

Non-linear Optical Responses of Low-Density Lipoprotein are Associated with Intima-Media Thickness of Carotid Artery in Athletes

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Abstract We investigated the association between the degree of oxidative modification of LDL particles by non-linear optical response of LDL (Z-scan technique) and the presence of subclinical atherosclerosis in different segments of the carotid artery. We recruited high-intensity athlete runners ($n = 44$) and controls ($n = 51$) to participate in the study. The carotid intima-media thickness (cIMT), interleukin 10 (IL-10), TNF-alpha, and the non-linear optical responses of LDL particle (Z-scan) were assessed. In athletes, the mean cIMT differed between genders, with higher values observed in female athletes compared to male athletes ($P < 0.05$). Higher mean values for cIMT were seen in the right carotid arteries of female athletes as compared to female controls ($P < 0.05$). Higher levels of TNF-alpha and IL-10 were found in athletes ($P < 0.05$). Yet, $\Delta\Gamma_{pv}$ (transmittance curve) of Z-scan in athletes was higher than in the non-athletes, indicating less

oxidation in LDL particles of athletes ($P < 0.05$). There was an inverse association between the $\Delta\Gamma_{pv}$ and cIMT in the right internal carotid segments ($\beta = -0.163$, $P < 0.05$) in all subjects, and between the VO_{2max} and the mean cIMT ($\beta = -0.003$, $P < 0.05$) in male subjects. The present study shows that the Z-scan technique enabled to detect less oxidative modifications in LDL particles from athletes. This effect was associated with cIMT in a gender-dependent mode.

Keywords Z-scan · Oxidized low-density lipoprotein · Athletes · Subclinical atherosclerosis

Introduction

Assessment of subclinical atherosclerosis by carotid intima-media thickness (cIMT) is independently associated with ischaemic cardiovascular and cerebrovascular events [1, 2]. Exercise promotes beneficial effects in cardiovascular risk factors for atherosclerosis by reducing insulin resistance and inflammation markers and modulating different mechanisms linked to the atherogenic process [3, 4]. These mechanisms include improvement in the capacity of the enzymatic antioxidant system, with the possibility of protecting low-density lipoprotein (LDL) particles from oxidative modification. Under certain conditions, LDL particles can be modified by oxidative agents and act as atherogenic agents. These agents activate the immune cells that interact with the endothelium, thus promoting the formation of atherosclerotic plaques [5].

Epidemiological studies have shown that the levels of oxidized LDL (oxLDL) depend on both cardiorespiratory and muscular fitness [6, 7]. It also seems that endurance training reduces oxidation of LDL particles [8–10].

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Yet, information about the effects of prolonged exercise on the progression of carotid atherosclerosis is divergent. Data on cIMT in athletes are scarce and dissonant regarding the effects of exercise on the atherosclerotic plaques in different carotid segments and other biomarkers associated with cardiovascular risk [10, 11]. In this context, there are few studies addressing the chronic effects of exercise in subclinical atherosclerosis and markers of LDL modification, especially in female athletes. Increased cIMT is a known independent predictor of cardiovascular events in the general population [12, 13]. However, the predictive value of this parameter in endurance athletes is unclear. Moreover, the possible relationship between phenotype of LDL particle, markers of cardiovascular disease, and subclinical atherosclerosis of the carotid artery in athletes was not stated in previous studies.

The aim of the present study was to investigate the effects of endurance training on subclinical atherosclerosis in different segments of the carotid artery (using B-mode ultrasound) and the degree of oxidative modification in LDL particles (by non-linear optical techniques). We hypothesized that the degree of oxidation of LDL particles in endurance athletes and non-athlete subjects of both genders can be associated with thickened carotid artery segments.

Methods

Subjects

Forty-four healthy athletic runners who performed intense training and fifty-one non-athletic controls were selected. The groups were matched for gender and age, and had no history of cardiovascular disease or risk factors for atherosclerosis, inflammatory diseases, and other comorbidities. Demographic, clinical, and biochemical data were obtained.

The study was conducted according to the ethical standards of the institutional committee on human experimentation, and the study protocol was approved by the local ethics committee. Written informed consent was obtained from all participants before the study was initiated.

Inclusion and Exclusion Criteria

Athletic runners of both genders aged 20–40 years were eligible if they had completed at least four half-marathons or eight 10-km races during the preceding 2 years. Exclusion criteria included diabetes mellitus, hypertension, renal failure, history of heart disease, musculoskeletal disorders, psychiatric disease, autoimmune diseases, hypertriglyceridemia, or recent infectious disease.

