



## Research paper

# A comprehensive assessment of impact of aerobic exercise on vascular and liver function in 50 male athletes: A 2-year follow-up

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### ABSTRACT

**Introduction:** Regular physical activity is associated with a lower risk for all-cause mortality, and the increase in death risk reduction was largest for vigorous exercise. However, vascular adaptation to excessive training could alter normal physiology and promote an increased cardiovascular risk in elite athletes.

**Aim:** In this study we investigated standardized surrogate markers of cardiovascular risk and subclinical atherosclerosis, such as IMT, pulse wave velocity, augmentation index, assessed at carotid and femoral arteries, and endothelial function, in 50 elite male athletes, before and after sport seasons, on a 2-year follow-up.

**Material and methods:** The study population consisted of 50 healthy Caucasian male elite athletes and 40 healthy male controls. We performed ultrasound vascular assessment to assess carotid and femoral intima media thickness and compliance during a 2-year follow up.

**Results and discussion:** We reported a slower increase of carotid IMT of athletes in comparison to controls. Femoral compliance of athletes was higher than control compliance after 2 year follow up.

**Conclusions:** The worsening of femoral pulse-wave velocity and stiffness values at femoral arteries could be the result of a sport specific phenomenon. Physical activity differentially affects vascular beds in elite athletes, and in elicited district by strenuous exercise, we reported the progression of early markers of atherosclerosis.

## 1. INTRODUCTION

Regular physical activity is associated with a lower risk for all-cause mortality,<sup>1</sup> and the increase in death risk reduction was largest for vigorous exercise. However, vascular adaptation to excessive training could alter normal physiology and promote an increased cardiovascular risk in elite athletes.

Studies in endurance sports have shown that vigorous physical activity can induce changes on the elasticity of the vascular structures and can modulate the production of NO by the endothelium, in order to stimulate the body's adaptation.<sup>2,3</sup>

Moreover, several researches focused on the role of aerobic exercise in influencing pattern of different surrogate markers of cardiovascular risk, such as endothelial function, arterial stiffness and subclinical atherosclerosis; the debate is very intense and conflicting data were reported.

Elite sports could alter vascular structure and modify the stress response of vessel walls, and evidence on the progression of these vascular structural changes and how they may affect cardiovascular risk over time, are still insufficient and controversial. Evaluating the effects of long-term exposure to high-intensity training among professional runners on subclinical atherosclerosis, Bittencourt et al. showed that male athletes presented lower intima media thickness (IMT) at common carotid arteries, without differences in IMT between female athletes and controls.<sup>4</sup> In a meta-analysis by Ashor et al., authors observed a significant improvement in pulsewave velocity (PWV) and augmentation index (AIX) in response to aerobic exercise intervention.<sup>5</sup> Heffernan et al. did not find significant differences in carotid IMT values in exercise trained and age-matched, sedentary men with pre-hypertension.<sup>6</sup>

The metabolic profile is actually considered an important ground of research, and, in literature, we are seeing a growing interest in the study of fatty liver disease as an indispensable indicator of cardiovascular risk. Non-alcoholic fatty liver disease (NAFLD), defined as the accumulation of liver fat over 5% per-liver-weight, is associated to the increase in systemic atherosclerotic burden.<sup>7</sup>

Clinical observations indicated that NAFLD might be an independent risk factor for coronary artery disease, and, moreover, non-alcoholic steato-hepatitis (NASH) may increase atherosclerotic and cardiovascular risks by local over-expression of inflammatory mediators, endothelial damage, and regulators of blood pressure. Several evidences suggested that NAFLD could be considered a multi-system disease,<sup>8</sup> which concurs to pathogenesis of cardiovascular disease.<sup>9</sup> Indeed, NAFLD showed a strong relationship with metabolic syndrome, and NAFLD patients have a higher risk of death than the general population, mainly due to cardiovascular disease.<sup>10</sup> For these reasons, the presence of NAFLD could be an early indicator of atherosclerotic burden and cardiovascular risk in elite sports. However, current evidences regarding the presence of hepatic steatosis in athletes are scarce, being a topic largely unexplored.

