Positive association of pro-oxidative stress markers with adipose mass markers in pre- and early-pubertal boys

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OBJECTIVES

BACKGROUND

Obesity: A modern day epidemic characterized by increased pro- and reduced anti-oxidation, as well as increased adipocytokines and inflammatory markers even in children

Puberty: A maturation period in human development.

Exercise: A potent stimulus of pro- and anti-oxidation mechanisms.

Oxidative stress: A state of imbalance between pro- and anti- oxidation within the cell.

Pro-oxidation: Mitochondrial and non-mitochondrial mechanisms, which generate reactive oxygen and nitrogen species (RONS).

Anti-oxidation: An adaptive activation of enzymatic and/or non-enzymatic mechanisms, which scavenge pro-oxidants and their products within cells and in extracellular body fluids.

Adipokines or adipocytokines: Cell signaling proteines (cytokines) secreted by the adipose tissue.

Inflammatory markers: Signaling proteins secreted in response to inflammation.

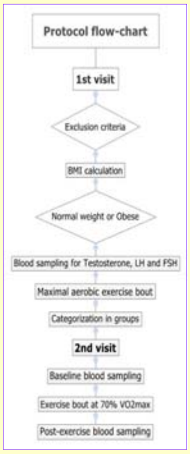
OBJECTIVE

To investigate the association of adipocytokines with markers of oxidative stress at baseline and their change and associations during exercise in normal weight and obese pre- and early-pubertal boys.

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 +

METHODS



Design: Cross-sectional human experimental study

Setting: University ergophysiology laboratory and endocrine unit.

Participants: 76 healthy pre-pubertal, and early pubertal, normal weight and obese boys. (TABLE 1)

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.

Protocol flow-chart

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

Maximal oxygen consumption (VO2max): Participants had their 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)

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.
Immediately after the exercise bout a second (post-exercise) blood sampling was performed.

Main Outcome Measures:

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).

Adipocytokines: adiponectin, leptin, NGAL and RBP4.

Inflammatory markers: hsCRP and hsIL-6.

RESULTS

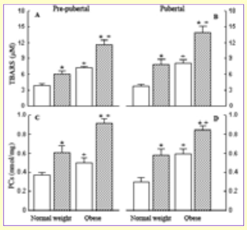


Figure 1: Markers of pro-oxidation: TBARS and PCs, concentrations (mean±SE) at baseline (white bars) and post-exercise (at 70% VO2max) (shaded bars) in pre-pubertal (panels A and C, respectively) and pubertal (panels B and D, respectively) normal weight and obese subjects.

All Figures: Measurements were compared with Repeated Measures ANOVA followed by LSD Fischer's post-hoc test (P<0.05)
* Denotes significant difference (P<0.05) between baseline and post-exercise 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.

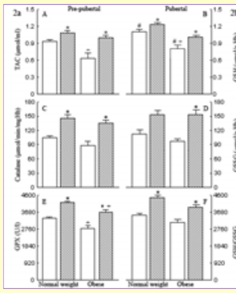


Figure 2: Markers of anti-oxidation: TAC, catalase and GPX activity values (mean±SE) at baseline (white bar) and post-exercise (at 70% VO2max) (shaded bar) in pre-pubertal (panels A, C and E, respectively) and early pubertal (panels B, D and F, respectively) normal weight and obese subjects.

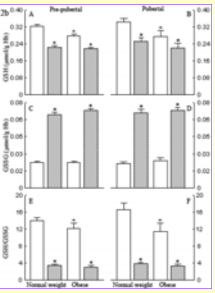


Figure 3: Markers of anti-oxidation: GSH, GSSG concentrations and GSH/GSSG ratio (mean±SE) at baseline (white bars) and post-exercise (at 70% VO2max) (shaded bars) in pre-pubertal (panels A, C and E, respectively) and early pubertal (panels B, D and F, respectively) normal weight and obese subjects.

