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Impact of physical activity on redox status and nitric oxide bioavailability in nonoverweight and overweight/obese prepubertal children

Laura Leite-Almeida [a], Manuela Morato [b], Dina Cosme [c,d], Joana Afonso [c,d], José C. Areias [e,f], António Guerra [e,g], Alberto Caldas Afonso [a,h,i], António Albino-Teixeira [c,d], Teresa Sousa [c,d,1], Liane Correia-Costa [a,h,i,*,1]

[a] Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Portugal
[b] Laboratory of Pharmacology, Department of Drug Sciences & LAQV@REQUIMTE, Faculty of Pharmacy of Porto, University of Porto, Portugal
[c] Department of Biomedicine - Unit of Pharmacology and Therapeutics, Faculty of Medicine of University of Porto, Portugal
[d] MedInUP - Center for Drug Discovery and Innovative Medicines, University of Porto, Porto, Portugal
[e] Department of Gynecology-Obstetrics and Pediatrics, Faculty of Medicine of University of Porto, Portugal
[f] Division of Pediatric Cardiology, Integrated Pediatric Hospital, Centro Hospitalar Universitário São João, Porto, Portugal
[g] Division of Pediatric Nutrition, Integrated Pediatric Hospital, Centro Hospitalar Universitário São João, Porto, Portugal
[h] EPIUnit – Instituto de Saúde Pública, Universidade do Porto, Portugal
[i] Department of Pediatric Nephrology, Centro Materno-Infantil Do Norte, Centro Hospitalar Universitário Do Porto, Porto, Portugal

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ABSTRACT

Nutritional status might contribute to variations induced by physical activity (PA) in redox status biomarkers. We investigated the influence of PA on redox status and nitric oxide (NO) production/metabolism biomarkers in nonoverweight and overweight/obese prepubertal children. We performed a cross-sectional evaluation of 313 children aged 8–9 years (163 nonoverweight, 150 overweight/obese) followed since birth in a cohort study (Generation XXI, Porto, Portugal). Plasma total antioxidant status (P-TAS), plasma and urinary isoprostanes (P-Isop, U-Isop), urinary hydrogen peroxide (U–H_2O_2), myeloperoxidase (MPO) and plasma and urinary nitrates and nitrites (P-NOx, U-NOx) were assessed, as well as their association with variables of reported PA quantification (categories of PA frequency (>1x/week and ≤1x/week)and continuous PA index (obtained by the sum of points)) in a questionnaire with increasing ranks from sedentary to vigorous activity levels. U-NOx was significantly higher in children who presented higher PA index scores and higher PA frequency. Separately by BMI classes, U-NOx was significantly higher only in nonoverweight children who practiced PA more frequently ($p = 0.037$). In overweight/obese children, but not in nonoverweight, P-TAS was higher among children with higher PA frequency ($p = 0.007$). Homeostasis model assessment index (HOMA-IR) was significantly lower in more active overweight/obese children, but no differences were observed in nonoverweight children. In the fully adjusted multivariate linear regression models for P-TAS, in the overweight/obese group, children with higher PA frequency presented higher P-TAS. In the U-NOx models, U-NOx significantly increased with PA index, only in nonoverweight children. Our results provide additional evidence in support of a protective effect of physical activity, in nonoverweight by increasing NO bioavailability and in overweight/obese children by enhancing systemic antioxidant capacity and insulin sensitivity. These results highlight the importance of engaging in regular physical exercise, particularly among overweight/obese children, in which a positive association between oxidant status and cardiometabolic risk markers has been described.

1. Introduction

Oxidative stress results from an imbalance between the production of reactive oxygen species (ROS) and their elimination by antioxidant defenses. The shift in favor of a pro-oxidant status potentially leads to macromolecular damage and disruption of redox signaling and control, playing an important role in the development of many chronic and

* Corresponding author. Instituto de Saúde Pública da Universidade do Porto (ISPUP), Rua das Taipas nº 135, 4050-600, Porto, Portugal.
E-mail address: lianecosta@icbas.up.pt (L. Correia-Costa).
[1] Both authors contributed equally to the supervision and scientific revision of this paper.

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degenerative diseases [1]. Available evidence from our research group and others suggests that oxidative stress plays a critical role, since the early phase of life, in children with obesity and associated complications [2,3].

A growing body of literature has reported a significant increase of pro- and anti-oxidant status biomarkers after exercise in adults [4,5]. Chronic exposure to pro-oxidants due to exercise is believed to upregulate antioxidant mechanisms, thus decreasing the magnitude of exercise-induced oxidative stress [6,7]. Additionally, one of the most important molecular consequences of regular physical activity (PA) seems to be the increase in nitric oxide (NO) bioavailability, which is known to be associated with vasodilation and improved vascular function [8].

Few researches have addressed the impact of PA on redox status in children and conflicting results have been reported. Most studies corroborate the adaptation of antioxidant systems to PA [9–15] but some authors reported higher values of oxidative markers in physically active children [16,17]. This conflicting body of evidence might suggest that other factors, such as characteristics of the PA, fitness, body weight and pubertal status may influence the PA-induced responses [5].

