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

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Abstract

Obesity is a multifactorial pathophysiological condition with an imbalance in biochemical, immunochemical, redox status and genetic parameters values. We aimed to estimate the connection between relative leucocyte telomere lengths (rLTL) – biomarker of cellular ageing 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 sexes. The results suggested that rLTL are significantly shorter in obese, compared with lean group ($P < 0{\cdot}01$). Negative correlation of rLTL with total oxidant status (TOS) (Spearman's $\rho = -0{\cdot}365$, $P < 0{\cdot}001$) as well as with C-reactive protein (Spearman's $\rho = -0{\cdot}363$, $P < 0{\cdot}001$) 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 BMI > 30 kg/m^2 according to logistic regression analysis was PCA-derived antioxidant factor's score (OR: 1·66, 95th CI 1·05–2·6, $P = 0{\cdot}029$). PCA analysis confirmed that oxidative stress importance in biological ageing is caused by obesity and its multiple consequences related to prooxidants augmentation and antioxidants exhaustion and gave us clear signs of disturbed cellular homoeostasis deepness, even before any overt disease occurrence.

Keywords: **Childhood obesity: Oxidative stress: Inflammation: Relative leucocyte telomere length**

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 socio-economic elements play pivotal roles in the complex aetiology of obesity. The ramifications of childhood obesity extend into adulthood, predisposing individuals to heightened risks of ailments like diabetes and CVD[2]. The hallmark of obesity is the expansion of adipose tissue, a heterogeneous amalgamation of adipocytes, stromal pre-adipocytes, immune cells and endothelium[3]. Adipocytes initiate the endocrine secretion of a myriad of cell signalling molecules adipokines, including adiponectin, leptin, inflammatory chemokines such as TNF-α, IL-6, and angiogenic and vasoactive molecules such as vascular endothelial growth factor and angiotensin II[4]. Elevated concentrations of IL-6 and free fatty acids lead to decreased nitric oxide, impeding vasodilation and promoting the up-regulation of adhesion molecules, alongside immune infiltration into vessel walls[4]. Endothelial dysfunction, characterised by increased inflammatory biomarkers like C-reactive protein[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

Abbreviations: AOPP, advanced oxidation protein products; IMA, ischaemia-modified albumin; LTL, leucocyte telomere length; PAB, prooxidant–antioxidant balance; PCA, principal component analysis; rLTL, relative leucocyte telomere length; ROS, reactive oxygen species; SHG, sulfhydryl group; SOD, superoxide dismutase activity; TAS, total antioxidant status; TOS, total oxidant status; UA, uric acid.

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reactive oxygen species (ROS), hypoxia-inducible factor 1-α and vascular endothelial growth factor production[8,9].

Low-grade inflammation and the generation of ROS emerge as key factors accelerating cellular ageing, 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, characterised by systemic low-grade inflammation and ROS production, directly impacts DNA integrity and telomere length. Importantly, even in paediatric 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 leucocyte 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 (OS) 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.

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 utilised, including serum/plasma and peripheral blood mononuclear cells, were collected using two types of vacutainer blood collection tubes – one with a clot activator and the other with EDTA anticoagulant.

To determine the necessary number of study participants, we utilised G*Power software version 3.1.9.4 (Universität Kiel, Germany). The sample size calculation was based on a two-tailed test with $\alpha = 0{\cdot}05$ and $\beta = 0{\cdot}2$, aiming to reject the null hypothesis. In total, the study involved 107 subjects in the obese group and forty-two subjects in the control group, ensuring a calculated power greater than 0·800. The *post hoc* power of the 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 the alternative hypothesis where obese children have shorter relative leucocyte telomere length (rLTL) as main parameter of our study[12]. Following a medical examination by a paediatric endocrinologist, the diagnosis of obesity without additional co-morbidities 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 at the University Children's Hospital. Venepuncture 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.

