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ORIGINAL ARTICLE

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In early pubertal boys, testosterone and LH are associated with improved anti-oxidation during an aerobic exercise bout

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Abstract

Purpose To investigate the association of the hypothalamic-pituitary-testicular (HPT) axis with pro- and anti- oxidation, in relation to puberty and obesity in boys, before and after an aerobic exercise bout.

Methods This is a cross-sectional human observational study of 92 healthy normal-weight, obese pre- and early- pubertal boys that underwent a blood sampling, before, and after an aerobic exercise bout at 70% VO_2max, until exhaustion. LH, FSH, total testosterone (tT) and markers of pro- (TBARS and PCs) and anti- (GSH, GSSG, GPX, catalase, TAC) oxidation were measured.

Results Baseline LH, FSH, and tT concentrations were greater in early, than in pre- pubertal boys, independently of weight status. Post-exercise, LH concentrations decreased in early pubertal boys while FSH concentrations did not change in any of the studied groups. Baseline and post-exercise tT concentrations were lower in obese than in normal-weight early pubertal boys, while baseline and post-exercise LH and FSH concentrations did not differ between these groups. Post-exercise tT concentrations increased in early pubertal obese boys. Baseline LH, FSH and tT concentrations correlated positively with baseline anti-oxidation markers concentrations in pre-pubertal boys. Baseline tT concentrations correlated positively with the increase of TAC concentrations in early pubertal normal-weight boys. In all boys, baseline LH concentrations were the best positive predictors for the exercise-associated increase of TAC concentrations.

Conclusions It appears that the HPT axis maturation during puberty (in particular its LH and testosterone components) is positively associated with the increase of anti-oxidation during a bout of aerobic exercise.

Keywords HPT axis · obesity · puberty · LH, FSH, testosterone

Introduction

Puberty is a maturation period in human development, when sexual characteristics and reproductive competence are developed [1]. In boys, it is characterized by changes of the dynamically regulated hypothalamic-pituitary-testicular (HPT) axis [2]. In pubertal boys, testosterone secretion increases in parallel to testicular volume [3]. Obesity in

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childhood and especially after the onset of puberty is associated with a relative hypogonadal state [4]. Obesity is characterized by lower total testosterone (tT) concentrations, probably due to insulin resistance-associated decreased concentrations of SHBG secretion [5]. Testosterone aromatization to estradiol in the adipose tissue results in decreased free testosterone concentrations [4].

Oxidative stress is a state of imbalance between pro- and anti- oxidation within the cell [6]. Pro-oxidation refers to mitochondrial and non-mitochondrial mechanisms, which generate reactive oxygen and nitrogen species [6]. Anti-oxidation is the adaptive activation of enzymatic, and non-enzymatic mechanisms, that counterbalance pro-oxidation by activating the scavengers of pro-oxidants, and their products within cells, and in extracellular body fluids [6, 7]. In the past we have shown that early puberty is associated with an increase of antioxidant capacity, as compared to pre-puberty, in, both, normal-weight, and obese boys [8]. Obese individuals present with increased pro-oxidation and decreased anti-oxidation as compared to normal-weight individuals, even in childhood and in the transition to early puberty [8, 9].

Reactive oxygen, and nitrogen species-induced damage of enzymes may result in decreased steroidogenic capacity of cultured Leydig cells [10]. Total antioxidant capacity (TAC) is significantly lower in adult men with decreased testosterone, when compared with that of normal controls, while a strong positive correlation between TAC, and testosterone concentrations exists [11]. In adult men, anti-oxidation is lower in isolated hypogonadism than in normogonadism, an effect that is reversed after physiologic testosterone replacement [12]. Conversely, supraphysiological doses of testosterone downregulate gene expression of eNOS and consequently the formation of NO; this mechanism might be involved in the detrimental cardiovascular side effects of testosterone abuse [13].

An acute exercise bout interferes with HPT axis homeostasis [14]. Luteinizing hormone (LH) concentrations decrease after acute aerobic exercise in adolescent boys, while follicle stimulating hormone (FSH) concentrations remain unchanged after exercise [15, 16]. Testosterone concentrations increase after acute exercise bouts. This increase correlates with both chronological age and pubertal status, and is more evident in adult males [17]. It has been suggested that training exercise protocols of variable intensity may result to direct, or indirect lactate-induced testosterone increase [18, 19]. An acute bout of aerobic exercise represents a potent stimulus of energy substrate use in the mitochondrion and the cytosol that leads to the activation of pro- and the recruitment of anti- oxidation [20]. In the past we have shown that acute exercise stimulates pro- and anti- oxidation in pre- and early pubertal boy [8].

To investigate the hypothesis of putative associations of gonadotrophins and tT concentrations with the increase of anti-oxidant capacity from pre- to early puberty, ninety-two pre- and early pubertal normal-weight and obese boys were studied at baseline and after an acute bout of aerobic exercise at 70% VO_2max. The latter was employed as a stimulus of energy substrate use and testosterone secretion.