Given the above, our aim was to study the association between PA, oxidative stress and NO bioavailability in nonoverweight and overweight/obese prepubertal children. We also explored the influence of PA on cardiometabolic risk parameters in these two groups of children. The redox status and NO bioavailability biomarkers chosen have been widely used in experimental and clinical studies in the field of cardiometabolic diseases and there is strong evidence regarding their relevance in the pathophysiology of these conditions [18–23].

Abbreviations

ABMP	Ambulatory Blood Pressure Monitoring
BMI	Body Mass Index
CI	Confidence Intervals
HDL	High Density Lipoprotein
HOMA-IR	Homeostasis Model Assessment of Insulin Resistance
hsCRP	high sensitivity C-Reactive Protein
MAP	Mean Arterial Pressure
MPO	Myeloperoxidase
NO	Nitric Oxide
NOS	Nitric Oxide Synthase
eNOS	endotelial Nitric Oxide Synthase
iNOS	inducible Nitric Oxide Synthase
nNOS	neuronal Nitric Oxide Synthase
P-Isop	Plasma Isoprostanes
P-NOx	Plasma Nitrates and Nitrites
P-TAS	Plasma Total Antioxidant Status
PA	Physical Activity
ROS	Reactive Oxygen Species
U–H2O2	Urinary Hydrogen Peroxide
U-Isop	Urinary Isoprostanes
U-Nox	Urinary Nitrates and Nitrites
USA	United States of America
WHO	World Health Organisation

2. Methods

2.1. Study design and sample

We studied children aged 8–9 years that have been followed since birth in a previously established cohort study (Generation XXI, Porto, Portugal) [24]. The children included in this cohort are believed to be representative of the population of Northern Portugal, as a very broad catchment area was included, and the participation proportion was high (at birth, 92% of the mothers invited accepted to participate). From the original cohort (n = 8647), 4590 children attended a face-to-face follow-up visit at 7 years of age including an anthropometric evaluation and blood sample collection, thus being eligible for the ObiKid project - a specific project aiming to clarify the impact of childhood obesity and associated comorbidities on the kidney [25]. We defined a minimum sample of 300 children for the ObiKid project's main objective; assuming that about 35% would be excluded due to refusal to participate, exclusion criteria or incomplete information, 463 children were preselected to be consecutively screened according to the date of their 7-years-old evaluation: 16 could not be contacted, 32 refused to participate, 23 although willing to participate were unable to schedule the study visits during the recruitment period and 68 met exclusion criteria [4 chronic diseases (genetic, renal or metabolic), 1 chronic usage of medication (affecting blood pressure or glucose or lipid metabolism), 51 with residence more than 30 km away from the study site, and 6 pairs of twins]. We enrolled 324 participants, but for the present analysis we additionally excluded 11 children due to incomplete evaluation, such as absence of blood or urine sample for oxidative stress and NO production markers determination. Children included in the final analysis (n = 313) were fairly representative of the eligible children with respect to sex, weight, height, systolic/diastolic blood pressure, and parental education level at the follow-up visit at 7 years of age.

2.2. Data collection and variables definition

The study visits took place at the Public Health and Forensic Sciences and Medical Education Department, Faculty of Medicine of the University of Porto. Anthropometric and general physical examination were performed, according to standard procedures and as previously reported [26]. Body mass index (BMI) was calculated and BMI-for-age values were classified according to the World Health Organization (WHO) reference data for BMI z-score into the following categories: nonoverweight (≤+1SD, including only 1 child with thinness), overweight (>1SD and ≤+2SD) and obesity (>2SD) [27]. Ambulatory blood pressure monitoring (ABPM) for 24 h was performed in all children with a portable non-invasive oscillometric blood pressure recorder (Spacelabs Healthcare, model 90207, Snoqualmie, Washington, USA). The non-dominant arm was used in all children with a cuff size appropriate to the child's arm circumference. Blood pressure measurements were taken automatically at 20-min intervals during the daytime and at 30-min intervals during the nighttime. A minimum monitoring duration of 24 h with gaps of less than 2 h was required for acceptance; 5 exams were excluded of the ABPM analysis due to insufficient readings. All readings were used to calculate mean 24 h, day and night mean arterial pressure (MAP), systolic and diastolic blood pressure, by the SpaceLabs software.

2.3. Sample collection and processing

A venous blood sample was collected after an overnight fast of at least 8 h and analyzed for glucose, insulin, lipids, high sensitivity C-reactive protein (hsCRP), myeloperoxidase (MPO), plasma isoprostanes (P-Isop), nitrates and nitrites (P-NOx) and total antioxidant status (P-TAS). Insulin resistance was determined using the homeostasis model assessment index (HOMA-IR) [28]. All participants collected a 24-h

urine sample, which was analyzed for urinary isoprostanes (U-Isop), hydrogen peroxide (U–H_2O_2) and nitrates and nitrites (U-NOx). All children's parents received information on the correct methods of 24-h urine collection and, upon sample delivery, compliance was rechecked by a brief questionnaire. The samples were considered valid if urinary creatinine was within the range of 11.3–28.0 mg/kg/day (according to age- and sex-specific reference values [29]) and if urinary volume was over 300 mL; based on these criteria 15 urine samples were excluded from the analysis.

