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Childhood obesity accelerates biological ageing: is oxidative stress a link?

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Abbreviations: rLTL, relative leukocyte telomere lenght; TOS, total oxidant status; PCA, principal component analysis; BMI, body mass index; OR, odds ratio; Cl, confidence level

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Abstract: Obesity is multifactorial pathophysiological condition with an imbalance in biochemical, immunochemical, redox status and genetic parameters values. We aimed estimate connection between relative leukocyte telomere lengths (rLRL) - biomarker of cellular aging with metabolic and redox status biomarkers values in a group of obese and lean children. The study includes 110 obese and 42 lean children and adolescents, both genders. The results suggested that rLTL are significantly shorter in obese, compared to lean group ($p<0,01$). Negative correlation of rLTL with total oxidant status, TOS (Spearman's $\rho = -0.365$, $p<0.001$) as well as with C reactive protein (Spearman's $\rho= -0,363$, $p<0.001$) were observed. Principal component analysis (PCA) extracted three distinct factors (i.e. principal components) entitled as: prooxidant factor with 35% of total variability; antioxidant factor with 30% of total variability and lipid antioxidant – biological ageing factor with 12% of the total variability. The most important predictor of body mass index (BMI) >30kg/m^2 according to logistic regression analysis was PCA derived antioxidant factor's score (OR: 1.66, 95th Cl: 1.05-2.6, p=0.029). PCA analysis confirmed oxidative stress importance in biological ageing caused by obesity and its multiple consequences related to prooxidants augmentation and antioxidants exhaustion and gave us clear signs of disturbed cellular homeostasis deepness, even before any overt disease occurrence.

Keywords: childhood obesity; oxidative stress; inflammation; relative leukocyte telomere length

1. Introduction

Childhood obesity has become an escalating concern, impacting over 340 million children and adolescents globally, with a prevalence surge from 4% to over 18% in the last four decades[1]. Contributing factors such as dietary habits, sedentary lifestyle, familial predisposition, and socioeconomic elements play pivotal roles in the complex etiology of obesity. The ramifications of childhood obesity extend into adulthood, predisposing individuals to heightened risks of ailments like diabetes and cardiovascular diseases[2]. The hallmark of obesity is the expansion of adipose tissue, a heterogeneous amalgamation of adipocytes, stromal preadipocytes, immune cells, and endothelium[3]. Adipocytes initiate the endocrine secretion of a myriad of cell-signaling molecules adipokines, including adiponectin, leptin, inflammatory chemokines such as tumor necrosis factor-alpha (TNF-alpha), interleukin-6 (IL-6), and angiogenic and vasoactive molecules such as vascular endothelial growth factor (VEGF) and angiotensin II[4]. Elevated concentrations of IL-6 and free fatty acids lead to decreased nitric oxide (NO), impeding vasodilation and promoting the upregulation of adhesion molecules, alongside immune infiltration into vessel walls[4]. Endothelial dysfunction, characterized by increased inflammatory biomarkers like C-reactive protein (CRP)[5], is intimately associated with obesity-related complications. The intricate blood capillary network in adipose tissue, vital for nutrient and oxygen exposure[6], faces disruption due to an excess of vasoactive molecules, resulting in blood vessel constriction. This disruption can potentially compromise macrophage function, impeding the removal of necrotic adipocytes[7]. The development of hypoxia in adipose tissue triggers a vicious cycle, leading to an increase in reactive oxygen species, hypoxia-inducible factor 1-alpha (HIF1-α), and VEGF production[8, 9].

Low-grade inflammation and the generation of reactive oxygen species (ROS) emerge as key factors accelerating cellular aging, as evidenced by telomere length shortening. Telomeres, composed of hexanucleotide sequences (TTAGGG) at chromosome ends, undergo shortening after each cell division, culminating in cellular dysfunction upon reaching a critical point, leading to genomic instability and cell death[10]. Obesity, characterized by systemic low-grade inflammation and ROS production, directly impacts deoxyribonucleic acid (DNA) integrity and telomere length. Importantly, even in pediatric age, adiposity parameters, such as BMI and hip to waist ratio, display a negative association with telomere length[11].

The main objective of this research is to enhance our comprehension of leukocyte telomere shortening length, metabolic, and redox status in children and adolescents who are obese. The study places a particular emphasis on identifying differences in emerging biochemical parameters representing common activity of inflammation and oxidative stress across individuals with varying degrees of obesity. The study aims to provide a more profound understanding and clarification of the metabolic pathways responsible for the onset and advancement of obesity.

