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Anti-oxLDL autoantibodies and their correlation with lipid profile and nutritional status in adolescents

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Abstract

Objective: To investigate whether levels of autoantibodies to oxidized LDL (anti-oxLDL) in the plasma of adolescents correlates with their anthropometric measurements and lipid profiles.

Methods: The study enrolled 150 adolescents aged between 10 and 15 years, recruited from the obesity clinic at Universidade Federal de São Paulo (SP) and from public schools in Piracicaba, SP, Brazil. Anthropometric measurements such as body mass index and waist and arm circumferences were used to classify the adolescents as having healthy weight, overweight or obesity. Colorimetric enzymatic methods were used for biochemical lipid profile analysis and ELISA was used to determine anti-oxLDL autoantibody levels.

Results: Analysis of anthropometric variables indicated that the obese group's profile was abnormal compared to the healthy weight and overweight groups ($p < 0.01$), indicating cardiovascular risk. Analysis of the lipid profiles demonstrated statistically significant differences in concentrations of total cholesterol ($p = 0.011$), HDL-cholesterol ($p = 0.001$) and LDL-cholesterol ($p < 0.042$) between the healthy weight group and the obese group. Analysis of plasma anti-oxLDL autoantibodies demonstrated that the overweight ($p = 0.012$) and obese groups ($p < 0.001$) had higher values than the healthy weight group. There were also correlations between anti-oxLDL autoantibody levels and anthropometric variables.

Conclusions: In adolescents the presence of anti-oxLDL autoantibodies and metabolic changes to the lipid profile vary in proportion with anthropometric parameters, which makes anti-oxLDL concentration a potential biochemical indicator of risk of metabolic syndrome.

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Introduction

Obesity is a chronic, multifactorial disease, characterized by excessive accumulation of adipose tissues around the body, in which both calorie intake and energy expenditure are dependent not only on genetic and physiological factors but also on cultural, social and psychological variables which determine both the quantity and the quality of nutritional intake.[1] In Brazil, recent decades (1974-1996) have seen an increase in the prevalence of overweight/obesity in the general population (24.8%).[2] The prevalence among adolescents has also increased and in 2002 and 2003, 18.7% of this section of the population were overweight/obese.[3] This subset, therefore, is exhibiting an epidemic of behavior putting it at risk of obesity and its complications,[4] especially because these abnormalities can persist into adulthood, increasing the

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risk of metabolic syndrome (MS). The most common metabolic complications include glucose intolerance, diabetes Type II, hypertension and dyslipidemia,[5] all of which are intimately related to oxidative stress.[6]

In this context, in addition to being intrinsic components of the genesis of obesity, oxidative modifications to biomolecules and, in particular, to the low density lipoprotein (oxLDL), have been identified as a primary proatherogenic factor.[7] According to Miller et al.,[7] the products generated (malondialdehyde, conjugated dienes, hydroperoxides) have an elevated cytotoxic, chemotactic and proinflammatory potential.[8] The immunogenic capacity of oxLDL is also of note, since the particle and its autoantibodies have been identified in both atherosclerotic lesions[8,9] and in circulation.[8,10] Despite these observations, the role played by anti-oxLDL autoantibodies in the development of risk factors associated with MS in adolescents, particularly obese adolescents, has so far been little investigated. The cost, time, and specialized personnel needed to monitor biomarkers has stimulated the development and validation of alternative tools. Anthropometric parameters have been widely used in epidemiological studies to assess the nutritional status of adolescents since they provide a practical, rapid and low-cost method, although with certain limitations.[11]

Therefore, the objective of this study is to determine whether the concentration of anti-oxLDL autoantibodies in the plasma of adolescents correlates with the anthropometric measurements and lipid profile classically employed to assess risk of MS in adolescents.