Non-athletic individuals from the university community were invited to participate in the study as controls. The eligibility criteria included the following variables: age (20–40 years), practice of regular physical exercise of moderate or vigorous intensity (not more than 2 days/week), no smoking, absence of metabolic or infectious diseases, absence of cardiac disease (hypertension, previous or present acute coronary syndrome, heart failure, or renal failure), and good general health state.

Measurement of Carotid Intima-Media Thickness (cIMT)

The intima-media thickness of the carotid arteries (cIMT) was measured by ultrasonography with the patient in the supine position, following the guidelines of the American Society of Echocardiography Carotid Intima-Media Thickness Task Force [13]. High-resolution B-mode ultrasound images were scanned by a Hewlett-Packard SONOS 5500 (Hewlett-Packard-Phillips, Palo Alto, CA), equipped with a vascular software for two-dimensional imaging, colour and spectral Doppler ultrasound modes, internal electrocardiogram monitor, and linear-array transducer (with a frequency range 7.5–12.0 MHz). All studies were performed in temperature-controlled rooms (24–26 °C). Screening of the carotid artery was performed by a single reader trained for the protocol and blinded to the characteristics of the study subjects. Screening of the vessel wall was performed using a B-mode ultrasound in the longitudinal and transverse planes, visualizing the common carotid arteries, carotid bulb, and proximal internal carotid arteries, bilaterally. The cIMT measurements were evaluated by an experienced sonographer, following the recommendations of the American Society of Echocardiography Carotid Intimal-Medial Thickness in B-mode ultrasound [13] in a blinded fashion. The scans were recorded in S-VHS tapes and analysed offline by an image analyst who was unaware of subjects' characteristics. The maximal value for cIMT was used in the statistical analysis. The intra- and inter-sonographer variabilities in the cIMT measurements were 0.05 ± 0.02 and 0.07 ± 0.04 mm, respectively.

Cardiopulmonary Exercise Stress Testing

Assessment of maximum functional capacity of athletes and non-athletic controls was carried out by employing a cardiopulmonary test on a treadmill (Total Health Centurion 300, Micromed, Brasilia, DF, Brazil), with ramp protocol until exhaustion, starting at 5 km/h and a fixed slope (1 %), with 1-km/h increments up to 18 km/h, and then the slope was increased to 2 % up to the maximal voluntary exhaustion. The total exercise time varied in the range 8–17 min, as previously described [14]. All studies were performed in temperature-controlled rooms

(19–21 °C), with humidity in the range 50–60 %. The athletes were advised as to the diet before the test and were told not to exercise within 24 h prior to the test.

Heart rate was monitored throughout the test using a 12-lead electrocardiogram (ErgoPC Elite, Micromed, Brasilia, DF, Brazil). Blood pressure was measured using an aneroid-sphygmomanometer before the beginning of the test and during the exercise bout.

Breath-by-breath gas analyses were performed using silicone masks (Hans-Rudolph, Shawnee, KS) and gas analyser (Cortex Metalyzer 3B, Metasoft-Cortex, Micromed, Brasilia, DF, Brazil), before the beginning of the test, during the exercise bout, and up to the 6th min of recovery. Calibration of the gas analyser system was performed before each test. Oxygen uptake (VO_2) and carbon dioxide production (VCO_2) were determined using the respiratory gas exchange and a computerized system (OxyScan, Micromed, Brasilia, DF, Brazil). Peak oxygen uptake (VO_2 peak) was defined as the maximum VO_2 measured at the end of the exercise bout, when the athlete was no longer able to maintain the load as imposed by the ergometer. The anaerobic threshold [15] was determined by loss of linearity in the relationship between VCO_2 and VO_2 , at the point where the ventilatory equivalent of oxygen (VE/VO_2) or the end-tidal oxygen pressure (PetO_2) reached its lowest level before starting to increase during the test. The respiratory compensation point was determined at the point where the ventilatory equivalent of carbon dioxide (VE/VCO_2) reached its lowest level before starting to increase and where the end-tidal carbon dioxide pressure (PetCO_2) reached its maximum value before decreasing [16].

