Immunometabolic and Vascular Health Responses among High Endurance Trained Subjects

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ABSTRACT

In this study, we aimed to examine the impact of high endurance training on vascular health parameters and immune-endocrine responses against modified low-density lipoprotein (LDL) particles. This observational, cross-sectional study included high endurance-trained and healthy non-trained subjects. Vascular ultrasound was used to assess vascular health parameters based on carotid intima-media thickness and endothelial function (flow-mediated dilation). Enzyme-linked immunosorbent assays were used to measure interleukin (IL)-8 and IL-10, autoantibody isotypes anti-oxidized LDL (oxLDL) and anti-apolipoprotein B (ApoB-D) peptide. Plasma levels of the corticosterone and 17 α-hydroxyprogesterone hormones were analyzed by mass spectrometry. This study enrolled 96 subjects, of whom 44 were high endurance trained and 52 were healthy non-trained individuals. Smaller carotid intima-media thickness values were observed in the high-endurance trained than in the healthy non-trained males, while no differences were observed between female groups. Flow-mediated dilation measurements did not differ by training or sex. The humoral immune responses to IgG anti-oxLDL and IgM anti-ApoB-D autoantibodies showed an isotype imbalance between the high-endurance trained and the non-trained groups. Immunoendocrine parameters showed inverse correlations between 17 α-hydroxyprogesterone concentrations and carotid intima-media thickness measurements. Direct correlations were found between IL-10 concentrations and flow-mediated dilation measurements. Chronic high-endurance exercise modulates immune-endocrine and vascular health parameters, in a sex-dependent manner.

Introduction

Beneficial effects of physical activity in cardiovascular health and disease prevention are well known [1, 2]. However, early atherosclerosis or acute coronary syndrome in athletes or trained subjects remains not fully explored, and the possible effects of long-term high-endurance exercise on the atherosclerosis process are lacking or inconclusive [3, 4]. In humans, data about the effects of exercise intensity and volume on subclinical atherosclerosis is conflicting [5, 6].

Exercise may have beneficial effects on atherosclerosis, including metabolic and hormonal factors, due to increase in the enzymatic and non-enzymatic antioxidant activities to reduce tissue damage. Furthermore, exercise may affect immunological components, such as low-density lipoprotein (LDL) particle, as well as cellular and humoral responses [7].

The humoral response to oxidized LDL and atherosclerosis progression has been poorly investigated in athletes or high endurance-trained individuals [8]. The endurance training through metabolic and immunological adaptation could impact vascular and atherosclerosis modulation [9, 10].

Previously, we reported that postmenopausal hormone replacement therapy is associated with lower autoantibody titers against apoB sequence and LDL oxidized, as compared to controls without hormone therapy, and that this outcome may be associated with vascular function [11]. However, these parameters have not yet been evaluated in higher trained subjects.

Cytokine IL-8 acts via the stimulation of CXCR1 and CXCR2 receptors. CXRC2 is expressed by vascular endothelial cells and is responsible for IL-8 induced angiogenesis. It has been shown that exercise induces CXCR2 mRNA and protein expression in the vascular endothelial cells, which may be related to vascular function in trained subjects [12]. On the other hand, IL-10 is a knowledge cytokine related to B cell regulations and humoral responses, and chronic exercise may be modulating IL-10 expression [13]. However, there is a lack of evidence for higher trained subjects that the expression of IL-8 and IL-10 could be associated with vascular health and humoral immune response against autoantigens.

Given the limited evidence, this study aimed to investigate the possible differences in immune-metabolic responses and vascular health parameters between high-endurance trained and healthy non-trained subjects.

Materials and Methods

Subjects

We conducted an observational, cross-sectional study with non-random inclusions of high endurance trained (HET) and healthy, active non-trained (HNT) subjects. The individuals were matched for sex and age, and had no history of cardiovascular disease or classical risk factors for atherosclerosis, inflammatory diseases, and other comorbidities.