2. Experimental methods

This research was conducted at the University Children's Hospital Belgrade between June 2022 and April 2023. The study adhered to the principles outlined in the Declaration of Helsinki and received approval from the Institutional Ethical Board (Ethical Licence No. 16/25, 10.06.2022). The biological samples utilized, including serum/plasma and peripheral blood mononuclear cells (PBMC), were collected using two types of vacutainer blood collection tubes—one with a clot activator and the other with ethylenediaminetetraacetic acid (EDTA) anticoagulant.

To determine the necessary number of study participants, we utilized G*Power software version 3.1.9.4 (Universität Kiel, Germany). The sample size calculation was based on a 2-tailed test with α=0.05 and β=0.2, aiming to reject the null hypothesis. In total, the study involved 107 subjects in the obese group and 42 subjects in the control group, ensuring a calculated power greater than 0.800. The post-hoc power of study was calculated and 0.999 value was obtained. This outcome indicates that the sample size is likely sufficient to detect the specified effect size at the chosen significance level, with only 5% chance of making a Type I error, accepting alternative hypothesis where obese children have shorter rLTL as main parameter of our study[12]. Following a medical examination by a pediatric endocrinologist, the diagnosis of obesity without additional comorbidities was confirmed. The control group consisted of children without chronic diseases, exhibiting adequate height and weight for their age, and demonstrating good health status at the time of blood collection. The patients were recruited during routine health examinations in the University Children's Hospital. Venipuncture was performed during patients' routine medical checkups as prescribed by physicians. Sociodemographic and anthropometric data were collected by physicians subsequent to the signed informed consent obtained from either the children or their parents.

2.1 Measurement of biochemical parameters

The whole blood was centrifuged 10 minutes, at room temperature, at 3500 RPM on Rotofix 32 A type of centrifuge (Andreas Hettich GmbH & Co. KG, Tuttlingen, Germany) to obtain serum or plasma. After completing routine biochemical analyses, serum and plasma were stored at -80°C until analysis. The measured biochemical parameters, sample type, methods and analyzer type are presented in the supplementary Table S1. Basic biochemical parameters were used to calculate the following parameters: De Ritis ratio: aspartate aminotransferase (AST) / alanine aminotransferase (ALT), risk factor for cardiovascular disease (RFCVD: total cholesterol / high-density lipoprotein (HDL) cholesterol), index of atherosclerosis (IA): low-density lipoprotein (LDL) cholesterol / HDL cholesterol, homeostatic model assessment for insulin resistance HOMA-IR: Insulin * Glucose / 22.5 and hepatic steatosis index (HIS): 8 * ALT/AST + BMI.

2.2 The assessment of redox status parameters

The antioxidant activity of the test compounds was evaluated through the measurement of the following parameters: total antioxidant status (TAS), superoxide dismutase activity (SOD) and concentration of total sulfhydryl group (SHG) as protective parameters; total oxidative status (TOS), advanced oxidation protein products (AOPP), ischemia-modified albumin (IMA) and prooxidant-antioxidant balance (PAB) as damaging parameters. The concentrations of all parameters were measured using spectrophotometer analyzer Ilab 300Plus (Instrumentation Laboratory, Milan, Italy), while PAB, SHG and IMA were measured on ELISA reader, SPECTROstar Nano Microplate Reader, at 450 nm (BMG Labtech, Ortenberg, Germany). The measurement of TAS employed Erel's method[13], which entails the oxidation of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) in the presence of hydrogen peroxide, by all reducing substances in blood[14]. SOD activity was determined using the adrenalin method proposed by Misra and Fridovich[15]. The total concentration SHG in serum was determined through Ellman's method[16], involving the reaction of 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) with aliphatic thiol compounds. TOS were determined by modifying of colorimetric method, with oxidation of ferrous ion-o-dianisidine complex to ferric ion, proportional to total oxidants protection present in sample[17]. The concentration of AOPP was determined in reaction of oxidatively changed proteins with glacial acetic acid and potassium iodine[18]. IMA was determined spectrophotometrically, involving the reaction of serum albumin with cobalt chloride, which

unbound remainder reacts with dithiotreitol[19]. PAB values, representing the total prooxidant content remained after the reaction with antioxidants, were determined using a colorimetric method involving the simultaneous reaction of tetramethylbenzidine (TMB) with hydrogen peroxide and uric acid[20].