2.4. Inflammatory and oxidative stress biomarkers

All the standard laboratory analyses were performed in the Clinical Pathology Department of Centro Hospitalar São João, Porto, Portugal. All the plasma, serum and urine samples used for the determination of inflammatory and oxidative stress biomarkers were stored at - 80 °C until assayed.

MPO, oxidative stress and NO production/metabolism markers were assessed through commercial kits according to manufacturer instructions at the Department of Biomedicine – Unit of Pharmacology and Therapeutics, Faculty of Medicine of the University of Porto. Serum MPO was quantified by an immunoenzymatic assay (BioCheck, MPO Enzyme Immunoassay Test Kit, Oxis International Inc., Foster City, CA, USA).

The quantification of the overall antioxidant capacity may be more representative of the in vivo balance between oxidizing and antioxidant compounds than the evaluation of individual antioxidants [30]. P-TAS was evaluated by a spectrophotometric assay (Antioxidant Assay Kit; Cayman Chemical Company, Ann Arbor, Michigan, USA) that measures the combined antioxidant activities of water- and lipid soluble antioxidants, including vitamins, glutathione, uric acid, bilirubin, albumin, etc. This assay depends on the ability of the antioxidants present in the sample to inhibit the absorbance of the radical cations of 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid). The antioxidant capacity of the sample is compared with that of Trolox, a water-soluble tocopherol analogue, and is expressed as mM Trolox equivalents. This assay was chosen for its simple methodology, its requirement of a very small volume of sample (10 μL) and for its ability to measure the combined antioxidant activities of water- and lipid soluble antioxidants, including vitamins, glutathione, uric acid, bilirubin, albumin, etc, while other assays used for evaluating antioxidant status measure only thiol groups or other individual antioxidants and require larger volumes of samples, which can be a limitation when performing clinical studies in pediatrics [31].

Free isoprostanes were quantified in plasma (P-Isop) containing the preservatives butylated hydroxy toluene (BHT, 0.005% w/v) and indomethacin (10 μM) added before storage. A solid phase extraction using Sep-Pak Classic C18 and Sep Pak Vac-Silica columns (Waters® Corporation, Milford, MA, USA) was performed prior to the measurement of P-Isop by a competitive enzyme immunoassay (15-Isoprostane F2t ELISA Kit, Oxford Biomedical Research, Inc., Michigan, USA).

Total nitrates and nitrites (NOx) were evaluated in plasma (P-NOx) and urine (U-NOx) by a colorimetric assay (Nitrate/Nitrite Colorimetric Assay Kit; Cayman Chemical Company, Ann Arbor, Michigan, USA). Plasma samples were ultrafiltered before assay using 30-kDa filters. This colorimetric assay provides an accurate method for the measurement of total NOx in a simple two-step process, where nitrates are first converted into nitrites by nitrate reductase and then the Griess Reagent is added, forming a deep purple azo compound due to its reaction with nitrites. The absorbance of this azo chromophore is then photometrically measured at 540 nm.

Urinary excretion of H_2O_2 (U–H_2O_2) was evaluated by a microplate fluorimetric assay (Amplex Red Hydrogen Peroxide/Peroxidase Assay Kit, Molecular Probes, Alfagene, Carcavelos, Portugal).

Urinary isoprostanes (U-Isop) were quantified by a competitive enzyme immunoassay (Urinary Isoprostane ELISA Kit, Oxford Biomedical Research, Inc., Michigan, USA) in non-extracted urine containing BHT (0.005%, w/v) added before storage and incubated with β-glucuronidase prior to the assay since a significant amount of isoprostanes is excreted in urine conjugated with glucuronide [32].

Data concerning oxidative stress and markers of NO bioavailability have been recently used by our group as part of studies on the same cohort to evaluate the association of these biomarkers with cardiometabolic risk, renal function and angiotensinogen [3,21,33].

2.5. Physical activity parameters

PA was assessed by a questionnaire, adapted [34] from the original version [35] and previously used by Portuguese authors, and was determined to have good reliability with strong intra-class correlation coefficients [36,37]. The questionnaire had 5 questions, each 4 or 5-point scale: 1 – Does the child take part in organized sport outside school? (Never; Less than once a week; At least once a week; Almost every day); 2 – Does the child take part in non-organized sport outside school? (Never; Less than once a week; At least once a week; Almost every day); 3 – How many times per week does the child take part in sport or PA for at least 20 min outside school? (Never; Less than once a week; Between once a week and once a month; 2 or 3 times a week; 4 times a week or more); 4 – How many hours per week does the child usually take part in PA so much that he/she sweats or gets out of breath outside school? (Never; 30 min to 1 h; 2–3 h; 4–6 h; 7 h or more); 5 – Does the child take part in sports competition? (Never; No, but already had; Yes, at school; Yes, in a club). A PA index (PAI) was obtained according to the total sum of points (maximum 22), with increasing rank from sedentary to vigorously activity levels. Frequency of PA was dichotomized, according to the answers to question number 3, into once a week or less and more than once a week.