The HET group's eligibility criteria were endurance-trained individuals of both sexes age 20–40 years who had completed at least four half-marathons or eight 10-km races in the previous two years. The HNT group's inclusion criteria were individuals of both sexes age 20–40 years who regularly exercised at a moderate or vigorous intensity (no more than two days per week).

The exclusion criteria for both groups were history of diabetes mellitus, hypertension, chronic kidney disease, heart disease, musculoskeletal disorders, autoimmune diseases, dyslipidemias, or recent infectious disease.

Demographic, clinical data, and blood samples were collected at the inclusion visit defined in the protocol.

This 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.

Cardiopulmonary exercise stress testing

The subjects' maximum functional capacity was assessed using a cardiopulmonary test on a treadmill (Total Health Centurion 300; Micromed, Brasilia, DF, Brazil) with a 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 between 8 and 17 min, as previously described [14]. All assessments were performed in temperature-controlled rooms (19–21 °C), with a humidity of 50–60 %. The athletes were advised about the diet before the test and were told not to exercise within 24 h before the test.

Heart rate was monitored throughout the test using a 12-lead electrocardiogram (ErgoPC Elite; Micromed, Brasilia, DF, Brazil).

Breath-by-breath gas analyses were performed using silicone masks (Hans-Rudolph, Shawnee, KS, USA) and gas analyzer (Cortex Metalyzer 3B with Metasoft software; Micromed, Brasilia, DF, Brazil) before the test, during the exercise bout, and up to the 6th min of recovery. The gas analyzer system was calibrated before each test. Oxygen uptake (VO_2) and carbon dioxide production were determined using the respiratory gas exchange and a computerized system (OxyScan; Micromed, Brasilia, DF, Brazil). Peak VO_2 (VO_2 peak) was defined as the maximum VO_2 measured at the end of the exercise bout, and anaerobic and ventilatory thresholds were determined [15].

Vascular health parameters assessment

Two methods were used to evaluate vascular health: endothelial-dependent flow-mediated dilatation (FMD) and carotid intima-media thickness (cIMT).

Endothelial function and cIMT were measured using echocardiography with a SONOS 5500 Ultrasound System (Hewlett-Packard-Phillips; Palo Alto, CA, USA) equipped with vascular software for two-dimensional imaging, color and spectral Doppler ultrasound modes, internal electrocardiogram monitor, and linear-array transducer (with a frequency range 7.5–12.0 MHz).

Endothelial function was evaluated based on the FMD of the brachial artery using ultrasound [16]. The percent change in vessel diameter from the baseline value was calculated to determine the FMD and endothelium-independent dilatation. All assessments were performed blindly by the ultrasonographers. The intra- and inter-sonographer variability values were < 1 % and < 2 %, respectively.

The cIMT was assessed by ultrasonography of the carotid right and left arteries using the protocol recommended by the American Society of Echocardiography: Carotid Intima-Media Thickness in B-mode Ultrasound [17]. The ultrasonographers performing the measurements were blinded to the study group.

Blood biochemistry analysis

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 using the Friedewald equation when TG was < 400 mg/dL. Analysis of apolipoproteins A-1 (ApoA-1) and B (ApoB) was performed using Siemens reagent on a Siemens BNI nephelometer

(Siemens Healthcare Diagnostics, Newark, DE, USA). Glucose was measured using the glucose oxidase method (Hitachi-911; Boehringer Mannheim, Mannheim, Germany).