2.3 Measurement of rLTL

Peripheral Blood Mononuclear Cells (PBMC) were isolated from ethylenediamine tetraacetic acid (EDTA) blood using phosphate-buffered saline (PBS) and centrifugation with a Ficcol-Paque gel separator. After purification and removal of erythrocytes with lysis buffer, the PBMCs were stored at -80°C. DNA isolation was performed using the FlexiGene DNA kit, involving centrifugation, addition of FG2/protease mixture, vortexing, incubation, isopropanol introduction, centrifugation, ethanol rinsing, and air-drying of the DNA pellet. The final step included dissolving DNA in FG3 buffer with a 30-minute incubation at 65°C[21]. Leukocyte telomere length (LTL) was determined using Real-time polymerase chain reaction (PCR) with SYBR Green, using the stable albumin gene as a control. Results were presented as a ratio of the target gene's cDNA amount to the albumin gene's cDNA amount, chosen for its unaffected expression by physiological and pathological factors[22].

2.4 Statistical analysis

Data distribution was evaluated using Kolmogorov-Smirnov or Shapiro-Wilk tests, as deemed appropriate. Due to the predominantly non-normal distribution of most parameters, results for all variables are reported as medians with 25th and 75th percentile values. Inter-group comparisons utilized the nonparametric Mann-Whitney U test for two independent samples for all parameters, while the nonparametric Kruskal-Wallis test was employed for comparisons involving more than two groups. The correlation between variables was examined using Spearman's test. To streamline the analysis and reduce the number of variables under examination, PCA with varimax rotation was implemented. Factor extraction was determined based on eigenvalues greater than 1, with variables exhibiting factor loadings below 0.5 excluded from the analysis. Scores were computed for three factors identified by PCA, and these factors underwent subsequent binary logistic regression analysis to predict severe obesity (BMI >30 kg/m2). Statistical significance for all analyses was set at $p<0.05$. The statistical software package used for data analysis was SPSS for Windows 18.0 (SPSS, INC., Chicago, Illinois, USA).

3. Results

All biochemical parameters, concentrations and enzyme activities of the control group and obese children were measured in serum, and results are presented in Table 1.

Serum lipids were significantly higher in obese than in control group (except HDL-cholesterol), as well as the activity of ALT and AST enzymes. Glucose concentration and glycohemoglobin (HbA_{1c}) levels were also significantly higher in obese children, followed by increased insulin concentration. CRP, as an indicator of low-grade inflammation, was considerably elevated in the group of obese children compared to the control.

Calculated indexes, De Ritis ratio, RFCVD, IA and HOMA-IR, represent a better insight into the metabolic state and cardiovascular risk of the patients since their values depend on several different biochemical parameters. These indexes are schematically presented in Figure 1.

The concentrations of redox status parameters in obese children and adolescents and the control group are presented in Table 2.

Antioxidant parameters, specifically SOD and SHG, exhibited notably lower levels in obese children compared to the control group, while TAS demonstrated a significant increase in obese children. Conversely, all measured prooxidants showed significant elevation in obese children compared to the control group.

rLTL in obese and lean children, stratified by gender, is depicted in Figure 2. rLTL varied significantly between obese and lean children and adolescents (0.650 (0.451 - 0.980) vs 1.600 (1.425 - 1.776), $p<0.001$), with a noticeable gender-related difference. The obese group exhibited significantly shorter rLTL in both boys and girls compared to the gender-matched control group. There was no significant gender-based difference in rLTL within the obese or control groups. Spearman's correlation analysis between rLTL and children's age did not yield statistical significance (Spearman's $\rho = 0.210$, $p>0.05$).

To explore the relationship between rLTL and redox status parameters measured in this study, obese patients were categorized based on rLTL tertile values (Table 3). Results revealed significantly lower AOPP concentrations in the third tertile of the obese children and adolescents group compared to the second tertile group ($p < 0.01$). TAS levels in the group with the longest rLTL were higher compared to both the first and second tertile groups of obese children and adolescents ($p < 0.05$). Unexpectedly, the highest PAB values were

observed in the group with the longest rLTL (Table 3). Other measured biochemical parameters or calculated indices did not differ in rLTL sub-groups (data not shown).

Principal component analysis was applied to redox status parameters and rLTL. Sampling and model adequacy was confirmed by the Kaiser-Meyer-Olkin index (0.763) and Bartlett index of sphericity ($p<0.001$), respectively. Three extracted factors explained 77% of the variance in a group of examined combinations of parameters. The first factor ("Prooxidant factor") explained 35% of the total variance and was associated with positive loadings of TOS, AOPP, IMA and PAB. The second factor ("Antioxidant factor") explained 30% of the total variance and contained positive loadings of total sulphydryl groups and SOD, but negative loadings of TAS. The third factor ("Lipid antioxidant-biological ageing factor") explained 12% of the variance and included positive loadings of both PON1 and rLTL.

In order to test possible predictive capabilities of PCA-selected factors regarding obesity status, binary logistic regression analysis was applied with scores produced in the primary analysis, with BMI >30kg/m^2 as a state variable. Results presented in Table 4 revealed that the antioxidant factor, which compiled SOD, SHG and TAS, had the highest predictive potency towards BMI>30 kg/m^2, while the prooxidant factor was a slightly weaker obesity status predictor.