Materials and methods

Subjects

The summary of the exercise protocol is depicted in the participants flow chart (Fig. 1). All male subjects from the

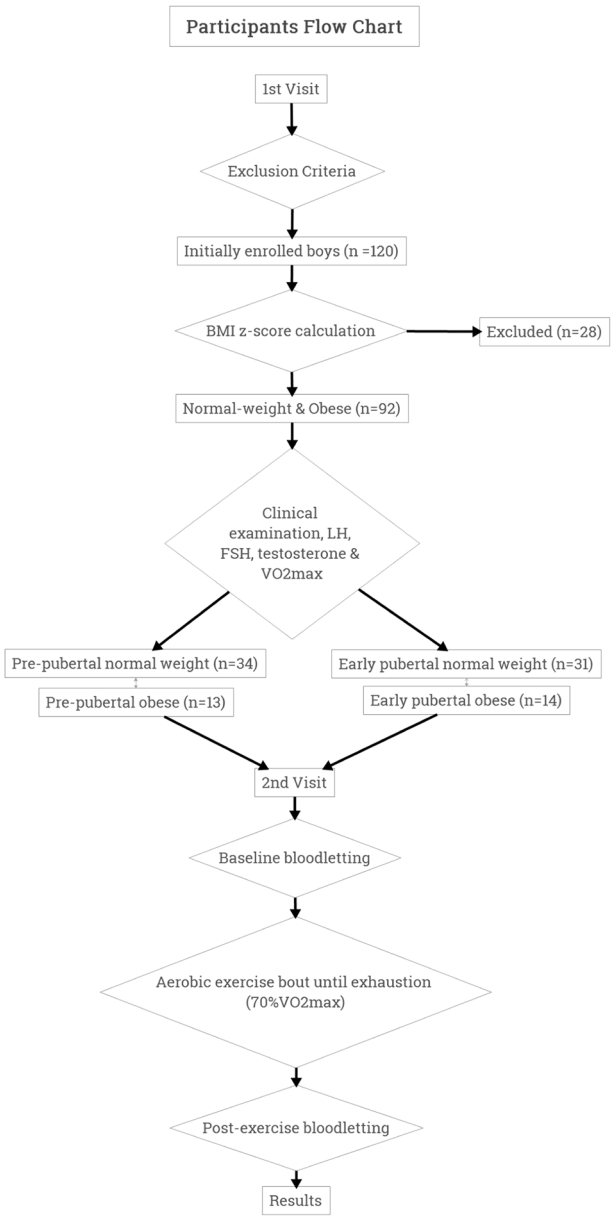

Fig. 1 Flow-chart of the selection of the study participants

Table 1 Anthropometric data in normal weight and obese pre- and early pubertal boys

	Pre-pubertal ($n = 47$)		Early pubertal ($n = 45$)	
	Normal weight ($n = 34$)	Obese ($n = 13$)	Normal weight ($n = 31$)	Obese ($n = 14$)
Age (yrs)	10.32 ± 0.24	10.43 ± 0.38	11.53 ± 0.22[a]	11.71 ± 0.33[a]
Height (m)	1.43 ± 0.03	1.38 ± 0.04	1.48 ± 0.02	1.43 ± 0.05
Weight (kg)	37.24 ± 2.24	55.74 ± 3.86[b]	45.57 ± 2.23	61.91 ± 2.63[b]
BMI (kg/m^2)	18.44 ± 0.64	28.26 ± 0.93[b]	20.57 ± 0.44	29.86 ± 0.49[b]
BMI z-score	0.16 ± 0.15	2.62 ± 0.34[b]	0.22 ± 0.18	2.91 ± 0.18[b]
Waist circumference (cm)	66.22 ± 1.45	89.35 ± 3.42[b]	75.87 ± 1.69	91.59 ± 1.98[b]
Hip circumference (cm)	75.3 ± 2.4	90.1 ± 4.1[b]	81.7 ± 1.9[a]	92.3 ± 1.9[b]
Waist to hip ratio	0.86 ± 0.01	0.95 ± 0.01[b]	0.89 ± 0.01	0.97 ± 0.01[b]
Waist to height ratio	0.49 ± 0.02	0.65 ± 0.03[b]	0.48 ± 0.01	0.66 ± 0.02[b]
VO_2max (l/min.)	1.88 ± 0.02	1.85 ± 0.03	1.92 ± 0.02	1.72 ± 0.02[b]

Measurements were compared among groups by employing factors ANOVA. Significant main effects were revealed by the Unequal N HSD *post-hoc* test. Statistical significance was set at ($P < 0.05$)

[a]Denotes significant difference between early pubertal and respective pre-pubertal boys

[b]Denotes significant difference between obese and respective normal weight boys

