Is Plasma Alpha-Tocopherol Associated with Electronegative LDL in Obese Adolescents?

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Summary Obesity has increased in children and adolescents. What is reflected in the early occurrence of cardiometabolic alterations, like hypertension and type 2 diabetes, where the oxLDL formation is stimulated. Various studies have shown that plasma α-tocopherol (α-TP) can protect LDL against oxidation. Nevertheless, the action of plasma α-TP in cardiovascular diseases remains controversial. We conducted a cross-sectional study to evaluate plasma α-TP and its impact on the concentration of LDL(−). Adolescents (n=150) of both sexes were classified into three groups: healthy weight (HW; 50%), overweight (OV; 22%), and obese (OB; 28%). Lipid profile, LDL(−), anti-oxLDL and anti-LDL(−) antibodies, CRP (ELISA) and plasma α-TP (HPLC) were analyzed. Demographic, anthropometric, and food intake data were evaluated. Crude and energy-adjusted intake of vitamin E in the OB group were higher than in the HW group (p<0.001). Crude and energy-adjusted vitamin E intakes were not correlated with plasma α-TP (r=−0.07; p=0.412 and r=−0.064; p=0.467, respectively). Subjects in the OB group had higher TC and LDL-C and lower HDL-C than in the HW and OV groups. C-reactive protein and anti-oxLDL antibodies changed as a function of BMI. The impact of obesity was reinforced by high values for LDL(−) and low content of plasma α-TP in comparison with the HW (p<0.001) and OV groups (p=0.03). This negative profile was maintained for the ratio between α-TP and TC or LDL-C. Plasma α-TP, α-TP/TC and α-TP/LDL-C were negatively associated with LDL(−) and other cardiometabolic risk factors (BMI, WC, AC and anti-oxLDL). Our results demonstrate that obesity in adolescents is associated with high levels of LDL(−) and low plasma α-TP content.

Key Words plasma alpha-tocopherol, electronegative low-density lipoprotein, obesity, adolescents

Obesity is characterized as an imbalance between energy intake and expenditure. This results in accumulation of adipose tissue, which is strongly influenced by genetic and environmental factors (1).

In the last decades, prevalence of obesity has increased in people of all ages. However, the rate of increase in children and adolescents has been more accelerated than in adults. In the last 30 y, obesity in the USA has almost tripled in children aged 2–5 y (5–14%) and adolescents aged 12–19 y (5–17%) and it has quadrupled in children aged 6–11 y (4–19%) (2). According to the Brazilian Institute for Geography and Statistics, overweight and obesity are present in 16.7 and 2.0% of Brazilian adolescents, respectively (3). Currently, the worldwide increase in childhood obesity is recognized and characterized as a global epidemic.

Beyond the implications for social and individual health in terms of emotional welfare (4), the effect of overweight on the physical health of adolescents has been widely recognized (5, 6). Development of obesity is reflected in the early occurrence of cardiovascular events like type 2 diabetes and hypertension (7, 8). Therefore, prevention of atherosclerosis and its consequences should begin in adolescence and young adulthood.

Furthermore, growing evidence suggests that oxidative stress is a common element in atherosclerosis, cardiovascular disease, and obesity (9–11). Martino et al. (12) showed that already in children oxidative stress may have an important role in the occurrence of premature atherosclerosis. In this context, electronegative low-density lipoprotein [LDL(−)] has been widely monitored in adults and now it is considered a potential biomarker associated with initiation and progression of atherosclerosis (13–16). Despite these observations, a limited number of studies have detected LDL(−) in children and adolescents (17, 18).

Regarding oxidized LDL (oxLDL) as a cardiovascular risk factor, various studies have shown that antioxidants, particularly α-tocopherol (α-TP), can protect LDL against oxidation (19). α-TP has been widely studied due to its antioxidant and non-antioxidant properties, and its cardioprotective potential role (20). However, no positive effect of α-TP supplementation on cardiometabo-
bolic risk in adults was observed (21). Conversely, many authors showed that α-TP either isolated or associated with other bioactive substances reduces oxidative stress and cardiometabolic risk (22, 23). Similarly, Hall et al. (24) concluded that impairment of α-TP status in hyperlipidemia could influence cardiovascular risk. However, a limited number of studies has evaluated the impact of α-TP on LDL(−) content.