4. Discussion

Obesity is characterized by disruptions in metabolic pathways, particularly those involving carbohydrates and fats. This study further explored the connection between obesity and the production of pro-inflammatory markers, which play a crucial role in the progression of oxidative stress (OS). Within this system, telomeres, essential components of DNA molecules, are vulnerable to direct impact from free radicals and pro-inflammatory molecules, leading to their shortening.

Obesity, both in adults and in children, is manifested by an increased insulin concentration and the presence of insulin resistance, which is reflected in this study (Figure 1). The mechanism of obesity development is strongly associated with development of insulin resistance. Dephosphorylating and deactivating multiple mitogen-activated protein kinase (MAPKs) is crucial in resistance development[8], but also an increased inhibitory serine phosphorylation of the insulin receptor or its substrates, can further promote the emergence of resistance[14]. While the result from a study performed by Bacha et al.[23] showed that obese children with a similar BMI can differ on the basis of the degree of insulin resistance, we

found a strong positive correlation between BMI and insulin concentration (Supplementary Table S2). Similar result were showed in paper of Martinović et all, in a group of Montenegrin children[24]. Excessive ingestion of nutrients activates Toll-like receptors (TLR) and the receptor for advanced glycation end-products (RAGE). TLR activation triggers the production of inflammatory cytokines and activates transcription factors nuclear factor kappa B (NF-κB) and activator protein 1 (AP-1). Continuous activation of these pathways play a crucial role in the development of metabolic inflammation[25]. Our results confirmed mild inflamation by a moderately elevated concentration of CRP. Accordingly, metabolic inflammation persistence leads to a redox status disturbance and the onset of overt oxidative stress.

The results obtained in our study suggested that patients have metabolic disorders, perceived through the elevated insulin, lipid parameters and central obesity, compared to lean children. Also, even if it was a group of generally healthy children, we noticed sub-clinical liver function impairment, with fat accumulation in the liver, evidenced by the presence of a HIS and HOMA-IR positive correlation. A strong negative correlation between BMI and De Ritis ratio (Supplementary Table S2), indicates possible risk of non-alcoholic fatty liver disease (NAFLD) development later in life[26, 27]. The mechanism of impaired liver function is reflected in increased *in vivo* fatty acids synthesis from excessive intake of carbohydrates in prolonged period. In this study, fasting glucose concentration of obese children was higher than in control children, although still in normal fasting reference range, which is comparable to the results of previous investigations, indicating normoglycemia in obese, otherwise healthy children and adolescents[28, 29]. Significantly increased HbA_1c levels of obese compared to lean children, even all values in the reference range, is an alarm considering that obese children's HbA1c median value equals 75^{th} percentile value of lean children. This suggests that glucose concentration persistently moves towards higher values, mostly due to deleterious effects of hyperglycemia in prolonged period of time. It is well-known that obesity increases the risk of having metabolic syndrome and cardiovascular diseases in later life, and since obese children will probably remain obese as adults, it is very important to keep the lipid status under control. A study conducted by Friedland[30] showed that obese children had lower HDL-cholesterol concentrations compared to control group, while total cholesterol, LDL-cholesterol and triglycerides concentrations were elevated compared to control group. Our results proved that obese children already have impaired homeostasis of lipid parameters. Additionally, we found increased values of RFCVD and IA in obese group.

