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ARTICLE

Profile of oxidant and antioxidant activity in prepubertal children related to age, gender, exercise, and fitness

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Abstract: Tissue damage resulting from oxidative stress induced by a pathological condition might have more serious consequences in children than in adults. Researchers have not yet identified particular markers — alone or in combination with others — of oxidative stress, or their role in pediatric diseases. The aim of this study was to identify gender-based biomarkers for measuring oxidative stress. Oxidative biomarkers were studied in 138 healthy Spanish children (85 boys, 53 girls) 7 to 12 years of age, at the prepubertal (Tanner I) stage, independent of body mass index (BMI), age, fitness (measured by 20-m shuttle run test), and physical activity (measured by participation in an after-school exercise program). The oxidative biomarkers measured were lipid peroxidation products, total nitrites, protein carbonyls, and oxidized glutathione (GSSG). The antioxidant biomarkers measured were total glutathione (TG), reduced glutathione (GSH), superoxide dismutase activity (SOD), and glutathione peroxidase activity. In the study population, height, weight, waist circumference, and BMI were lower in girls than in boys. For oxidative biomarkers, boys had higher levels of protein carbonyl than girls ($p < 0.001$). In spite of this, girls had higher levels of GSSG ($p < 0.001$) and TG ($p = 0.001$), and a lower GSH/GSSG ratio ($p < 0.001$) than boys. For the antioxidant response, girls had higher levels of SOD ($p = 0.002$) than boys. All analyses were adjusted for BMI, age, fitness, and physical activity. In conclusion, prepubertal girls had higher oxidative stress than boys, in addition to higher levels of SOD, independent of age, BMI, fitness, and physical activity.

Key words: paediatric, healthy, oxidative stress, gender, fitness, physical activity.

Résumé : Les dommages tissulaires causés par le stress oxydatif suscité par une condition pathologique semblent plus graves chez les enfants que chez les adultes. Les chercheurs n'ont pas encore identifié des marqueurs particuliers — isolés ou groupés — du stress oxydatif et leur fonction dans les maladies pédiatriques. Cette étude se propose d'identifier des biomarqueurs spécifiques au sexe pour la mesure de l'état de stress oxydant. On analyse les biomarqueurs du stress oxydant chez 138 jeunes Espagnols (85 garçons, 53 filles) âgés de 7 à 12 ans au stade prépubère (Tanner I), et ce, indépendamment de l'indice de masse corporelle (IMC), de l'âge, de la condition physique (mesurée par le test de navette sur 20 m) et la pratique de l'activité physique (« PA ») (mesurée d'après les inscriptions au programme d'exercice postscolaire). Les biomarqueurs du stress oxydant évalués sont les suivants : produits de la peroxydation lipidique (« LPO »), les nitrites totaux (« NOx »), groupements carbonyles dans les protéines (« PC ») et le glutathion oxydé (« GSSG »); les biomarqueurs antioxydants sont les suivants : glutathion total (« TG »), glutathion réduit (« GSH »), activité de la superoxyde dismutase (« SOD ») et activité de la glutathion peroxydase (« GPx »). Comparativement aux garçons, les filles présentent des valeurs plus faibles de taille, de poids, de tour de taille et d'IMC. Au sujet des biomarqueurs du stress oxydant, les garçons présentent des valeurs de PC plus élevées que les filles ($p < 0,001$). Néanmoins, les filles présentent de plus fortes valeurs de GSSG ($p < 0,001$) et de TG ($p = 0,001$) et un plus faible ratio GSH/GSSG ($p < 0,001$) comparativement aux garçons. Au sujet de la réponse antioxydante, les filles présentent des valeurs de SOD ($p = 0,002$) plus élevées que les garçons. Toutes les analyses prennent en compte l'IMC, l'âge, la condition physique et la pratique de l'activité physique. Les filles prépubères présentent un plus haut stress oxydatif que les garçons et, parallèlement, une plus grande activité de la SOD, et ce, indépendamment de l'âge, de l'IMC, de la condition physique et de la pratique de l'activité physique. [Traduit par la rédaction]

Mots-clés : pédiatrie, santé, stress oxydatif, sexe, condition physique, activité physique.

Introduction

An overproduction of free radicals can alter the endogenous antioxidant defense system, which has been associated with an increase in oxidative stress (OS). OS biomarkers determine the extent of oxidative injury (Jakus and Rietbrock 2004; Noiri and Tsukahara 2005); this occurs in many pathological processes and significantly contributes to disease mechanisms (Heitzer et al. 2001). The effects of oxidation can be predicted with OS biomarkers, which can be used to design appropriate interventions to prevent or alleviate oxidative damage (Fisher-Wellman and Bloomer 2009). The detection of more than 1 OS biomarker is crucial, because a single biomarker might yield misleading results (Tsukahara 2007).