Assessment of metabolic responses

Corticosterone and 17α-hydroxyprogesterone levels were evaluated using a LC-MS/MS method. Plasma samples (0.2 mL) were spiked with 0.2 mL of internal standard (17α-hydroxyprogesterone-d8 in methanol and zinc sulphate), vortexed and centrifuged. The supernatant was filtered and 50 µL were injected into a 1260 LC system (Agilent Technologies, Mississauga, ON, Canada). Chromatographic separation was performed on a Kinetex C18 column (100 × 3.0 mm, 2.6 µm, 100[a]; Phenomenex, Torrance, CA, USA) by gradient elution at a flow rate of 0.55 mL/min, 45°C, using water (mobile phase A) and methanol (mobile phase B). LC system was coupled to a triple-quad mass spectrometer (Sciex Qtrap 5500, Concord, ON, Canada) fitted with atmospheric pressure chemical ionization (APCI) source in positive mode (height 5 mm; curtain gas 28 psi; medium collision gas; temperature 500⁰ C; nebulizer gas 50 psi; auxiliary gas 50 psi). Compounds were monitored with selected-reaction monitoring at transitions 347.1/12.1 for corticosterone, 331.0/97.0 for 17α-hydroxyprogesterone and 339.2/100.1 for internal standard.

Measurements of cytokines

Plasma samples were stored at -70 °C until analyzed. Concentrations of Interleukin (IL)-8 and IL-10 were determined using commercially available enzyme-linked immunosorbent assay (ELISA) kits according to the information provided by the manufacturer (R&D Systems, Minneapolis, MN, USA).

Assessment of humoral immune responses against LDL particle

To determine the autoantibodies (Abs) to copper-oxidized LDL, we used an established enzyme-linked immunosorbent assay (ELISA) method as previously described [18, 19]. The evaluation of titers of anti-oxLDL Abs, isotype IgG and IgM (0.1 µg/ml and 10 µg/ml; purified human IgG and IgM, KPL, Kirkegaard & Perry Laboratories, Gaithersburg, Maryland, USA) were performed by a protocol previously established in our laboratory [20]. The buffer blank (phosphate-buffered saline (PBS) was used as control to compensate for intra-plate variation. Inter-plate imprecision was minimized by processing all the samples in the same time period. To minimize false positive results due to cross-reactivity with antigen naïve epitopes, antibody titers were expressed as the reactivity index (RI), calculated as RI = [(ODsample–ODsample blank)/(ODIgG or IgM-ODIgG or IgM blank)], where IgG or IgM were used as controls. Samples were run in duplicate and the variation within the duplicates did not exceed 5 % of the mean.

Assessment of humoral immune responses against apolipoprotein B

Quantification of anti-ApoB-D autoantibodies (Abs) was assessed in total plasma by ELISA method, as previously described [21]. To evaluate the immune response to the ApoB-D peptide, we also used ELISA. Briefly, we coated the plaques overnight with 10 µg/mL of the peptide (ApoB-D, is apoliprotein B-peptide fragment with 22 amino acids of conserved I region of LDL particle). After three cycles of wash, the plate of samples of volunteers was added (1/1000 in PBS). Three more cycles of wash were performed in sequence, and we added IgG or IgM antibodies to evaluate titers of anti-ApoB-D peptide Abs respectively (0.1 µg/ml and 10 µg/ml; purified goat anti-human IgG and IgM, KPL, Kirkegaard & Perry Laboratories, Gaithersburg, Maryland, USA). After two hours of incubation, the plaques were washed, and 3,3′,5,5′-tetramethylbenzidine (6.5 % in dimethyl sulfoxide; Sigma, St Louis, MO) and H_2O_2 (Sigma) diluted in citrate/phosphate buffer (0.1 mol/l; 250 µl; pH 5.5) were added (at room temperature) as enzyme substrate. The reaction was stopped by addition of H_2SO_4 (2 mol/l). The optical density (OD) of samples was measured at 450 *nm*. Autoantibody (Abs) titers were expressed as the reactivity index (RI), calculated as RI = [(OD sample–OD sample blank)/(OD IgG or IgM–OD IgG or IgM blank)] where the IgG or IgM antibodies were used as controls. Samples were run in duplicate and the variation within the duplicates did not exceed 5 % of the mean.

