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Serum adiponectin levels and cardiorespiratory fitness in nonoverweight and overweight Portuguese adolescents: The LabMed Physical Activity Study

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Abstract

Purpose: This study examined the independent associations between cardiorespiratory fitness and circulating adiponectin concentration in adolescents, controlling for several potential covariates.

Methods: This is a cross-sectional study in Portuguese adolescents. A sample of 529 (267 girls) aged 12-18 years were included and categorized as overweight and nonoverweight. Cardiorespiratory fitness was assessed by 20 meters shuttle run test. We measured serum adiponectin, high-sensitivity C-reactive protein, fasting glucose, insulin and HDL-cholesterol.

Results: After adjustment for age, sex, pubertal stage, adherence to the Mediterranean diet, socioeconomic status, body fat percentage, insulin resistance, HDL-cholesterol and C-reactive protein, regression analysis showed a significant inverse association between adiponectin and cardiorespiratory fitness in nonoverweight participants (B=-0.359; $p < .042$). Analysis of covariance showed a significant difference between the highest cardiorespiratory fitness Healthy zone (above healthy zone) and the Under and the Healthy cardiorespiratory fitness zones in nonoverweight adolescents ($p = .03$) ($F_{(2, 339)} = 3.156$, $p < .001$).

Conclusion: Paradoxically, serum adiponectin levels are inversely associated with cardiorespiratory fitness in nonoverweight, but not in overweight adolescents. In nonoverweight adolescents, those with highest levels of cardiorespiratory fitness (above healthy zone) presented lower levels of adiponectin compared with those in Under and Healthy cardiorespiratory fitness zones.

Disciplines

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Serum adiponectin levels and cardiorespiratory fitness in non-overweight and overweight Portuguese adolescents: the LabMed Physical Activity Study.

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ABSTRACT

Purpose: This study examined the independent associations between cardiorespiratory fitness and circulating adiponectin concentration in adolescents, controlling for several potential covariates. **Methods:** This is a cross-sectional study in Portuguese adolescents. A sample of 529 (267 girls) aged 12-18 years were included and categorized as overweight and non-overweight. Cardiorespiratory fitness was assessed by 20 meters shuttle run test. We measured serum adiponectin, high-sensitivity C-reactive protein, fasting glucose, insulin and HDL-cholesterol. **Results:** After adjustment for age, sex, pubertal stage, adherence to the Mediterranean diet, socioeconomic status, body fat percentage, insulin resistance, HDL-cholesterol and C-reactive protein, regression analysis showed a significant inverse association between adiponectin and cardiorespiratory fitness in non-overweight participants (B=-0.359; $p<0.042$). Analysis of covariance showed a significant difference between the highest cardiorespiratory fitness Healthy zone (above healthy zone) and the Under and the Healthy cardiorespiratory fitness zones in non-overweight adolescents (p=0.03) ($F_{(2, 339)} = 3.156$, $p<0.001$). **Conclusion:** Paradoxically, serum adiponectin levels are inversely associated with cardiorespiratory fitness in non-overweight, but not in overweight adolescents. In non-overweight adolescents, those with highest levels of cardiorespiratory fitness (above healthy zone) presented lower levels of adiponectin compared to those in Under and Healthy cardiorespiratory fitness zones.

KEYWORDS: Inflammatory biomarkers, adiposity, youth.

Introduction

Cardiovascular disease is the leading cause of mortality(13). Individuals with cardiovascular disease usually become symptomatic only in late life, but the underlying process of cardiovascular disease has its onset during childhood and often related to an inflammatory process (27). Recently, several metabolic biomarkers have emerged. Cytokines such as adiponectin, leptin, Interleukin 6 and phase acute proteins such as high-sensitivity CRP (hs-CRP), complement factors C3 e C4 have been found to be involved in the low-grade inflammation process (40) and associated with metabolic risk factors in adolescents (5).