Methods

The study enrolled 150 adolescents of both sexes (60% girls and 40% boys) with a mean age of 12.8±1.29 years. One hundred and ten of these adolescents (73.3%) were recruited public schools in Piracicaba, SP, Brazil, while 26.7% (n = 40) were selected from the obesity clinic at the Universidade Federal de São Paulo (UNIFESP), in São Paulo (SP), Brazil.

This was a cross-sectional study of adolescents aged from 10 to 15 years selected from the obesity clinic at UNIFESP and from public schools in the town of Piracicaba (SP). The sample size was calculated for a randomized study using three factors: age (four levels), sex (two levels: male and female) and group (three levels: healthy weight, overweight and obesity). Power Analysis Sample Size[12] software was used to determine the sample size necessary for a minimum power of 80%, a significance level (p) of 0.05 and resolution to detect a minimum difference between the mean values of around three units. The program estimated that each group must contain a minimum 48 individuals.

Children were excluded if their body mass index (BMI) was below the third percentile[13] for age and sex, if they had a clinical diagnosis of a chronic disease (diabetes mellitus, hypertension or kidney diseases), if they were on continuous medication or if they were participating in any other research protocol. All of the individuals' parents or guardians were fully informed about the study, after which they signed consent forms. The study was approved by the Ethics Committee at the Public Health Faculty of Universidade de São Paulo (no. 1223) and met the National Human Research Ethics Commission guidelines.[14]

Height and weight were measured using and Alturexata Stadiometer (TBW Brasil®, Brazil) and a *Control* digital balance (Plenna®, Brazil). These measurements were used to calculate BMI, which was in turn classified against the growth curves published by the Centers for Disease Control and Prevention - CDC[13] for sex and age, according to the cutoff points, by percentile, for healthy weight, overweight and obesity. Parameters published by Fernández[15] and Frisancho,[16] were used to classify the adolescents according to their waist circumference (WC) and arm circumference (AC), respectively. Cardiovascular risk was determined based on WC as proposed by Fernández ($p \geq 90$).[15]

After 12-15 h fasting, 20 mL of blood was taken for the biochemical analyses. Colorimetric enzymatic methods were used to determine lipid profiles. Concentrations of total cholesterol (TC), cholesterol associated with HDL and triglycerides were determined by direct analysis. Concentrations of cholesterol associated with LDL and VLDL were determined using Friedwald's formula.[17]

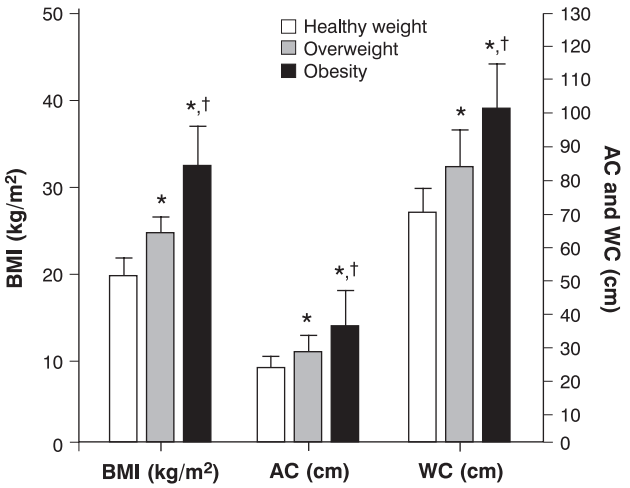
Anti-oxLDL autoantibodies were determined using ELISA, with results presented at an equivalent absorbance relative to IgG anti-oxLDL. The standard curve was created using total human IgG (23.4 to 0.183 ug/mL).