This parameters indicates the impaired lipid homeostasis and the existence of a risk for cardiovascular disease occurrence in adulthood[31]. Our study showed a modest increase in triglycerides concentration, which is still significantly higher than in control group. This finding differed from the results of Brzeziński et al., who indicated a higher triglycerides concentration than in our study, without significant difference compared to the control group[32]. Consistent low-grade inflammation, as one of the main obesity characteristics, is influenced by the activation of the innate immune system in adipose tissue, which promotes pro-inflammatory state and oxidative stress[33]. Among the most important enzymatic antioxidants are SOD, glutathione peroxidase, and catalase[34]. As part of this study, we determined the activity of SOD enzyme and concentrations of TAS and SHG as non-enzymatic representatives. Total SOD activity measured in subject's serum is presented in the largest part by extracellular SOD isoform, which has Cu2+ and Zn2+ microelements in the active site. Obese children's total SOD activity was significantly lower compared to lean children ($p<0.001$, Table 2). Ozata and colleagues observed a significantly lower zinc concentration in obese individuals compared to their healthy counterparts[35]. It is well-established that maintaining a normal zinc concentration is crucial for the proper activity of SOD enzyme, as we demonstrated through the decreased values of SOD[36]. Recent research has indicated that zinc deficiency may compromise the functioning of numerous enzymes, potentially leading to the development of obesity. Furthermore, zinc deficiency is implicated in contributing to leptin resistance, a hormone with the potential to inhibit eating behaviors, primarily through an elevated production of neuropeptide Y in the hypothalamus[36]. Beside this mechanism, optimal zinc concentrations are important for adequate insulin activity, preventing development of insulin resistance[35]. Increase in TAS in obese children could potentially be attributed to concurrent hyperuricemia in comparison to their lean counterparts. Hyperinsulinemia or insulin resistance, present in obesity, may contribute to a disruption in the glycolytic pathway, resulting in the accumulation of ribose-5-phosphate, a significant substrate for uric acid production. Furthermore, the elevated levels of uric acid (UA) in obese individuals are likely linked to the compromised excretion of UA from the renal distal tubules, a consequence of UA absorption at the proximal tubular region[37]. Research findings have illustrated that increased leptin levels in obesity can also exacerbate the development of hyperuricemia[38, 39]. A comparable scenario is anticipated during the determination of PAB, given that uric acid represents the antioxidant component in the assessment of PAB[40]. The results presented in Table 2, regarding TOS, AOPP and IMA, as prooxidants measured in this

study, unequivocally indicate that oxidative stress is an indispensable obesity companion, which burden is a consequence of obesity *per se*, but its long-term presence, at the same time, could be a cause of future metabolic disturbances[41, 42]. Low-grade inflammation and altered production of free radicals make insulin resistance even more pronounced due to the fact that pancreatic β-cells are especially vulnerable to oxidative stress. Our study showed metabolic triad existence: insulin resistance, increased BMI values and disturbed redox balance. The importance of the glutathione system and the total sulfhydryl groups' concentration has been well documented in redox homeostasis maintenance, as well as in the case of ample oxidant species production, like in obesity[43]. Ucar et al.[44] showed that glutathione system imbalance with decreased liver glutathione concentration and simultaneous low SOD and glutathione transferase activity can cause liver damage. Our results showed that obese children had significantly lower SHG concentrations compared to the control group. An important parameter that indicates the degree of functional liver damage and disease progression is IMA, which we found significantly increased in obese children. IMA manifests an albumin binding disorder, although liver could preserve normal synthetic capacity, and albumin is in the reference range. The impaired binding of albumin can be one of the early signs of impaired liver function, making this parameter an important early biomarker[45]. The second protein product of oxidative stress influence, AOPP, is considered a reliable marker for protein damage estimation, primarily assayed in renal disease patients[18]. Previous research indicates that obesity leads to regulatory proteins' oxidation by free radicals involved in the expression and synthesis of insulin receptors[46]. We have found significantly higher AOPP concentration in obese children compared to lean ones, with a significant correlation between this oxidative damage biomarker and insulin resistance degree, and with BMI.

Telomere length is known to be one of the indicators of cellular ageing. Telomeres protect the genome from nucleolytic degradation and unnecessary recombination. Telomere length is the largest at birth, and many pathophysiological processes and diseases can lead to its accelerated shortening[47]. Further research has shown that accelerated telomere shortening is associated with the onset of a large number of conditions, such as cardiovascular disease, diabetes mellitus, cancer and obesity[48]. Studies done on obese adult subjects showed a significant shortening of the LTL compared to its age and sex-matched regular-weight counterparts[49]. Many studies confirmed telomere shortening due to obesity, even in a pediatric population, which is also found in our investigation[50, 51]. There is a great inter-individual variation in LTL, with gender differences confirmed in some studies, according to

which females have 0.1 – 0.3 kb longer telomeres than males[52]. Conversely, our results proved that obesity leads equally to the shortening of the telomere length, regardless of gender. The low-grade inflammation persistence, a well-known obesity feature, leads directly to the shortening of LTL, affecting DNK integrity[53]. Obesity promotes the formation of reactive oxygen species and cytokines which could ignite inflammation and cell ageing, representing a vicious circle of mutually potentiated events, wich we proved through the strong negative correlation between CRP concentration and rLTL. A strong negative correlation between TOS and rLTL (Supplementary Table S4) in obese children implicates oxidative stress role in telomere shortening.