Statistical analyses

Categorical variables are expressed as *n* (%) and compared using Chi-square test. Numerical variables are expressed as the mean with standard error (SE) or median with interquartile range (IQR). The normality of their distribution was assessed using the Kolmogorov-Smirnoff test. Since the distributions of IL-8 and IL-10 concentrations were non-Gaussian, their values were log-transformed prior analysis. The numerical variables were compared between HET and HNT subjects using the independent-samples *t*-test or Mann-Whitney U test. Correlations between autoantibodies, interleukins, and hormones were assessed using Spearman or Pearson correlation coefficients. All analyses were performed using the Statistical Package for Social Science 17.0 software package (SPSS Inc., Chicago, IL, USA). Two-sided *P*-values < 0.05 were considered significant.

Results

Subjects characteristics

This study enrolled 96 subjects, 44 in the HET group and 52 in the HNT group. ▶ **Table 1** shows the demographic characteristic of the HET and HNT subjects. The age distribution did not differ significantly between groups. As expected, oxygen consumption by VO_2max analysis and body composition differed significantly between groups ($P < 0.001$).

Vascular heath parameters

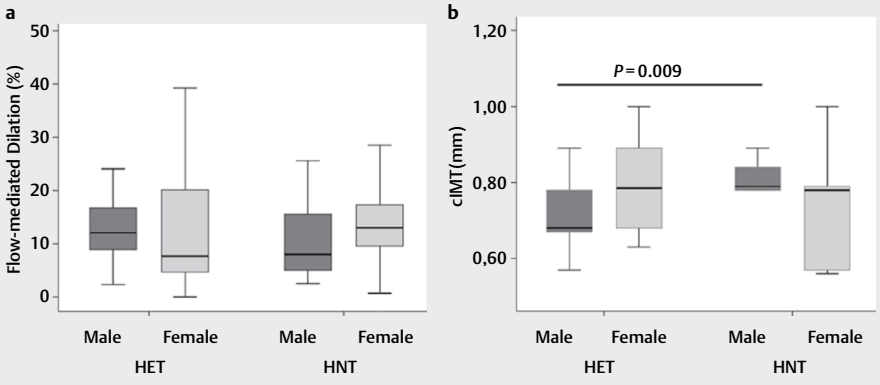
The FMD measurements did not differ significantly between the HET and HNT groups ($P = 0.831$; ▶ **Table 1**). In addition, they did not differ significantly between the HET and HNT subjects among males (12.2 ± 1.7 and 10.9 ± 1.9, respectively; $P = 0.624$) or females (12.9 ± 2.8 and 14.2 ± 1.7, respectively; $P = 0.701$; ▶ **Fig. 1a**).

The cIMT measurements did not differ significantly between the HET and HNT groups ($P = 0.547$). However, these values were significantly lower in male HET subjects (0.72 ± 0.09) than in male HNT

▶ **Table 1** Demographic, biochemistry and cardiovascular characteristics of subjects.

Variables	Overall (N = 96)	Healthy non-trained (N = 52)	High endurance-trained (N = 44)	*P*-values
Demographic characteristics				
Sex (M/F)	43/53	21/31	22/22	
Age (years), *mean, SE*	32.0 (7.5)	33.5 (8.0)	31.0 (6.5)	0.176
Fat mass (%), *mean, SE*	20.0 (9.0)	27.3 (6.0)	12.8 (4.5)	**<0.001**
VO_2 max (ml/Kg.min^{-1}), *mean, SE*	41.5 (6.5)	31.0 (6.5)	54.5 (6.5)	**<0.001**
Biochemistry parameters				
Glucose (mg/dL), *median, IQR*	87 (82–92)	89 (85–93)	85 (80–89)	**0.003**
Total cholesterol (mg/dL), *median, IQR*	165 (148–191)	176 (149–210)	156 (144–153)	**0.004**
LDL-c (mg/dL), *median, IQR*	93 (76–113)	105 (84–131)	81 (71–97)	**<0.001**
HDL-c (mg/dL), *median, IQR*	57 (48–70)	50 (46–61)	62 (55–72)	**<0.001**
Triglycerides (mg/dL), *median, IQR*	66 (49–81)	77 (61–102)	54 (42–66)	**<0.001**
ApoB/ApoA, *median, IQR*	0.50 (0.42–0.62)	0.58 (0.50–0.77)	0.45 (0.37–0.52)	**<0.001**
Creatinokinase (mg/dL), *median, IQR*	140 (90–248)	99 (72–140)	232 (158–334)	**<0.001**
Vascular health markers				
Carotid Intima-Media Thickness *(mm), mean, SE*	0.76 (0.11)	0.76 (0.12)	0.75 (0.10)	0.547
Flow-mediated dilatation *(%),mean, SE*	12.8 (9.5)	13.05 (8.5)	12.60 (10.35)	0.831