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Tsukahara (2007) states that under normal physiological conditions, younger people (especially children) are more likely to be exposed to higher concentrations of reactive oxygen species and higher total nitrite and nitrate concentrations (as a marker of nitric oxide formation) than older people. Moreover, Casado et al. (2007) found that pathogenesis and the evolution of numerous diseases at this age are associated with oxidative damage induced by reactive oxygen species. This is related to the need in infants for subsequent tissue growth to match somatic growth, and survival rates in children are normally higher. Furthermore, the use of antioxidants has presented new therapeutic perspectives for diseases that are related to enhanced OS (Tsukahara 2007).

Women appear to have greater resistance to inflammatory and oxidative processes than men (Kerksick et al. 2008), which might influence the prevalence and severity of certain diseases — especially cardiovascular diseases (Miller et al. 2007). In fact, the gender longevity gap is associated with lower OS (Ali et al. 2006; Pepe et al. 2009).

In animal models, females have been shown to have higher concentrations of antioxidants and greater resistance to oxidative damage (Bureau et al. 2003). Similarly, higher levels of glutathione peroxidase (GPx) (Rush and Sandiford 2003) and lower levels of lipid and DNA oxidation (Pansarasa et al. 2000) have been reported in young women, compared with men (Proteggente et al. 2002). However, it has been reported that levels of glutathione (GSH) are higher in newborn baby girls than in boys (Lavoie and Chessex 1997), suggesting that females have some type of protection against oxidant insults at birth. Moreover, there is no convincing evidence of an existing correlation between OS and some diseases (Pavlova et al. 2005), and few evaluation studies are available on the oxidative status of healthy children (Tsukahara 2007).

In contrast, obesity-related alterations in OS markers have been reported to be largely gender-independent in adolescents (Oliver et al. 2010). Physical fitness is known to exert independent positive effects on oxidative homeostasis, regardless of adiposity status (Kasapis and Thompson 2005). However, because the oxygen flow into working muscles might be greater in children, exercise-induced OS response might be higher in children than in adults (Cooper et al. 2004). It has been reported that acute aerobic exercise induces OS, whereas regular aerobic exercise is associated with a decrease in oxidants and an increase in antioxidants (Cakir-Atabek et al. 2010).

Therefore, we evaluated, in prepubertal boys and girls, the status of a series of oxidative markers (lipid peroxidation products, total nitrite and nitrate concentration, and protein carbonyl), oxidized glutathione (GSSG), and antioxidant biomarkers (total glutathione (TG), reduced GSH, superoxide dismutase activity (SOD), and GPx). Our aim was to analyze whether these biomarkers are influenced by age, body mass index (BMI), cardiorespiratory fitness, and (or) physical activity (PA).

Materials and methods

Subjects and design

We encouraged 450 children from 2 local elementary schools in Spain to participate in the study. Originally, 156 prepubertal children volunteered, but some did not meet the inclusion criteria (the absence of pubertal development, no disease, no long periods of rest after illness, and no use of medication that alters blood pressure or metabolism), some decided not to participate or not complete the study, and in some, vein puncture blood sampling was not possible. After these exclusions, our study cohort consisted of 138 healthy children (85 boys, 53 girls), 7 to 12 years of age, at the prepubertal (Tanner I) stage.

The study was conducted at the Department of Paediatrics. Written informed consent was obtained from parents or legal guardians, and the study procedures were verbally explained to all children. Ethical approval of the study was given from the local human research committee and the hospital ethics committee. Study methodologies conformed to the standards set by the Declaration of Helsinki.

Physical examination and anthropometric and blood pressure measurements

Anamnesis was assessed and a physical examination was conducted, which included the evaluation of sexual maturity according to Tanner's 5-stage scale (Tanner 1962). Prepubertal (Tanner I) stage was confirmed with the identification of appropriate plasma sex hormone levels. Anthropometric measurements (weight, height, BMI) were taken using standard techniques. Systolic and diastolic blood pressure were measured by the same person, using a random-zero sphygmomanometer (Dinamap V-100), on the right arm of the subjects while they were seated. An average of 3 consecutive measurements was used for analysis.

Evaluation of fitness and physical activity

A validated scale developed by Olds et al. (2006) was used to measure fitness after a 20-m shuttle run test; it is described in detail elsewhere (Léger et al. 1988). This test is one of the most commonly used field tests to assess fitness in children and adolescents. After participants were taught how to do the test correctly, they ran as long as possible back and forth across a 20-m space. A specified audio signal protocol was used to dictate pace, which was increased by 0.5 km·h^{-1} each minute until running speed reached 8.0 km·h^{-1}. Subjects were allowed to voluntarily withdraw from the test after being verbally encouraged to perform maximally during each assessment. The test is completed when the participant fails to reach the end lines concurrent with the audio signals on 2 consecutive occasions. The last lap completed was considered to be the fitness level of that individual for the raw variable obtained. The z-scores from the shuttle run test are relative to all children of the same age and sex from all countries.

All children were enrolled in an after-school exercise program. To estimate PA, we established whether they participated at least 3 times per week for at least 1 year or whether they were sedentary. A short test, based on the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) validated questionnaire (Pianta 2007), was used to obtain information about the PA and sedentary habits of the children. In addition, information elicited from program staff was used in the assignment of children to the active or sedentary group. These after-school programs are designed to encourage children to take up some PA and to counteract the increase in sedentary habits in children. All physically active children in this study participated in similar activities, which were driven by professional staff from the 2 schools.