A large number of parameters determined as part of this study indicate explicit relation between obesity and the disturbed balance of biochemical and redox status parameters, associated with shortened TL. In order to better understand how specific groups of parameters are associated with obesity, we applied principal component analysis. In a group of obese subjects, redox status parameters and rLTL explained 77% of the total variation, and extracted factors were as follows: prooxidant factor (35% of total variability), antioxidant factor (20% of total variability) and finally, PON1 and rLTL as Lipid antioxidant – biological ageing factor (12% of the total variability). A similar approach to data for a better understanding of the origin and development of the pathophysiological process in a group of acute myocardial infarction patients is presented in the study by Vuković Dejanović et al[54]. It has been shown in an animal model that paraoxonase 1 (PON1) and rLTL are both affected by lipid peroxidation[55] which was also evident in our group of obese patients. Prooxidant biomarkers dominance in obese subjects (co-opting 35% of explainable variation) is expectable and confirms oxidative stress involvement in obesity-related comorbidities[48]. Antioxidant factor explained a smaller percent of variability in obese children, but after scoring performed in PCA this factor showed the most significant predictive capability for BMI>30kg/m^2, which is the value near the II class of obesity. Negative β value in binary logistic regression analysis of this antioxidant factor speaks in favor of exhausted antioxidant protection in obese subjects caused by its constant challenge by reactive oxidant species and/or its pathophysiological diminishing or dysfunction as characteristics of increased adiposity in the prolonged time frame.

5. Conclusion

This study underscores that pediatric obesity is associated with telomere shortening and heightened oxidative stress, accounting for significant percent of the total variability in the prooxidant factor, as identified through PCA and logistic regression. Obesity, being a complex and multifactorial condition, disrupts a spectrum of biomarkers, emphasizing the imperative for interventions such as dietary regulation, insulin resistance therapy, and heightened physical activity. Subsequent phases of this research will entail evaluating alterations in biomarker concentrations post-intervention to gauge the effectiveness of these interventions in ameliorating the overall health and biological aging of the patients.

Supplementary Materials: Table S1; Table S2; Table S3: Table S4.

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Table 1. Socio-demographic, anthropometric and biochemical parameters of control and obese children and adolescents.

Parameter	**Control group** n= 42	**Obese group** n=107	**p**
Age (years)	14.0 (7.7 - 15.2)	12.5 (8.7 - 17.0)	0.041
Gender (male/female)	22/20	51/56	> 0.05
BMI (kg/m^2)	17.4 (15.7 - 20.6)	31.8 (28.1 - 32.9)	< 0.001
Waist circumference (cm)	74.1 (67.2 - 80.3)	108.5 (95.7 - 118.7)	0.011
Hip circumference (cm)	90.3 (79.9 - 100.3)	112 (91.5 - 123.1)	0.009
Glucose (mmol/L)	4.2 (3.9 - 4.7)	5.5 (5.1 - 5.6)	< 0.001
Total cholesterol (mmol/L)	3.90 (3.62 - 4.70)	4.50 (3.78 - 5.03)	0.045
HDL cholesterol (mmol/L)	1.60 (1.23 - 1.95)	1.27 (1.04 - 1.56)	0.002
LDL cholesterol (mmol/L)	1.90 (1.60 - 2.75)	2.58 (2.33 - 3.12)	0.005
TGL (mmol/L)	0.66 (0.47 - 0.90)	0.90 (0.51 - 1.02)	0.002
Albumin (g/L)	40 (36 - 42)	39 (34 - 43)	0.050
ALT (U/L)	36 (30 - 40)	44 (31 - 59)	0.003
AST (U/L)	23 (18 - 30)	28 (25 - 32)	0.030
CRP (mg/L)	1.1 (0.6 - 1.6)	5.8 (1.6 - 9.4)	< 0.001
Insulin (μIU/mL)	9.7 (8.0 - 10.6)	19.3 (14.5 - 26.3)	< 0.001
HbA_{1c} (%)	5.0 (4.9 - 5.3)	5.3 (5.1 - 5.4)	0.011
HIS	27.5 (26.8 - 28.9)	48.9 (42.7 - 53.8)	< 0.001

Data were analyzed by nonparametric Mann-Whitney U test. Abbreviations: HDL, high-density lipoprotein ; LDL, low-density lipoprotein ; TGL, triglycerides; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CRP, C reactive protein; HIS, hepatic steatosis index;

Table 2. Concentrations of redox status parameters in control and obese group.

Parameter	**Control group**	**Obese group**	**p**
TAS (mmol/L)	568 (280 - 778)	715 (648 - 766)	<0.001
SOD (U/L)	141 (138 - 144)	92 (80 - 110)	<0.001
SHG (mmol/L)	0.550 (0.443 - 0.721)	0.312 (0.241 - 0.386)	<0.001
TOS (mmol/L)	67 (54 - 82)	100 (77 - 107)	0.005
AOPP (μmol/L)	49.6 (44.7 - 52.8)	77.6 (61.4 - 92.3)	<0.001
IMA	0.154 (0.134 - 0.176)	0.723 (0.574 - 0.830)	<0.001
PAB (HK)	109 (93 - 120)	147 (140 - 159)	<0.001

Data were analyzed by nonparametric Mann-Whitney U test. Abbreviations: TAS, total antioxidant status; SOD - superoxide dismutase; SHG, total concentration of sulfhydryl group; TOS, total oxidative status; AOPP, advanced oxidation protein products; IMA, ischemia-modified albumin; PAB, prooxidant-antioxidant balance.