Results are presented in the form of means and standard deviations for analyses collected in triplicate for each study variable. Normality was tested using the Kolmogorov-Smirnov test ($p > 0.05$), while analysis of variance (ANOVA) and the Kruskal-Wallis test were used to compare differences between groups. Spearman's test was used to establish correlations between variables. The significance level was set at $p < 0.05$ for all analyses. The Statistical Package for the Social Sciences® (SPSS, version 10.0) was used for these analyses.[18]

Results

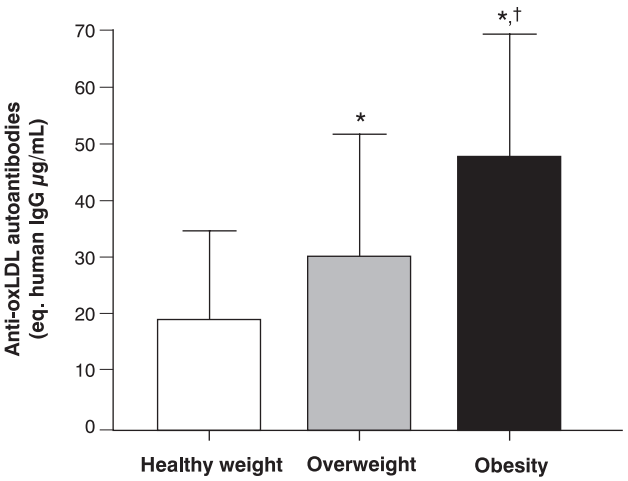
The adolescents were divided into three groups based on their anthropometric parameters (BMI): healthy weight (HW, n = 75), overweight (OW, n = 33) and obese (OB, n = 42). According to the results obtained, the groups did not exhibit statistical differences in terms of sex ($p = 0.299$) or age ($p = 0.301$).

Figure 1 illustrates the mean values plus standard deviations for BMI, WC and AC by nutritional status. There were statistical differences ($p < 0.01$) between the mean BMI observed in the groups HW (19.1±2.18 kg/m^2), OW



AC = arm circumference; BMI = body mass index; WC = waist circumference.
* Vs. healthy weight group.
† Vs. overweight group.

Figure 1 - Anthropometric parameters and anti-oxLDL autoantibody concentration in the plasma (ANOVA, significance level p < 0,05), according to nutritional status (São Paulo, SP, Brazil, 2004-2006)



* Vs. healthy weight group.
†Vs. overweight group.

Figure 2 - Anthropometric parameters and anti-oxLDL autoantibody concentration in the plasma (Kruskal-Wallis, significance level p < 0,05), according to nutritional status (São Paulo, SP, Brazil, 2004-2006)

(24.2±1.87 kg/m^2) and OB (30.8±4.55 kg/m^2). This classification of the adolescents by BMI was confirmed by the analysis of waist circumference, where it was demonstrated that the OB group had values (99.5±13.91 cm) above the 90th percentile (P90), indicating that this population is at cardiovascular risk. The difference between the OB group and the HW and OW groups was statistically significant (68.9±7.36 and 82.3±11.11 cm, respectively; p < 0.01). A similar profile was observed in the analysis of AC measurements, with the obese adolescents (34.7±10.54 cm) having higher values than the HW and OW groups (22.8±2.57 and 27.7±4.48 cm; p < 0.01).

Analysis of the lipid profiles indicated that the differences between the HW and OB groups were statistically significant for TC (p = 0.011), HDL-cholesterol (p = 0.001) and LDL-cholesterol (p = 0.042). However, the OW group exhibited values that were similar to both the HW and the OB groups. All three groups were similar in terms of VLDL-cholesterol and triglycerides (p = 0.126 and 0.126, respectively) (Table 1).

Assessment of plasma anti-oxLDL autoantibody concentrations demonstrated that both the OW (p = 0.012) and OB (p < 0.001) groups had higher levels than the HW group and were different from each other (p = 0.005) (Figure 2).