2.6. Ethics

The ObiKid study was approved by the Ethics Committee of Centro Hospitalar São João, E.P.E. and Faculty of Medicine of the University of Porto and complies with the Helsinki Declaration, the guidelines for the ethical conduct of medical research involving children [38] and the current national legislation. Written informed consent from parents (or their legal substitute) and verbal assent from children was obtained, concerning information and biological samples gathering.

2.7. Statistical analysis

Statistical analysis was performed using IBM® SPSS® Statistics 24.0. The distribution of oxidative stress, NO production/metabolism markers and cardiometabolic risk variables by categorical variables of PA and classes of BMI is shown in box plot graphs and in tables, respectively, and compared using Kruskal-Wallis tests. Linear multivariate regression models were fitted to identify PA variables independently associated with P-TAS or U-NOx (the only oxidative/NO production markers significantly associated with PA in the univariate analysis), defined as the dependent variables. The results are presented separately by BMI z-score classes because the effect of PA variables on each of the oxidative stress parameters was found to be different in these groups (p for interaction: PA index 0.037/frequency of PA 0.014 and PA index 0.144/frequency of PA 0.356 for P-TAS and U-NOx, respectively). The models were adjusted for sex, age (in months), 24-h MAP, HOMA-IR, hs-CRP and non-HDL cholesterol. P values were considered statistically significant if < 0.05.

3. Results

A total of 313 children (53% male) with a mean (SD) age of 8.8 (0.2) years old were included in the present analysis. Clinical characteristics and values of biochemical parameters separately for nonoverweight (n

= 163) and overweight/obese children (n = 150, of whom 89 overweight and 61 obese) are shown in Table 1. Overweight/obese children presented significantly higher 24-h and nighttime MAP. Regarding analytical parameters, overweight/obese children presented higher levels of total cholesterol, non-HDL-cholesterol, triglycerides, hs-CRP, insulin and insulin resistance. Overweight/obese children presented significantly higher median values of U-Isop, U-NOx and MPO, when compared to nonoverweight children (Table 1). There were no significant differences on P-TAS, P-Isop, P-NOx and U–H_2O_2 values between overweight/obese and nonoverweight children.

Table 1
General characteristics, oxidative stress and physical activity parameters by classes of body mass index (nonoverweight and overweight/obese).

	WHO BMI z-score classification[a]		
	Nonoverweight n = 163	Overweight/Obese n = 150	*p*
Demography and anthropometry			
Age (months)	105.1 ± 3.0	105.4 ± 2.8	0.300
Male sex	83 (51%)	83 (55%)	0.085
Weight (kg)	27.7 ± 3.3	38.9 ± 6.8	<0.001
Height (cm)	131.1 ± 5.4	135.7 ± 5.9	<0.001
BMI (kg/m^2)	16.1 ± 1.2	21.0 ± 2.5	<0.001
BMI z-score	0.0 ± 0.7	2.0 ± 0.7	<0.001
24-h MAP (mmHg)	81.1 ± 4.3	82.5 ± 5.6	0.012
24-h MAP z-score	0.4 ± 0.9	0.6 ± 1.0	0.018
Daytime MAP (mmHg)	84.7 ± 4.6	85.9 ± 6.0	0.070
Daytime MAP z-score	0.1 ± 0.9	0.2 ± 1.0	0.129
Nighttime MAP (mmHg)	73.3 ± 4.8	75.0 ± 5.7	0.006
Nighttime MAP z-score	0.5 ± 0.9	0.8 ± 0.9	0.009
Physical activity			
PA Index	4 (4–9)	4 (4–9)	0.061
Frequency of PA			0.176
≤1/week	82 (50.3%)	64 (42.7%)	
>1/week	81 (49.7%)	86 (57.3%)	
Biochemical parameters			
Total cholesterol (mg/dL)	156.4 ± 24.8	162.3 ± 26.7	0.045
HDL-cholesterol (mg/dL)	54.7 ± 10.7	53.0 ± 9.7	0.152
Non-HDL-cholesterol (mg/dL)	101.8 ± 21.5	109.3 ± 24.7	0.004
Triglycerides (mg/dL)	53.2 ± 19.7	65.0 ± 31.2	<0.001
Glucose (mg/dL)	85.7 ± 5.4	86.2 ± 5.2	0.455
Insulin (μIU/mL)	5.7 ± 2.5	8.11 ± 4.1	<0.001
HOMA-IR	1.12 (0.82–1.44)	1.47 (1.13–1.97)	<0.001
hs-CRP (mg/L)	0.0 (0.0–0.4)	0.6 (0.2–1.4)	<0.001
Oxidative Stress parameters/markers			
P-TAS (m_M Trolox equivalents)	1.0 (0.8–1.2)	1.0 (0.6–1.2)	0.243
U–H_2O_2 (nmol/day)	705.1 (333.5–1312.2)	719.2 (397.3–1351.0)	0.700
P-Isop (ng/ml)	0.16 (0.10–0.33)	0.20 (0.11–0.41)	0.050
U-Isop (ng/day)	1143.7 (831.8–1456.2)	1305.7 (1052.9–1788.8)	<0.001
P-NOx (nmol/mL)	9.8 (8.0–12.3)	9.5 (7.6–12.4)	0.766
U-NOx (μmol/day)	498.3 (397.8–712.8)	680.8 (531.6–885.9)	<0.001
MPO (ng/mL)	29.1 (18.6–46.4)	51.9 (29.0–76.5)	<0.001

The values presented are mean ± standard deviation, median (25th percentile-75th percentile) or n (%).