Table 3. Redox status parameters according to telomere tertile sub-groups in obese children and adolescents.

Parameter	**rLTL first tertile 0.413 (0.313 - 0.445)**	**rLTL second tertile 0.683 (0.610 - 0.738)**	**rLTL third tertile 1.10 (0.958 - 1.205)**	**P**
AOPP	68.2 (51.3 - 85.8)	68.9 (57.9 - 81.1)	50.3 (45.9 - 64.6)[bb]	0.023
PAB	99 (91-107)	104 (78-119)	115 (97 – 122)[a]	0.050
TAS	970 (843 - 1065)	958 (830 - 1077)	1046 (1009 - 1120)[a,b]	0.048

Data were analyzed by nonparametric Mann-Whitney U test. One letter in superscript: $p<0.05$; two letters in superscript: $p<0.01$; a - difference between first tertile rLTL; b - difference between second tertile rLTL.

Table 4. PCA extracted factors among redox status parameters and rLTL and subsequent univariant binary logistic regression analysis of PCA extracted factors for obesity status (BMI >30 kg/m^2). Abbreviations: B, unstandardized regression weight; SE, variation of unstandardized regression weight; OR , odds ratio; Cl, confidence interval.

Factorial analysis		**Univariant binary logistic regression analysis**			
Factor	**Variables with Loadings**	**B (SE)**	**Wald coefficient**	**OR (95% CI)**	**P**
Prooxidant factor (35%)	TOS 0.954 AOPP 0.849 IMA 0.847 PAB 0.649	0.504 (0.231)	4.8	1.66 (1.05-2.60)	0.029
Antioxidant factor (30%)	SHG 0.871 TAS -0.845 SOD 0.827	-0.748 (0.225)	0.937	0.47 (0.29-0.78)	0.003
Lipid antioxidant-biological ageing factor (12%)	PON1 0.729 rLTL 0.570	-0.076 (0.213)	0.126	0.93 (0.61-1.41)	0.927

Abbreviations: B, unstandardized regression weight; SE, variation of unstandardized regression weight; OR , odds ratio; Cl, confidence interval.