Therefore, in view of the potential role of LDL(−) in the development of atherosclerosis and the controversial action of α-TP in cardiovascular risk, our goal was to investigate the influence of overweight and obesity on α-TP plasma levels in adolescents and to establish a possible association with electronegative LDL.

MATERIALS AND METHODS

Study population. This cross-sectional study included adolescents selected from public schools located in the cities of Piracicaba and Sao Paulo, SP, Brazil. Adolescents (n=150) of both sexes, aged 10–15 y old were included in the study. According to the classification of body mass index (BMI), as defined by the Centers for Disease Control (25), the adolescents were put into 3 groups: healthy weight (HW), overweight (OV), and obese (OB). Adolescents with BMI values below the 3rd percentile of the CDC classification were not included in the study. The study protocol was approved by the Ethics Committee (School of Public Health, University of Sao Paulo; Proc. #1223) and followed the recommendations of the National Council for Health on Ethics in Research with Humans. All parents of the adolescent signed a written consent to participate in the study.

The sample size for a randomized study was established using three factors: age (five levels), sex (two levels: male and female), and group (three levels: healthy weight, HW; overweight, OV; and obese, OB). The test power was ≥80% and the alpha level of significance was set as <0.05 to detect a minimum difference of about 3 units between the average values for the extracts.

Anthropometric measurements. Height and weight were determined by a stadiometer (AlturaExata, TBW Brazil, Sao Paulo, SP, Brazil) and a digital balance (Control, Plenna, Sao Paulo, SP, Brazil), respectively. BMI (weight/height^2) was calculated and the nutritional status was classified according to the CDC growth curves (25) for sex and age. Arm (AC) and waist circumferences (WC) were assessed using a 1 mm-precision flexible and inelastic tape (TBW Brazil®, Sao Paulo, SP, Brazil).

Food frequency questionnaire (FFQ). A validated FFQ was used to determine the energy, macronutrient, and vitamin E intake (26). Interviewers were provided with a food instruction booklet to collect descriptions of foods consumed by the interviewees and their respective amounts. Each food description was compared with relevant food probes in the food instruction booklet. The respondents used measurement guides to approximate the amount of food eaten. All interviews were conducted by dieticians. Vitamin E was expressed in crude and energy-adjusted form as proposed by Willett and Stampfer (27).

Blood samples and lipid analysis. After a 12-h fast, blood samples (20 mL) were collected and centrifuged for plasma separation (3,000 rpm, 10 min, 4˚C). Concentrations of total cholesterol (TC), HDL-cholesterol (HDL-C), and triglycerides (TG) were determined by colormetric methods using commercial kits (Labtest Diagnostica, Lagoa Santa, MG, Brazil). LDL-cholesterol (LDL-C) concentration was determined by the Friedewald equation [(LDL-cholesterol) = (total cholesterol) − (HDL-cholesterol) − (triacylglycerol/5)]. The TC/HDL-C and LDL-C/HDL-C ratios were calculated.

C-reactive protein. C-reactive protein concentration (CRP; ng/mL) was determined by ELISA (Diagnostic Systems Laboratories, Inc., Webster, TX, USA). The C-reactive protein was analyzed in a subsample (n=88), matched for BMI, sex, and age.

Electronegative LDL analysis. Detection of LDL(−) was performed by ELISA as standardized by Damasceno et al. (28). Microtiter plates (Costar, model 3690, Cambridge, MA, USA) were coated (overnight, 4˚C) with plasma diluted (1:500, v/v) in carbonate-bicarbonate buffer (0.1 M, pH 9.6). After this period, the plasma solutions were discarded and the plates were blocked with skimmed milk (5%) in PBS (2 h, 37˚C). The plates were washed with PBS-Tween-20 (0.05%, four times) and incubated again (2 h, 37˚C) with anti-LDL(−) monoclonal antibody (MAb 3D1036; INPI #2637/2002; 1.0 μg/mL; 50 μL/well). Horseradish peroxidase-conjugated polyclonal goat anti-mouse IgG antibody (Rockland Immunochemicals for Research, Gilbertsville, PA, USA; 50 μL/well) thereafter diluted (1:5,000, v/v) in skimmed milk (1% in PBS) was added to the wells and incubated (2 h, 37˚C). Afterward, the plates were washed and the substrate, containing 3,3′,5,5′-tetramethylbenzidine (TMB; 250 μL), phosphate-citrate buffer (10.0 mL; pH 4.2), and hydrogen peroxide (10.0 μL), was added to the wells (50.0 μL/well) and reactivity was blocked with H2SO4 (2 mL). After 30 min of reaction, colour intensity was evaluated by light absorption (Bio-Tek Instruments, Winooski, VT, USA; λ=450 nm). Analyses were done in triplicate for all samples and results are shown as [(Abs_Sample − Abs_Background) × Titer].