Measurement of biochemical parameters

The whole blood was centrifuged 10 min, at room temperature, at 3500 RPM on Rotofix 32 A type of centrifuge (Andreas Hettich GmbH & Co. KG) 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 analyser type are presented in online 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 CVD (RFCVD: total cholesterol/HDL-cholesterol), index of atherosclerosis (IA): LDL-cholesterol/HDL-cholesterol, homoeostatic model assessment for insulin resistance (HOMA-IR): Insulin × Glucose/22·5 and hepatic steatosis index (HIS): 8 × ALT/AST + BMI.

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 oxidant status (TOS), advanced oxidation protein products (AOPP), ischaemia-modified albumin (IMA) and prooxidant–antioxidant balance (PAB) as damaging parameters. The concentrations of all parameters were measured using spectrophotometer analyser Ilab 300Plus (Instrumentation Laboratory), while PAB, SHG and IMA were measured on ELISA reader, SPECTROstar Nano Microplate Reader, at 450 nm (BMG Labtech). The measurement of TAS employed Erel's method[13], which entails the oxidation of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) 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 of SHG in serum was determined through Ellman's method[16], involving the reaction of 5,5′-dithiobis-(2-nitrobenzoic acid) with aliphatic thiol compounds. TOS were determined by modifying the 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 the 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 with hydrogen peroxide and uric acid (UA)[20].

Measurement of relative leucocyte telomere length

Peripheral blood mononuclear cells were isolated from EDTA blood using PBS and centrifugation with a Ficcol-Paque gel separator. After purification and removal of erythrocytes with

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lysis buffer, the peripheral blood mononuclear cells were stored at −80°C. DNA isolation was performed using the FlexiGene DNA kit, involving centrifugation, the 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-min incubation at 65°C[21]. Leucocyte telomere length (LTL) was determined using real-time 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].

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 utilised the non-parametric Mann–Whitney *U* test for two independent samples for all parameters, while the non-parametric 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, principal component analysis (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 $P < 0{\cdot}05$. The statistical software package used for data analysis was SPSS for Windows 18.0 (SPSS, Inc.).

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 the 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. C-reactive protein, as an indicator of low-grade inflammation, was considerably elevated in the group of obese children compared with 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 Fig. 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 with the control group, while TAS demonstrated a significant increase in obese children. Conversely, all measured prooxidants showed significant elevation in obese children compared with the control group.

rLTL in obese and lean children, stratified by sex, is depicted in Fig. 2. rLTL varied significantly between obese and lean children and adolescents (0·650 (0·451–0·980) *v.* 1·600 (1·425–1·776), $P < 0{\cdot}001$), with a noticeable sex-related difference. The obese group exhibited significantly shorter rLTL in both boys and girls compared with the gender-matched control group. There was no significant sex-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 $\rho = 0{\cdot}210$, $P > 0{\cdot}05$).

To explore the relationship between rLTL and redox status parameters measured in this study, obese patients were categorised 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 with the second tertile group ($P < 0{\cdot}01$). TAS levels in the group with the longest rLTL were higher compared with both the first and second tertile groups of obese children and adolescents ($P < 0{\cdot}05$). 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 subgroups (data not shown).

PCA was applied to redox status parameters and rLTL. Sampling and model adequacy were confirmed by the Kaiser–Meyer–Olkin index (0·763) and Bartlett index of sphericity ($P < 0{\cdot}001$), 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 paraoxonase 1 (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 > 30 kg/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.

Discussion

Obesity is characterised 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

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Table 1. Sociodemographic, anthropometric and biochemical parameters of control and obese children and adolescents

	Control group (*n* 42)		Obese group (*n* 107)		
Parameter	Median	25th–75th percentile	Median	25th–75th percentile	*P*
Age (years)	14·0	7·7–15·2	12·5	8·7–17·0	0·041
Sex (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
TAG (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

ALT, alanine aminotransferase; AST, aspartate aminotransferase; CRP, C-reactive protein; HIS, hepatic steatosis index.
Data were analysed by non-parametric Mann–Whitney *U* test.