BMI, body mass index; HDL, high density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; hs-CRP, high sensitivity C-reactive protein; MAP, mean arterial pressure; MPO, myeloperoxidase; PA, physical activity; P-Isop, plasma isoprostanes; P-NOx, plasma nitrates and nitrites; P-TAS, plasma total antioxidant status; U–H_2O_2, urinary hydrogen peroxide; U-Isop, nitrates and nitrites; U-NOx, urinary nitrates and nitrites.

[a] BMI z-score classes were defined according to the World Health Organization criteria [27].

The distribution of oxidative and NO production/metabolism markers by categories of PA in all children is presented in Table 2. Values of U-NOx were significantly higher in children who presented higher PA index scores and in children who were physically active more frequently. No differences were found in the values of other oxidative and NO production/metabolism markers by categories of PA.

The distribution of oxidative stress and nitric oxide (NO) production/metabolism markers by tertiles of PA index and the two categories of PA frequency, separately in nonoverweight and overweight/obese children, is shown in Fig. 1. Significant differences were only found for U-NOx and P-TAS values. In the nonoverweight, but not in the overweight/obese children, U-NOx values were significantly different across PA index tertiles (1st tertile 620.3 (478.9–800.8), 2nd tertile 851.4 (500.8–1320.4), 3rd tertile 742.6 (577.6–980.8) mmol/day, p = 0.022) and children who practiced PA more than once a week presented higher values of U-NOx (774.5 (545.8–1167.0) vs. 622.6 (489.7–919.8) mmol/day, in the groups >1x/week vs. ≤1x/week, respectively, p = 0.037). In overweight/obese children, but not in nonoverweight children, P-TAS concentrations were significantly different across PA index tertiles (1st tertile 1.11 (0.79–1.42), 2nd tertile 1.28 (0.72–1.84), 3rd tertile 1.23 (1.07–1.64) mM Trolox equivalents, p = 0.020). P-TAS concentrations were significantly higher among children with higher PA frequency, when compared to children with lower PA frequency (1.23 (1.05–1.68) vs. 1.12 (0.72–1.46) mM Trolox equivalents, p = 0.007), only in the overweight/obese group.

The distribution of cardiometabolic risk variables (24-h MAP and HOMA-IR) by parameters of PA (PA index tertiles and PA frequency categories), separately in nonoverweight and overweight/obese children, is displayed in Table 3. PA had no significant impact on 24-h MAP in nonoverweight and overweight/obese children. However, HOMA-IR values were significantly lower in more active children, both in those with higher PA index scores and higher PA frequency.

In Table 4, multivariate linear regression models for P-TAS and U-NOx, in nonoverweight and overweight/obese children, adjusted for age, sex, 24-h MAP, HOMA-IR, hs-CRP and non-HDL cholesterol are presented. In the overweight/obese group, children with higher PA frequency (>1x/week) presented higher P-TAS, by +0.16 (0.01–0.31) mM Trolox equivalents. In the U-NOx models, only in nonoverweight children, U-NOx significantly increased by +44.1 (15.4 a 72.9) nmol/mL per unit of PA index.

4. Discussion

The major findings of our study were that PA was associated with increased U-NOx values in nonoverweight children, and with increased P-TAS and decreased insulin resistance in overweight/obese children.

NO has a wide range of reported functions, from blood vessel dilation and regulation of cardiac and renal function to nerve signaling and bactericidal effects [39,40]. It can be produced by three different NO synthases (NOS) [39,41]. Neuronal NOS (nNOS) and endothelial (eNOS) are constitutive enzymes, with the first being expressed not only in central and peripheral nerves, but also in skeletal, cardiac and vascular muscle cells, epithelial cells of several organs, kidney macula densa cells and pancreatic islet cells, and the second being mostly expressed in endothelial cells, where it contributes to vasodilation and vascular protection. The third isoform is inducible (iNOS); it is expressed in various cells but only in the presence of a proinflammatory stimulus [41]. Nitrates and nitrites are NO metabolites and reflect its bioavailability [20,42]. Although in the present study we were not able to differentiate the sources of the increased U-NOx values in nonoverweight children, it has been described that the stress induced by regular PA upregulates the production of NO by eNOS, therefore increasing NO bioavailability and improving vasodilation [8]. In a meta-analysis involving overweight and obese children in 2015, PA was reported to be associated with improved vascular function, assessed by conduit artery flow-mediated dilation, a noninvasive index of preclinical

Table 2
Distribution of oxidative stress and nitric oxide (NO) production/metabolism markers by physical activity (PA) parameters (PA index tertiles and PA frequency categories) in all children.