Sampling and biochemical analysis

Blood samples were collected between 0900–0930 h, after a 12-h fasting period, while the children were at rest. An indwelling venous line was used to draw a 3-mL sample in tubes containing 1 mg·mL^{-1} EDTA-K3 as the anticoagulant (for plasma and erythrocytes). Samples were placed in chilled tubes, and were stored in containers with ice and kept in the dark. Particular care was taken to avoid exposure to air, light, and ambient temperature. Within 1 h of extraction, centrifugation at 3500*g* for 10 min was used to separate plasma from erythrocytes. Aliquots of supernatant (1 mL) were immediately frozen to −82 °C until analysis, 1 month later.

Determination of OS and antioxidant biomarkers

Lipid peroxidation products

Plasma malondialdehyde (MDA) and 4-hydroxyalkenals (4-HDA) were estimated in accordance with the method described by Erdelmeier et al. (1998). Briefly, a chromogenic reagent reacts with MDA+4-HDA at 45 °C, and yields a stable chromophore with maximum absorbance at 586 nm.

Protein carbonyls

Plasma protein carbonyl concentrations were measured in accordance with the method described by Levine et al. (1990). Samples were incubated with 2,4-dinitrophenylhydrazine in HCl for 60 min. Proteins were then precipitated from the solution, using 500 µL of trichloroacetic acid (20%). Subsequently, proteins were washed with a solution of ethanol and ethyl acetate (1:1 v/v), and dissolved in 1 mL of guanidine hydrochloride (6 mol·L^{-1}) at 37 °C. Carbonyls were evaluated in a spectrophotometer (UV-1630; Shimadzu) at a wavelength of 360 nm (Luo and Levine 2009).

Total nitrites (nitrites and nitrates)

Total nitrite and nitrate concentrations were used as markers of nitric oxide levels, and were assayed in plasma in accordance the Griess method (Ricart-Jané et al. 2002). This assay uses the determination of nitrite as an indicator of nitric oxide production in biological samples. Nitric oxide is transformed into nitrate and nitrite. It is common practice to use either enzymatic or chemical reduction to convert all nitrates in a sample into nitrite, and to measure total nitrite as an indicator of nitric oxide production. When nitrate reduction was completed, total nitrite was spectrophotometrically determined using the Griess reaction. Reaction was monitored at 540 nm. Absorbance (in g·mL^{-1}) was evaluated in a spectrophotometer (UV-1603; Shimadzu).

TG, GSSG, and GSH

TG and GSH levels were evaluated in red blood cells, using the Bioxytech GSH-420 and GSH-400 kits, respectively, from BIOXYTECH aop-490 (Oxis International, Portland, Ore., USA). The combined action of the antioxidants in the sample leads to the reduction of Cu^{2+} to Cu^{+}. Thus, the chromogenic reagent results in a Cu^{+} complex with absorbance at 490 nm (Price et al. 2006). The determination of TG levels was based on the formation of a chromophoric thione with absorbance at 420 nm. The GSH concentration is based on a reaction that leads to the formation of a chromophore with absorbance at 400 nm (Rahman et al. 2006). GSSG levels were calculated by subtracting GSH from TG.

SOD and GPx

SOD activity in erythrocytes was determined using a colorimetric assay kit from BioVision Research Products (Mountain View, Calif., USA). SOD catalyzes the dismutation of the superoxide anion into hydrogen peroxide and molecular oxygen. The relation between the superoxide anion reduction rate and xanthine oxidase activity is linear and is inhibited by SOD. Therefore, the inhibitory activity of SOD is determined using a colorimetric method.

GPx activity in red blood cells was evaluated in accordance with the method of Flohé and Günzler (1984), using the glutathione peroxidase assay kit (Cayman Chemical). This assay is based on the oxidation of NADPH to NADP^{+}, which is catalyzed by a limited concentration of GSH reductase, with maximum absorbance at 340 nm. Activity was measured on the basis of the formation of GSSG from the GPx-catalysed oxidation of GSH by H_2O_2, coupled with NADPH consumption, in the presence of exogenously added GSH reductase, with maximum absorbance at 340 nm.

Statistical analysis

Data are expressed as means ± SD. Normal data distribution was assessed with the Shapiro–Wilk test. Homogeneity of variances was estimated using Levene's test. The group means for continuous variables with normal distribution were compared using Student's *t* test in unpaired samples; variables with asymmetric distribution were compared using the Mann–Whitney *U* test.

Finally, differences between boys and girls were determined using analysis of covariance (ANCOVA) after adjustment for age, BMI, fitness, and PA. All statistical analyses were performed using the Statistical Package for Social Science software (PASW Statistics 18, SPSS Inc., Chicago, Ill., USA).