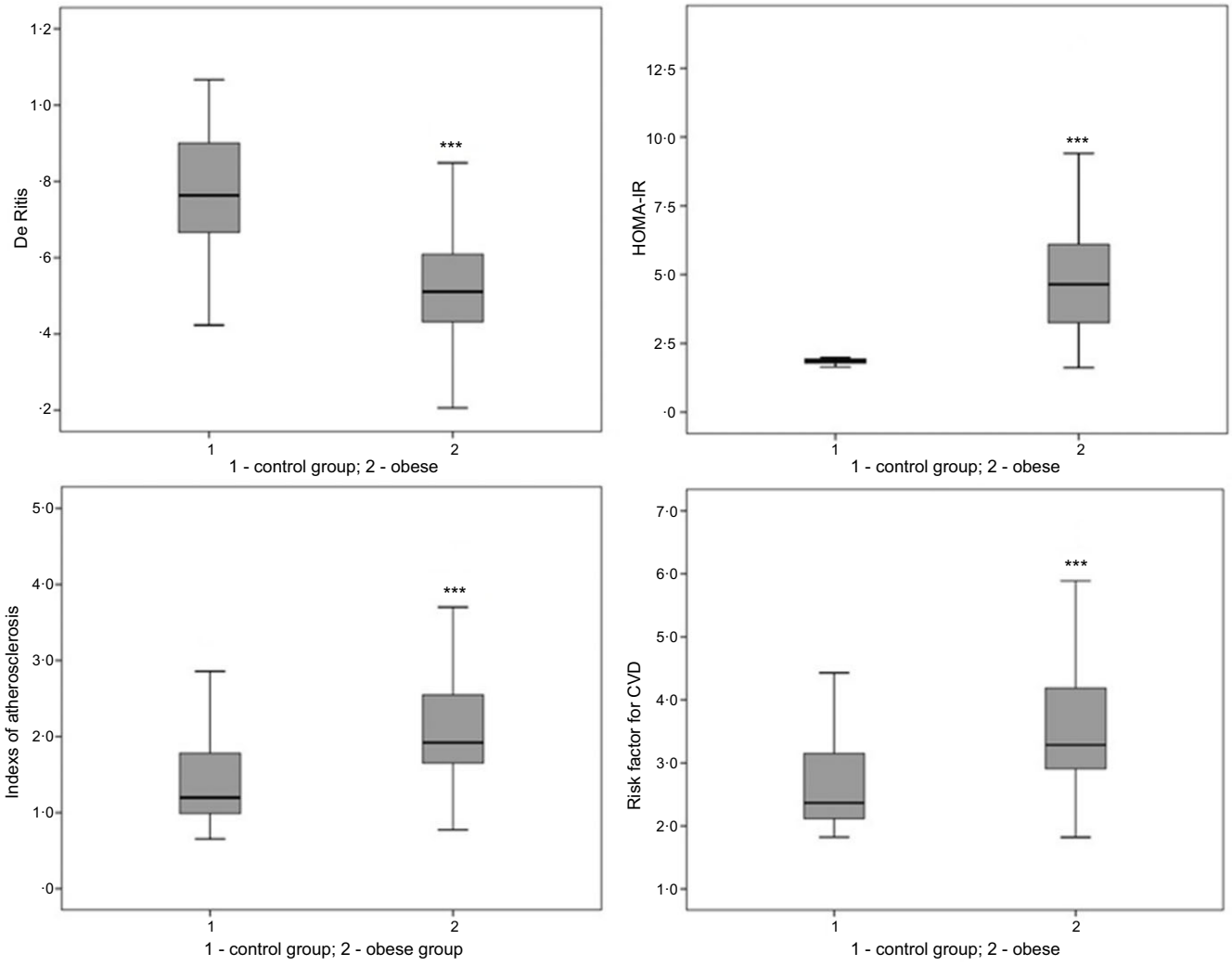

Fig. 1. De Ritis, HOMA-IR, risk factor for CVD, and index of atherosclerosis values in obese children and adolescents and control group. Data were analysed by non-parametric Mann–Whitney *U* test. ***Statistically significant difference between control and obese group, $P < 0{\cdot}001$. HOMA-IR, homoeostatic model assessment for insulin resistance.