Abbreviations: SE, standard error; 95 % IQR, interquartile range; ApoB/ApoA, apolipoprotein B/apolipoprotein A.



▶ **Fig. 1** **a.** Box plots representing the median and interquartile ranges of the flow-mediated dilation (FMD) of the brachial artery expressed in percentual values, by sex and group. **b.** Boxplots representing the median and interquartile ranges of the carotid intimal medial-thickness (cIMT) expressed in millimeters, evaluated by non-invasive echocardiography method, by sex and group.; The sex groups are male (*dark gray*) and female (*light gray*) *HET, high endurance trained subjects; HNT, health non-trained subjects.*

subjects (0.81 ± 0.09; *P* = 0.009). However, they were similar between female HET (0.78 ± 0.10) and HNT (0.73 ± 0.13) subjects (*P* = 0.265; ▶ **Fig. 1b**).

Immune and metabolic assessment

Regarding humoral immune responses, titers of immunoglobulin G (IgG) autoantibodies against oxidized LDL (oxLDL) were significantly lower in the HET group than in the HNT group (*P* = 0.020). Nonetheless, titers of immunoglobulin (IgM) autoantibodies against oxLDL were similar among groups (*P* = 0.530). Titers of IgG autoantibodies against the ApoB-D peptide were similar between groups, while titers of IgM autoantibodies against the ApoB-D were higher in the HET group than in the HNT group (*P*<0.001) (▶ **Table 2**).

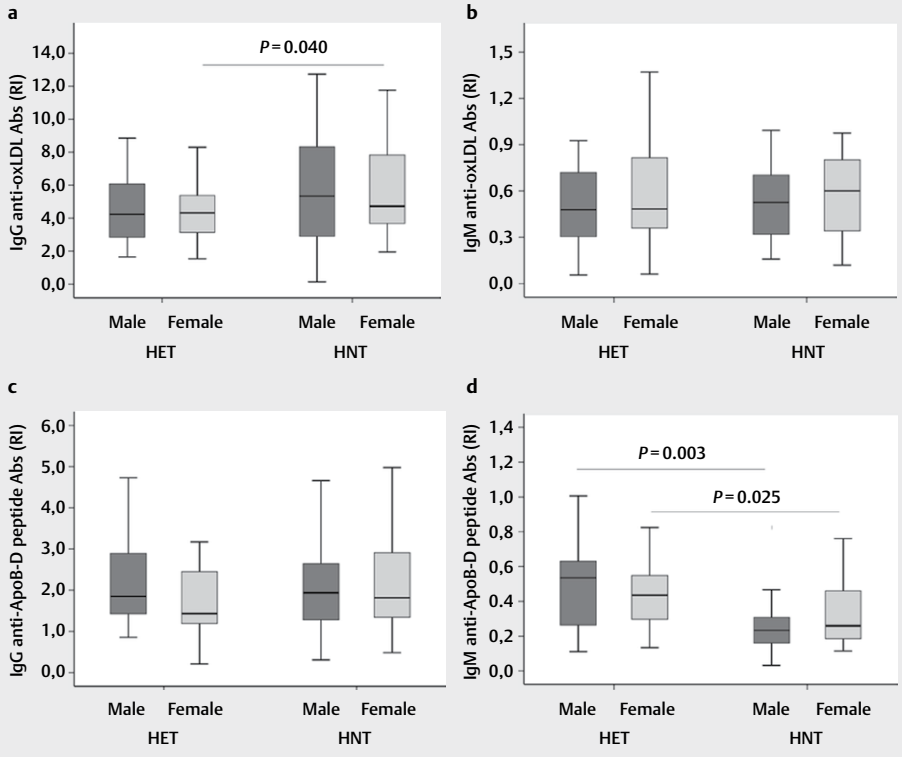
Sex-based analyses of immune responses showed that titers of IgM autoantibodies against the ApoB-D peptide were higher in male HET subjects (0.53, IQR = 0.25–0.66) than in male HNT subjects (0.23, IQR = 0.15–0.31; *P* = 0.003). Similarly, titers of IgM autoantibodies against the ApoB-D peptide were higher in female HET subjects (0.43, IQR = 0.29–0.58) as compared to female HNT subjects (0.25, IQR = 0.18–0.46; *P* = 0.025). In addition, in the female group, lower titers of IgG autoantibodies against oxLDL were found in HET compared with the HTN groups (*P* = 0.040). Likewise, titers of IgM autoantibodies against oxLDL did not differ between male and female HET and HNT subjects (▶ **Fig. 2**).

