Anti-oxidation improves in early puberty in normal weight and obese boys, in positive association with exercise stimulated growth hormone secretion

George Paltoglou1,2, Ioannis G. Fatouros3, George Valsamakis2, Maria Schoina3, Alexandra Avloniti 3, Athanasios Chatzinikolaou3, Antonis Kambas3, Dimitris Draganidis3, Aimilia Mantzou4, Maria Papagianni5, Christina Kanaka-Gantenbein2, George P. Chrousos2, George Mastorakos1

Institutions:

1. Endocrine Unit, Second Department of Obstetrics and Gynecology, Aretaieion Hospital, Athens University Medical School, Athens, Greece.
2. First Department of Pediatrics, "Aghia Sofia" Children's Hospital, Athens University Medical School, Athens, Greece.
3. School of Physical Education and Sports Sciences, Democritus University of Thrace, Komotini, Greece
4. Endocrine Unit, Evgenidion Hospital, Athens University, Athens, Greece.
5. Pediatric Endocrinology Unit, Third Department of Pediatrics, Hippokrateion Children's Hospital, Aristotle University Medical School, Thessaloniki, Greece.

ABSTRACT

Oxidative stress in humans has been associated with obesity. Puberty is a maturation period characterized mainly by changes of the growth hormone (GH) and the gonadotrophin hormones secretion. To investigate the possible association of the GH and the hypothalamic-pituitary-gonadal (HPG) axes before and during early puberty, with the pro- and anti- oxidation mechanisms 76 healthy, pre-pubertal normal weight (N=28), pre-pubertal obese (N=11), early pubertal normal weight (N=25) and early pubertal obese (N=12) male pupils of the 5th and 6th grades of an elementary school were studied at baseline and after a sub-maximal exercise protocol (on a stationary cycle ergometer) at 70% VO2max. All subjects underwent blood sampling before and after this exercise bout for measurement of pro- [thiobarbituric acid reactive substances (TBARS) and protein carbonyls (PC)s] and anti- [glutathione (GSH) and the oxidized glutathione disulfide (GSSG), the GSH/GSSG ratio, the enzymes glutathione peroxidase (GPX) and catalase and total antioxidant capacity (TAC)] oxidation markers and hormones (GH, IGF1, IGFBP3, FSH, LH, and testosterone).

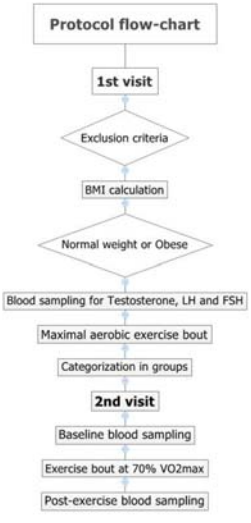
Introduction

Oxidative stress: defines a state of imbalance between pro- and anti- oxidation within the cell. Oxidative stress in humans has been associated with obesity and resulting co-morbidities. Childhood obesity has been associated with oxidative stress even before co-morbidities occur.
Pro-oxidation: refers to mitochondrial and non-mitochondrial mechanisms, which generate reactive oxygen and nitrogen species (RONS).
Anti-oxidation: refers to the adaptive activation of enzymatic and/or non-enzymatic mechanisms, which scavenge pro-oxidants and their products within cells and in extracellular body fluids.

For the measurement of oxidative we use:
Markers of pro-oxidation: Thiobarbituric acid reactive substances (TBARS) and protein carbonyls (PC)s.
Markers of anti-oxidation: Glutathione (GSH) and oxidized glutathione disulfide (GSSG), the enzymes glutathione peroxidase (GPX) and catalase and the so-called total antioxidant capacity (TAC).

Obesity: A modern day epidemic characterized by increased pro- and reduced anti-oxidation, even in children
Puberty: a maturation period in human development, characterized by changes in the dynamically regulated hypothalamic-growth hormone (GH)-insulin like growth factor (IGF)1 and hypothalamic-pituitary-gonadal (HPG) axes.
Children with GH deficiency demonstrate decreased production of anti-oxidation markers, which increase upon GH replacement therapy 12. In addition, in adults with GH deficiency endothelial dysfunction is common and associated with the increased levels of pro-oxidation markers, which decrease following GH replacement therapy.
Previous studies have suggested that anti-oxidation in humans matures with age.
Exercise: represents a potent stimulus of pro- and anti-oxidation mechanisms, the GH axis (potentiated along puberty) and various components of the HPG axis.

To investigate the pro- and anti- oxidation mechanisms in pre- and early puberty regarding the presence of obesity as well as their possible association with the GH and HPG axes, seventy-six pre- and early pubertal normal weight and obese boys were studied at baseline and after an acute bout of aerobic exercise at 70% of maximal oxygen consumption (VO2max). Markers of the hormonal axes, of pro- and anti- oxidation were measured.