5th (born between 2005 and 2006) and 6th (born between 2006 and 2007) grades of an elementary school of a sub-urban Greek town of Thrace, Greece were recruited for this study. Exclusion criteria included: (a) exercise additional to that included in the school time-table; (b) dietetic intervention in the six months preceding this study; (c) medical history that included diabetes, dyslipidemia, cardiovascular disease, hypertension, overt hypothyroidism, or any other endocrine condition such as Klinefelter syndrome, or other known chronic metabolic pathology. In total, 120 male subjects were initially screened for this study. Following inclusion, body mass index (BMI) was calculated and compared to the standard BMI curves for the Greek pediatric population, according to the International Obesity Task Force (IOTF) criteria, in order to select among them normal-weight and obese boys [21, 22]. Normal-weight, or obese boys were considered those that had a projected BMI value for the age of 18 years lower than 25 kg/m^2 or between 30 and 35 kg/m^2, respectively [22]. At the end, 92 healthy normal-weight ($n = 65$) and obese ($n = 27$) boys, from the initially recruited 120 boys, were selected and included in this study (Fig. 1). Then, they had their plasma tT concentrations assessed. Those with tT concentration lower than 0.2 ng/ml, had testes less than 4 ml and were classified as pre-pubertal [Tanner stage I: 47 boys; mean ± SD; aged 10.43 ± 0.35 years old], while those with tT concentration greater than 0.2 ng/ml, had testes equal or greater than 4 ml and were classified as early pubertal [37 boys (Tanner stage II: 30 boys; Tanner stage III: 7 boys); aged 11.63 ± 0.23 years old), respectively] [23, 24]. By combining, both, characteristics (BMI, and pubertal status) four groups of boys were formed: (a) pre-pubertal normal-weight boys ($n = 34$), (b) pre-pubertal obese boys ($n = 13$), (c) early pubertal normal-weight boys ($n = 31$) and (d) early pubertal obese boys ($n = 14$). Their characteristics are presented in Table 1.

Protocol

The study was conducted in accordance with the Declaration of Helsinki and approved by the National and Kapodistrian University of Athens Medical School designated ethics committee. The aims and the procedure of the study were fully disclosed to the parents or legal guardians of the participants, and informed, written consent was obtained from them while boys gave verbal consent before the participation in the study. The study took place in a university laboratory of ergophysiology in two visits, performed within two weeks from the date of initial recruitment.

During the first visit all, initially recruited, boys ($n = 120$) underwent a clinical examination by a certified paediatrician (G.P.). Body weight was measured to the nearest 0.1 kg (Beam Balance 710, Seca, Birmingham, UK), on a digital scale, with boys wearing light clothes, without shoes. Barefoot standing height was measured to the nearest 0.1 cm (Stadiometer 208, Seca, Hanover, MD) while BMI and BMI z-scores were calculated [22]. Blood samples were drawn for testosterone measurements from all boys for the determination of the pubertal development. Maximal oxygen consumption (VO_2max) of the selected boys was measured employing a graded exercise test until maximum exercise tolerance on a stationary cycle ergometer (Monark 834E, Sweden) according to a previously described protocol [25]. VO_2max was measured with an automated online pulmonary gas exchange system that employs open-circuit spirometry *via* continuous breath-by-breath analysis

(averaged every 30 s) (SensorMedics 2900c, SensorMedics Corporation, USA). Throughout testing, and during recovery heart rate, 12-lead electrocardiogram, blood pressure, and ratings of perceived exertion were continuously monitored. VO_2max was attained if either a subject reached exhaustion (a pedalling rate <60 revolutions/min), or respiratory exchange ratio was ≥1.10 or a VO_2 plateau was observed (<2 mL/kg/min) despite further increases of the workload or heart rate exceeded 200 beats/min.

After a 2-week period, normal-weight and obese boys were called in for a second visit. A baseline blood sampling was performed followed by 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 previously measured VO_2max. This bout of exercise was completed successfully by all subjects. A second, post exercise blood sampling was performed at the end of the exercise bout.

Assays

Blood was collected into EDTA tubes, or tubes containing SST-Gel and subsequently centrifuged for plasma and serum separation, respectively. Red blood cells collected after plasma separation were lysed, and the lysate was used for the analysis of catalase activity, glutathione (GSH) concentration and oxidized glutathione disulfide (GSSG) concentration [8]. Serum was used for hormonal analysis, measurement of thiobarbituric acid reactive substances (TBARS) concentration, protein carbonyls (PCs) concentration, and total anti-oxidant capacity (TAC) concentration [8]. Whole blood was used for the measurement of glutathione peroxidase (GPX) activity [8]. Samples were stored in multiple aliquots (serum, and lysate samples at −80 °C, and whole blood at −20 °C), were protected from light and auto-oxidation, and were thawed, once, before analysis. All assays were performed in duplicate and the mean value was recorded.

Oxidative stress markers

Markers of pro- (TBARS and PCs) and anti- (GSH, GSSG, GPX, catalase, TAC) oxidation were measured, as previously reported [8].

Hormones assays

Follicle stimulating hormone, LH, and tT were measured with a solid-phase, competitive chemiluminescent enzyme immunoassay (Immulite 2000, Siemens, Germany). Intra- and inter- assay coefficients of variation and assay sensitivities were 2.9%, 4.1%, and 0.1 mIU/ml for FSH, 3.04%, 6.6%, and 0.05 mIU/ml for LH, and 11.7%, 13.0% and 0.15 ng/ml for testosterone, respectively.

