kg/m^2) vs. 24.56 ± 1.76 kg/m^2 in controls (p-value < 0.0001). Stratification in obesity classes was: 38 patients (44.70%) - class I obesity, 31 patients (36.47%) – class II obesity, 16 patients (18.83%) – class III obesity (Fig. 1).

FORT values were increased in obese patients vs. controls (3.09 ± 0.36 mmol/L vs. 2.03 ± 0.14 mmol/L, p-value<0.001). FORT values based on obesity class were: class I obesity = 2.95 ± 0.27 mmol/L, class II obesity = 3.18 ± 0.36 mmol/L and class III obesity = 3.26 ± 0.41 mmol/L vs. 2.03 ± 0.14 mmol/L in controls, p-value<0.0001 for all obesity classes vs. controls. FORD values were decreased in obese patients vs. controls (0.69 ± 0.15 mmol/L vs. 1.27 ± 0.13 mmol/L, p-value<0.0001). FORD values based on obesity class were: class I obesity = 0.74 ± 0.13 mmol/L, class II obesity = 0.65 ± 0.14 mmol/L and class III obesity = 0.63 ± 0.16 mmol/L vs. 1.27 ± 0.13 mmol/L in controls, p-value<0.0001 for all obesity classes vs. controls (Table 1).

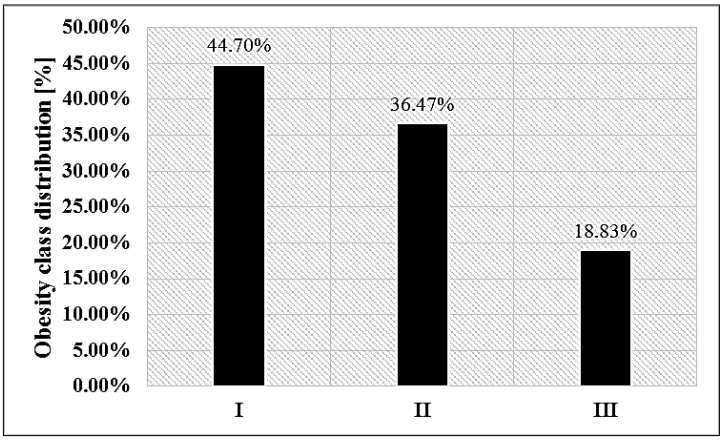

Fig. 1. Distribution of the study group based on obesity classes

Table 1
FORT VALUES WERE INCREASED AND FORD VALUES WERE DECREASED IN OBESE SUBJECTS VS. CONTROLS.

	Obese patients		Controls	p-value
FORT [mmol/L]	Overall	3.09 ± 0.36	2.03 ± 0.14	<0.001
	Class I obesity	2.95 ± 0.27		
	Class II obesity	3.18 ± 0.36		
	Class III obesity	3.26 ± 0.41		
FORD [mmol/L]	Overall	0.69 ± 0.15	1.27 ± 0.13	<0.0001
	Class I obesity	0.74 ± 0.13		
	Class II obesity	0.65 ± 0.14		
	Class III obesity	0.63 ± 0.16		

Fibrinogen levels were increased in obese patients vs. controls: 405.78 ± 36.11 mg/dL vs. 267.97 ± 36.64 mg/dL in controls, p-value<0.0001. CRP levels were increased in obese patients vs. controls: 6.75 ± 0.79 mg/dL vs. 3.24 ± 0.58 mg/dL in controls, p-value<0.0001. Serum ferritin levels were increased in obese patients vs. controls 321.30 ± 18.85 ng/mL vs. 158.60 ± 59.09 ng/mL in controls, p-value<0.0001. We recorded positive weak correlations between FORT – CRP (r = 0.03) and FORT – ferritin (r = 0.03), as well as a very weak correlation between FORT - fibrinogen (r = 0.06). The correlation between FORD – fibrinogen was very weak and negative (r = -0.06). We did not record significant differences in terms of NLR in obese subjects vs. controls (2.46 ± 0.96 vs. 2.31 ± 0.26, p=0.4036). In obese subjects, we found a positive weak correlation between FORT and BMI (r = 0.38), and very weak positive correlations FORT - NLR (r = 0.07) and FORT - age (r = 0.07).