	P-TAS	U–H_2O_2	P-Isop	U-Isop	P-NOx	U-NOx	MPO
PA Index	p = 0.339	p = 0.198	p = 0.913	p = 0.598	p = 0.601	**p = 0.014**	p = 0.358
1st tertile	1.18	1110.87	0.35	1557.01	11.94	722.69	52.42
	(0.96–1.50)	(665.16–1885.21)	(0.21–0.64)	(1249.85–2397.41)	(9.62–15.27)	(540.71–969.59)	(33.82–87.43)
2nd tertile	1.22	1552.11	0.40	1824.89	12.20	887.99	60.60
	(0.99–1.64)	(685.71–2151.61)	(0.16–0.60)	(1208.09–2430.07)	(9.68–17.24)	(556.62–1340.07)	(38.02–96.39)
3rd tertile	1.28	1320.50	0.36	1557.62	12.65	922.02	62.55
	(1.03–1.71)	(781.48–2474.63)	(0.22–0.58)	(1189.55–2104.50)	(9.49–17.25)	(647.41–1165.33)	(35.19–92.96)
PA Frequency	p = 0.158	p = 0.757	p = 0.383	p = 0.405	p = 0.665	**p = 0.019**	p = 0.565
≤1x/week	1.20	1430.77	0.38	1740.26	12.34	740.76	57.43
	(0.94–1.53)	(701.20–2062.07)	(0.20–0.60)	(1226.36–2583.45)	(9.57–15.27)	(546.64–1083.45)	(36.99–89.97)
>1x/week	1.24	1285.92	0.35	1575.85	12.43	885.86	61.52
	(1.03–1.69)	(724.42–2180.67)	(0.19–0.58)	(1189.57–2145.61)	(9.71–17.66)	(595.25–1232.07)	(36.45–94.55

MPO, myeloperoxidase (in ng/mL); PA, physical activity; P-Isop, plasma isoprostanes (in ng/mL); P-NOx, plasma nitrates and nitrites (in nmol/mL); P-TAS, plasma total antioxidant status (in mM Trolox equivalents); U–H_2O_2, urinary hydrogen peroxide (in nmol/day); U-Isop, urinary isoprostanes (in ng/day); U-NOx, urinary nitrates and nitrites (in μmol/day).
The values presented are median (25th percentile-75th percentile).

atherosclerosis, and this improvement was believed to be related to an upregulation of the NO vasodilator system [43]. The increase in eNOS is apparently induced by arterial shear stress and leads to an improvement in endothelial function [8,44,45]. The results of that study are in contrast with our present observations since we detected a significant impact of PA on U-NOx values in nonoverweight children, but not in overweight and obese children. However, in a previous study evaluating the same population of children, we reported a significant increase of U-NOx values in the overweight/obese group, along with an increase in oxidative stress/proinflammatory markers, probably reflecting an enhanced production of NO by iNOS which could inhibit eNOS activity and impair endothelium-mediated vasodilation [3,46,47]. Thus, the lack of detection of a significant effect of PA on NO bioavailability in these overweight/obese children could have resulted from the fact that they had already higher U-NOx values. Additionally, it could have resulted from difficulties in discriminating the sources of NO with the methodologies used. Nevertheless, we detected a positive impact of PA on insulin resistance in overweight and obese children, evidenced by significantly lower values of HOMA-IR in those that were more physically active. This improvement in insulin sensitivity could have been caused, at least in part, by an enhancement of eNOS-derived NO production since eNOS seems to play a major role in the regulation of systemic glucose metabolism and insulin delivery to peripheral tissues [48–50]. On the contrary, NO and other reactive nitrogen species derived from iNOS promote hyperglycemia, hyperinsulinemia and insulin resistance [48]. Thus, it is conceivable that the lower values of HOMA-IR in overweight and obese children that practiced more PA might be associated with an increased eNOS-derived NO production, along with a reduction of iNOS activity.

In addition to its impact on NO bioavailability, regular PA also appears to have a stimulatory effect on antioxidant systems [8]. In adults, this association is well-documented [4,5] and is believed to be part of a physiological response to the repeated oxidative stimulus of PA [51–53]. In children, there is some evidence of a similar phenomenon [9–15], but studies reporting lower antioxidant levels in athletes, independently of the load of PA, also exist [16,17]. Our study reinforces the hypothesis of an enhanced antioxidant response in association with PA, although this was only observed in overweight/obese children. In line with our results, several interventional and cross-sectional studies in children and adolescents have found increased antioxidant defenses levels or a decrease of oxidative stress markers in those submitted to different programs of exercise or in those reporting regular PA [9,10,12–15,54–57]. In contrast to our results, there is an interventional study [58] and a report [59] of higher prooxidant and lower antioxidant values in obese children after exercise, while others report a lack of differences in redox markers levels [60]. Specifically, in prepubertal children, the evidence is also scarce, but a previous study, comparing high and low fitness groups reported higher antioxidant levels in the high fitness group [11].