Anti-LDL(−) and anti-oxLDL antibodies analyses. Electronegative LDL was isolated by FPLC using ion exchange high pressure liquid chromatography monitored at 280 nm (Shimadzu, Tokyo, Japan) and a 7.0 mm x 35.0 mm UNO Q-1 column (Bio-Rad Inc., Hercules, CA). A 1.0 mL sample was injected and eluted at 1.0 mL/min with helium-spared and pressurized Tris-HCl 0.01 M, pH 7.2, starting buffer. After 5 min, a NaCl gradient from 0 to 0.3 M in Tris-HCl buffer was eluted over the remaining 45 min. The peaks were collected in tubes containing 1.0 mL EDTA, 5.0 μM phenylmethylsulfonyl fluoride, 10.0 μM benzamidine, 10.0 μg/mL aprotinin and 100.0 μg butylated hydroxytoluene that were immersed in an ice bath. LDL(−) was diluted (10 μg/mL, v/v) in carbonate-bicarbonate buffer (0.01 M, pH 9.6) and used to coat the plates (Costar, model 3690). Solutions containing non-adsorbed par-
Instruments) and reactivity was blocked with H2SO4 (250.0 mL, pH 4.2), and hydrogen peroxide (10.0 mL). After 18 h, the plates were washed with Tween-20 (0.05% in PBS; 2 h, 37˚C) and plasma samples diluted (1 : 1,600, v/v) in skimmed milk solution (1% in PBS) were added. After this period, anti-human IgG antibody (Rockland Immunochemicals, USA) at a flow rate of 1 mL/min. Results of plasma antioxidant concentrations were calculated in terms of human IgG equivalents. The same protocol was used for anti-oxLDL analysis, except that LDL(−) was replaced with oxLDL (LDL incubated with 10 mM CuSO4, 18 h, 37˚C) and plasma samples were diluted (1 to 400, 800, and 1,000, v/v, for plasma from subjects in groups HW, OV, and OB, respectively).

Plasma alpha-tocopherol (α-TP) analysis. Concentration of α-TP in plasma was determined by high performance liquid chromatography (HPLC) using a fluorescence detector (λex = 295 and λem = 325 nm; LaChrom L-7480, Merck-Hitachi, Kyoto, Japan). Antioxidants were extracted with a methanol : hexane (1:3, v/v) solution under protection from light according to Moriel et al. (29). After solvent evaporation, the pellet was resuspended in HPLC mobile phase (methanol : acetonitrile : chloroform, 35 : 35 : 30, v/v/v) containing lithium perchlorate (20 mM). All samples were filtered (22 µm, Millipore, São Paulo, SP, Brazil) and manually injected (20 µL) into the chromatographic column. Antioxidants present in the samples were quantified using synthetic α-TP (Sigma Aldrich, St. Louis, MO, USA) as an external standard. Multiple-level calibration curves were constructed using the Class LC-10 program in the chromatography workstation. Analyses were performed in the LC-10 AT VP chromatography system (Shimadzu) with a C18 column (250×4.6 mm, 5-µm, 100-Å; Varian Microsorb-MV, Varian, Lake Forest, CA, USA), at a flow rate of 1 mL/min. Results of plasma α-TP concentration are shown as crude form and α-TP/LDL-C ratios.

Statistical analyses. After evaluation of data distribution by the Kolmogorov-Smirnov test (p>0.05), differences between groups were determined by the ANOVA and Kruskal-Wallis tests. When significant differences were detected, post-hoc analyses were made by the Tukey test for paired samples or Mann-Whitney test for unpaired samples. The parametric variables were shown as means (standard deviation) and non-parametric variables as median (interquartile range). The nutrient intake was tested using log transformed data. The $\chi^2$ test was used for anti-IgG equivalents.

Table 1. Demographic, anthropometric and biochemical profile of adolescents according to BMI.