Statistical analyses

All participants completed the two visit and sampling protocol (no missing data). All variables were normally distributed. Results are reported as mean ± SE. Statistical significance was set at $P < 0.05$. All variables assessed at pre- (baseline) and post- exercise were compared by employing repeated-measures analysis of variance test (ANOVA). Differences of variables measured only at baseline were evaluated by employing factorial ANOVA. Significant main effects were tested by Unequal N HSD *post-hoc* test. Within groups correlations of baseline value, and the absolute changes between baseline and post- exercise values (Δ), were evaluated by the Pearson's R coefficient. To further examine inter-subject variability and reduce the chance of spurious correlations, multiple regression analysis of all subjects taken as a single group was used to test potential significant linear regressions among the changes (Δ) of LH, FSH and testosterone axis and the respective Δ of pro- and anti- oxidation markers. To investigate for potential predictors a standard forward, multiple stepwise regression model was employed. All statistical evaluations were performed with the STATISTICA 8 software (STATSOFT, USA). The STROBE cross sectional checklist was employed for writing this report [26].

Results

Baseline and post-exercise concentrations of LH, FSH and tT

The baseline and post-exercise concentrations of LH, FSH and tT in the four groups of boys studied, as well as their statistical comparisons are reported in Fig. 2.

When baseline and post-exercise concentrations of these parameters were compared with all boys taken as a single group, post-exercise concentrations of LH were significantly lower than their respective baseline concentrations (mean ± SE; 0.66 ± 0.13 mIU/ml and 0.54 ± 0.09 mIU/ml, respectively; $P < 0.05$).

Baseline and post-exercise concentrations of pro- (TBARS and PCs) and anti- (GSH, GSSG, GPX, catalase and TAC) oxidation markers

The baseline and post-exercise concentrations of pro- (TBARS, and PCs) and anti- (GSH, GSSG, GPX, catalase and TAC) oxidation markers in the four groups of boys

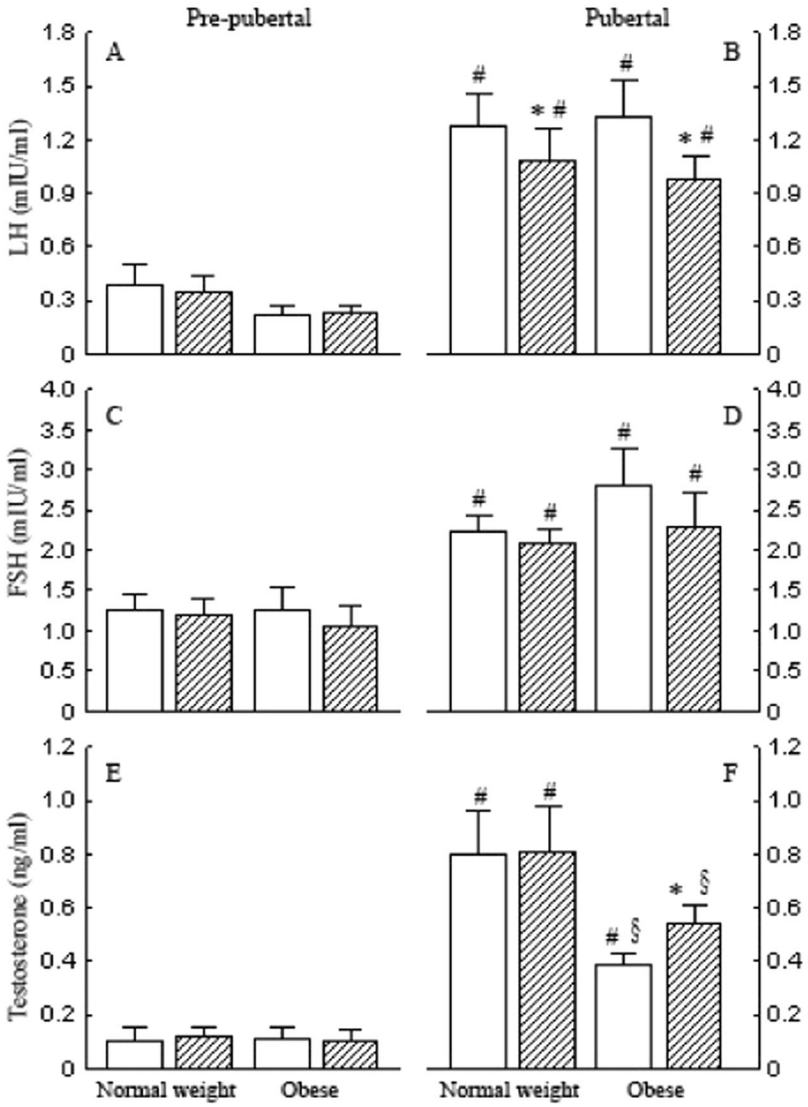

Fig. 2 Follicle stimulating hormone (FSH), luteinizing hormone (LH) and total testosterone concentrations (mean ± SE) at baseline (white bar) and post-exercise (shaded bar) in pre- (**a**, **c** and **e**, respectively) and early (**b**, **d** and **f**, respectively) pubertal normal weight and obese boys. * 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 boys; # denotes significant difference ($P < 0.05$) between early pubertal and respective pre- pubertal boys

studied, as well as their statistical comparisons are reported in Table 2.