In a previous study of our group, involving the same overweight/obese children analyzed in the present work, we observed higher values of oxidative stress and NO bioavailability markers in relation to fat accumulation which were associated with a worse cardiometabolic profile and altered kidney function [3]. Therefore, our findings of an increased antioxidant capacity among those with higher PA levels, in the same group of overweight/obese children, would most probably translate into a protective cardiometabolic effect. Indeed, we observed that overweight/obese children more physically active exhibited significantly lower insulin resistance, assessed by HOMA-IR. This reduction of HOMA-IR is in accordance with the results of other authors who found that an increase of antioxidant capacity, induced by antioxidant supplementation, attenuated insulin resistance and endothelial dysfunction in young overweight adults [61]. This is not surprising since oxidative stress has been associated with reduced endothelial-derived NO bioavailability due to enhanced quenching of NO by ROS or to the uncoupling of eNOS [62,63]. Furthermore, as previously referred, eNOS impairment leads to the dysregulation of systemic glucose metabolism and insulin sensitivity [48].

Our results corroborate the evidence supporting the role of PA in improving glucose, insulin homeostasis and cardiovascular profile [64–69].

One of the major strengths of our study is the large population-based sample of prepubertal children and the extensive evaluation of oxidative stress markers and cardiometabolic risk factors, while the cross-sectional design of the present analysis is an important limitation to point out. However, considering that our sample was selected from a birth cohort with regular evaluations, we aim to further investigate prospectively the studied association. Also, as a limitation, our study population is composed of children born in the Northern region of Portugal, most of them of Western European ancestry, which might limit the ability to generalize our results to other ancestries or ethnic groups. Since very few studies exist on the associations studied, we believe that our results should be replicated in other populations in order to confirm our findings and further explore the mechanisms underlying the differences found across PA groups in children with different nutritional status.

In conclusion, our study supports the importance of engaging in regular physical exercise, particularly among overweight/obese children, in which the activation of an adequate antioxidant response and the improvement in insulin sensitivity could play a critical role in