Adiponectin is a protein abundantly expressed in adipose tissue, and it is known to have insulin-sensitizing, anti-inflammatory, and cardioprotective effects (32). Adiponectin is considered to have “healthy effects” because unlike all other adipokines, its expression and serum levels are decreased in obese subjects(31). Adiponectin have been associated with an adherence to a Mediterranean dietary pattern (12), insulin resistance(11), hs-CRP (38), adiposity (16), whereas it was positively associated with HDL-cholesterol (33, 38) and it is considered an independent factor inversely associated with cardiovascular disease (2). However, there is some emerging evidence suggesting that high circulating adiponectin concentrations may play a paradoxical role in the pathogenesis of cardiovascular disease. Recently, investigations showed that high serum adiponectin concentration is associated with an increased risk of cardiovascular disease and total mortality in adulthood(6). Indeed, there is a lack of knowledge on this adiponectin paradox (19).

Cardiorespiratory fitness is a powerful marker of current and future health status during childhood and adolescence (30, 34) and is an important predictor of all-cause mortality and cardiovascular disease mortality (18). It has been suggested that cardiorespiratory fitness is one of the most important risk factor for cardiovascular disease, and therefore it should be included in definitions of metabolic syndrome (3).

Paradoxically, recent investigations have showed that adiponectin concentration are inversely associated with physical activity (28), muscular fitness (1), and cardiorespiratory fitness (5, 25) in adolescents. However, studies in adolescents are scarce. Martinez-Gomez and colleagues (25) found an inverse association between cardiorespiratory fitness and adiponectin in 198 Spanish adolescents, independently of age, sex, pubertal status and waist circumference. Likewise, a study of 413 Danish adolescents reported an inverse association between cardiorespiratory fitness and adiponectin after adjusting for adiposity, sex and pubertal stage (5). On the other hand, a study carried out in 192 Scottish adolescents found no association (4). However, none of these studies analyzed their sample in relation neither metabolic nor weight status. Such analyses might be able to highlight multiple key factors that can influence the relationship of adiponectin with cardiorespiratory fitness. Furthermore, intervention studies have mainly been conducted with obese, diabetics or subjects with metabolic syndrome(10). Recently, Choi and colleagues have suggested that the measurement of adiponectin concentration and BMI together could be an additional predictive marker of survival among the elderly (6). In addition, another study showed that high circulating adiponectin concentration may be an indicator of decreased physical performance, in older adults (17). However, in order to dissect the context-dependent and roles of adiponectin, studies in subjects with different metabolic or weight status (non-overweight and overweight subjects) are required.

To date, the association between cardiorespiratory fitness and circulating adiponectin levels in adolescents remains poorly understood and the studies have often overlooked potential confounders in their analysis. Furthermore, we are not aware of any previous study examining the relationship between serum adiponectin and cardiorespiratory fitness separately in overweight and non-overweight controlling for dietary patterns and other important potential confounders such as pubertal status, adiposity, HDL-cholesterol, inflammation, socioeconomic status and insulin resistance. Thus, the aim of the present study was to examine the associations between cardiorespiratory fitness and serum adiponectin levels in non-overweight and overweight adolescents, controlling for the above-mentioned covariates.

Methods

Study Design and Sample

The current report is part of the "Longitudinal Analysis of Biomarkers and Environmental Determinants of Physical activity (LabMed Physical Activity Study)", a school-based prospective cohort study carried out in four Portuguese cities from the North Region. Detailed description of sampling and recruitment approaches, data collection, analysis strategies have been described elsewhere (29). In short, baseline data was collected in the fall of 2011, for 1,229 apparently healthy adolescents, i.e., participants without any medication or medical diagnose of physical or mental impairment, aged 12 to-18 years. Of the 1229 adolescents that agreed to participate in the LabMed study, 534 accepted to undergo blood collection. Five individuals were excluded due to hs-CRP values >10 mg/L, which may be indicative of acute inflammation or illness. Thus, leaving 529 adolescents (267 girls, 262 boys, mean age

14.3±1.7 years) as the final sample for the present report. Power analysis was calculated *post hoc* and it was higher than 0.8 for multiple regression analysis and ANCOVA.

The LabMed Physical Activity Study was conducted in accordance with the Helsinki Declaration for Human Studies and approved by the Portuguese Data Protection Authority (#1112434/2011) and the Portuguese Ministry of Science and Education (0246200001/2011). All participants were informed of the study's goals, and written informed consent was obtained from participating adolescents and their parents or legal guardians.