Regarding hormones, both plasma corticosterone (*P* = 0.028) and 17 α-hydroxyprogesterone (*P* = 0.047) concentrations were

▶ **Table 2** Autoantibodies anti-oxLDL, anti-ApoB-D peptide, immunological and hormonal measurements of subjects.

Variables	Healthy non-trained (52)	High endurance-trained (44)	*P*-values
Autoantibodies			
IgG anti-oxLDL Abs, RI, *median, (IQR)*	4.80 (3.50–8.30)	4.32 (2.85–6.00)	**0.029**
IgM anti-oxLDL Abs, RI, *median, (IQR)*	0.55 (0.30–0.80)	0.47 (0.36–0.47)	0.530
IgG anti-ApoB-D Abs, RI, *median, (IQR)*	1.85 (1.30–2.70)	1.71 (1.20–1.65)	0.682
IgM anti-ApoB-D Abs, RI, *median, (IQR)*	0.25 (0.17–0.35)	0.46 (0.28–0.62)	**<0.001**
Cytokines			
IL-8, pg/mL, *median, (IQR)*	6.62 (3.44–20.40)	5.00 (3.72–17.50)	0.781
IL-10, pg/mL, *median, (IQR)*	2.12 (1.05–2.20)	2.25 (2.05–2.38)	**0.014**
Immune cells			
Leucocytes, cell/mm³, *median, (IQR)*	5.97 (5.31–7.00)	5.59 (5.70–6.30)	**0.041**
Basophils, cell/mm³, *median, (IQR)*	0.05 (0.03–0.06)	0.03 (0.02–0.05)	**0.011**
Monocytes, cell/mm³, *median, (IQR)*	0.31 (0.25–0.36)	0.23 (0.20–0.28)	**<0.001**
Hormones			
Corticosterone, ng/dL, *median, (IQR)*	168.4 (92.0–246.2)	237.5 (167.3–382.7)	**0.028**
17 α-hydroxyprogesterone, ng/dL, *median, (IQR)*	57.0 (33.5–76.9)	86.2 (45.4–145.1)	**0.047**

Abbreviations: Abs, autoantibodies; RI, reactivity index; 95 % IQR, interquartile range.