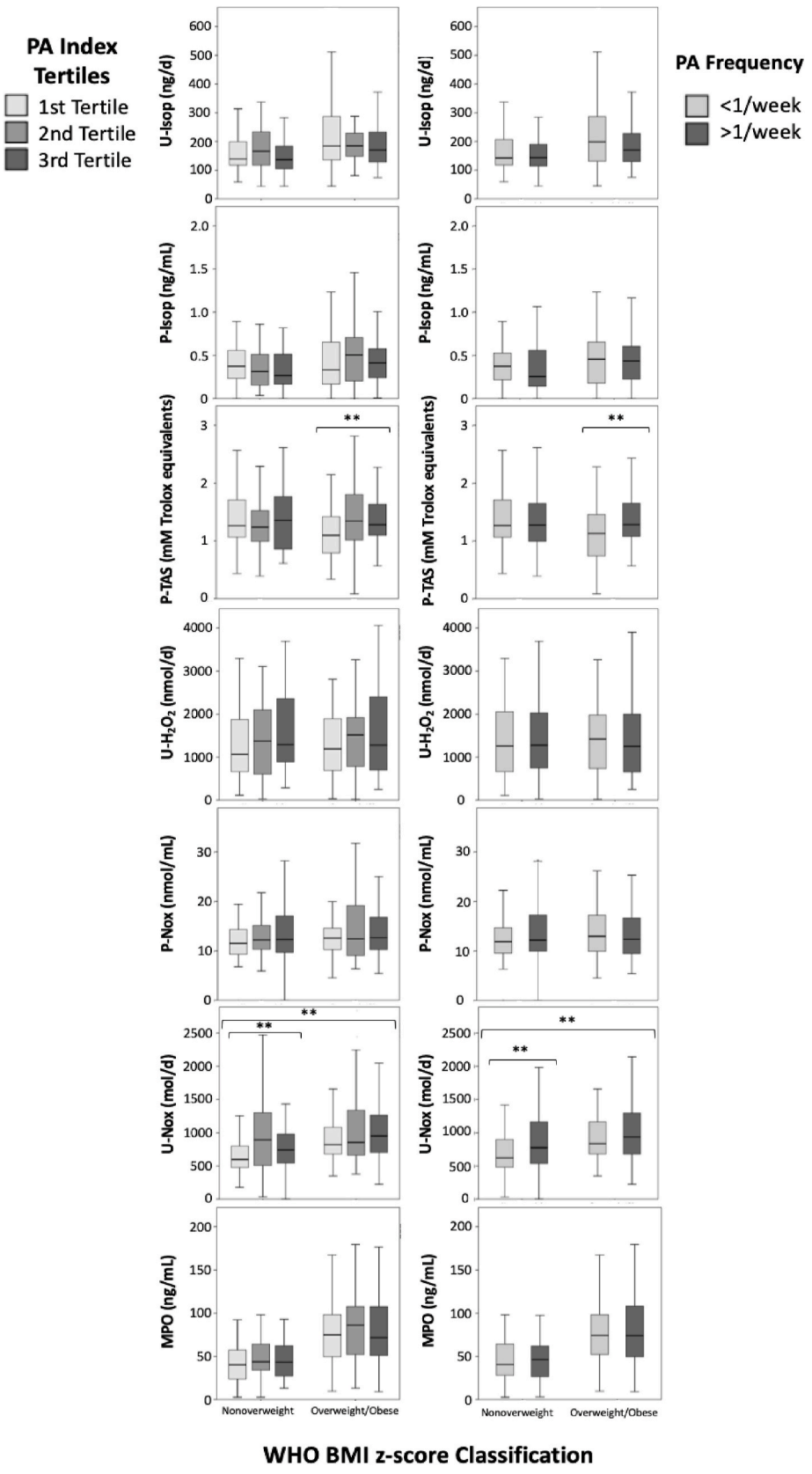

Fig. 1. Distribution of oxidative stress and nitric oxide (NO) production/metabolism markers by classes of physical activity (PA) parameters (PA index tertiles and PA frequency categories) separately in nonoverweight and overweight/obese children. **p < 0.005 Oxidative stress/NO bioavailability markers data are expressed as medians and 25th, 75th percentiles.

Table 3
Distribution of cardiovascular risk variables (24-h MAP and HOMA-IR) by physical activity (PA) parameters (PA index tertiles and PA frequency categories) and BMI classes (nonoverweight and overweight/obese).

	24-h MAP z-score	HOMA-IR
PA Index		
Nonoverweight	p = 0.803	p = 0.200
1st tertile	0.28 (−0.22–1.09)	1.22 (0.96–1.45)
2nd tertile	0.36 (−0.25–0.92)	1.12 (0.91–1.48)
3rd tertile	0.16 (−0.17–0.82)	1.00 (0.75–1.27)
Overweight/Obese	p = 0.101	**p = 0.020**
1st tertile	0.43 (−0.18–1.26)	1.76 (1.19–2.73)
2nd tertile	0.76 (0.18–1.40)	1.48 (1.22–1.99)
3rd tertile	0.41 (0.02–0.89)	1.45 (1.04–1.74)
PA Frequency		
Nonoverweight	p = 0.741	p = 0.207
≤1x/week	0.27 (−0.32–0.87)	1.16 (0.96–1.41)
>1x/week	0.27 (−0.17–0.83)	1.07 (0.77–1.36)
Overweight/Obese	p = 0.484	**p = 0.010**
≤1x/week	0.55 (0.02–1.32)	1.67 (1.24–2.66)
>1x/week	0.49 (0.08–0.95)	1.45 (1.09–1.79)

The values presented are median (25th percentile-75th percentile).
BMI, body mass index; HOMA-IR, homeostasis model assessment of insulin resistance; MAP, Mean Arterial Pressure; PA, physical activity.

Table 4
Multivariate linear regression models for P-TAS and U-NOx, in nonoverweight and overweight/obese children.

P-TAS models				
	Nonoverweight		Overweight/obese	
	Adjusted β (95% CI)	p	Adjusted β (95% CI)	p
Model 1 - PA index	−0.01 (−0.03 to 0.01)	0.221	0.02 (0.00–0.04)	0.050
Model 2 - Frequency of PA (>1x/week)	−0.10 (−0.23 to 0.04)	0.150	0.16 (0.01–0.31)	**0.037**
U-NOx models				
	Nonoverweight Adjusted β (95% CI)	**p**	**Overweight/obese Adjusted β (95% CI)**	**p**
Model 1 - PA index	44.13 (15.39–72.86)	**0.003**	12.67 (−9.97 to 35.32)	0.270
Model 2 - Frequency of PA (>1x/week)	225.58 (−2.98 to 454.14)	0.053	125.36 (−64.00 to 314.73)	0.193

The values presented are adjusted linear regression coefficients (β) and 95% confidence intervals (95% CI), estimated by multivariate linear regression models with P-TAS or with U-NOx as the dependent variables. The models were adjusted for 24-h MAP, HOMA-IR, hs-CRP, non-HDL cholesterol, as well as for sex and age (months). The results are presented separately by BMI z-score classes because the effect of physical activity variables on each of the oxidative stress parameters was found to be different in these groups (p for interaction: PA index 0.037/frequency of PA 0.014 and PA index 0.144/frequency of PA 0.356 for P-TAS and U-NOx, respectively).
BMI, body mass index; HOMA-IR, homeostasis model assessment of insulin resistance; hsCRP, high sensitivity C-reactive protein; MAP, Mean Arterial Pressure; PA, Physical Activity; P-TAS, plasma total antioxidant status; U-NOx, urinary nitrates and nitrites.

counteracting oxidative stress and the consequent rise in cardiometabolic risk. Nonetheless, the clinical relevance of our findings needs to be further clarified, especially in the long term.

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Declaration of competing interest

None.

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