Correlations among baseline concentrations of LH, FSH and tT concentrations with baseline pro- and anti- oxidation markers concentrations and their respective changes (Δ) within groups

Statistically significant correlations among baseline LH, FSH and tT concentrations, with baseline pro-, and anti-oxidation markers concentrations in pre-pubertal normal-weight and obese boys, are reported in Fig. 3. Statistically significant correlations among baseline LH, FSH and tT concentrations, with the changes (Δ) of pro-, and anti- oxidation markers concentrations in pre- and early pubertal normal weight and in pre-pubertal obese boys, are reported in Fig. 4. In pre-pubertal obese boys baseline LH correlated negatively with ΔGSH ($P < 0.05$, $R = -0.82$), while in early pubertal normal-weight boys baseline testosterone correlated positively with ΔGSH/GSSG ($P < 0.05$, $R = 0.73$). In early pubertal obese boys, no statistically significant correlations were found among all parameters studied.

Multiple regression analyses of LH, FSH and tT concentrations and their baseline to post-exercise changes (Δ) with baseline pro- and anti- oxidation markers concentrations and their respective changes (Δ) in all boys

When multiple regression analyses were performed with all boys taken as a single group, significant positive correlations were found: between baseline and post-exercise either LH or FSH or tT concentrations ($P < 0.05$, $r = 0.97$; $P < 0.05$, $r = 0.94$ and $P < 0.05$, $r = 0.98$, respectively); at baseline: between LH and FSH ($P < 0.05$, $r = 0.61$), LH and tT ($P < 0.05$, $r = 0.60$), FSH and tT concentrations ($P <$

Table 2 Markers of pro- (TBARS and PCs) and anti- (GSH, GSSG, GPX, catalase and TAC) oxidation

	Pre-pubertal		Early pubertal	
	Normal-weight ($n = 34$)	Obese ($n = 13$)	Normal-weight ($n = 31$)	Obese ($n = 14$)
TBARS (μmol/l)				
Baseline	3.73 ± 0.41	7.18 ± 0.26[b]	3.16 ± 0.53	5.68 ± 0.76
Post-Exercise	6.06 ± 0.62[c]	11.63 ± 0.86[b,c]	7.53 ± 1.59[c]	10.14 ± 1.30[c]
PCs (nmol/l)				
Baseline	0.36 ± 0.03	0.50 ± 0.05	0.34 ± 0.07	0.39 ± 0.06
Post-Exercise	0.61 ± 0.08[c]	0.91 ± 0.04[b,c]	0.60 ± 0.07[c]	0.65 ± 0.07[a,c]
TAC (μmol/ml)				
Baseline	0.94 ± 0.03	0.63 ± 0.10[b]	1.12 ± 0.07[c]	0.82 ± 0.08[a,b]
Post-Exercise	1.07 ± 0.04[c]	1.00 ± 0.04[c]	1.25 ± 0.02[b]	1.06 ± 0.03[c]
Catalase (μmol/min/mg Hb)				
Baseline	101.65 ± 4.67	87.58 ± 8.39	117.58 ± 13.14	94.92 ± 6.46
Post-Exercise	145.38 ± 7.48[c]	134.88 ± 6.23[c]	162.48 ± 11.09[c]	138.32 ± 7.68[c]
GSH (μmol/g Hb)				
Baseline	0.33 ± 0.01	0.28 ± 0.01[b]	0.31 ± 0.01	0.31 ± 0.01
Post-Exercise	0.23 ± 0.01[c]	0.22 ± 0.01[c]	0.23 ± 0.02[c]	0.23 ± 0.02[c]
GSSG (μmol/g Hb)				
Baseline	0.024 ± 0.001	0.024 ± 0.002	0.021 ± 0.003	0.024 ± 0.001
Post-Exercise	0.069 ± 0.003[c]	0.073 ± 0.002[c]	0.067 ± 0.006[c]	0.068 ± 0.002[c]
GSH/GSSG				
Baseline	14.29 ± 0.74	12.20 ± 2.67	16.32 ± 2.67	13.57 ± 1.42
Post-Exercise	3.34 ± 0.22[c]	3.00 ± 0.14[c]	3.49 ± 0.29[c]	3.51 ± 0.37[b]
GPX (U/l)				
Baseline	3350.85 ± 68.69	2804.11 ± 143.94[b]	3329.40 ± 189.35	3227.33 ± 97.72
Post-Exercise	4202.00 ± 60.02[c]	3671.44 ± 161.67[b,c]	4261.40 ± 234.30[c]	4065.22 ± 132.31[c]

All measured variables assayed at baseline and post- exercise were compared by employing repeated-measures ANOVA. Significant main effects were revealed by the Unequal N HSD *post-hoc* test. Statistical significance was set at ($P < 0.05$)

[a]Denotes significant difference between early pubertal and respective pre-pubertal boys

[b]Denotes significant difference between obese and respective normal weight boys

[c]Denotes significant difference of post-exercise from the respective baseline measurements

0.05, $r = 0.37$); in post-exercise: between LH and FSH ($P < 0.05$, $r = 0.70$), LH and tT ($P < 0.05$, $r = 0.71$), FSH and tT concentrations ($P < 0.05$, $r = 0.59$).

Multiple regression analysis revealed statistically significant negative correlation between ΔLH with ΔTBARS concentrations ($P < 0.05$; $r = -0.41$).