Results

Anthropometric differences were found between girls and boys. Prepubertal girls were younger than prepubertal boys, and height, weight, waist circumference, and BMI were lower in girls than in boys. Blood pressure was also lower in girls (Table 1). T1

Differences in oxidative and antioxidant biomarkers were observed between sexes before adjustment for age, BMI, fitness, and PA (Table 2). For oxidant biomarkers, after adjustment, levels of protein carbonyl were higher in boys than in girls (5.27 vs. 1.74 nmol·L^{-1}; $p < 0.001$), but there were no differences between boys and girls in lipid peroxidation products (0.22 vs. 0.21 µmol·L^{-1}; $p = 0.632$) or total nitrite and nitrate concentration (14.63 vs. 14.67 µmol·L^{-1}; $p = 0.975$). T2

For the antioxidant response, levels of TG ($p = 0.001$) (Fig. 1) and SOD were lower in boys than in girls (Fig. 2). F1 F2

In contrast, GSSG levels were higher in girls than in boys, and the GSH/GSSG ratio was lower in girls (Fig. 1). No differences were found in GPx (Fig. 2).

Discussion

The main results of this study suggest that there is an independent effect of gender on OS, even at a prepubertal age. Girls had higher levels of TG, GSSG, and SOD than boys, and a lower GSH/GSSG ratio.

Research on prepubertal subjects or newborns is scarce (Casado et al. 2007; Lavoie and Chessex 1997); most studies are focused on the adult population and are not gender-based (Picot et al. 1992). Our study comprises a larger sample of children than previous studies.

Although we found anthropometric differences between the sexes, these probably do not have an influence on OS. In fact, our analysis was adjusted for age and BMI to eliminate such effects. In addition, to eliminate the effect of puberty on OS (Pérez-Navero et al. 2009), we focused on prepubertal-stage children (7 to 12 years of age) rather than on age-matched groups; previous studies did not consider the physical development of infants (Erden-Inal et al. 2002).

It has been proposed that good fitness (Santos-Silva et al. 2001) and moderate exercise have an antioxidant effect (Gomez-Cabrera et al. 2008; Llorente-Cantarero et al. 2012). Some authors have found that boys and girls do not differ in the redox response to training (Cavas and Tarhan 2004; Kabasakalis et al. 2009) or acute exercise (Nikolaidis et al. 2007). Similarly, we found no differences in OS biomarkers between boys and girls in relation to cardiorespiratory fitness and PA levels.

TG is a low-molecular-mass, thiol-containing tripeptide (glutamic acid, cysteine, glycine). It plays a major role in the detoxification of a wide range of chemicals. It acts as a cofactor for the enzyme peroxidase, serving as an indirect antioxidant that donates electrons. It also exhibits nonselenium-dependent GPx activity against organic hydroperoxides (Guemouri et al. 1991). High TG activity has been observed during the first year of life; it decreases and remains constant in childhood, adulthood, and old age (Picot et al. 1992). The results of our study are in agreement with those obtained by Habif et al. (2001), who found that females had higher levels of TG than males.

GSH is converted to GSSG by seleno-dependent GPx. GSSG is subsequently reduced back to GSH by GHS reductase. These 2 GSH-dependent coupled enzymes (GPx and GSH reductase) maintain the GSH/GSSG ratio within the cell, and an imbalance in this ratio generates OS (Al-Turk et al. 1987). Erden-Inal et al. (2002) found no difference between females and males in GSH or GSSG biomarkers. However, they found, in the 28 subjects they studied (2 to 11 years of age), that females had lower GSH/GSSG ratios than

Table 1. Demographic, anthropometric, and blood pressure measurements in prepubertal boys and girls.

Parameter	Girls, $n = 53$	Boys, $n = 85$	p
Age (y)	8.78±0.90	10.16±0.97	<0.001
SBP (mm Hg)	115.20±13.64	125.89±12.65	<0.001
DBP (mm Hg)	63.92±10.36	69.14±9.15	0.003
Weight (kg)	36.94±9.14	45.89±12.84	<0.001
Height (cm)	138.41±6.31	147.19±9.97	<0.001
BMI (kg·m^{-2})	19.08±3.68	20.83±3.74	0.010
WC (cm)	63.62±9.11	69.56±11.09	0.002
CRF, low (%)	60.4	37.8	0.170
Non-PA practice (%)	52.8	34.5	0.520

Note: For SBP, data are in 90th to 95th percentile in girls vs. >95th percentile in boys; for DBP, data are in the 75th percentile in girls vs. the 90th percentile in boys. The 90th percentile is 1.28 SD, the 95th percentile is 1.645 SD, and the 99th percentile is 2.326 SD over the mean in boys and girls (Working group on High Blood Pressure in Children and Adolescents 2004). Statistical significance after application of Student's *t* test (or Mann–Whitney *U* test) to data expressed as means ± SD. For CRF and PA, statistical significance after application of the chi square test. Data are expressed as percentages. BMI, body mass index; CRF, cardiorespiratory fitness; DBP, diastolic blood pressure; PA, physical activity; SBP, systolic blood pressure; WC, waist circumference.

Table 2. Plasma and erythrocyte levels of oxidative stress biomarkers in prepubertal boys and girls before adjustment for age, body mass index, fitness, and physical activity.