Analyses of Blood Biochemical Components and Serum Lipids

Creatinine was analysed using the rate-blanked compensated Jaffe method on an autoanalyser (Roche, COBAS-C311, Mannheim, Germany). Serum total cholesterol (TC), high-density lipoprotein-cholesterol (HDL-C), and triglycerides (TG) were determined enzymatically (Opera Bayer, Leverkusen, Germany). LDL-cholesterol (LDL-C) was estimated by the Friedewald equation when TG was <400 mg/dL [17]. Glucose was measured using the glucose oxidase method (Hitachi-911, Boehringer, Mannheim, Germany), and glycated haemoglobin (HbA1c) was measured using high-performance liquid chromatography (HPLC).

Determination of Cytokines and High-Sensitivity C-Reactive Protein

Plasma samples were stored at -70 °C until analysed. Concentrations of interleukin (IL)-10 and tumour necrosis factor-alpha (TNF- α) were determined using commercially

available enzyme-linked immunosorbent assay (ELISA) kits according manufacturer's instruction (R&D Systems, Minneapolis, MN). High-sensitivity C-reactive protein (hs-CRP) was determined by nephelometry (R100 Analyser, Boehringer, Mannheim, Germany).

Assessment of Non-linear Optical Responses of LDL Particle

Separation of LDL Fraction

Blood samples (30 mL) drawn from fasting (12 h) subjects were utilized in the non-linear optical measurements with the Z-scan technique (Z-scan) [18, 19]. Plasma was obtained after centrifugation ($1000\times g$; 4 °C; 15 min) and supplemented with benzamidine (2 mM), gentamicin (0.5 %), chloramphenicol (0.25 %), phenylmethylsulfonyl fluoride (PMSF) (0.5 mM), and aprotinin (0.1 unit/mL). Low-density lipoprotein particles ($1.006 < d < 1.063$ mg/mL) were isolated by sequential ultracentrifugation ($100,000\times g$; 4 °C), using an ultracentrifuge rotor (Hitachi, Japan) as described by Havel et al. [20] The particles were dialysed (4 °C) against phosphate-buffered saline (PBS; pH 7.4) with ethylenediaminetetraacetic acid (EDTA; 0.01 %), and the LDL solution was sterilized by filtration (0.22 μm -pore; Millipore, Darmstadt, Germany). The lipoprotein concentration was quantified (BCA kit; Pierce, Rockford) using bovine serum albumin (BSA) as protein standard.

Z-scan Technique (Z-scan)

Depending on the time scale of the laser-light pulse employed, different physical phenomena can be studied. The Z-scan technique (Z-scan) allows investigating the non-linear optical properties of transparent and weakly absorbing materials, including aqueous solutions of LDL. Especially in these solutions, it was shown that pulsed laser-light at millisecond time scale induces the formation of a thermal lens [21]. Moreover, such optical response was shown to be critically dependent on the oxidative state of LDL: the smaller the non-linear optical response, the stronger the oxidative state of the particle. The experimental set-up used in the Z-scan measurements was composed of a continuous-wave (CW) Nd:YVO₄ green laser beam (model Verdi V10, Coherent, Santa Clara, CA) with Gaussian profile. The laser beam ($\lambda = 532$ nm) was chopped (17 Hz) and focused using a Z-scan lens (diameter: 25.4 mm; focal distance: 150 mm). The Rayleigh length (z_0) was 3.8 ± 0.2 mm [21], and the power of the laser-light incident on the samples was 122 ± 2 mW. A mechanical chopper provided laser pulses (30 ms) followed by a time lag (30 ms) without light. A silicon

photodetector (model PDA36A, THORLABS, Newton, NJ) positioned in the far-field limit collected the transmitted light. Optical glass (in-between flat parallel; slab geometry) plates were utilized to confine the samples containing LDL solution. The samples were moved along the laser beam direction (z -axis), passing by the focal point of the Z-scan lens. At each sample position, the transmitted light $I(t, z)$ was measured in the vicinity of the beam waist position ($z = 0$). At every sample position, ten independent measurements were performed. Sample thickness was constant (200 μm) and all the measurements were performed at room temperature (22 $^{\circ}\text{C}$).

The values for transmittance ($\Gamma_N(z)$) of the normalized laser-light were calculated as follows:

$$\Gamma_N(z) = \frac{I(t = 30 \text{ ms}, z) - I_B}{I(t = 0 \text{ ms}, z) - I_B},$$

where I_B is the intensity of background light. This normalization procedure was applied to obtain $\Gamma_N(z)$, which gave the typical peak-to-valley-shaped curve as a function of z . In previous studies, we showed that amplitude of the peak-to-valley (p - v) distance is a characteristic of the oxidation state of LDL particles. The values for p - v amplitude as measured in the curve for native LDL particles are higher than those for oxidized LDL [19, 21]. In the limit of complete oxidation of LDL particles, the characteristic p - v shape is lost, and the $\Gamma_N(z)$ curve becomes flat. For each sample, a polynomial curve was fitted to the normalized values for Γ_N as a function of z and the p - v distance was calculated.