Measures

Anthropometrics

Body height was measured to the nearest 0.1 cm in bare or stocking feet with the adolescent standing upright against a portable stadiometer (Seca213, Hamburg, Germany). Body weight was measured to the nearest 0.10 kg, lightly dressed, with no shoes, using a portable electronic weight scale (Tanita Inner Scan BC532, Tokyo, Japan)(24). Body mass index (BMI) was calculated from the ratio of body weight (kg) to body height (m^2). Participants were categorized as non-overweight (including underweight and normal weight participants) , overweight (including overweight and obese participants) according to Cole's cut-offs(7, 21).Percentage of body fat was measured with bioelectrical impedance with a frequency current of 50 kHz (Tanita Inner Scan BC 532, Tokyo, Japan). Participants were asked to fast overnight for at least 10 hours. After the assessors manually introduced the age, sex and height into the scale system, the participants stood on the scale with light clothes and bared foot(39).

Pubertal stage

Participants self-assessed their pubertal stage of secondary sex characteristics (breast and pubic hair development in girls and genital and pubic hair development in boys ranging from stage I to V), according to the criteria of Tanner and Whitehouse (35).

Socioeconomic Status

Adolescents' socioeconomic status was assessed with the Family Affluence Scale(8). The answers were summed and socioeconomic status was computed as a continuum variable to perform the statistical analyses.

Blood Sampling

Blood samples were obtained from each subject early in the morning, following a 10-hour overnight fast by venipuncture from the antecubital vein. The samples were stored in sterile blood collection tubes in refrigerated conditions (4° to 8°C), and then sent to an analytical laboratory for testing according to standardized procedures; (i) hs-CRP, latex enhanced immunoturbidimetric assay (Siemens ADVIA 1800, Erlangen, Germany); (ii) HDL-Cholesterol, Precipitation of the Apolipoprotein B containing lipoproteins with dextran-magnesium-chloride (Siemens Advia 1600/1800 Erlangen, Germany); (iii) Adiponectin, ELISA (Plate Reader); (iv) Glucose, Hexokinase method (Siemens Advia 1600/1800 Erlangen, Germany);(v) Insulin, Chemiluminescence immunoassay (Siemens ACS Centaur System, Erlangen, Germany). All assays were performed in duplicate according to the manufacturers' instructions. The homeostatic model assessment (HOMA) was calculated as the product of basal glucose and insulin levels divided by 22.5, and was used as a proxy measure of insulin resistance(26).

Adherence to the Mediterranean diet

To assess the degree of adherence to the Mediterranean diet the KIDMED index (Mediterranean Diet Quality Index for children and adolescents) was used (36). The index is based on a 16-questions self-administered, which sustain the principles of the Mediterranean dietary patterns, as well as, those that undermine it. The final results of index varied between 0 and 12 points. The questions that have one negative connotation in relation to Mediterranean diet were equal to (-1), the questions that constitute positive aspect were equal to (+1). A continuum variable was computed to perform the statistical analyses.

Cardiorespiratory Fitness

Cardiorespiratory fitness was assessed with the 20-metre Shuttle Run Test (20 m SRT)(22). This test requires participants to run back and forth between two lines set 20 m apart. Running speed started at 8.5 km/h and increased by 0.5 km/h each minute, reaching 18.0 km/h at minute 20. A detailed description of this test can be found elsewhere(22).

The test was performed once, and the number of shuttles performed by each participant was recorded to posterior calculation of VO_{2max} using Léger's equations (22). Adolescents were also classified in three groups according to the age and sex-specific cut-off points of FITNESSGRAM criteria, as "Under", "Healthy" and "Above" healthy zones (41).

Statistics Analysis

Data analysis was performed with the Statistical Package for the Social Sciences for Windows (Version 21.0 SPSS Inc., Chicago, IL). All the variables were checked for normality, hs-CRP, HDL-cholesterol, body fat percentage, adiponectin and cardiorespiratory fitness were not normally distributed therefore were transformed using the natural logarithm. Descriptive data are presented as means and standard deviation. Independent Two-tailed *t*-Tests for continuous variables and Chi-square for categorical variables, respectively, were used to examine differences between weight status categories. We found no significant interaction effect for sex (e.g., sex x cardiorespiratory fitness), thus all data are presented for boys and girls together.