Biomarker	Girls	Boys	p
PC	2.00±1.05	4.94±2.93	<0.001
LPO	0.21±0.11	0.22±0.11	0.554
GSH	22.63±3.52	22.52±3.83	0.572
TG	29.01±3.88	25.17±4.95	<0.001
GSSG	6.39±2.33	2.65±1.83	<0.001
GSH/GSSG	4.99±5.36	14.1±12.65	<0.001
SOD	5.43±4.21	2.28±1.85	<0.001
NOx	15.08±3.01	14.27±5.43	0.082
GPx	0.14±0.14	0.25±0.47	0.316

Note: Statistical significance after application of Student's *t* test (or Mann–Whitney *U* test) to data expressed as mean ± SD. GPx, glutathione peroxidase; GSH, reduced glutathione; GSH/GSSG, reduced glutathione/oxidized glutathione ratio; GSSG, oxidized glutathione; LPO, lipid peroxidation products; NOx, total nitrites; PC, proteins carbonyl; SOD, superoxide dismutase; TG, total glutathione.

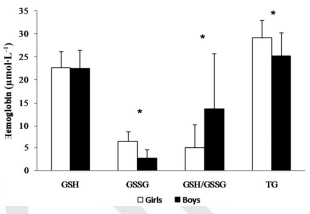

Fig. 1. Levels of oxidized glutathione (GSSG), reduced glutathione (GSH), reduced GSH/GSSG ratio, and total GSH (TG) in erythrocytes in prepubertal boys and girls. Data are expressed as means ± SD. *, Statistical significance after analysis of covariance adjusted for age, body mass index, fitness, and physical activity ($p < 0.001$).

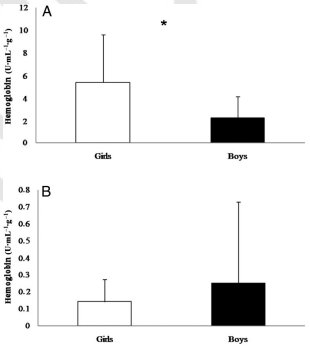

Fig. 2. Levels of (A) superoxide dismutase (SOD) and (B) glutathione peroxidase (GPx) activity in erythrocytes in prepubertal boys and girls. Data are expressed as means ± SD. *, Statistical significance after analysis of covariance adjusted for age, body mass index, fitness, and physical activity ($p < 0.001$).

males. The results obtained by Erden-Inal et al. (2002) match what we found in our larger sample; GSH/GSSG ratios were lower in girls than in boys, and GSSG levels were higher.

Our results suggest that healthy girls are more prone to OS than boys, although boys had higher protein carbonyl levels. There are different types of protein oxidative modification, and there is no single universal marker for protein oxidation (Dalle-Donne et al. 2003). However, the ratio of reduced GSH to GSSG is an indicator of cellular health, with reduced GSH constituting up to 98% of cellular GSH under normal conditions. Therefore, the GSH/GSSG ratio is reduced in some diseases and is an excellent biomarker of cellular redox potential (Owen and Butterfield 2010). In boys, the significant increase in the GSH/GSSG ratio, resulting from lower GSSG levels, suggests that boys have a lower oxidant status.

The body has developed a complex defense strategy to minimize the damaging effects of oxidants. Central to this defense are antioxidant enzymes, which include SOD and GPx (Franco et al. 2007). A study conducted in a Turkish population established that age, gender, and physical exercise are associated with SOD and GPx (Ozbay and Dülger 2002). That study found no gender-based differences in any antioxidant enzyme; however, there were higher levels of SOD and GPx in children, adolescents, and adults than in elderly people after acute exercise, and lower levels of SOD and GPx in adults. In our study, levels of SOD were higher in girls than in boys, although no differences were found in GPx levels. These results were not dependent on age, exercise, or cardiorespiratory fitness. Other studies in children have observed elevated erythrocyte SOD activity (Aydin et al. 2001), which could be a compensatory mechanism against superoxide radical overproduction. This protective mechanism has also been observed in some disorders characterized by the presence of OS, such as obe-

sity (Erdeve et al. 2004) and atherosclerosis (Sierakowska-Fijalek et al. 2008) in children.

The role gender plays in physiological changes in the oxidation system is still not clear. In neonates, it has been suggested that gender-related differences exist in the maturation of the enzymatic systems in different tissues (Lavoie and Chessex 1997). Nikolaidis et al. (2007) describe the possible influence of different factors, such as age, environment, and lifestyle, on both sexes. However, gender is the only interference variable that has been shown to have an independent relation with the production of OS. Further studies are needed to assess the influence of age and sex on OS, and to try to understand the physiological mechanism that induces this situation.

Conclusion

Prepubertal girls have more OS than boys, and higher levels of SOD, independent of age, BMI, fitness, and PA. Future paediatric research could provide information about the influence of gender on OS mechanisms.