Statistical Analyses

Categorical variables are herein expressed as n (%) and they were compared using the Pearson's Chi square test. Numerical variables are expressed as means or medians, followed by standard error (SE) and interquartile range (IQR), respectively. Normality of distribution was assessed by the Kolmogorov–Smirnov test. Distribution of concentration values for IL-10, TNF- α , LDL-C, TG, and hs-CRP was not Gaussian and they were thus log-transformed prior to analysis. The values for numerical variables of athletes and non-athletes were compared using the independent sample t test or Mann–Whitney test for non-parametric variables. Interaction between values of cIMT and those of other variables was analysed using the Spearman's or Pearson's correlation tests. Variables identified to have a significant interaction were also tested with multiple linear regression analyses, with mean cIMT or maximal IMT (for the right internal carotid segment) as dependent variables. These analyses were performed in all subjects (model 1) or only in males of both groups (model 2). All tests were performed using the SPSS 17.0 software

package (Statistical Package for Social Science, SPSS Inc., Chicago, IL). Two-sided p values <0.05 were considered statistically significant.

Results

Characteristics of Subjects

All athletes ($n = 44$) trained 14.1 ± 1.8 h/week and ran 122.1 ± 4.6 km/week (mean \pm SD). The female group trained 15.0 ± 1.4 h/week and ran 126.1 ± 6.4 km/week. Male runners trained 13.2 ± 1.7 h/week and ran 119.3 ± 6.5 km/week. The training time and distance run per week is representative of running training.

The characteristics of study participants are shown in Table 1. As expected, the values for aerobic capacity and anthropometric data showed differences between groups. The values for diastolic blood pressure in athletes were lower than in the non-athletes. The lipid profile showed a favourable effect of exercise, as seen by the lower values for total- and LDL-cholesterol and TG, and higher values for HDL-C in athletes when compared with controls. Lower values for blood glucose and hs-CRP and higher values for creatine kinase (CK) were observed in athletes as compared with non-athletes.

Table 1 shows that the values for IL-10 and TNF- α in athletes were higher than in the non-athletes. When these variables were compared between genders, the values for hs-CRP in female athletes were lower than in the female controls ($P < 0.05$). Regarding male subjects, the values for IL-10 in athletes were higher than in the non-athletes ($P < 0.05$). These results are shown in Table 2.

Non-linear Optical Responses of LDL

The non-linear optical properties of LDL assessed by Z-scan are shown in Tables 1 and 2. The values for peak-to-valley distance in athletes were higher than in controls ($P = 0.04$), and in male athletes as compared to male controls ($P = 0.04$). Figure 1a shows a dot-plot of normalized laser-light transmittance of LDL solution samples as a function of z position (Z-scan), and the characteristic peak-to-valley distance ($\Delta\Gamma_{pv}$) for athletes (dark dots) and non-athlete controls (white dots). Figure 1b shows the bar graphs with mean values and standard errors (SE) for $\Delta\Gamma_{pv}$ in the Z-scan for athletes (dark bars) and non-athletes (grey bars).

Measurements of cIMT

Table 3 shows the values for cIMT by gender and group. In athletes, differences between cIMT of different carotid