Obesity can lead to oxidative stress *via* several biochemical mechanisms but other factors such as chronic inflammation, elevated lipid levels, postprandial ROS generation, low antioxidant defenses, hyperglycemia, mineral or vitamin deficiencies and endothelial dysfunction are main contributors nevertheless [10]. Furukawa *et al.* revealed that elevated levels of fatty acids from the adipose tissue of obese mice and from cultured adipocytes increased oxidative stress levels *via* NADPH oxidase activation which in turn enhanced local and systemic generation of pro-inflammatory adipocytokines, i.e. IL-6, TNF-α, IL-1β, monocyte chemotactic protein-1 (MCP-1), adiponectin and plasminogen activator inhibitor-1 (PAI-1) [19]. In obese subjects, the overexpression of pro-inflammatory cytokines is also generated by the activation of the c-Jun N-terminal kinase [9]. The most important cytokines in obesity are IL-6, the inflammasome-activated IL-1β and TNF-α, since these molecules are responsible for the chronic inflammation in obese subjects [1]. The inflammasome is an innate immune cell sensor activated by ROS, hyperglycemia, lipopolysaccharides, uric acid etc. which initiates the inflammatory response by activating a NOD-like receptor (NLRP3) which promotes, in the fat tissue, the activation of T-cells by mechanisms mediated by macrophages [9, 20]. Nishimura *et al.* concluded that CD8+ T-cells are key actors in the initiation and propagation of inflammation *via* enlarged adipocytes found in the fat tissue. In turn, CD8+ T-cells that are activated by the enlarged adipocytes recruit and activate macrophages in the fat tissue [21]. On the other hand, the obesity-related inflammatory response is linked with a change in the macrophages' phenotype, particularly in the fat tissue located in the viscera. Under the influence of lipopolysaccharides and IFN-γ, these cells release pro-inflammatory cytokines (IL-6, TNF-α) and generate ROS [17]. As previously mentioned, the increased levels of oxidative stress and pro-inflammatory cytokines in obese subjects might also be related to the hypoperfusion and subsequent hypoxia of the adipose tissue due to the progressive enlargement of adipocytes [15]. Thus, one important link in explaining the crosstalk between obesity, oxidative stress and chronic inflammation might be the overexpression of pro-inflammatory cytokines [9].

In obese subjects, the free fatty acid accumulation is responsible for the activation of several serine kinases with a pro-inflammatory activity (c-Jun N-terminal kinase, IκB kinase etc.). As an effect, fat tissue is stimulated to release IL-6 which, in turn, will enhance the production and secretion of CRP (a very sensitive inflammation marker) by liver cells [22]. Previous research has shown that there is a correlation between abdominal fat deposits, the elevated risk of cardiovascular events and CRP levels [9]. Some studies have shown that TNF-α is expressed and secreted by adipocytes and that the adipose body mass might mediate the relationship between chronic inflammation and obesity. Independently of the BMI, abdominal fat is linked to increased values of CRP; on the same hand, also independently of the BMI, increased values of high-sensitivity CRP are linked to visceral obesity [9, 17, 23].

Iron is a transition metal necessary for normal cell growth and proliferation. However, excessive amounts can catalyze the production of toxic ROS *via* the Fenton reaction, inducing oxidative tissue damage. Ferritin is an acute-phase protein whose expression can be up-regulated by inflammatory processes, infections, excessive production of ROS and uncontrolled proliferation of cells [24]. Serum ferritin levels are indicators of the total amount of iron stored in the body and increased serum ferritin concentrations are associated with various metabolic risk factors, including insulin resistance [25].

Uric acid is one of the most important endogenous antioxidants in the plasma, but its effects are controversial, since during generation of uric acid, ROS are produced. Xanthine oxidoreductase inhibition in obesity is associated with vascular alterations, cell differentiation, foam cell formation and insulin resistance [26-28].

Neutrophil-to-lymphocyte ratio (NLR) is an easy to measure laboratory parameter that can reflect the levels of inflammation of a certain subject. Studies have shown that it is a useful prognostic factor of morbidity and mortality in several types of cardiovascular disorders or malignancies, and as a marker of infection, inflammation or postoperative complications [29-30]. According to Forget *et al.*, non-geriatric, healthy adults should have a NLR between 0.78 and 3.53 [31].

It is noteworthy to mention that, in susceptible individuals, including obese subjects, increased levels of oxidative stress and inflammation may contribute to an increased risk of developing solid and haematological cancers [32-34].