References

Ali, S.S., Xiong, C., Lucero, J., Behrens, M.M., Dugan, L.L., and Quick, K.L. 2006. Gender differences in free radical homeostasis during aging: shorter-lived female C57BL6 mice have increased oxidative stress. Aging Cell, 5: 565–574. doi:10.1111/j.1474-9726.2006.00252.x. PMID:17129217.

Al-Turk, W.A., Stohs, S.J., el-Rashidy, F.H., and Othman, S. 1987. Changes in glutathione and its metabolizing enzymes in human erythrocytes and lymphocytes with age. J. Pharm. Pharmacol. 39: 13–16. doi:10.1111/j.2042-7158.1987.tb07154.x. PMID:2880977.

Aydin, A., Orhan, H., Sayal, A., Ozata, M., Sahin, G., and Isimer, A. 2001. Oxidative stress and nitric oxide related parameters in type II diabetes mellitus: effects of glycemic control. Clin. Biochem. 34: 65–70. doi:10.1016/S0009-9120(00)00199-5. PMID:11239518.

Bureau, I., Gueux, E., Mazur, A., Rock, E., Roussel, A.M., and Rayssiguier, Y. 2003. Female rats are protected against oxidative stress during copper deficiency. J. Am. Coll. Nutr. 22: 239–246. PMID:12805251.

Cakin-Atabek, H., Demir, S., PinarbaSili, R.D., and Gündüz, N. 2010. Effects of different resistance training intensity on indices of oxidative stress. J. Strength Cond. Res. 24: 2493–2497. doi:10.1519/JSC.0b013e3181dcfd11. PMID:20802287.

Casado, A., de la Torre, R., and López-Fernández, M.E. 2007. Copper/zinc superoxide dismutase activity in newborns & young people in Spain. Indian J. Med. Res. 125: 655–660. PMID:17642501.

Cavas, L., and Tarhan, L. 2004. Effects of vitamin–mineral supplementation on cardiac marker and radical scavenging enzymes, and MDA levels in young swimmers. Int. J. Sport Nutr. Exerc. Metab. 14: 153–166. PMID:15318388.

Cheeseman, K.H. 1993. Lipid peroxidation in biological systems. *In* DNA and free radicals. *Edited by* B. Halliwell and O.I. Auroma. Ellis Harward, London, UK.

Cooper, D.M., Nemet, D., and Galassetti, P. 2004. Exercise, stress, and inflammation in the growing child: from the bench to the playground. Curr. Opin. Pediatr. 16: 286–292. doi:10.1097/01.mop.0000126601.29787.39. PMID:15167015.

Dalle-Donne, I., Rossi, R., Giustarini, D., Milzani, A., and Colombo, R. 2003. Protein carbonyl groups as biomarkers of oxidative stress. Clin. Chim. Acta, 329: 23–38. doi:10.1016/S0009-8981(03)00003-2. PMID:12589963.

Erdelmeier, I., Gérard-Monnier, D., Yadan, J.C., and Chaudière, J. 1998. Reactions of *N*-methyl-2-phenylindole with malondialdehyde and 4-hydroxyalkenals. Mechanistic aspects of the colorimetric assay of lipid peroxidation. Chem. Res. Toxicol. 11: 1184–94. doi:10.1021/tx9701802. PMID:9778315.

Erden-Inal, M., Sunal, E., and Kanbak, G. 2002. Age-related changes in the glutathione redox system. Cell Biochem. Funct. 20: 61–66. doi:10.1002/cbf.937. PMID:11835271.

Erdeve, O., Sikkar, Z., Kocatürk, P.A., Dallar, Y., and Kavas, G.O. 2004. Antioxidant superoxide dismutase activity in obese children. Biol. Trace. Elem. Res. 98: 219–228. doi:10.1385/BTER:98:3:219. PMID:15131319.

Esterbauer, H., and Cheeseman, K.H. 1987. Lipid peroxidation: Pathological implications. Chem. Phys. Lipids, 45: 105–107.

Fisher-Wellman, K., and Bloomer, R.J. 2009. Acute exercise and oxidative stress: a 30 year history. Dyn. Med. 8: 1. doi:10.1186/1476-5918-8-1. PMID:19144121.

Flohé, L., and Günzler, W.A. 1984. Assays of glutathione peroxidase. Methods Enzymol. 105: 114–121. doi:10.1016/S0076-6879(84)05015-1. PMID:6727659.

Franco, M.C., Kawamoto, E.M., Gorjão, R., Rastelli, V.M., Curi, R., Scavone, C., et al. 2007. Biomarkers of OS and antioxidant status in children born small for gestational age: evidence of lipid peroxidation. Pediatr. Res. 62: 204–208. doi:10.1203/PDR.0b013e3180986d04. PMID:17597662.

Gomez-Cabrera, M.C., Domenech, E., and Viña, J. 2008. Moderate exercise is an antioxidant: upregulation of antioxidant genes by training. Free Radic. Biol. Med. 44: 126–131. doi:10.1016/j.freeradbiomed.2007.02.001. PMID:18191748.