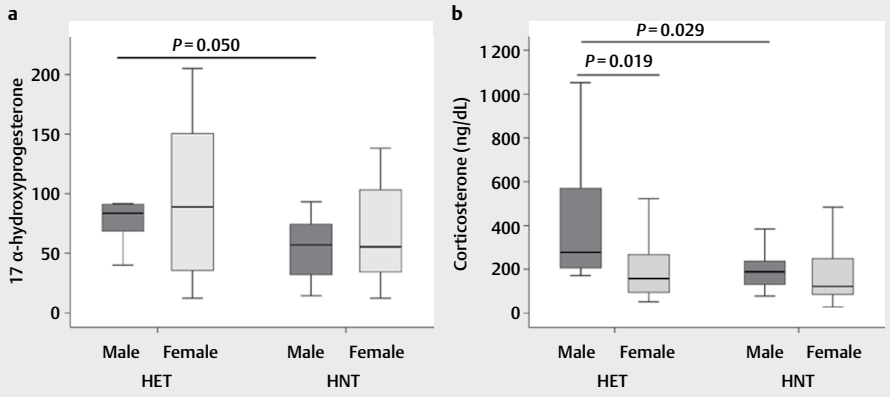


▶ **Fig. 2** **a.** Box plots representing the distribution of IgM anti-oxLDL; **b.** IgG anti-oxLDL; **c.** IgG anti-ApoB-D-peptide; **d.** IgM anti-ApoB-D autoantibody titers in the total plasma, expressed as RI, by sex and group. The sex groups are male (*dark gray*), and female (*light gray*). *HET, high endurance-trained subjects; HNT, health non-trained subjects.* RI, reactivity index.

higher in the HET group than in the HNT group (▶ **Table 2**). Similarly, 17 α-hydroxyprogesterone ($P = 0.050$; ▶ **Fig. 3a**) and corticosterone ($P = 0.029$; ▶ **Fig. 3b**) plasma concentrations were higher in male HET subjects than in male HNT subjects, whereas corticosterone ($P = 0.019$; ▶ **Fig. 3b**) plasma concentrations were higher in female HET subjects than in female HET subjects.

Correlations between immunoendocrine responses and vascular health parameters

Correlations between immunoendocrine parameters and vascular health parameters were examined. Measurements of cIMT were inversely correlated with 17 α-hydroxyprogesterone hormone concentrations ($r = -0.37$; $P = 0.006$). In addition, FMD measurements



▶ **Fig. 3** **a.** Boxplots representing the median and interquartile range of the 17 α-hydroxyprogesterone plasma concentrations, expressed as ng/dL, by sex and group. **b.** Boxplots representing the median and interquartile range of corticosterone plasma concentrations, expressed as ng/dL, by sex and group. Sex groups are male (*dark gray*) and female (*light gray*). *HET, high endurance trained subjects; HNT, health non-trained subjects.*

▶ **Table 3** Correlations between immune-endocrine parameters with FMD and cIMT.

Concentrations	cIMT		FMD	
	r	*P-value*	*r*	*P-value*
IgG anti-oxLDL Abs	−0.02	0.854	−0.07	0.492
IgM anti-oxLDL Abs	0.05	0.610	−0.06	0.561
IgG anti-ApoB-D Abs	0.05	0.612	0.04	0.677
IgM anti-ApoB-D Abs	−0.03	0.722	−0.04	0.245
IL-8	−0.18	0.094	−0.04	0.724
IL-10	−0.09	0.391	0.22*	0.049
Corticosterone	−0.12	0.364	0.08	0.577
17 α-hydroxyprogesterone	0.37*	0.006	−0.04	0.757

Abbreviations: cIMT, carotid intimal medial-thickness; FMD, flow-mediated dilatation, Abs, autoantibodies; r, Pearson correlation; *Significant, $P<0.05$.

were directly correlated with the IL-10 concentrations (r = 0.22; $P = 0.049$; ▶ **Table 3**). Titers of autoantibodies against oxLDL and the ApoB-D peptide were not correlated with the vascular health parameters, FMD and cIMT.

Discussion

In this study, we demonstrated that long-term high-endurance training might affect the humoral responses against LDL particles and the immune dominant region of ApoB, concomitant with changes in metabolic pathways associated with health vascular parameters, in a sex-dependent manner.

The sex differences in the humoral immune response findings might be due to sex hormones exerting suppressive effects on both humoral and cellular responses. A previous study showed that female high trained subjects presented higher levels of these sex hormones than female HNT subjects [22]. However, our findings did not show sex-based differences in hormone concentrations associated with immune responses, contrasting with previous results in non-trained subjects [23].