When the variables were correlated, we found that, irrespective of study group, anti-oxLDL autoantibody levels had a positive and significant correlation with BMI (r = 0.49; p < 0.001), WC (r = 0.44; p < 0.001) and AC (r = 0.44; p < 0.001). Figure 3 illustrates the correlation between anti-oxLDL autoantibodies and WC. When the correlation between

Table 1 - Adolescents' lipid profiles, by nutritional status (São Paulo, SP, Brazil, 2004-2006)*†

	HW (n = 75) Mean	OW (n = 33) Mean	OB (n = 42) Mean
Total cholesterol	130.7±26.79	136.8±31.14	148.1±33.29‡
HDL-cholesterol	39.6±9.64	36.2±6.83	32.6±11.90‡
LDL-cholesterol	89.5±26.11	96.9±30.89	103.0±28.99‡
VLDL-cholesterol	14.2±8.17	16.6±10.26	16.9±8.65
Triglycerides	71.2±40.85	83.2±51.31	84.3±43.24

HW = healthy weight; OB = obese; OW = overweight.
* Values in mg/dL.
† Significant differences were determined using ANOVA with a significance level of p < 0,05.
‡ Vs. healthy weight group.

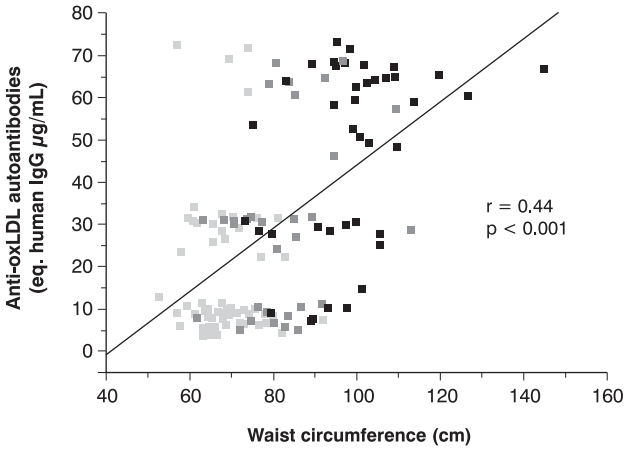


Figure 3 - Correlation between anti-oxLDL autoantibodies and waist circumference (São Paulo, SP, Brazil, 2004-2006)

anti-oxLDL autoantibodies and lipid profile was investigated, a correlation was observed with LDL-c ($r = 0.205$ and $p < 0.01$). Table 2 lists the values of the correlations between lipid profile and anthropometric profile.

Discussion

Obesity is a public health problem whose prevalence is growing in the adolescent population. This being so, our objective was to investigate possible correlations between classic anthropometric and biochemical parameters and the concentration of anti-oxLDL autoantibodies associated with obesity.

When the concentration of TC and LDL-cholesterol were compared between the three groups of adolescents, an increase proportional to BMI was observed, where the OB group exhibited the highest serum lipid concentrations. In contrast, an inverse association was observed between HDL-cholesterol and BMI, although all groups had values below the recommendation. Therefore, we observed that the results obtained in this study confirm earlier studies that have observed a positive association between TC and BMI, and reduced HDL-cholesterol levels in obese adolescents.[19] Despite the many studies that have indicated that the majority of adolescents are within acceptable limits for TC and LDL-cholesterol, an elevated percentage are borderline (150-169 and 100-129 mg/dL, respectively), indicating the potential risk to which this population is subject.[20] In our study, we found that irrespective of nutritional status approximately 12.0% were over the ideal LDL level, rising to 15.2 and 21.4% for the overweight and obese children, respectively.

In addition to the relationship between lipid profile and anthropometry observed in our study, Weiss et al.[21] have demonstrated that obesity is correlated with insulin resistance ($r = 0.31$ and $p < 0.001$), fasting glucose ($r = 0.08$ and $p = 0.08$) and post prandial glucose ($r = 0.12$ and $p = 0.007$). Similar results were observed in a study by Sur et al.,[22] who described an increase in BMI simultaneous with increases in TC and LDL-cholesterol. In our study, we found similar correlations, providing evidence of the direct relationship between nutritional status and risk factors associated with coronary artery disease. The correlations observed, although significant, were weak ($r < 0.5$), indicating that other factors influence weight gain, such as genetic and environmental factors and physical activity. In addition to this possibility, it is probable that the correlation between these variables was weakened due to the majority of adolescents in our study (88.0%) having lipid profiles within acceptable limits and 50% of the sample being overweight or obese.