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

Parameter	Control group		Obese group		*P*
	Median	25th–75th percentile	Median	25th–75th percentile	
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

TAS, total antioxidant status; SOD, superoxide dismutase; SHG, total concentration of sulfhydryl group; TOS, total oxidant status; AOPP, advanced oxidation protein products; IMA, ischaemia-modified albumin; PAB, prooxidant–antioxidant balance.
Data were analysed by non-parametric Mann–Whitney *U* test.

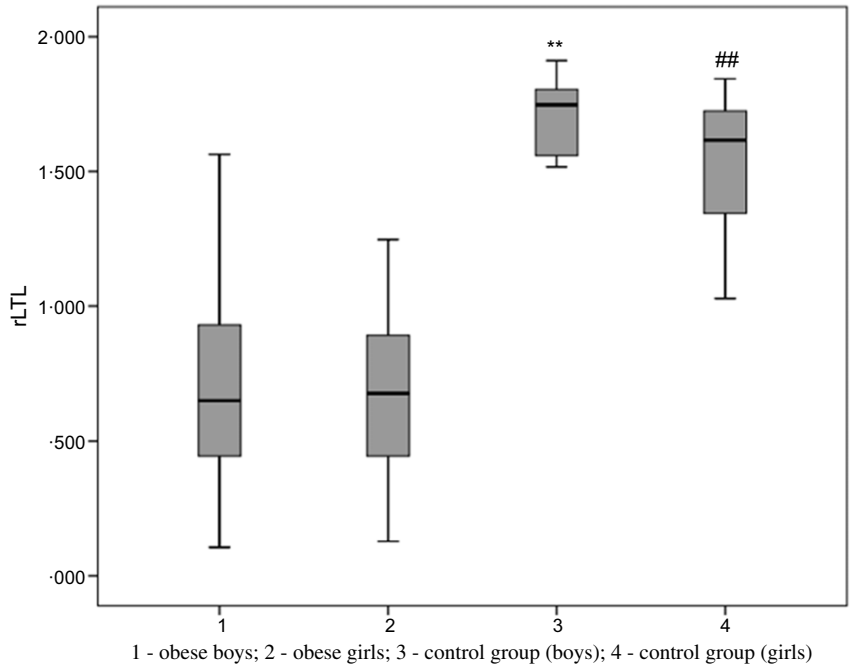

Fig. 2. rLTL in obese and control (lean) children and adolescents divided by sex. Data were analysed by non-parametric Mann–Whitney *U* test. ** – statistically significant difference in rLTL between obese and lean boys, $P < 0{\cdot}01$; ## – statistically significant difference in rLTL between obese and lean girls, $P < 0{\cdot}01$. rLTL, relative leucocyte telomere length.

role in the progression of 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 (Fig. 1). The mechanism of obesity development is strongly associated with the development of insulin resistance. Dephosphorylating and deactivating multiple mitogen-activated protein kinase 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 (online Supplementary Table S2). Similar results were showed in the 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. Toll-like receptor activation triggers the production of inflammatory cytokines and activates transcription factors NF-κB and activator protein 1. Continuous activation of these pathways play a crucial role in the development of metabolic inflammation[25]. Our results confirmed mild inflammation by a moderately elevated concentration of C-reactive protein. Accordingly, metabolic inflammation persistence leads to a redox status disturbance and the onset of overt OS.

The results obtained in our study suggested that patients have metabolic disorders, perceived through the elevated insulin, lipid parameters and central obesity, compared with lean children.

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Table 3. Redox status parameters according to telomere tertile subgroups in obese children and adolescents

	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)		
Parameter	Median	25th–75th percentile	Median	25th–75th percentile	Median	25th–75th percentile	*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

rLTL, relative leucocyte telomere length; AOPP, advanced oxidation protein products; PAB, prooxidant–antioxidant balance; TAS, total antioxidant status.
Data were analysed by non-parametric 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.