<table>
<thead>
<tr>
<th></th>
<th>HW (n=75)</th>
<th>OV (n=33)</th>
<th>OB (n=42)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male, n (%)a</td>
<td>27 (36)</td>
<td>12 (36)</td>
<td>21 (50)</td>
</tr>
<tr>
<td>Female, n (%)a</td>
<td>48 (64)</td>
<td>21 (64)</td>
<td>21 (50)</td>
</tr>
<tr>
<td>Age, ya</td>
<td>12.8 (1.3)</td>
<td>12.7 (1.2)</td>
<td>13.1 (1.4)</td>
</tr>
<tr>
<td>BMI, kg/m²c</td>
<td>19.1 (2.2)</td>
<td>24.2 (1.9)*</td>
<td>31.8 (4.5)***</td>
</tr>
<tr>
<td>WC, cm²</td>
<td>68.9 (7.3)</td>
<td>82.3 (11.1)*</td>
<td>99.5 (13.9)**</td>
</tr>
<tr>
<td>AC, cm²</td>
<td>22.7 (2.6)</td>
<td>27.7 (4.5)**</td>
<td>34.8 (4.5)**</td>
</tr>
<tr>
<td>TC, mg/dLc</td>
<td>130.6 (26.8)</td>
<td>136.8 (31.1)*</td>
<td>148.1 (33.3)*</td>
</tr>
<tr>
<td>LDL-C, mg/dLc</td>
<td>89.4 (26.1)</td>
<td>96.9 (30.9)</td>
<td>103.0 (29.0)*</td>
</tr>
<tr>
<td>HDL-C, mg/dLc</td>
<td>39.5 (9.6)</td>
<td>36.2 (6.8)</td>
<td>32.5 (11.9)*</td>
</tr>
<tr>
<td>TG, mg/dLc</td>
<td>71.2 (40.8)</td>
<td>83.2 (51.3)</td>
<td>84.3 (43.2)</td>
</tr>
<tr>
<td>TC/HDL-C</td>
<td>3.5 (1.1)</td>
<td>3.9 (1.1)</td>
<td>5.3 (3.1)****</td>
</tr>
<tr>
<td>LDL-C/HDL-Cc</td>
<td>2.4 (1.0)</td>
<td>2.8 (1.0)</td>
<td>3.8 (2.7)*</td>
</tr>
<tr>
<td>LDL(−), Abs×1,000c</td>
<td>437.1 (171.2)</td>
<td>531.1 (216.2)*</td>
<td>520.3 (189.3)*</td>
</tr>
<tr>
<td>Anti-oxLDL, µg/mLb</td>
<td>10.2 (8.2–30.3)</td>
<td>30.6 (9.5–46.2)*</td>
<td>58.2 (28.4–66.1)**</td>
</tr>
<tr>
<td>Anti-LDL(−), µg/mLb</td>
<td>49.8 (43.8–52.8)</td>
<td>50.7 (45.9–52.7)</td>
<td>48.6 (44.5–54.4)</td>
</tr>
<tr>
<td>α-TP, mol/Lb</td>
<td>0.144 (0.116–0.486)</td>
<td>0.120 (0.104–0.132)*</td>
<td>0.119 (0.104–0.131)*</td>
</tr>
<tr>
<td>CRP, ng/mLc</td>
<td>6.63 (1.38)</td>
<td>7.83 (1.44)*</td>
<td>8.25 (1.01)*</td>
</tr>
</tbody>
</table>

*a p<0.05 vs HW group. **p<0.05 vs OV group.

a Chi-Square test.
b Kruskal-Wallis and Mann-Whitney post-hoc test: (median and interquartile range).
c ANOVA and Tukey post-hoc test: mean (SD).

* HW, healthy weight; OV, overweight; OB, obese; WC, waist circumference; AC, arm circumference; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; Anti-oxLDL, antibody to oxidized LDL; Anti-LDL(−), antibody to electronegative low density lipoprotein; CRP, C-reactive protein; Antibodies are given as anti-IgG equivalents.
α-Tocopherol and LDL(−) in Obese Adolescents

Table 2. Dietary intake of adolescents according to BMI.