Table 2: Adipocytokine and inflammatory markers data in normal weight and obese, pre- and early pubertal subjects.
Measurements were compared with Repeated Measures ANOVA followed by LSD Fischer's post-hoc test (P<0.05)
* Denotes significant difference (P<0.05) between baseline and post-exercise 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.

		Pre-pubertal (n=39)		Early pubertal (n=37)	
		Normal weight (n=28)	Obese (n=11)	Normal weight (n=25)	Obese (n=12)
Adiponectin	Baseline	19.60±2.77	10.31±1.32 +	17.14±4.01	12.37±1.56 +
	Post-exercise	18.86±2.29	10.61±1.17 +	19.37±4.49	12.27±1.44 +
Leptin	Baseline	5.099±2.417	11.505±2.752 +	6.134±2.043	11.594±2.547 +
	Post-exercise	5.363±2.745	10.317±1.957 +	3.898±0.982	10.201±3.634 +
NGAL	Baseline	70.50±6.67	68.25±8.25	74.01±6.56	71.37±6.08
	Post-exercise	67.82±8.53	62.31±5.67	71.06±5.61	77.37±5.38
RBP4	Baseline	15.12±0.57	16.34±1.61	16.69±0.93	16.66±0.55
	Post-exercise	16.07±1.19	17.36±1.78	18.29±0.67 *	17.68±0.81
hsCRP	Baseline	0.74±0.43	1.78±0.46 +	0.53±1.15	1.66±0.27 +
	Post-exercise	0.69±0.50	1.84±0.48 +	0.54±1.45	1.64±0.37 +
hsIL-6	Baseline	1.30±0.30	3.36±0.89 +	1.62±0.33	1.96±0.47
	Post-exercise	1.23±0.36	3.55±0.84 +	1.93±0.31	1.97±0.26

Correlations between baseline concentrations of pro- and anti- oxidation markers and either of adipocytokines or of inflammatory markers.
In pre-pubertal normal weight and in pubertal normal weight and obese subjects, no significant correlations were found. In pre-pubertal obese subjects hsIL-6 concentrations correlated positively with PCs concentrations (P<0.05, r = 0.83), while hsCRP concentrations correlated negatively with Catalase concentrations (P<0.05, r = – 0.77).

Correlations between the changes (Δ) of pro- and anti- oxidation markers concentrations and either adipocytokines or inflammatory markers before and after an acute bout of aerobic exercise.
In pre pubertal normal-weight subjects, ΔCatalase correlated positively with ΔRBP4 concentrations (P<0.05, r = 0.63). In pre-pubertal obese subjects ΔCatalase correlated positively with ΔAdiponectin (P<0.05; r = 0.87) concentrations. ΔPCs correlated positively with ΔhsIL-6 (P<0.05; r = 0.87) concentrations. In early pubertal normal weight subjects, no significant correlations were found.. In early pubertal obese subjects, ΔGSSG concentrations correlated negatively with ΔhsIL-6 concentrations (P<0.05; r = – 0.96).

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 NGAL, RBP4, Adiponectin, Leptin, hsCRP and hsIL-6, all taken as independent variables.
Baseline leptin was the best positive predictor (P<0.05; b= 0.538) for post-exercise concentrations of TBARS. Baseline hsIL-6 was the best positive predictor (P<0.05; b= 0.451) for post-exercise concentrations of PCs and the best negative predictor (P<0.05; b=–0.37) for post-exercise concentrations of GSH/GSSG. Baseline Adiponectin was the best positive predictor (P<0.05; b= 0.419), for post-exercise concentrations of Catalase.

CONCLUSIONS

Pro-oxidation markers, inflammatory adipocytokines and leptin are increased in obese compared to normal-weight pre- and early- pubertal boys whereas anti-oxidation markers are increased in normal weight boys. Post-exercise both pro- and anti- oxidative stress markers change significantly in normal and obese boys.
In all subjects pro-and anti- oxidation markers are positively and negatively correlated, respectively, with inflammatory adipocytokines and leptin, a marker of adipose mass. These findings indicate the deleterious association of pro-oxidation with adipose tissue in pre- and early- pubertal boys.
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.

References

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