Forward stepwise regression analysis

To investigate for potential predictors of post-exercise concentrations of pro- and anti- oxidation markers (each one taken separately as dependent variable) among baseline BMI z-score, waist to height ratio, waist to hip ratio, VO_2max, FSH, LH and tT (all taken as independent variables), a standard forward stepwise regression model was employed. It revealed that baseline LH concentrations were the best positive predictors ($P < 0.05$, $b = 0.41$) for post-exercise TAC concentrations.

Discussion

We found that baseline and post-exercise LH and FSH concentrations did not differ between normal-weight and obese pre- and early pubertal boys. In the past, a similar finding has been reported in obese and normal-weight adult men [16, 27]. However, in this study, both baseline and post-exercise tT concentrations were lower in obese than in normal-weight early pubertal boys. Obese boys present with decreased testosterone concentrations across all pubertal stages [4]. In childhood and adolescent obesity, decreased testosterone concentrations are attributed to estradiol-

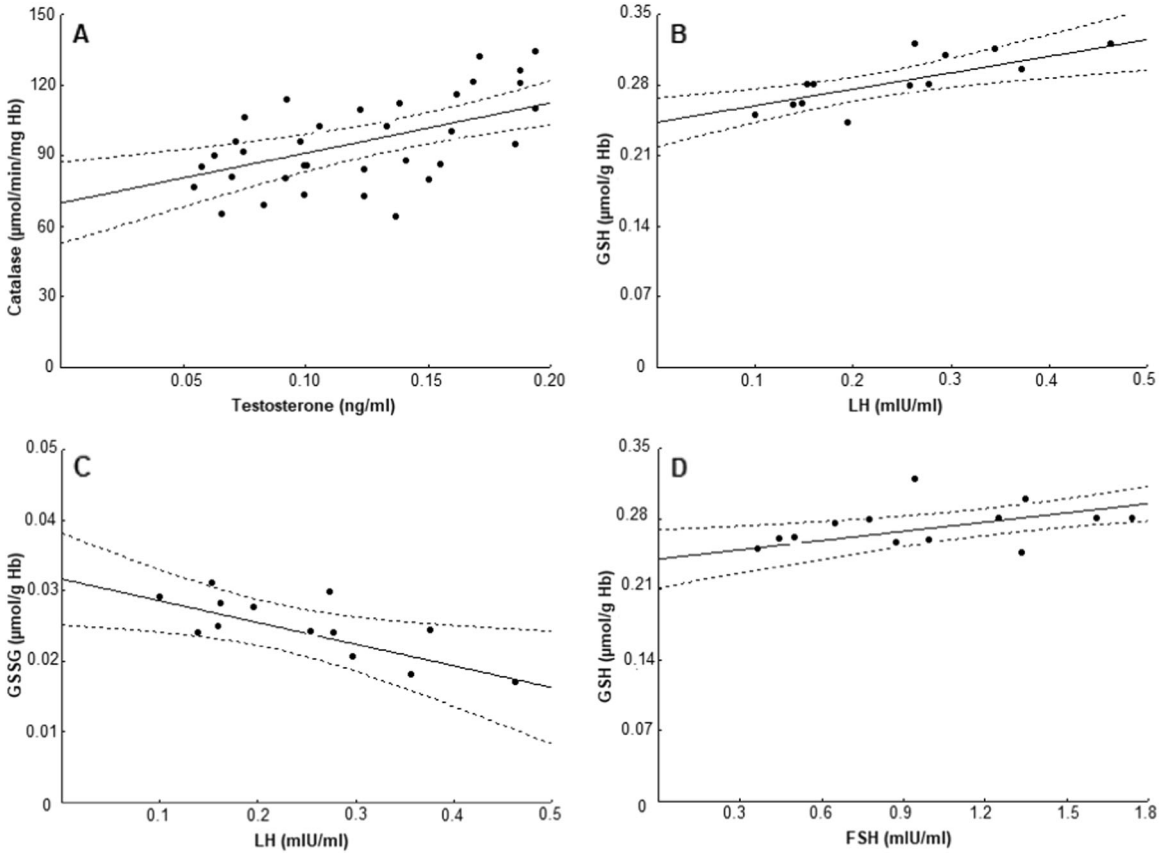

Fig. 3 Significant correlations between baseline total testosterone concentrations and baseline catalase activity in pre-pubertal normal weight boys (**a**, $R = 0.80$); between baseline LH and GSH (**b**, $R = 0.92$) or GSSG (**c**, $R = -0.85$) concentrations in pre-pubertal obese boys; and between baseline FSH and GSH concentrations (**d**, $R = 0.90$) in pre-pubertal obese boys. Correlations were evaluated by Pearson's correlation test. The level of significance was set at $P < 0.05$

induced LH inhibition, hyperinsulinemia-induced decreased SHBG concentrations, and/or leptin-induced paracrine inhibition of intra-testicular steroidogenesis [4, 5]. Circulating estradiol concentrations are increased due to testosterone aromatization in the adipose tissue while, recently we reported increased leptin concentrations in a similar cohort of pre-pubertal and pubertal obese boys [28]. Of note, baseline LH, FSH, and tT concentrations were, as expected, greater in early pubertal than in pre-pubertal boys, independently of the BMI status [3]. When all boys were taken as a whole, baseline concentrations of LH, FSH, and tT correlated significantly to each other confirming the known dynamic balance among these three hormones.