Linear regression models were performed separately for non-overweight and overweight adolescents and for the total sample to determine the associations between serum adiponectin and cardiorespiratory fitness, adjusted for age, sex, pubertal stage, socioeconomic status, adherence to the Mediterranean diet, HOMA-IR, HDL-cholesterol, hs-CRP and body fat percentage. Unstandardized regression coefficients were used to express the B coefficients of the regression analyses.

Analysis of covariance (ANCOVA) with Bonferroni post-hoc multiple comparison tests were used to assess the differences of serum adiponectin levels across healthy fitness zones of cardiorespiratory fitness in non-overweight and overweight adolescents. Covariates included were age, sex, pubertal stage, socioeconomic status, adherence to the Mediterranean diet, HOMA-IR, HDL-cholesterol, hs-CRP and body fat percentage. A *P* value less than .05 was regarded as significant.

Results

Descriptive characteristics of the participants are presented in **Table 1**. Overweight subjects had higher body fat percentage, hs-CRP and insulin resistance whereas non-overweight subjects had higher levels of serum adiponectin, HDL-cholesterol and cardiorespiratory fitness (*p*<0.001 for all).

INSERT TABLE 1

Regression analysis (**Table 2**), showed a significant inverse association between adiponectin and cardiorespiratory fitness (B=-0,359; *p*<0.042), after adjustments for age, sex, pubertal stage, socioeconomic status, adherence to the Mediterranean diet, HOMA-IR, HDL-cholesterol, hs-CRP and body fat percentage in non-overweight adolescents. For the overweight group no significant association were found. For the total sample, after adjustments for age, sex, pubertal stage, socioeconomic status, adherence to the Mediterranean diet, HOMA-IR, HDL-cholesterol, hs-CRP no association were found between cardiorespiratory fitness and adiponectin, however when body fat percentage was included (model 4), this result became significant (B=-0,370; *p*<0.030).

INSERT TABLE 2

ANCOVA (**Fig.1**) adjusted for age, sex, pubertal stage, socioeconomic status, adherence to the Mediterranean diet, HOMA-IR, HDL-cholesterol, hs-CRP and body fat percentage showed significant difference in adiponectin levels across cardiorespiratory fitness healthy zones in non-overweight. The Highest cardiorespiratory fitness zone (Above healthy zone) showed lower levels of serum adiponectin compared to the Under and Healthy cardiorespiratory fitness zones (p=0.03) ($F_{(3, 339)}$= 3.156, *p*=0.044). In the overweight group, no significant results were found.

INSERT FIGURE

Discussion

This study shows that adiponectin levels are inversely associated with cardiorespiratory fitness in non-overweight adolescents even after adjusting for several potential confounders. We also found significant differences in adiponectin levels according to the cardiorespiratory fitness healthy zones in non-overweight adolescents. These results suggest that cardiorespiratory fitness may have an important impact on adiponectin levels in non-overweight adolescents independently of several confounders. We are not aware of any study that has analyzed the relationship between cardiorespiratory fitness and levels of serum adiponectin independent of several cardiometabolic markers such as hs-CRP, HDL-cholesterol, insulin resistance and adherence to a healthy dietary pattern.

Low cardiorespiratory fitness is referred to as an independent risk factor for development of cardiovascular disease and is a strong predictor for all-cause mortality, in addition, it is considered one of the most powerful markers of health (30). The current physical activity guidelines for children and adolescents recommend that most of the daily physical activity should be aerobic to develop a healthy cardiovascular system due to its health-related benefits, including the prevention in metabolic disease and cardiovascular disease risk factors (42). On the other hand, adiponectin has been proposed to be a cytokine with protective properties, but surrounded by controversy (10, 14, 19). In addition, recently, it has emerged interesting data showing that adiponectin is not exclusively released from adipocytes but also being produced and released by skeletal muscle (20, 23),which has been showed to be an important peripheral target tissue for adiponectin to exert its metabolic effects (23).