<table>
<thead>
<tr>
<th></th>
<th>HW (n=75)</th>
<th>OV (n=33)</th>
<th>OB (n=42)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Crude</td>
<td>EAN</td>
<td>Crude</td>
</tr>
<tr>
<td>Energy, kcal/d</td>
<td>3,042 (1216)</td>
<td>3,294 (1647)</td>
<td>3,647 (1693)</td>
</tr>
<tr>
<td>Proteins, g/d</td>
<td>95.7 (41.5)</td>
<td>94.5 (15.1)</td>
<td>110.3 (13.6)</td>
</tr>
<tr>
<td>Lipids, g/d</td>
<td>113.3 (53.1)</td>
<td>110.3 (13.6)</td>
<td>116.3 (71.1)</td>
</tr>
<tr>
<td>Carbohydrates, g/d</td>
<td>428.9 (163.8)</td>
<td>423.9 (35.5)</td>
<td>482.1 (226.8)</td>
</tr>
<tr>
<td>Vitamin E, mg/d</td>
<td>5.2 (3.8)</td>
<td>6.3 (3.7)</td>
<td>6.3 (3.7)</td>
</tr>
</tbody>
</table>

Values are mean (SD). *p<0.05 vs HW group.
ANOVA and Tukey post-hoc test: variables were tested using log form.
HW, healthy weight; OV, overweight; OB, obese; EAN, energy-adjusted nutrient.

RESULTS

Table 1 shows the demographic, anthropometric and biochemical profile of the adolescents (n=150) included in the study. Regarding BMI classification, groups HW (n=75, 50%), OV (n=33, 22%), and OB (n=42, 28%) did not show differences in age (p=0.265) or gender (p=0.296). As expected, BMI data confirm that the three groups were different (p<0.001). The anthropometric profile reinforced significant differences by the WC and AC between groups.

Lipid profile analysis indicated that the OB group shows higher concentrations of TC (p=0.011) and LDL-C (p=0.042), and lower values of HDL-C (p=0.001), in comparison to HW group. However, TG content exhibited similar profiles for the three groups (p=0.132). The OV group did not show differences between lipid variables when compared with the HW and OB groups. TC/ HDL-C and LDL-C/HDL-C ratios in OB group were higher than those in HW group (p<0.001 and p<0.001, respectively).

Fig. 1. (A) Distribution of plasma LDL(−) and (B) plasma α-tocopherol (α-TP), according to BMI of adolescents. For α-TP: Kruskal-Wallis and Mann-Whitney post-hoc test. For LDL(−): ANOVA and Tukey post-hoc test. * vs HW (p<0.001). HW, healthy weight; OV, overweight; OB, obese; ▲, 1st percentile; ▼, 99th percentile; ■, mean.
respectively). However, crude and energy-adjusted vitamin E intakes were not correlated with plasma α-TP ($r = -0.07; p = 0.412$ and $r = -0.064; p = 0.467$, respectively).

Figure 1 shows the distribution of LDL(−) and α-TP contents in the plasma of adolescents, according to their BMI. LDL(−) content in adolescents with overweight and obesity was higher than the content for adolescents in the HW group, but similar between them. Coefficients of intra-assay (4.5%) and inter-assay (2.9%) confirm validation of the ELISA used in the present study. Inversely, the values for plasma α-TP concentration in the HW group proved to be higher than those in the OV ($p = 0.003$) and OB groups ($p < 0.001$), which were similar. Regarding α-TP concentration, differences between groups remain unchanged after the analyses of plasma α-TP/TC and plasma α-TP/LDL-C ratios. The values of the plasma α-TP/LDL-C ratio in the OV and OB groups were lower than that of the HW group. A similar profile was shown for the plasma α-TP/TC ratio, where the ratios of the OV ($p = 0.011$) and OB groups ($p < 0.001$) were reduced in comparison to the HW group (Fig. 2).

Table 3 shows values for correlations between the variables, independent of the values for BMI of the adolescents. Plasma α-TP data showed negative correlation with LDL(−) ($r = -0.53; p < 0.001$), and this correlation was maintained in plasma α-TP/TC ratio versus LDL(−) ($r = -0.38; p < 0.001$) and α-TP/LDL-C versus LDL(−) ($r = -0.45; p < 0.001$). Significant correlations between LDL(−) levels and BMI ($r = 0.22; p < 0.009$), WC ($r = 0.17; p = 0.047$), LDL-C ($r = 0.23; p = 0.005$), TG ($r = 0.22; p = 0.009$), and anti-oxLDL antibodies ($r = 0.40; p < 0.001$) were obtained.