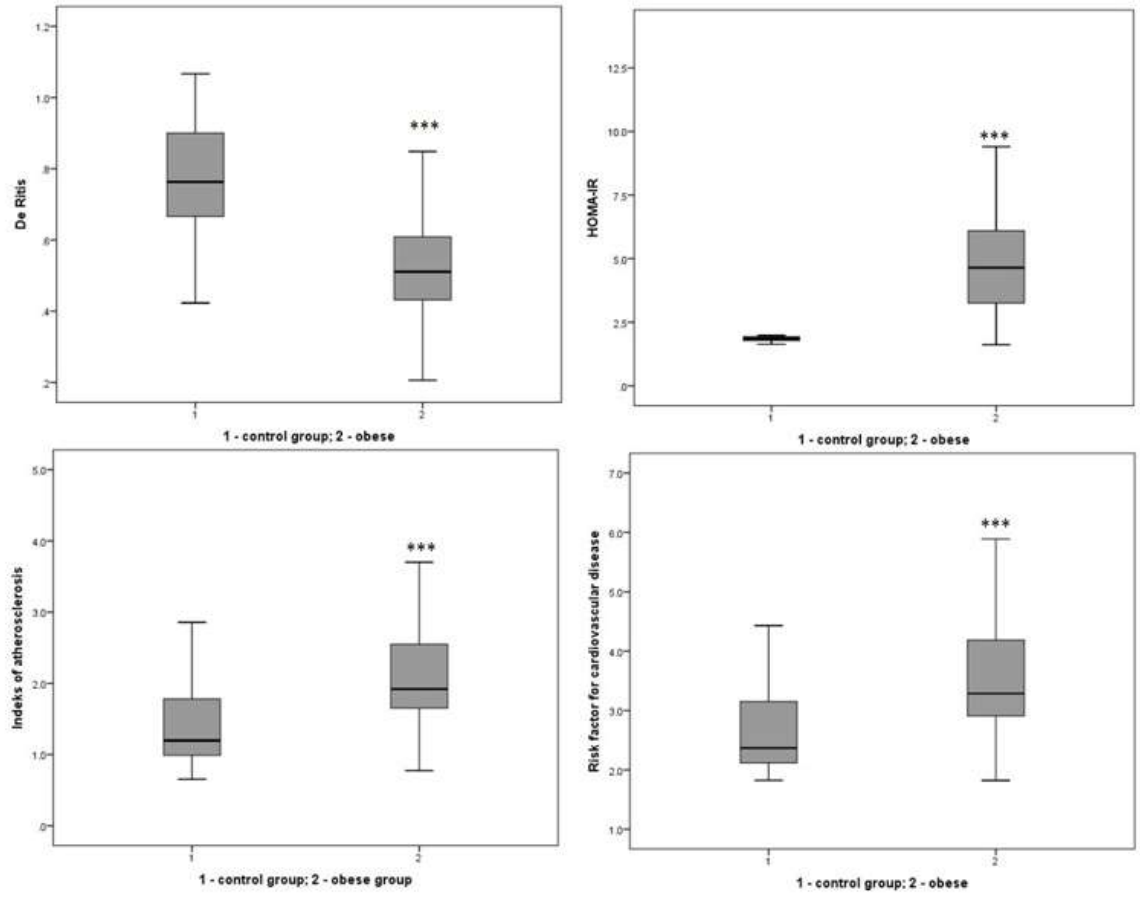

Figure 1. De Ritis, HOMA-IR, risk factor for cardiovascular diseases an index of atherosclerosis values in obese children and adolescents and control group. Data were analyzed by nonparametric Mann-Whitney U test. Abbreviations: ***, statistically significant difference between control and obese group, $p<0.001$

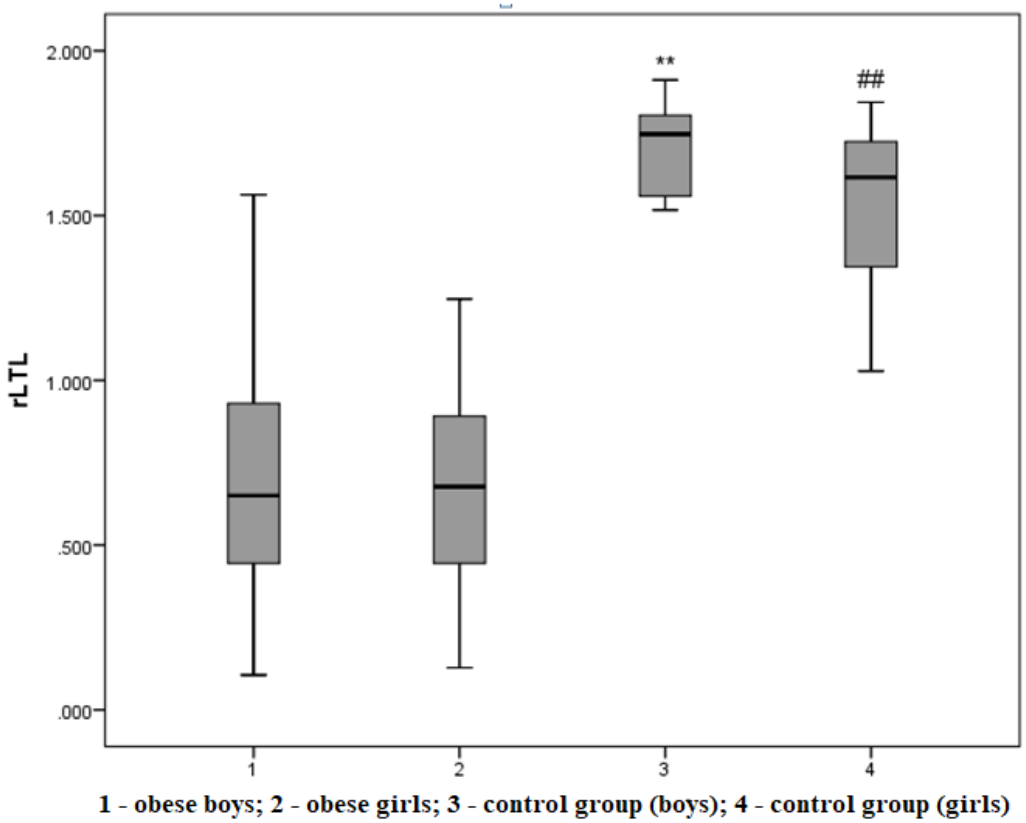

Figure 2. rLTL in obese and control (lean) children and adolescents divided by gender. Data were analyzed by nonparametric Mann-Whitney U test. Abbreviations: **, statistically significant difference in rLTL between obese and lean boys, $p<0.01$; ## - statistically significant difference in rLTL between obese and lean girls, $p<0.01$