The paradoxical inverse association between cardiorespiratory fitness and adiponectin in adolescents found in our study is, however, consistent with two previous cross-sectional studies (5, 25). A recent finding among Spanish adolescents (AFINOS study)(25) showed an inverse association between adiponectin and cardiorespiratory fitness after controlling for age, sex, pubertal stage and waist circumference and the authors suggested that adiponectin secretion is inhibited in adolescents with normal insulin sensitivity and potentially due to fitness levels. Similarly, Bugge and colleagues(5) reported a negative association between VO2peak and adiponectin after adjusting for fatness. However, none of these studies examined the relationship between fitness and adiponectin stratified by weight status. In this line of thought it is important to mention a recent intervention study in adults that demonstrated that obese and overweight subjects, after 12 months of regular exercise, without changes in the body fat had an improvement in cardiorespiratory fitness, with an unexpected significant reduction in the levels of serum adiponectin in 15% and 18%, respectively (15). In the light of this and based on our findings it seems that cardiorespiratory fitness and weight status may play a key role in the levels of serum adiponectin. Furthermore, our results confirm and to a certain degree extend on the previous findings from cross-sectional studies in adolescents. Not only by stratifying our analysis according to the weight status of the participants, but also by including several others potential confounders such as adherence to a Mediterranean dietary pattern and insulin resistance.

Adiponectin have shown to be strongly associated with insulin resistance (16) and is a potential marker of type 2 diabetes (14). Moreover, it is known that insulin sensitivity is affected by dietary factors and adiponectin is recognized as having insulin sensitizing properties (11, 16). Previous studies reported that a greater adherence to the Mediterranean-type diet enhances adiponectin levels in healthy subjects (12) and that omega-3 supplementation or daily intake of fish and a low calorie-diet increased adiponectin levels (37). Importantly of this discussion, in the present report the association of serum adiponectin with cardiorespiratory fitness remained significant even after the models were adjusted for HOMA-IR and adherence to a Mediterranean diet in non-overweight adolescents.

HDL-cholesterol and hs-CRP have been proposed in several studies as important metabolic risk factors. Recently, investigations showed a positive correlation between HDL-cholesterol and adiponectin (33, 38). Moreover it was reported that adiponectin had an inverse relationship with markers of inflammation such as hs-CRP (38) in adolescents. Interestingly, the associations between adiponectin and cardiorespiratory fitness remained in non-overweight subjects even after HDL-cholesterol and hs-CRP were included as covariates in the regression models.

A recent review observed that currently, most of the knowledge on the role of adiponectin is driven from studies including only obese subjects (10). Indeed, it is already clear that the circulating adiponectin levels are decreased in overweight subjects, several studies, as well as our results support this. However, it is also known that adiponectin is also segregated by different cells, isoforms and have different receptors, which are not yet fully understood(10). Our results were stratified by weight status, and to a further analysis, we have also included a more accurate variable of fatness (body fat percentage) in the regression models to evaluate it's potential effect, which did not changed the results.

We showed significant differences between Under and Healthy cardiorespiratory fitness healthy zones with the highest cardiorespiratory fitness zone (Above healthy zone) in non-overweight participants. Adolescents in non-overweight condition with a high cardiorespiratory fitness level has been referenced with several related health benefits (30). Nonetheless, based on the present data and existing literature, it is difficult to explore the potential clinical relevancy of the difference on adiponectin serum levels observed between the higher cardiorespiratory fitness zone (Above healthy zone) with the others cardiorespiratory fitness healthy zones (Under and Healthy cardiorespiratory fitness zones) in non-overweight apparently healthy adolescents. However, high levels of adiponectin and low BMI have been associated with increased all-cause and cardiovascular mortality in adulthood (6). Indeed, it is known that adiponectin is also expressed by different tissue such as cardiomyocytes, bone-forming cells, pituitary cells and skeletal muscle (23). However, the degree to which non-adipose tissue sources contribute to increased adiponectin in chronic diseases, has yet not been determined (19). In addition, our results highlight the multifaceted and controversial immunometabolic actions of adiponectin (10). Future experimental studies in human and animal models should be carried out to understand the role of different isoforms and receptors of this protein.

The strengths of our study include the inclusion of potential important confounding variables in our analysis. In addition, adolescence is a period of natural changes in several metabolic systems such as sex hormones and body composition, which may confound the results (40) and all the analyses were controlled for sex, age and pubertal stage. We also classified the participants in three groups according to the age and sex-specific cut-off points for cardiorespiratory fitness. The cardiorespiratory fitness tests used in our study were based on previous studies which have shown to be valid, reliable and feasible for health monitoring purposes in adolescents (35) .