Also, even if it was a group of generally healthy children, we noticed subclinical 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 (online Supplementary Table S2) indicates a possible risk of non-alcoholic fatty liver disease 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 normoglycaemia in obese, otherwise healthy children and adolescents[28,29]. Significantly increased HbA_1c levels of obese compared with lean children, even all values in the reference range, is an alarm considering that obese children's HbA_{1c} median value equals 75th percentile value of lean children. This suggests that glucose concentration persistently moves towards higher values, mostly due to deleterious effects of hyperglycaemia in prolonged period of time. It is well known that obesity increases the risk of having metabolic syndrome and CVD 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 with the control group, while total cholesterol, LDL-cholesterol and TAG concentrations were elevated compared with the control group. Our results proved that obese children already have impaired homoeostasis of lipid parameters. Additionally, we found increased values of RFCVD and IA in the obese group. These parameters indicate the impaired lipid homoeostasis and the existence of a risk for CVD occurrence in adulthood[31]. Our study showed a modest increase in TAG concentration, which is still significantly higher than in the control group. This finding differed from the results of Brzeziński *et al.*, who indicated a higher TAG concentration than in our study, without significant difference compared with 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 OS[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 with lean children ($P < 0·001$, Table 2). Ozata and colleagues observed a significantly lower Zn concentration in obese individuals compared with their healthy counterparts[35]. It is well established that maintaining a normal Zn 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 Zn deficiency may compromise the functioning of numerous enzymes, potentially leading to the development of obesity. Furthermore, Zn deficiency is implicated in contributing to leptin resistance, a hormone with the potential to inhibit eating behaviours, primarily

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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)

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

PCA, principal component analysis; rLTL, relative leucocyte telomere length; B, unstandardised regression weight; SE, variation of unstandardised regression weight OR, odds ratio; CI, confidence interval; TOS, total oxidant status; AOPP, advanced oxidation protein products; IMA, ischaemia-modified albumin; PAB, prooxidant–antioxidant balance; SHG, total concentration of sulfhydryl group; TAS, total antioxidant status; SOD, superoxide dismutase; PON1, paraoxonase 1.

through an elevated production of neuropeptide Y in the hypothalamus[36]. Beside this mechanism, optimal Zn concentrations are important for adequate insulin activity, preventing the development of insulin resistance[35]. An increase in TAS in obese children could potentially be attributed to concurrent hyperuricemia in comparison with 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 UA production. Furthermore, the elevated levels of 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 UA 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 OS is an indispensable obesity companion, which burden is a consequence of obesity, 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 OS. Our study showed a metabolic triad existence: insulin resistance, increased BMI values and disturbed redox balance. The importance of the glutathione system and the total SHG's concentration has been well documented in redox homoeostasis 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 with 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 OS 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 with 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 CVD, diabetes mellitus, cancer and obesity[48]. Studies done on obese adult subjects showed a significant shortening of the LTL compared with its age- and sex-matched regular-weight counterparts[49]. Many studies confirmed telomere shortening due to obesity, even in a paediatric population, which is also found in our investigation[50,51]. There is a great inter-individual variation in LTL, with sex 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 sex. The low-grade inflammation persistence, a well-known obesity feature, leads directly to the shortening of LTL, affecting deoxyribonucleic acid (DNK) integrity[53]. Obesity promotes the formation of ROS and cytokines which could ignite inflammation and cell ageing, representing a vicious circle of mutually potentiated events, which we proved through the strong negative correlation between C-reactive protein concentration and rLTL. A strong negative correlation between TOS and rLTL (online Supplementary Table S4) in obese children implicates OS role in telomere shortening.

A large number of parameters determined as part of this study indicate an 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

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PCA. 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 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 OS involvement in obesity-related co-morbidities[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 > 30 kg/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 favour of exhausted antioxidant protection in obese subjects caused by its constant challenge by ROS and/or its pathophysiological diminishing or dysfunction as characteristics of increased adiposity in the prolonged time frame.

Conclusion

This study underscores that paediatric obesity is associated with telomere shortening and heightened OS, accounting for a 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, emphasising 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 ageing of the patients.

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The authors declare no conflict of interest.

The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Ethical Board (Ethical Licence No. 16/25, 10.06.2022).

Informed consent was obtained from all subjects involved in the study.

Supplementary material

For supplementary material/s referred to in this article, please visit https://doi.org/10.1017/S0007114524000898

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