Table 1 Clinical parameters of athletes and non-athlete controls

Variables	Overall (95)	Athletes (44)	Non-athletes (51)	<i>p</i> values*
Age (years)	32.2 (0.7)	31.1 (0.09)	33.3 (1.2)	0.17
Male <i>n</i> (%)	41 (43)	21 (45)	20 (40)	0.18
Body mass (kg)	64.3 (1.5)	56.8 (1.1)	72.2 (2.4)	<0.001
Height (m)	1.67 (0.01)	1.66 (0.01)	1.67 (0.01)	0.87
Body mass index (kg/m ²)	22.9 (0.4)	20.2 (0.2)	25.8 (0.6)	<0.001
Fat mass (%) ^a	19.9 (0.9)	12.8 (0.6)	27.3 (0.9)	<0.001
Heart rate (beats/min)	63 (1.3)	58 (1.4)	70 (1.8)	<0.001
VO _{2max} (mL kg ⁻¹ min ⁻¹)	41.6 (1.74)	54.5 (1.28)	31 (1.17)	<0.001
Anaerobic threshold (mL kg ⁻¹ min ⁻¹)	27.4 (1.29)	37.2 (0.86)	19.4 (0.85)	<0.001
Week run (km)	NA	127 (4)	NA	NA
Systolic blood pressure (mmHg)	109 (1.57)	109 (1.8)	108 (2.7)	0.72
Diastolic blood pressure (mmHg)	68 (1.2)	66 (1.9)	72 (1.5)	0.02
Median cIMT (mm)	0.65 (0.01)	0.64 (0.01)	0.65 (0.01)	0.72
Maximal right common carotid (mm)	0.61 (0.01)	0.60 (0.01)	0.61 (0.02)	0.98
Maximal right carotid bulb (mm)	0.67 (0.01)	0.67 (0.01)	0.67 (0.01)	0.86
Maximal right internal carotid (mm)	0.64 (0.01)	0.64 (0.01)	0.63 (0.02)	0.85
Maximal left common carotid (mm)	0.63 (0.01)	0.62 (0.01)	0.64 (0.02)	0.29
Maximal left carotid bulb (mm)	0.70 (0.01)	0.69 (0.01)	0.70 (0.02)	0.64
Maximal left internal carotid (mm)	0.65 (0.01)	0.65 (0.01)	0.65 (0.01)	0.87
Glucose (mg/dL)	88 (0.8)	85 (1.07)	90 (1.2)	<0.005
HbA1c (%)	5.4 (0.3)	5.3 (0.04)	5.4 (0.05)	0.56
Creatinine (mg/dL)	0.91 (0.01)	0.91 (0.01)	0.90 (0.02)	0.66
Total cholesterol (mg/dL)	172 (3.6)	159 (4)	183 (5.5)	0.001
LDL-C (mg/dL) [#]	101 (4.6)	81 (71–97)	105 (84–131)	<0.001
HDL-C (mg/dL)	59 (1.5)	65 (2.2)	54 (2)	0.001
Triglycerides (mg/dL) [#]	66 (49–61)	54 (42–66)	77 (61–102)	<0.001
Creatine kinase (U/L)	186 (13.8)	263 (20.6)	118 (12.4)	<0.001
Hs-CRP (mg/L) [#]	1.0 (0.4–2.2)	0.7 (0.4–1.1)	1.3 (0.4–2.9)	<0.01
Leucocytes/1000 (cells/mm ³)	6.08 (0.15)	5.78 (0.21)	6.35 (0.20)	0.05
TNF- α (mg/dL) [#]	3.40 (3.16–3.92)	3.45 (3.22–4.40)	3.29 (2.90–3.80)	0.01
IL-10 (mg/dL) [#]	2.17 (1.90–1.35)	2.25 (2.05–2.35)	2.12 (1.05–2.05)	0.01
$\Delta\Gamma_{pv}$ (Z-scan) ^a	0.62 (0.03)	0.69 (0.6)	0.53 (0.04)	0.04

P < 0.05; Student's *t* test. Results are shown as mean (standard error, SE) or as [#] median and interquartile range (IQR)

HbA1c glycated haemoglobin, *Hs-CRP* high-sensitivity C-reactive protein

* Comparison between athletes and controls

^a Bioelectric impedance

artery segments were observed between genders, with mean cIMT in females higher than in males. In the group of non-athletes, females had lower values for cIMT in the right common (*P* = 0.02), right internal (*P* = 0.04), and left common (*P* < 0.01) carotid segments than males.

Analysis of groups by gender showed that the values for cIMT in non-athlete males were higher than in athletes in the right common (*P* = 0.02) and left common (*P* < 0.01) carotid segments, and left carotid (*P* = 0.01) bulb. Among females, the values for cIMT in the right internal carotid of athletes were higher than those of non-athlete controls (*P* = 0.04).

In all segments of the carotid arteries, the mean values for cIMT in female athletes were higher than those in the group of male athletes (*P* = 0.04), but did not differ from those of non-athlete females (*P* = 0.11). In the group of male athletes, the mean values for cIMT were lower than those of non-athlete males (*P* < 0.01) (Table 3).