Our results should be interpreted with the understanding of some limitations. First, our cross-sectional design does not allow us to establish causality. Second, we measured serum adiponectin levels which result several adipose and non-adipose tissues(10), so it can be argued to what extent cardiorespiratory fitness level may contribute to these levels. Nonetheless, it has been shown that high circulating adiponectin concentration may be an indicator of decreased physical performance, in older adults (17).

We didn't include physical activity as a confounder variable in our analyses due cardiorespiratory fitness has been already reported to be associated with several metabolic outcomes independently of physical activity levels (9). Moreover, cardiorespiratory fitness is a surrogate or a consequence of physical activity levels (i.e. although the genetic component of Vo2max, it is assumed that one's become more fit if perform more physical activity). In our regression models we have been included variables that previous literature has shown to be correlated with the dependent variable (adiponectin).

In conclusion, this cross-sectional study shows that serum adiponectin levels are inversely associated with cardiorespiratory fitness in non-overweight, but not in overweight adolescents. In non-overweight adolescents, those with a cardiorespiratory fitness level above the healthy zone had lower levels of adiponectin compared to those under and in the healthy cardiorespiratory fitness zones. These results suggest that variability in adiponectin levels among non-overweight adolescentes seems to be explained by the levels of cardiorespiratory fitness and that cardiorespiratory fitness and weight status have an important impact on adiponectin levels.

Conflict of interest statement

The authors declare that they have no conflicts of interest.

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Appendices, Tables, Figure Legends

Table 1. Participants' characteristics.

Characteristics	Mean (SD) Non-Overweight (n=381)	Overweight (n=148)	Total sample (n=529)
Age (year)	14.40(±1.76)	14.13(±1.63)	14.33(±1.73)
Weight (kg)	50.38(±9.54)	67.43(±11.97)[a]	55.15(±12.81)
Height (cm)	160.23(±9.96)	160.3(±8.61)	160.27(±9.59)
BMI (kg/m^2)	19.46(±2.14)	26.07(±3.70)[a]	21.31(±3.84)
Body Fat (%)[b]	17.59(±6.42)	28.59(±7.47)[a]	20.67 ± 8.34
Adiponectin (mg/L)[b]	12.01(±5.40)	10.56(±5.44)[a]	11.61(±5.45)
Hs-CRP (mg/L)[b]	0.78 (±1.75)	1.37(±2.12)[a]	0.95(±1,88)
Vo2max ($mL\cdot kg^{-1}\cdot min^{-1}$)	49.61(±25.61)	32.74(±19.99)[a]	42.01 ± 6.80
Insulin resistance (HOMA-IR)[b]	2.85(±1.43)	5.00(±9.74)[a]	3.45(±5.38)
HDL-C (mg/dL)[b]	55.80(±12.02)	50.78(±10.97)[a]	54.39(±11.95)
KIDMED Index	7.07(±1.98)	7.20(±2.13)	7.11(±2.05)
Socioeconomic Status (FAS)	6.40(±1.76)	6.38(±1.54)	6.40(±1.70)
Pubertal status% A: ≤III/ IV/ V	40.9/ 45.2/ 13.9	37.8/ 50.7/ 11.5	40.1/ 46.7/ 13.2
Pubertal status% B: ≤III/ IV/ V	28.3/50.7 / 21.0	29.7/ 46.6/ 23.7	28.7/ 49.5/ 21.8
CRF healthy zones			
Under	105	86	191
Healthy	184	42	226
Above	53	5	58

[a] Significantly different from non-overweight, p<0.001 (Independent Two-tailed *t*-Tests for continues variables or Chi-square for categorical variables); BMI, body mass index; hs-CRP, High-sensitivity C-reactive Protein; KIDMED Index, adherence to the Mediterranean diet index; HDL-C ,high density lipoprotein cholesterol, FAS, Family Affluence Scale Pubertal stage-A – breast development in girls; genital development in boys. Pubertal stage-B – pubic hair development.