Guemouri, L., Artur, Y., Herbeth, B., Jeandel, C., Cuny, G., and Siest, G. 1991. Biological variability of superoxide dismutase, glutathione peroxidase and catalase in blood. Clin. Chem. 37: 1932. PMID:1934468.

Habif, S., Mutaf, I., Turgan, N., Onur, E., Duman, C., Özmen, D., et al. 2001. Age and gender dependent alterations in the activities of glutathione related enzymes in healthy subjects. Clin. Biochem. 34: 667–671. doi:10.1016/S0009-9120(01)00279-X. PMID:11849628.

Heitzer, T., Schlinzig, T., Krohn, K., Meinertz, T., and Münzel, T. 2001. Endothelial dysfunction, oxidative stress, and risk of cardiovascular events in patients with coronary artery disease. Circulation, 104: 2673–2678. doi:10.1161/hc4601.099485. PMID:11723017.

Jakus, V., and Rietbrock, N. 2004. Advanced glycation end-products and the progress of diabetic vascular complications. Physiol. Res. 53(2): 131–142. PMID:15046548.

Kabasakalis, A., Kalitsis, K., Nikolaidis, M.G., Tsalis, G., Kouvelas, D., Loupos, D., et al. 2009. Redox, iron, and nutritional status of children during swimming training. J. Sci. Med. Sport. 12: 691–696. doi:10.1016/j.jsams.2008.05.005. PMID:18768362.

Kasapis, C., and Thompson, P.D. 2005. The effects of physical activity on serum C-reactive protein and inflammatory markers: a systematic review. J. Am. Coll. Cardiol. 45: 1563–1569. doi:10.1016/j.jacc.2004.12.077. PMID:15893167.

Kerksick, C., Taylor, L., Harvey, A., and Willoughby, D. 2008. Gender-related differences in muscle injury, oxidative stress, and apoptosis. Med. Sci. Sports. Exerc. 40: 1772–1780. doi:10.1249/MSS.0b013e31817d1cce. PMID:18799987.

Lavoie, J.C., and Chessex, P. 1997. Gender and maturation affect glutathione status in human neonatal tissues. Free Radic. Biol. Med. 23: 648–657. doi:10.1016/S0891-5849(97)00017-7. PMID:9215830.

Léger, L.A., Mercier, D., Gadoury, C., and Lambert, J. 1988. The multistage 20 meter shuttle run test for aerobic fitness. J. Sports Sci. 6: 93–101. doi:10.1080/02640418808729800. PMID:3184250.

Levine, R.L., Garland, D., Oliver, C.N., Amici, A., Climent, I., Lenz, A.G., et al. 1990. Determination of carbonyl content in oxidatively modified proteins. Methods Enzymol. 186: 464–478. doi:10.1016/0076-6879(90)86141-H. PMID:1978225.

Llorente-Cantarero, F.J., Gil-Campos, M., Benito-Sillero, J.D., Muñoz-Villanueva, M.C., Túnez, I., and Pérez-Navero, J.L. 2012. Prepubertal children with suitable fitness and physical activity present reduced risk of oxidative stress. Free Radic. Biol. Med. 53: 415–420. doi:10.1016/j.freeradbiomed.2012.05.017. PMID:22634054.

Luo, S., and Levine, R.L. 2009. Methionine in proteins defends against oxidative stress. FASEB J. 23: 464–472. doi:10.1096/fj.08-118414. PMID:18845767.

Miller, A.A., De Silva, T.M., Jackman, K.A., and Sobey, C.G. 2007. Effect of gender and sex hormones on vascular oxidative stress. Clin. Exp. Pharmacol. Physiol. 34: 1037–1043. doi:10.1111/j.1440-1681.2007.04732.x. PMID:17714091.

Nikolaidis, M.G., Kyparos, A., Hadjicharalambous, M., Panou, N., Samaras, L., Jamurtas, A.Z., et al. 2007. Acute exercise markedly increases blood oxidative stress in boys and girls. Appl. Physiol. Nutr. Metab. 32: 197–205. doi:10.1139/h06-097. PMID:17486140.

Noiri, E., and Tsukahara, H. 2005. Parameters for measurement of oxidative stress in diabetes mellitus: applicability of enzyme-linked immunosorbent assay for clinical evaluation. J. Investig. Med. 53: 167–175. doi:10.2310/6650.2005.00403. PMID:15974243.

Olds, T., Tomkinson, G., Léger, L., and Cazorla, G. 2006. Worldwide variation in the performance of children and adolescents: an analysis of 109 studies of the 20-m shuttle run test in 37 countries. Sports Sci. 24: 1025–1038. doi:10.1080/02640410500432193. PMID:17115514.

Oliver, S.R., Rosa, J.S., Miller, G.L., Pourtello, A.M., Borntrager, H.I., Heydari, S., et al. 2010. Increased oxidative stress and altered substrate metabolism in obese children. Int. J. Pediatr. Obes. 5: 436–444. doi:10.3109/17477160903565163. PMID:20233149.

Owen, J.B., and Butterfield, D.A. 2010. Measurement of oxidized/reduced glutathione ratio. Methods Mol. Biol. 648: 269–277. doi:10.1007/978-1-60761-756-3_18. PMID:20700719.