Association of cIMT, Z-scan, and Oxygen Consumption

Multiple linear regression analyses of all study subjects revealed that the maximal values for cIMT in the right internal

Table 2 Values for interleukins, C-reactive protein, and LDL phenotype in athletes and non-athletes, by gender

Variables	Athletes		<i>p</i> values ^a	Non-athletes		<i>p</i> values ^b	<i>p</i> values ^c	<i>p</i> values ^d
	Male [21]	Female [22]		Male [20]	Female [31]			
hs-CRP (mg/L)	0.9 (0.3–1.3)	0.6 (0.3–1.0)	0.51	1.8 (0.5–2.8)	1.3 (0.4–4.2)	0.95	0.10	0.04
TNF- α (mg/dL)	3.56 (3.24–4.72)	3.37 (3.22–4.18)	0.41	3.40 (3.07–3.70)	3.20 (2.84–3.86)	0.81	0.10	0.08
IL-10 (mg/dL)	2.25 (2.07–2.38)	2.25 (2.04–2.38)	0.71	2.12 (1.98–2.17)	2.13 (0.96–2.32)	0.99	0.02	0.14
$\Delta\Gamma_{pv}$ (Z-scan)	0.62 (0.45–0.93)	0.65 (0.36–1.00)	0.76	0.32 (0.23–0.76)	0.56 (0.45–0.69)	0.15	0.04	0.62

hs-CRP high-sensitivity C-reactive protein, TNF- α tumour necrosis factor-alpha, IL-10 Interleukin 10

^{a,b} Comparison between genders, within group

^c Comparison between male groups

^d Comparison between female groups. Mann–Witney test

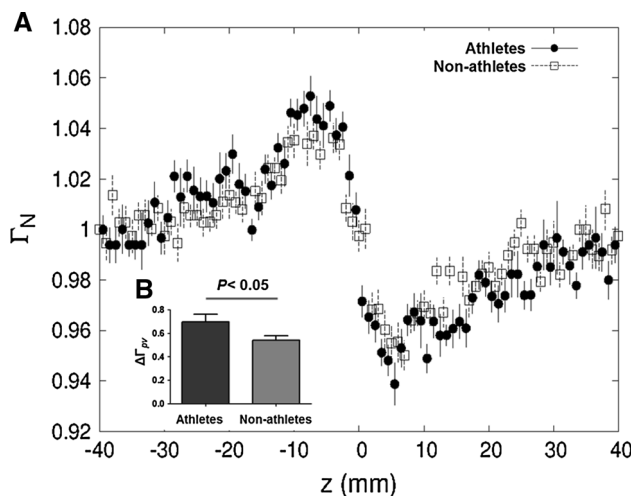


Fig. 1 a Dot-plots of normalized laser-light transmittance at each z position (Z-scan) for solutions of LDL samples showing median values and interquartile range (IQR) of the peak-valley distance ($\Delta\Gamma_{pv}$) as measured in athletes (dark dots) and non-athletes (white dots). Dots represent means values and lines represent standard errors (SE). (b) Bar graph representing means and SE for $\Delta\Gamma_{pv}$ in the Z-scan of samples of LDL from athletes (dark bars) and non-athletes (grey bars). High values for peak-valley distance ($\Delta\Gamma_{pv}$) were observed in athletes

carotid segments were negatively associated with the $\Delta\Gamma_{pv}$ coefficient of the Z-scan ($\beta = -0.163$, $P = 0.02$), which determines the LDL particle phenotype (Supplementary Table 1). In the multiple linear regression analyses by gender, an inverse association between the mean values for cIMT and maximal oxygen consumption ($\beta = -0.003$; $P = 0.005$) was found in males, but not in females (data not shown).

Discussion

In the present study, athletes and non-athlete controls exhibited differences between the values for cIMT in different segments of the carotid arteries, according to gender. Interestingly, the non-linear optical properties of LDL, as

shown by the Z-scan, indicated a low degree of modification in highly trained athletes, thus suggesting that their LDL particles have more components that prevent oxidative damage than those of non-athletes. The LDL phenotypes, as assessed by Z-scan, were shown to be associated with the maximal values for IMT in the right internal carotid segments of all subjects in the study. However, physical fitness, as assessed by the values of VO_{2max} , was associated with oxidized LDL only in males. The oxidation of the LDL particle may be one of the components that contribute to the progression of atherosclerosis, a condition that can be reflected in beneficial effects of exercise in immune response and endothelial function [22].

However, the chronic effect of endurance training seems to distinctly influence the intimal median thickening with regard to gender. Surprisingly, female athletes showed higher cIMT values and higher median cIMT as compared to male athletes and non-athletes.