[b]Values were natural log-transformed before analysis, but non-transformed values are presented

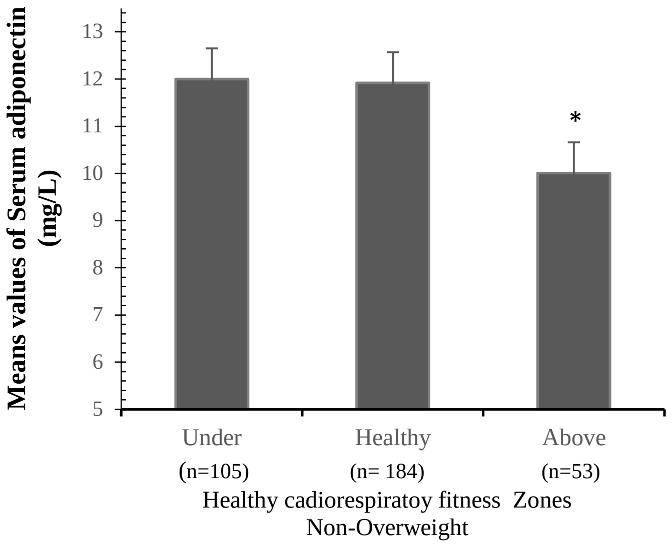
Table 2. Unstandardized regression coefficients examining the association of cardiorespiratory fitness with adiponectin in Non-Overweight and Overweight adolescents.

Cardiorespiratory Fitness[a]	Adiponectin[a]								
	Non-Overweight			Overweight			All		
	R^2	B	*P* value	R^2	B	*P* value	R^2	B	*P* value
Model 1	0.056	-0.662	<0.001	0.007	-0.051	0.869	0.013	-0.353	0.005
Model 2	0.113	-0.343	0.025	0.008	-0.088	0.809	0.059	-0.030	0.842
Model 3	0.143	-0.342	0.040	0.098	-0.206	0.546	0.145	-0.087	0.545
Model 4	0.141	-0.359	0.042	0.098	-0.281	0.466	0.170	-0.370	0.030

β: Unstandardized coefficients.
[a]Values were natural log-transformed before analysis
Model 1- Unadjusted model.
Model 2 - Adjusted for age, gender, pubertal stage, socioeconomic status, Adherence to the Mediterranean Diet, HOMA-IR.
Model 3 - Model 2 plus adjustment for HDL-Cholesterol, hs-CRP.
Model 4 - Model 3 plus adjustment for Body Fat percentage (Final adjusted model)

FIGURE: 1

* Significantly different from Under and Healthy zones in Non-Overweight (p= 0.03).
Figure 1: Serum adiponectin levels across cardiorespiratory fitness zones in non-overweight and overweight adolescents. The Bars represent adjusted means and 95% confidence interval, with age, sex, pubertal stage, socioeconomic status, Adherence to the Mediterranean Diet, HOMA-IR, HDL-C, hs-CRP, Body Fat percentage as confounders.



* Significantly different from Lowest and Thru zones ($p<0.05$)

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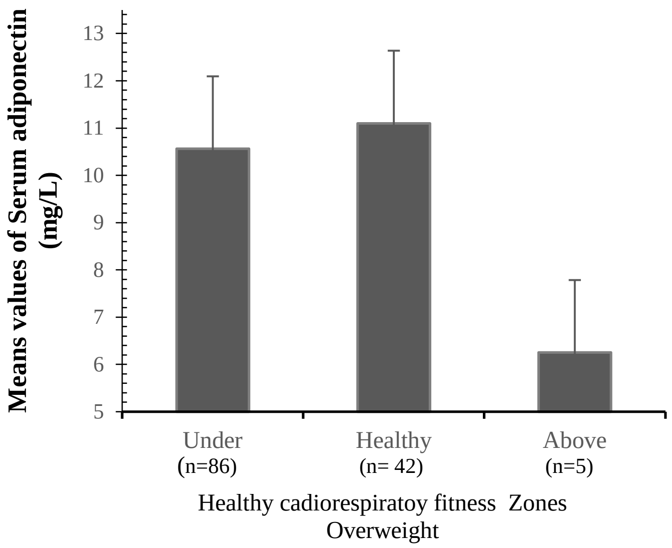

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