Being overweight predisposes individuals to the risk of cardiovascular diseases, influencing changes to lipid and glucose metabolism and arterial blood pressure.[23] Therefore, recognition of these changes is at once both indicative of cardiovascular risk and of obesity-associated morbidity.[20] Despite the studies described above, Siemianowicz et al.[24] demonstrated that children with an elevated risk of cardiovascular diseases based on family history do not exhibit the classic biochemical atherosclerosis risk factors. Consequently it is important to identify other biochemical markers which could indicate global risk of cardiovascular diseases.

It is in this context that oxidative modifications to LDL have become an important target for monitoring, particularly of atherosclerotic processes.[25] These observations suggest that detection of these particles, and their autoantibodies, may

Table 2 - Correlations between lipid profile variables and anthropometric profile variables (São Paulo, SP, Brazil 2004-2006)

	Total cholesterol		LDL-cholesterol		HDL-cholesterol	
	r	p	r	p	r	p
BMI (kg/m^2)	0,236	0,004*	0,215	0,008*	-0,244	0,003*
WC (cm)	0,272	0,001*	0,166	0,044*	-0,138	0,095
AC (cm)	0,251	0,002*	0,108	0,19	-0,063	0,448

AC = arm circumference; BMI = body mass index; WC = waist circumference.
* Significant differences were determined using Spearman's correlation with a significance level of $p < 0,05$.

offer an important biochemical marker associated with oxidative process *in vivo*.[26] Since monitoring of oxLDL demands sophisticated methods (high performance liquid chromatography/fast performance liquid chromatography, antibodies), its application is still relatively limited to experimental studies. In contrast, analysis of autoantibodies has emerged as a low-cost tool that is rapid precise and specific.

According to Young & McEneny,[26] anti-oxLDL autoantibodies are related to the progression of atherosclerosis. There are reports that children with healthy weights have elevated anti-oxLDL autoantibody production, exhibiting even higher values than adults.[27] In the same way, Barros et al.[10] detected lower anti-oxLDL antibody levels in hypercholesterolemic children and adolescents with or without a family history of hypercholesterolemia and coronary artery disease, when compared with a control group. Although other studies have described different profiles from that observed in our study, Liuba et al.[28] demonstrated that acute inflammation in children is accompanied by pro-atherogenic changes in lipids, together with an increase in anti-oxLDL antibodies, suggesting the development of atherosclerosis. More recently, Rodenburg et al.[29] found that children with a family history of hypercholesterolemia had higher levels of autoantibodies (isotype IgG and IgM) and the immunocomplexes anti-apoB and anti-malondialdehyde (isotype IgM). Therefore, there is consensus on the involvement of anti-oxLDL antibodies, although the impact of their increasing or decreasing on chronic diseases such as obesity and atherosclerosis still demands further investigation. Despite this, and in line with the results found in our study, it is probable that an increase in anti-oxLDL autoantibodies is an early marker of cardiovascular risk in obese adolescents. In our study, the fact that production of anti-oxLDL autoantibodies is in line with increasing BMI offers the perspective of interaction between biochemical and anthropometric parameters, both aimed at assessing the cardiovascular risk of adolescents. Although the number of studies involving production of anti-oxLDL autoantibodies in adolescents is low, according to Binder et al.,[25] immunoresponse plays an important role in atherogenesis and associated diseases (obesity and diabetes), suggesting that these particles are involved in the progression of these diseases.

Therefore, the results presented here confirm the presence of anti-oxLDL autoantibodies in adolescents and establish their relationship with anthropometric and biochemical parameters used to monitor the MS.

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