Our group has previously addressed oxidative modifications of the LDL particle, using the Z-scan, and has shown a relationship between the degree of oxidation of LDL and markers of atherosclerosis [23]. The Z-scan parameters can indicate physical and chemical changes in LDL particles, which are intrinsically lost by a decrease in the antioxidant content of the particle core [24].

For the first time, this study shows that LDL particles from athletes have physicochemical characteristics different from those of non-athletes. In previous studies, the level of physical activity or the effects of exercise were shown to modify LDL oxidation and alter the antibody response to specific epitopes of oxidized LDL [7, 25, 26]. However, these results have biases related to different methods used to assess the oxidation process within the particle.

It is also possible that products derived from LDL oxidation can activate the inflammatory response [27] or favour platelet aggregation [28]. Exercise can reduce modification in LDL particles, thus down-regulating the

Table 3 Values for carotid intimal-medial thickness in athletes and non-athletes, by gender

Variables	Athletes		<i>p</i> values ^a	Non-athletes		<i>p</i> values ^b	<i>p</i> values ^c	<i>p</i> values ^d
	Male [21]	Female [20]		Male [20]	Female [26]			
Maximal right common carotid (mm)	0.58 (0.01)	0.64 (0.02)	0.08	0.67 (0.02)	0.56 (0.03)	0.02	0.02	0.06
Maximal right carotid bulb (mm)	0.64 (0.02)	0.70 (0.02)	0.11	0.71 (0.02)	0.65 (0.02)	0.16	0.05	0.24
Maximal right internal carotid (mm)	0.61 (0.02)	0.67 (0.01)	0.06	0.68 (0.02)	0.60 (0.02)	0.04	0.08	0.04
Maximal left common carotid (mm)	0.60 (0.01)	0.63 (0.02)	0.31	0.71 (0.02)	0.60 (0.02)	<0.01	<0.01	0.44
Maximal left carotid bulb (mm)	0.67 (0.02)	0.71 (0.02)	0.18	0.75 (0.02)	0.60 (0.03)	0.05	0.01	0.33
Maximal left internal carotid (mm)	0.62 (0.01)	0.67 (0.02)	0.31	0.65 (0.03)	0.65 (0.02)	0.96	0.39	0.54
Mean of cIMT (mm)	0.62 (0.01)	0.67 (0.01)	0.04	0.69 (0.02)	0.62 (0.02)	0.03	<0.01	0.11

Results are shown as mean (\pm standard error, SE)

^{a,b} Comparison between genders, within groups

^c Comparison between male groups

^d Comparison between female groups. Independent-sample *t* test

inflammatory signalling cascade [22] and decreasing platelet activation [29, 30]. These mechanisms are crucial and result in atherothrombotic processes that lead to cardiovascular events [31].

Regarding subclinical carotid atherosclerosis, conflicting cIMT data in athletes have been reported and the effects of exercise seem inconclusive [10, 11]. We have shown herein that gender modulates cIMT. We have also shown that the mechanisms developed by athletes, but not by the non-athlete controls, can modify the natural course of atherosclerosis, as seen in different regions of the carotid artery. In addition, the modality of exercise can differently modulate the atherosclerotic process because of specific metabolic demands required by different sports modalities.

Interestingly, the values for cIMT in female athletes were higher than those found in male athletes and non-athletes. Data on the menstrual cycle, menopause, or amenorrhoea periods were not obtained from female participants aged less than 40 years. These conditions can have important immunomodulatory effects in atherosclerotic processes. Although in a previous study Moreau et al. [32] stated that endurance training could prevent an increase in the values for IMT in postmenopausal women, our results indicate the opposite direction. We believe that in trained females, hormone modulation can occur, especially after chronic and intense exercise. There is an evidence of exercise inducing amenorrhoea and this effect can reflect in cardiovascular disease markers. Female subjects have a protective factor for cardiovascular disease that is lost after menopause. These metabolic alterations may explain the exercise-induced amenorrhoea associated with unfavourable cardiovascular parameters observed in female-trained subjects.

Age is a powerful risk factor for atherosclerosis, but age-associated increase and the absolute values for cIMT

have shown to be smaller in middle-aged and older adults who practise regular aerobic-endurance exercises. These facts may explain the delay in atherosclerosis development and its outcomes in those who practise regular exercise [33, 34]. Regular training could also explain that the subjects in our study do not show carotid artery thickening that corresponds to atherosclerotic plaque formation.