Conclusions

In our study, we found decreased levels of antioxidants and increased levels of reactive oxygen species and inflammation markers in obese subjects vs. healthy controls. The culprits and the pathophysiological events leading to obesity-induced chronic inflammation, as well as the relationship between cytokines or inflammation and obesity indices, are still not fully understood. Further studies are needed to investigate the crosstalk between obesity, oxidative stress and inflammation.

References

1.MARSEGLIA, L., MANTI, S., D'ANGELO, G., NICOTERA, A., PARISI, E., DI ROSA, G., GITTO, E., ARRIGO, T., Int. J. Mol. Sci., **16**, nr. 1, 2015, p. 378.
2.CHEN, S.J., YEN, C.H., HUANG, Y.C., LEE, B.J., HSIA, S., LIN, P.T., PLoS One, **7**, nr. 9, 2012, p. e45693.
3.STEPIEN, M., STEPIEN, A., WLAZEL, R.N., PARADOWSKI, M., BANACH, RYSZ, J., Lipids Health Dis., **13**, nr. 29, 2014, p. 1.
4.PANENI, F., CONSTANTINO, S., COSENTINO, F., World J. Diabetes, **6**, nr. 2, 2015, p. 326.
5.VAN DER SCHUEREN, B., VANGOITSENHOVEN, R., GEERAERT, B., DE KEYZER, D., HULSMANS, M., LANNOO, M., HUBER, H.J., MATHIEU, C., HOLVOET, P., Int. J. Obes. (Lond.), **39**, nr. 8, 2015, p. 1254.
6.McMURRAY, F., PATTEN, D.A., HARPER, M.E., Obesity (Silver Spring), **24**, nr. 11, 2016, p. 2301.
7.HOLVOET, P., Verh. K. Acad. Geneeskd. Belg., **70**, nr. 3, 2008, p. 193.
8.MISCHUK, V. G., GRYGORUK, G. V., STUPNYTSKA, H. Y., LEVCHUK, R. D., Arch. Balk. Med. Union, **53**, nr. 3, p. 324.
9.ELLULU, M.S., PATIMAH, I., KHAZA'AI, H., RAHMAT, A., ABED, Y., Arch. Med. Sci., **13**, nr. 4, 2017, p. 851.
10.MANNA, P., JAIN, S.K., Metab. Syndr. Relat. Disord., **13**, nr. 10, 2015, p. 423.
11.KHAN, N.I., NAZ, L., YASMEEN, G., Pak. J. Pharm. Sci., **19**, nr. 1, 2006, p. 62.
12.GAMAN, M. A., EPINGEAC, M. E., GAMAN, A. M., Rev. Chim. (Bucharest), **70**, no. 3, 2019, p. 977.
13.ORTEGA, R.M., RODRIGUEZ-RODRIGUEZ, E., APARICIO, A., JIMENEZ-ORTEGA, A.I., PALMEROS, C., PEREA, J.M., NAVIA, B., LOPEZ-SOBALER, A.M., Int. J. Vitam. Nutr. Res., **82**, nr. 2, 2012, p. 121.
14.WHEATCROFT, S.B., WILLIAMS, I.L., SHAH, A.M., Diabet. Med., **20**, nr. 4, 2003, p. 255.
15.FERNANDEZ-SANCHEZ, A., MADRIGAL-SANTILLAN, E., BAUTISTA, M., ESQUIVEL-SOTO, J., MORALES-GONZALEZ, A., ESQUIVEL-CHIRINO, C., DURANTE-MONTIEL, I., SANCHEZ-RIVERA, G., VALADEZ-VEGA, C., MORALES-GONZALEZ, J. A., Int. J. Mol. Sci., **12**, nr. 5, 2011, p. 3117.
16.GOMES, A. L., TCHEKALAROVA, J. D., ATANASOVA, M., MACHADO, K.D., RIOS, M. A. D., PAZ, M. F. C. J., GAMAN, M. A., GAMAN, A. M., YELE, S., SHILL, M. C., KHAN, I. N., ISLAM, M. A., ALI, E. S., MISHRA, S. K., ISLAM, M. T., MUBARAK, M. S., LOPES, L. D., MELO-CAVALCANTE, A. A. D., Biomed. Pharmacother., **106**, nr. 2018, 2018, p. 1686, DOI: 10.1016/j.biopha.2018.07.121.