**DISCUSSION**

The results shown in the present study confirm that obesity in adolescents is associated with reduced plasma α-TP concentration and high LDL(−) content. These variables showed negative association, confirming the α-TP action on oxidative stress. These observations partly explain the high degree of association between adiposity and cardiovascular risk in obese adults, and it is likely that a similar correlation is present in overweight and obese adolescents.

Our results are strengthened by the fact that obese adolescents exhibited higher values for TC and LDL-C and lower values for HDL-C as compared to adolescents with low BMI, evidencing the first signal of cardiovascular risk. Similar results were described, in which adolescents with high BMI also had elevated TC and TG and low HDL-C concentrations (30–32). Cardiovascular heart diseases have different risk factors, and lipid profile is probably the most investigated of them. In a recent review, Haney et al. (30) verified that factors such as age, sex, and ethnic group have significant effects on lipid levels. However, these factors were similar among the groups studied herein.

**Table 3. Correlations between plasma LDL(−) and α-tocopherol (α-TP) with anthropometric and biochemical parameters.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>LDL(−)</th>
<th>α-TP</th>
<th>α-TP/TC</th>
<th>α-TP/LDL-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI, kg/m²</td>
<td>$0.22^b$</td>
<td>$&lt;0.009$</td>
<td>$-0.42^a$</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>WC, cm</td>
<td>$0.17^a$</td>
<td>$0.047$</td>
<td>$-0.32^a$</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>AC, cm</td>
<td>$0.10^b$</td>
<td>$0.215$</td>
<td>$-0.30^a$</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>TC, mg/dL</td>
<td>$-0.02^b$</td>
<td>$0.800$</td>
<td>$-0.04^a$</td>
<td>$6.80$</td>
</tr>
<tr>
<td>LDL-C, mg/dL</td>
<td>$0.23^b$</td>
<td>$0.005$</td>
<td>$-0.37^a$</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>TG, mg/dL</td>
<td>$0.22^b$</td>
<td>$0.009$</td>
<td>$-0.01^a$</td>
<td>$8.84$</td>
</tr>
<tr>
<td>LDL(−), Abs×1,000</td>
<td>$-0.53^a$</td>
<td>$&lt;0.001$</td>
<td>$-0.53^a$</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Anti-oxLDL, μg/mL</td>
<td>$0.40^a$</td>
<td>$&lt;0.001$</td>
<td>$-0.70^a$</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Anti-LDL(−), μg/mL</td>
<td>$-0.03^b$</td>
<td>$0.668$</td>
<td>$-0.10^2a$</td>
<td>$0.252$</td>
</tr>
<tr>
<td>CRP, ng/mL</td>
<td>$0.31^b$</td>
<td>$&lt;0.001$</td>
<td>$-0.24^a$</td>
<td>$0.001$</td>
</tr>
</tbody>
</table>

$^a$Spearman’s and $^b$ Pearson’s correlation test.
LDL(−), electronegative LDL; TC, total cholesterol; LDL-C, LDL-cholesterol; WC, waist circumference; AC, arm circumference; TG, triglycerides; Anti-oxLDL, antibody to oxidized LDL.
In addition to the lipid changes found in adolescents with obesity, our results showed high CRP content, confirming the inflammatory background of obesity (9). Caballero et al. (32) showed that obese Hispanic adolescents had higher CRP, intracellular adhesion molecule, plasminogen-activated inhibitor-1, tissue plasminogen activator, and white blood cell count when compared with lean adolescents. Roh et al. (33) evaluated obese adolescents and observed inflammatory characteristics similar to those of obese adults. In addition, Kelishadi et al. (34) observed that CRP in adolescents increased with abdominal obesity, and this elevation was associated with high levels of oxidative biomarkers (malondialdehyde and conjugated dienes). Our results demonstrated that CRP increased as a function of BMI, WC, and AC, which have been used to evaluate adiposity. The inflammatory and pro-oxidant profile in obese adolescents was strengthened by the presence of high levels of anti-oxLDL antibodies when compared with health weight or overweight individuals. Recently, Sanches et al. (35) detected anti-oxLDL antibodies in obese adolescents and observed that their levels were positively correlated with LDL-C, WC, and BMI values. In this context, Herder et al. (36) found that low-grade immune activation is associated with obesity in adolescents. Therefore, these findings further expand previous data on the presence of the dyslipidemia, low-grade inflammation and oxidative reactions in obese adolescents.