Exercise interferes acutely and chronically with all hypothalamic pituitary axes either, directly, or indirectly, resulting in perturbations of their homeostasis [14, 29]. In this study, after an acute bout of aerobic exercise, LH concentrations decreased in early pubertal boys, as previously shown in children, adolescents, and adults [15, 16]. This decrease might be related to either direct and/or indirect suppression of GnRH pulse frequency and amplitude induced by β-endorphin secreted as a cleavage product of POMC at the arcuate nucleus during acute aerobic treadmill exercise [14, 30]. Normally, LH β-subunit is produced following high frequency-high amplitude GnRH secretion, leading to LH synthesis in the gonadotroph cells [31]. Hypothalamic GnRH secretion is suppressed by β-endorphin, a phenomenon reversed by naltrexone, an anti-opioid drug [32, 33]. Luteinizing-hormone is stored in secretory granules, until stimulation by GnRH [34–36]. The suggested suppression of GnRH frequency, and amplitude during aerobic treadmill exercise, should lead to increased FSH synthesis [16]. The production of FSH β-subunit is the rate limiting step of FSH synthesis [37–39]. In the present study, baseline and post-exercise FSH concentrations were similar in pre- and early puberty in normal-weight and obese boys. The absence of FSH increase following exercise, might be due to the short duration of the applied exercise bout.

Furthermore, in this study, in early pubertal obese boys tT concentrations increased post-exercise (as compared to baseline concentrations), in contrast to LH decrease. Regardless of the kind of sport, maximal, or submaximal exercise (5–30 min) normally results in significant increases

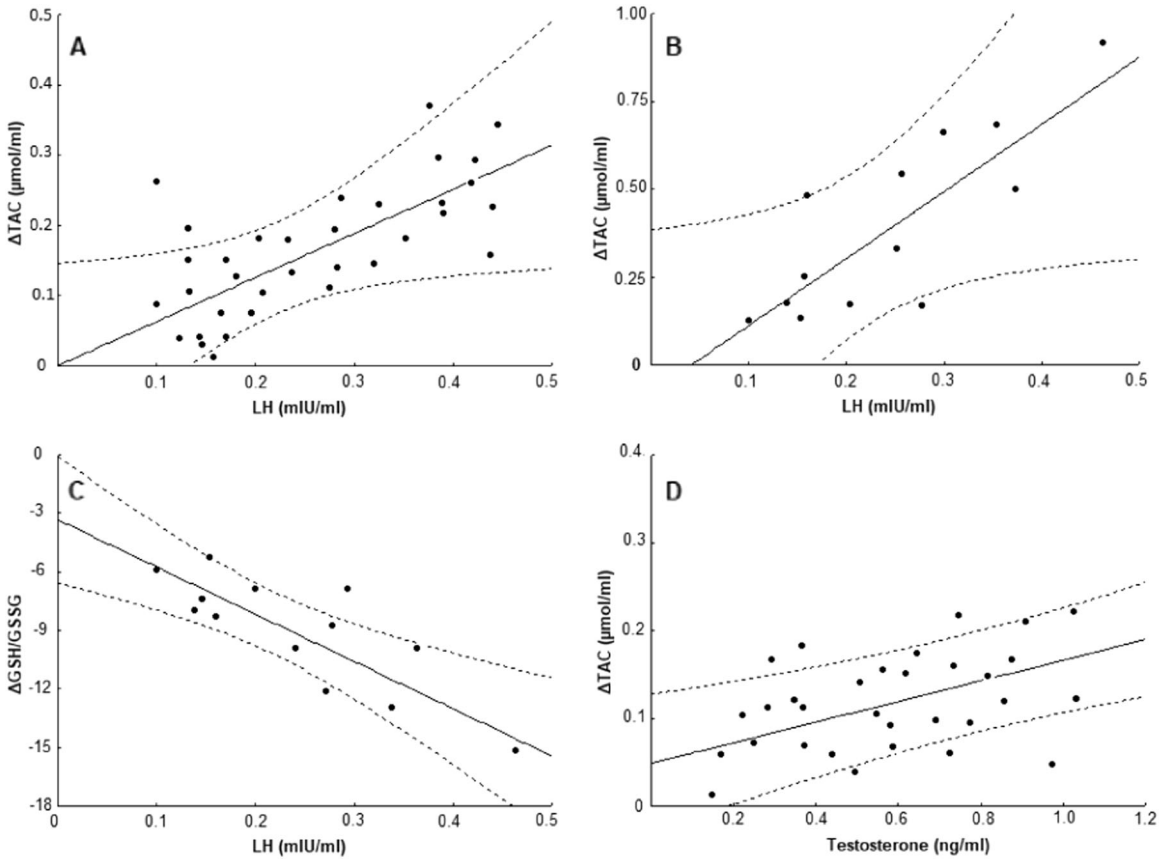

Fig. 4 Significant correlations between baseline LH concentrations and the change (Δ) of total anti-oxidant capacity (ΔTAC) in pre-pubertal normal weight (**a**, $R = 0.63$) and obese (**b**, $R = 0.82$) boys; between baseline LH and the change (Δ) of GSSG (ΔGSSG) in pre-pubertal obese boys (**c**, $R = -0.93$); and between total testosterone and ΔTAC (**d**, $R = 0.86$) in early pubertal normal-weight boys. Correlations were evaluated by Pearson's correlation test. The level of significance was set at $P < 0.05$