17.RODRIGUEZ-HERNANDEZ, H., SIMENTAL-MENDIA, L.E., RODRIGUEZ-RAMIREZ, G., REYES-ROMERO, M.A., Int. J. Endocrinol, **2013**, nr. 2013, 2013, p. 678159.
18.GAMAN, M. A., DOBRICA, E. C., PASCU, E. G., COZMA, M. A., EPINGEAC, M. E., GAMAN, A. M., PANTEA, S. A., BRATU, O. G., DIACONU, C. C., J. Mind Med. Sci., **6**, nr. 1, 2019, p. 157. DOI: 10.22543/7674.61.P157161.
19.FURUKAWA, S., FUJITA, T., SHIMABUKURO, M., IWAKI, M., YAMADA, Y., NAKAJIMA, Y., NAKAYAMA, O., MAKISHIMA, M., MATSUDA, M., SHIMOMURA, I., J. Clin. Invest., **114**, nr. 12, 2004, p. 1752.
20.STIENSTRA, R., TACK, C.J., KANNEGANTI, T.D., JOOSTEN, L.A., NETEA, M.G.,
Cell Metab., **15**, nr. 1, 2012, p. 10.
21.NISHIMURA, S., MANABE, I., NAGASAKI, M., ETO, K., YAMASHITA, H., OHSUGI, M., OTSU, M., HARA, K., UEKI, K., SUGIURA, S., YOSHIMURA, K., KADOWAKI, T., NAGAI, R., Nat. Med., **15**, nr. 8, 2009, p. 914.
22.ROCHA, V.Z., LIBBY, P., Nat. Rev. Cardiol., **6**, nr. 6, 2009, p. 399.
23.LAPICE, E., MAIONE, S., PATTI, L., CIPRIANO, P., RIVELLESE, A.A., RICCARDI, G., VACCARO, O., Diabetes Care, **32**, nr. 9, 2009, p. 1734.
24.BRESGEN, N., EKCL, P.M., Biomolecules, **5**, nr. 2, 2015, p. 808.
25.PADWAL, M.K., MURSHID, M., NIRMALE, P., MELINKERI, R.R., J. Clin. Diagn. Res., **9**, nr. 9, 2015, p. BC11.
26.EPINGEAC, M. E., GAMAN, M. A., DIACONU, C. C., GAD, M., GAMAN, A. M., Rev. Chim. (Bucharest), **70**, no. 6, p. 2241.
27.BATTELLI, M.G., POLITO, L., BOLOGNESI, A, Atherosclerosis, **237**, nr. 2, 2014, p. 562.
28.MOISA, C., GAMAN, M. A., PASCU, E. G., DRAGUSIN, O. C., ASSANI, A. D., EPINGEAC, M. E., GAMAN, A. M., Arch. Balk. Med. Union, **53**, nr. 1, 2018, p. 70.
29.PROCTOR, M.J., MORRISON, D.S., TALWAR, D., BALMER, S.M., FLETCHER, C.D., O'REILLY, D.S., FOULIS, A.K., HORGAN. P.G., MC MILLAN, D.C., Eur. J. Cancer, **47**, nr. 17, 2011, p. 2633.

30.KAHRAMANCA, S., OZGEHAN, G., SEKER, D., GOKCE, E.I., SEKER, G., TUNC, G., KUCUKPINAR, T., KARGICI, H., Ulus. Travma. Acil. Cerrahi. Derg., **20**, nr. 1, 2014, p. 19.
31.FORGET, P., KHALIFA, C., DEFOUR, J.P., LATINNE, D., VAN PEL, M.C., DE KOCK, M., BMC Res. Notes, **10**, nr. 1, 2017, p. 12.
32.MOISA, C., GAMAN, M. A., DIACONU, C.C., ASSANI, A.D., GAMAN, A. M., Arch. Balk. Med. Union, **53**, nr. 4, 2018, p. 529. DOI: 10.31688/ABMU.2018.53.4.07.
33.TOYOKUNI, S., Arch. Biochem. Biophys., **595**, nr. 2016, 2016, p. 46.
34.CANDIDO, J., HAGEMANN, T., J. Clin. Immunol., **33**, nr. Suppl 1, 2013, p. S79

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