Ozbay, B., and Dülger, H. 2002. Lipid peroxidation and antioxidant enzymes in Turkish population: relation to age, gender, exercise, and smoking. Tohoku J. Exp. Med. 197: 119–124. doi:10.1620/tjem.197.119. PMID:12233785.

Pansarasa, O., Castagna, L., Colombi, B., Vecchiet, J., Felzani, G., and Marzatico, F. 2000. Age and sex differences in human skeletal muscle: role of reactive oxygen species. Free. Radic. Res. 33: 287–293. doi:10.1080/10715760000301451. PMID:10993482.

Pavlova, E.L., Lilova, M.I., and Savov, V.M. 2005. Oxidative stress in children with kidney disease. Pediatr. Nephrol. 20: 1599–1604. doi:10.1007/s00467-005-1990-x. PMID:16001281.

Pepe, H., Balci, S.S., Revan, S., Akalin, P.P., and Kurtoglu, F. 2009. Comparison of oxidative stress and antioxidant capacity before and after running exercises in both sexes. Gend. Med. 6: 587–595. doi:10.1016/j.genm.2009.10.001. PMID: 20149009.

Pérez-Navero, J.L., Benítez-Sillero, J.D., Gil-Campos, M., Guillén-del Castillo, M., Tasset, I., and Túnez, I. 2009. Changes in oxidative stress biomarkers induced by puberty. An. Pediatr. 70: 424–428. doi:10.1016/j.anpedi.2009.01.019. PMID: 19375988.

Pianta, R.C., and ICHD, N 2007. Developmental science and education: the NICHD study of early child care and youth development findings from elementary school. Adv. Child Dev. Behav. 35: 253–296. doi:10.1016/B978-0-12-009735-7.50012-8. PMID:17682328.

Picot, I.C., Trivier, J.M., Nicole, A., Sinet, P.M., and Thevenin, M. 1992. Age correlated modifications of copper-zinc superoxide dismutase and glutathione related enzyme activities in human erythrocytes. Clin. Chem. 38: 66. PMID: 1733608.

Price, J.A., Samy, C.G., and Shevlin, D. 2006. Application of manual assessment of oxygen radical absorbent capacity (ORAC) for use in high throughput assay of "total" antioxidant activity of drugs and natural products. J. Pharmacol. Toxicol. Methods, 54: 56–61. doi:10.1016/j.vascn.2005.11.002. PMID:16337142.

Proteggente, A.R., England, T.G., Rehman, A., Rice-Evans, C.A., and Halliwell, B. 2002. Gender differences in steady-state levels of oxidative damage to DNA in healthy individuals. Free Radic. Res. 36: 157–162. doi:10.1080/10715760290006475. PMID:11999383.

Rahman, I., Kode, A., and Biswas, S.K. 2006. Assay for quantitative determination of glutathione and glutathione disulfide levels using enzymatic recycling method. Nat. Protoc. 1: 3159–3165. doi:10.1038/nprot.2006.378. PMID: 17406579.

Ricart-Jané, D., Llobera, M., and López-Tejero, M.D. 2002. Anticoagulants and other preanalytical factors interfere in plasma nitrate/nitrite quantification by the Griess method. Nitric Oxide, 6: 178–185. doi:10.1006/niox.2001.0392. PMID:11890742.

Rush, J.W., and Sandiford, S.D. 2003. Plasma glutathione peroxidase in healthy young adults: influence of gender and physical activity. Clin. Biochem. 36: 343–351. doi:10.1016/S0009-9120(03)00039-0. PMID:12849865.

Santos-Silva, A., Rebelo, M.I., Castro, E.M., Belo, L., Guerra, A., Rego, C., et al. 2001. Leukocyte activation, erythrocyte damage, lipid profile and oxidative stress imposed by high competition physical exercise in adolescents. Clin. Chim. Acta, 306: 119–126. doi:10.1016/S0009-8981(01)00406-5. PMID:11282102.

Sierakowska-Fijalek, A., Fijalkowski, P., Blaszczyk, J., Baj, Z., Stepień, M., Wosik-Irenbeck, M., et al. 2008. Estimation of selected parameters antioxidant barrier and lipid parameters in children with atherosclerosis risk factors. Pol. Merkur. Lekarski, 24: 14–17. PMID:18619141.

Tanner, J.M. 1962. Growth at adolescente. Blackwell, Oxford, UK.

Toyokuni, S. 1999. Reactive oxygen species-induced molecular damage and its application in pathology. Pathol. Int. 49: 91–102. doi:10.1046/j.1440-1827.1999.00829.x. PMID:10355961.

Tsukahara, H. 2007. Biomarkers for oxidative stress: clinical application in pediatric medicine. Curr. Med. Chem. 14: 339–351. doi:10.2174/092986707779941177. PMID:17305536.

Working group on High Blood Pressure in Children and Adolescents. 2004. National High Blood Pressure Education Program. Pediatrics, 114(2): 555–576. PMID:15286277.