In the present study, LDL(−) content changed as a function of BMI, AC, and WC, reinforcing the notion that oxidative reactions are involved in obesity. However, the levels of anti-LDL(−) antibodies did not show differences between groups. Although the mean values presented a slight increase in the OV group, the statistical analysis does not show significant differences. An explanation of a possible higher generation of LDL(−) in OV group in relation to OB group could be linked to: (I) increase of immune-complexes generation. This possibility was reinforced by the similar results of anti-LDL(−) between the groups shown in our study; (II) oxidative modification more intense in LDL of obese adolescents. This possibility was indirectly reinforced by increased values of anti-oxLDL. Regarding that, as the evaluation of oxidative stress in adolescents is sparsely described in the literature, this possibility needs more investigation. Grosso et al. (37) evaluated the immune response to LDL(−) in mice and observed a similar profile. According to these authors, low bioavailability of antibodies to LDL(−) is correlated to generation of high immune-complex to LDL(−). Inversely, Barros et al. (17) did not find differences in the concentrations of LDL(−) of children and adolescents with familial hypercholesterolemia antecedents when compared with the normocholesterolemic individuals. Contrary to oxLDL, LDL(−) has pro-atherogenic characteristics consistent with different mechanisms of origin: oxidative modifications, PAF-AH activity, non-enzymatic glycosylation, changes in lipoprotein catabolism, enrichment of non-esterified fatty acids, cross-reaction with haemoglobin, and other mechanisms not yet identified that could be able to change the structural and the functional characteristics of the LDL particle (38, 39).

Regarding the possible oxidative origin of LDL(−), plasma α-TP levels could change generation of this particle and its impact on cardiovascular risk. Our results confirm this hypothesis showing that values for plasma α-TP concentration in obese adolescents are lower than in healthy-weight and overweight adolescents. In the context of obesity, these results are consistent with three possibilities: (I) adolescents have a low consumption of α-TP-rich food, (II) they are subjected to high generation of free radicals and oxidation of plasma α-TP, and (III) adiposity reduces levels of plasma α-TP by non-oxidative mechanisms. In fact, environment factors, such as diet, could have influence on plasma α-TP. In the present study, crude and energy-adjusted vitamin E intake was positively correlated with BMI; however, it was not correlated with plasma α-TP. Plasma α-TP content is not influenced only by vitamin E intake: possibly exogenous (sex, diet, drugs) and endogenous (obesity, oxidative stress, lipid metabolism) factors too are associated with weak or absent correlation between intake and plasma content (40). These observations reinforce the validity of plasma α-TP content evaluated in our study. Despite the precise mechanisms underlying the association of obesity with low plasma α-TP not being completely understood (41), high anti-oxLDL antibodies and LDL(−) content and low plasma α-TP monitored strengthen the idea that obese adolescents are under oxidative stress. Additionally, Mafra et al. (42) reported that patients undergoing haemodialysis exhibited a significant decrease in LDL(−) content after α-TP supplementation. Similar results were previously published by Carpenter et al. (43) who showed that supplementation with an adequate amount of α-TP can inhibit LDL oxidation. Previously, Hodis et al. (44) observed that α-TP supplementation significantly raised its plasma levels and reduced LDL(−) content, but did not change the intima-media thickness in the carotid artery. On the other hand, Pereira et al. (45) reported that the effect of treatment with simvastatin in addition to α-TP did not reduce LDL(−) content when compared with simvas-tatin alone. Later, Codóner-Franch et al. (46) verified similar plasma α-TP content in children with or without comorbidities for metabolic syndrome and children with healthy BMI. However, our results were reinforced by a previous study in which it was observed that obese children have higher malondialdehyde, shorter lag phase, and lower α-TP levels than non-obese children (47). These authors proposed that the fat mass determines a greater degree of oxidative stress.

**CONCLUSIONS**

Our results showed that obese in adolescents have a profile (dyslipidemia, inflammation, immune activation, and oxidative reactions) similar to that observed in adults, but with lower intensity. Obese adolescents, showed low plasma levels of α-TP and high concentrations of LDL(−). This profile reinforces the antioxidant role of α-TP in lipid-soluble compartment and, particu-
larly in LDL particles. Therefore, obese adolescents have a high cardiometabolic risk.

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α-Tocopherol and LDL(–) in Obese Adolescents

107