The practice of vigorous exercise by subjects without risk factors for cardiovascular disease reduces the progression of atherosclerosis [34], and a reduction in mortality risk is also observed when recommendations for both moderate activity and vigorous exercise are followed [31]. Reduction in the values for cIMT with intense exercise appears to be associated with reduction in the levels of inflammatory markers [35]. However, elevated levels of the inflammatory marker TNF- α were shown in athletes as compared to non-athletes. Moreover, CRP levels showed an inverse pattern in athletes and non-athletes. This is evidence that a systemic inflammatory component is lacking in the study population. Elevation in the levels of TNF- α in athletes can be associated with muscle metabolic activity [22].

Other important finding of this study is that female athletes showed higher cIMT, without association with elevated concentration of either CRP or TNF- α . The possible participation of other mechanism involved in such thickening in the carotid artery of female athletes was not evaluated. Inflammatory markers are associated with progression of atherosclerosis plaques in the general population [36, 37].

Subclinical atherosclerosis in the carotid arteries is associated with the presence and severity of coronary artery disease. Prevalence of non-coronary atherosclerosis in athletes was not yet reported, and we show herein that the values for cIMT in distinct carotid segments and the

variable degrees of LDL oxidation are different in male and female athletes. Vigorous exercise is recognized to promote changes in artery morphology [38], immune response [22], and oxidative burst [39], all key mechanisms for atherosclerosis development. However, other mechanisms appear to have relationship with atherosclerosis progression in female athletes. In females, the development of cardiovascular disease occurs on average 10 years later than in their male counterparts [40]. Sex differences in common CVD manifestations and in the pattern of atherosclerosis have long been known [41, 42]. For surrogate markers of atherosclerosis, sex differences have been reported for vascular function assessed by flow-mediated dilation (FMD) and intima-media thickness of carotid arteries (cIMT) [43, 44]. In addition, women are less likely than men to be informed about heart disease [45], receive less medical care, or preventive measures. Our findings suggest that chronic, intense exercise in females has a different impact on subclinical atherosclerosis and possibly these athlete females should be evaluated in a different manner.

It is well established that improvement in physical activity can control cardiovascular risk factors and can be used as non-pharmacological approach for cardiovascular diseases; however, previous studies showed that 17 % of athletes have dyslipidemia or had elevation in glycaemia, and 9 % present any cardiac disease [46]. Other study with 10 years of evaluation showed that athletes and amateur athletes >35-year old had high prevalence of hypertension and dyslipidemia in both genders [47]. However, conflicting cIMT data in athletes have been reported and the effects of exercise seem inconclusive. Autopsy and ultrasound studies have shown a close relationship between atherosclerosis in the carotid and coronary arteries and cross-section and prospective population-based studies have shown carotid IMT and plaque (area and echogenicity) as associated with first-ever myocardial infarction and cerebrovascular events [44, 48, 49]. However, there are no prospective studies addressing the effects of exercise in subclinical atherosclerosis morphology and progression.

Our athletes did not stop their training session in the previous day of study, what may explain the higher CK levels when compared with controls. However, these levels were in the normal range.

Our findings showed a new method for the identification of oxidized LDL particles in plasma. The results with this new physical approach that can detect structural and chemical modifications in LDL particle may be a useful tool in the assessment of oxidative modification in lipoproteins, as shown in our previous studies. We herein demonstrate that the peak-to-valley response of LDL particle is associated with subclinical atherosclerosis, in a special athlete group.

Study Limitations

We have paired each case to a control with the same social demographic and clinical characteristics. The groups of athletes and non-athletic controls were homogeneous, without any known risk factor for atherosclerosis, enabling us to address the effects of exercise solely to the development of carotid atherosclerosis. However, the hormones involved in the menstrual cycle, which are confounding variables in atherosclerosis development, were not reported herein. Female athletes can present amenorrhoea and/or premature menopause, and these conditions are known to modulate the atherosclerotic process.

Perspectives

Our study brings evidence of a chronic effect of exercise modulating atherosclerosis in different regions of the carotid artery. These gender-dependent effects are related to the degree of oxidation of LDL particles. The degree of oxidation of LDL particles, measured by Z-scan, can be used as a future marker for atherosclerosis in different population, especially in trained individuals. The effects of exercise on lipoproteins and atherosclerosis can be assessed by uniting the non-linear optical responses to oxidized LDL and ultrasound methods. Future investigation with longitudinal studies can provide insight on the amount of exercise that can promote changes in LDL particle and carotid atherosclerosis.

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