in testosterone concentrations in adult men, independently of LH, and FSH response [40]. The extent of response of testosterone to aerobic exercise remains unclear. It seems, however, that this anabolic hormone is more responsive to exercise of greater intensity and longer duration [41]. This dissociation between the secretory profiles of LH and testosterone during exercise suggests a non-LH dependent early increase of testosterone possibly attributed to, either, activation of alternative biochemical pathways in Leydig cells, such as chloride channels, or the suggested brain-testicular circuits [42–44]. Other suggested mechanisms responsible for the exercise-induced LH-independent testosterone secretion include exercise-related increased sympathetic activity, and nitric oxide-induced blood flow increase and vasodilation in the testis [45]. In the past, an increase of testosterone concentrations was shown in normal-weight adult males, following acute exercise bouts [17]. This increase parallels chronological age, and pubertal status and correlates with baseline testosterone concentrations [17].

In the present study, post-exercise tT concentrations increased only in obese pubertal boys who demonstrated lower baseline concentrations than normal weight pubertal boys. The absence of tT increase in normal-weight pubertal boys is possibly due to the relatively moderate intensity of the exercise bout. Sub-maximal moderate exercise (between 50 %, and 85 % of VO_2max) is the level of exercise intensity at which whole body fat oxidation increases. The latter plateaus at 70% VO_2max exercise intensity while reports state that body fat oxidation decreases at higher exercise intensity [46].

In this study, in all pre-pubertal boys, baseline LH, FSH and tT correlated with baseline concentrations of anti-oxidation markers. Furthermore, baseline LH and tT concentrations in pre-pubertal normal-weight and obese boys as well as in early pubertal normal-weight boys correlated positively with ΔTAC, a robust marker of anti-oxidation and negatively with ΔGS/GSSG a robust marker of pro-oxidation. In addition, in all studied boys, baseline LH concentrations were the best positive predictors for the exercise-associated increase of TAC. Also, in all boys ΔLH concentrations correlated negatively with ΔTBARS, a marker of pro-oxidation. Previously, in male adults with post-surgical hypogonadism, multiple markers of anti-

oxidation were down-regulated, while treatment with testosterone reversed these changes [12]. The present data suggest a role of the HPT axis in the anti-oxidation mechanisms with puberty progress. Interestingly, we have recently shown in pubertal normal-weight boys that baseline, and exercise-associated increases in growth hormone (GH) concentrations correlate positively with GSSG increase, another marker of anti-oxidation [8]. These results indicated that another major parameter of puberty (i.e. GH) is associated with the improvement of anti-oxidation in puberty [8]. Puberty represents a hallmark of the maturation process with increased growth rate and sexual maturation, which seemingly parallel the intensification of anti-oxidative mechanisms [6, 8, 47]. It is possible that the effect of exercise-related modifications of the pro- and anti-oxidative mechanisms upon the HPT axis might be exerted directly and/or indirectly *via* effects exerted upon other pituitary regulated hormone axes (such as the GH axis) by these exercise-related modifications of the oxidative mechanisms. The elucidation of these interactions is challenging, however the methodological approach needed in humans might be complicated and prone to confounding misinterpretations.

In summary, an acute bout of aerobic exercise at 70% VO_2max resulted in specific HPT axis changes in pre-, and early pubertal boys. At post-exercise, LH concentrations decreased in all early pubertal boys, while tT increased in early pubertal obese boys. Follicular stimulating hormone concentrations remained unchanged. Furthermore, baseline and post-exercise tT concentrations were lower in obese than in normal-weight early pubertal boys. Finally, in all pre-pubertal boys, baseline LH correlated positively with anti-oxidation markers, while in early pubertal normal-weight boys baseline tT correlated with anti-oxidation markers, suggesting a direct and/or indirect involvement of the HPT maturation during puberty regarding the progress of anti-oxidation. The relatively small sample size of subjects is a limitation of the study. Sampling at 1 and/or 2 post-exercise hours would provide useful data regarding the functional recovery of the HPT axis, given that endocrine responses to exercise are time-dependent, with different "windows" for each hormone involved in the physiologic response to exercise. In addition, while tT is a reliable measure of androgen activity, the measurement of bioavailable/free testosterone would be useful for finer evaluation in this type of studies.

In conclusion, it appears that the maturation of the HPT axis during puberty in boys is positively associated with increase of anti-oxidation. This association is stronger with LH and testosterone components of the HPT axis in this cohort of pre- and early pubertal boys. Further studies, including both, boys and girls, could investigate the potential beneficial effects of these hormones on the development of anti-oxidative defensive mechanisms in specific conditions.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with animals performed by any of the authors. All procedures performed in the study involving human participants had the approval of the National and Kapodistrian University of Athens Medical School designated ethics committee and were in accordance with the 1964 Helsinki declaration and its later amendments.

Informed consent The aims and the procedure of the study were fully disclosed to the parents or legal guardians of the participants, and informed, written consent was obtained from them while boys gave verbal consent before the participation in the study.

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