Subjects and Methods

Protocol

The study was approved by the Institutional Review Board and was conducted in accordance with the Declaration of Helsinki as revised in 1996. Informed written consent was obtained from the parent/guardians of each child while children gave verbal consent to participate in the study. The protocol was performed in two visits separated by two weeks in a university ergophysiology laboratory.

First visit (subject selection and maximal oxygen consumption measurement)

Exclusion criteria: a) exercise additional to that included in the school time-table, b) nutritional intervention within the six months preceding this study, c) history of diabetes, insulin resistance, dyslipidemia, cardiovascular disease, and hypertension or other known chronic pathology
Obesity: BMI calculation and comparing to the standard BMI curves for the greek pediatric population, according to the International Obesity Task Force (IOTF) criteria
Subjects were considered normal weight or obese when their projected BMI value for the age of 18 years was lower than 25 kg/m2 or between 30 and 35 kg/m2, respectively
Puberty: Subjects with testosterone concentration greater than 0.2 ng/ml were considered as early pubertal
Subjects' characteristics are shown in Table 1.
Maximal oxygen consumption (VO2max): Participants had their maximal oxygen consumption (VO2max) measured, by performing a graded exercise test until maximum exercise tolerance on a stationary cycle ergometer (Monark 834E, Sweden. Open-circuit spirometry *via* continuous breath-by-breath analysis (averaged every 30s) was used to measure VO2max with an automated online pulmonary gas exchange system (SensorMedics 2900c, SensorMedics Corporation, USA). Heart rate, 12-lead electrocardiogram, blood pressure and ratings of perceived exertion were monitored continuously throughout testing and during recovery. VO2max was attained if: a) subject reached exhaustion (a pedaling rate <60revolutions/min), b) respiratory exchange ratio was ≥1.10, c) a VO2 plateau was observed (<2mL/kg/min) despite further increases of the workload, d) heart rate exceeded 200 beats/min.

Second visit (Baseline sampling, aerobic exercise bout and post-exercise sampling)

During their second visit, a baseline blood sampling was performed and following that all participants completed successfully an acute bout of aerobic exercise on a stationary cycle ergometer (Monark 834E, Sweden) until exhaustion (a pedalling rate < 60 revolutions/min) at an intensity corresponding to 70% of their VO2max. After the exercise bout a second (post-exercise) blood sampling was performed.

Table 1: Anthropometric data in normal weight and obese, pre- and early pubertal subjects.
Measurements were compared with Factors ANOVA followed by LSD Fischer's post-hoc test (P<0.05)
+ denotes significant difference (P<0.05) between obese and respective normal weight subjects.
# denotes significant difference (P<0.05) between early pubertal and respective pre-pubertal subjects.

	Pre-pubertal (n=39)		Early pubertal (n=37)	
	Normal weight (n=28)	**Obese** (n=11)	**Normal weight** (n=25)	**Obese** (n=12)
Age (yrs)	10.46±0.27	10.77±0.36	11.30±0.26#	11.66±0.22
Height (m)	1.41±0.02	1.39±0.03	1.49±0.02	1.44±0.04
Weight (kg)	37.15±2.29	55.81±3.77 +	45.60±2.13	61.88±2.74 +
BMI (kg/m^2)	18.47±0.53	28.28±0.98 +	20.55±0.42	29.89±0.46 +
BMI z-score	-0.18±0.13	2.64±0.36 +	0.27±0.13	2.92±0.16 +
Waist circumference (cm)	66.17±1.44	89.31±3.35 +	74.91±1.75	92.60±2.51 +

* denotes significant difference (P<0.05) of post-exercise from the respective baseline concentrations;
+ denotes significant difference (P<0.05) between obese and respective normal weight subjects;
# denotes significant difference (P<0.05) between early pubertal and respective pre-pubertal subjects.

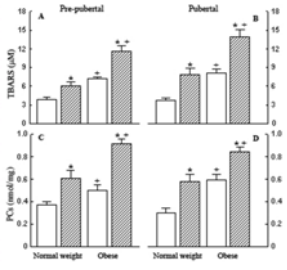


Figure 1: Markers of pro-oxidation: Thiobarbituric acid reactive substances (TBARS) and protein carobonyls (PCs), concentrations (mean±SE) at baseline (white bars) and post-exercise (at 70% VO2max) (shaded bars) in pre-pubertal (panels A and C, respectively) and pubertal (panels B and D, respectively) normal weight and obese subjects.