The high-intensity exercise might induce LDL particle modifications leading to epitopes recognized by autoantibodies [24]. Recent studies have shown that low to moderate exercise can elevate autoantibodies to virus protein-derivate [25] and improve influenza and COVID-19 vaccines [26, 27], indicating that the exercise intensity balance can improve antibodies against autoantigens and external agents.

Our previous studies showed that increased IgM anti-oxLDL titers correlate with better cardiovascular health associated with lower coronary artery disease burden and subclinical atherosclerosis [28, 29]. Interestingly, our results do not show differences in the natural immune response to LDL particles between groups. However, trained subjects showed higher IgM anti-ApoB-D peptide autoantibody titers compared to non-trained subjects. Recent studies have shown that higher titers of IgM autoantibodies against ApoB-derived peptides are associated with lower cIMT and less atherosclerosis severity in the general population [30, 31]. Our group has demonstrated that apoB-peptides can lead to endothelial dysfunction by altering endothelium permeability [32]. In addition, individuals with elevated titers of IgM autoantibodies against the ApoB-D peptide showed improved endothelial function, revealing associations between the natural immune response to the ApoB-D peptide and endothelium function [28].

This study did not demonstrate better endothelial function in HET subjects compared to HNT subjects. Interestingly, exercise promoted improved endothelial function via nitric oxide availability, reduced inflammation, and other neural activations [33]. However, high-intensity exercise might reduce the FMD with an increase of dilation between 1–24 h post-exercise, and normalization can occur 24–48 h after exercise cessation. These effects are intensity-, bout-, and exercise mode-dependent, with higher intensity leading to more sustained FMD reduction [34]. In this study, the HET subjects had trained with a 10–20 km run the day before recruitment for ultrasonography analysis, which might have impacted the FMD results.

Measurements of cIMT were similar between groups, although they differed significantly between male HET and HNT subjects.

Nevertheless, studies on the long-term effects of exercise on subclinical atherosclerosis have reported conflicting data [35, 36]. We believe exercise induces different morphological modifications inside atherosclerosis plaques that cannot be evaluated by measuring cIMT with ultrasonography techniques.

Interestingly, our study showed that the natural immune response was associated with hydroxyprogesterone in the HET subjects. This finding is consistent with previous studies showing that sex hormones modulate the synthesis of different immunoglobulin isotypes in B cells differently [37, 38]. However, the functional role of exercise in B cells and its relevance in cardiovascular disease is poorly investigated. Further studies on interrelation among the exercise, humoral response, and cardiovascular disease need to comprehensively examine the immunoendocrine pathways to support new therapeutics and future prevention.

Limitations

Our trial has other limitations that also merit consideration. First, due to its cross-sectional design, further randomized clinical trials are needed to reinforce our findings. Exercise could modulate humoral immune responses and provide improvements in vascular health. Second, previous exercise training might have affected FMD measurements and endocrine responses, impacting our results. Third, the open-label design might have led to reporting bias, although we attempted to control for ascertainment bias by masking all laboratories analyses, and the vascular health by endothelial-dependent flow-mediated dilatation and carotid intimal-medial thickness were measured by clinicians blinded to the study group subjects. Fourth, we included only high-endurance-trained subjects, without comparative moderate and sedentary groups. Highly trained subjects are frequently examined for performance, and health status may be in the gray zone related to risks for lesions/disease.

Conclusion

In conclusion, our results demonstrated that long-term high-endurance exercise subjects showed a sex-dependent immune-endocrine modulation compared to active non-trained subjects, reflecting substantial effects on vascular health parameters.

Author Contribuition

HARF, FAF, MCOI, AMFN: Concept and design of the study; CRB, AMM, LRS, CEFS: Performed the clinical and laboratory analysis; HARF: Performed statistical analysis; HARF, MG, FAF, and MCOI: Data analysis and interpretation the data; HARF and MG: Drafted the manuscript; all authors approved the final manuscript.

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Conflict of Interest

The authors declare no conflict of interest.

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