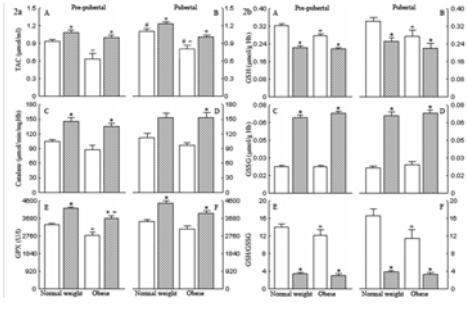


Figure 2A(left): Markers of anti-oxidation: Total antioxidant capacity (TAC), catalase and glutathione peroxidase (GPX) activity values (mean±SE) at baseline (white bar) and post-exercise (at 70% VO2max) (shaded bar) in pre-pubertal (panels A, C and E, respectively) and early pubertal (panels B, D and F, respectively) normal weight and obese subjects.
Figure 2B(right): Markers of anti-oxidation: Glutathione (GSH), oxidized glutathione (GSSG) concentrations and glutathione to oxidized glutathione ratio (GSH/GSSG) (mean±SE) at baseline (white bars) and post-exercise (at 70% VO2max) (shaded bars) in pre-pubertal (panels A, C and E, respectively) and early pubertal (panels B, D and F, respectively) normal weight and obese subjects.

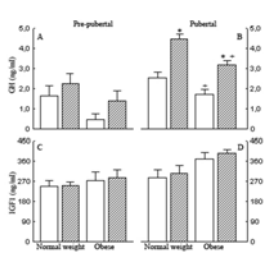


Figure 3: Growth hormone axis: Growth hormone (GH) and insulin-like growth factor (IGF)1 concentrations (mean±SE) at baseline (white bar) and post-exercise (at 70% VO2max) (shaded bar) in pre-pubertal (panels A and C, respectively) and early pubertal (panels B and D, respectively) normal weight and obese subjects.

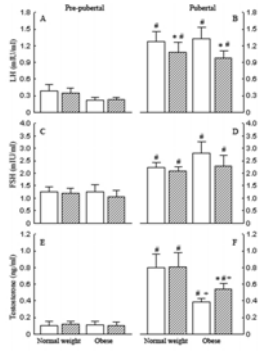


Figure 4: HPG axis: Follicle stimulating hormone (FSH), luteinizing hormone (LH) and testosterone concentrations (mean±SE) at baseline (white bar) and post-exercise (at 70% VO2max) (shaded bar) in pre-pubertal (panels A, C and E, respectively) and early pubertal (panels B, D and F, respectively) normal weight and obese subjects.

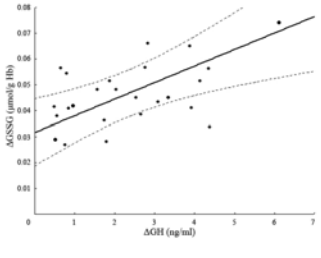


Figure 5: Statistically significant linear correlation in early pubertal normal weight subjects between ΔGH and ΔGSSG (P<0.05; r = 0.94).

PREDICTORS
In all subjects taken as a single group, forward stepwise regression analysis was employed to reveal potential predictors of the post-exercise concentrations of the pro- (PCs, TBARS) and the anti- (GSH, GSSG, GSH/GSSG ratio, Catalase, TAC) oxidation markers, each one taken as dependent variable, among baseline waist circumference, VO2max, GH, IGF1/IGFBP3 ratio, LH and testosterone, all taken as independent variables.
Baseline GH was the best negative predictor (P<0.05; b = – 0.37) for post-exercise concentrations of PCs
Baseline LH was the best positive predictor (P<0.05; b = 0.50) for post-exercise concentrations of TAC
Baseline waist circumference was the best negative and positive predictor for post-exercise concentrations of GPX (P<0.05; b = – 0.72) and TBARS (P<0.05; b = 0.74), respectively.

Discussion

Following an acute bout of aerobic exercise both pro- and anti- oxidation mechanisms are stimulated in normal weight and obese pre- and early pubertal boys. The anti-oxidant capacity of the organism improves with the onset of puberty. This might be related to the finding that GH is positively associated with anti-oxidation at baseline and post-exercise. Obese subjects demonstrate greater and lower pro- and anti- oxidation mechanisms, respectively, than normal-weight subjects, while also demonstrating lower GH concentrations. These observations provide on one hand a conceptual link between early pubertal obesity and increased pro-oxidation, highlighting the deleterious potential of obesity, while on the other hand they suggest the implication of pubertal physiological mechanisms in the maturation of anti-oxidation.
In conclusion, moderate acute aerobic exercise is a good model for the study of pro- and anti-oxidation mechanisms in children and adolescents. The suggested maturation of the anti-oxidation mechanisms during the transition to early puberty in humans should be studied further. Therefore, studies in the future need to investigate the interplay between exercise, onset of puberty and energy consumption and storage, especially regarding the increasing prevalence of obesity in the pediatric population.