## 2. AIM

In this study we investigated standardized surrogate markers of cardiovascular risk and subclinical atherosclerosis,<sup>11–13</sup> such as IMT, PWV, AIX, assessed at carotid and femoral arteries, and endothelial function,<sup>14</sup> in 50 elite male athletes, before and after sport seasons, on a 2-year follow-up.

Aims of our work were:

- (1) To assess the effects of chronic strenuous aerobic physical activity on markers of cardiovascular risk and vascular function;
- (2) To investigate the presence of any vascular sport-related effect at carotid and femoral arteries, assessing morphological and functional properties;
- (3) To evaluate the degree of NAFLD as marker of metabolic profile, in 50 elite male athletes, before and after sport seasons, on a 2-year follow-up.

At each time, results of elite athletes were compared to a control group.

## 2. MATERIAL AND METHODS

### 2.1. Study population

The study population consisted of 50 healthy Caucasian male elite athletes, playing soccer for at least  $11 \pm 0.3$  years, and 40 healthy male controls enrolled at the Office for Vascular Function Assessment, Careggi Hospital, Florence, between September and November 2010, for routine vascular function assessment, and followed for 2 years from the baseline. Data collection ended in November 2012.

Because findings described in the study were part of standard outpatient activity, it was not necessary to obtain institutional review board approval. All subjects gave written informed consent, and the investigation was performed in accordance with the Declaration of Helsinki.<sup>15</sup>

### 2.2. Study design and follow-up

Elite athletes were soccer players participating at a competitive level with different Italian football teams, who had engaged a competitive sport career for at least  $11.0 \pm 0.3$  years, and actively taking part in weekly soccer-specific training and competitions.

As required under Italian law, at each visit all athletes had obtained clinical eligibility for competitive sports by first-line investigators.

We eliminated from the study subjects reporting a long history of injuries as well as goalkeepers.

Control subjects included 40 healthy, sedentary males, not usually performing aerobic physical activity, who never had previously played sports at a competitive level or participated in aerobic exercise programs.

Other exclusion criteria comprised acute ill or pathological conditions, personal history of cardiovascular diseases, other systemic disorders such as diabetes, autoimmune diseases, or haematological disorders, and anxiety or depression. All subjects never smoked and were not alcoholics;

they showed normal weight. The presence of cardiovascular risk factors (CRFs) was assessed in each subject according to current guidelines.

Clinical assessment was performed in the morning, in a quiet room. Athletes completed the Paffenbarger Physical Activity Questionnaire,<sup>16</sup> in order to calculate average weekly hours of moderate and vigorous activity, and a medical questionnaire, in order to collect other clinically relevant data.

Resting heart rate (HR) and blood pressure, height, weight, body mass index (BMI) and waist circumference were measured; a 12-lead electrocardiogram (ECG) at rest was collected; and blood venous sampling (complete blood count, fasting glucose, lipid profile, kidney and liver function markers).

In the same day, we performed ultrasound assessment (IMT, PWV, and AIX; NAFLD), endothelial function evaluation (peripheral arterial tonometry) and ankle-brachial index (ABI). The same operator, who was blinded for the study population, performed the assessment.

$T_0$  evaluation was assessed before the 2010/2011 sport season;  $T_1$  and  $T_2$  evaluations were performed at the end of the 2010/2011 and 2011/2012 sport seasons, respectively. Clinical assessments of control subjects were performed during the same period, at each time. Clinical and instrumental assessment was performed at each time, with the exception of venous blood sampling and biochemical evaluation, and endothelial function assessment, performed at  $T_0$  and  $T_2$ . Study protocol was represented in Figure.

### 2.3. Ultrasound assessment

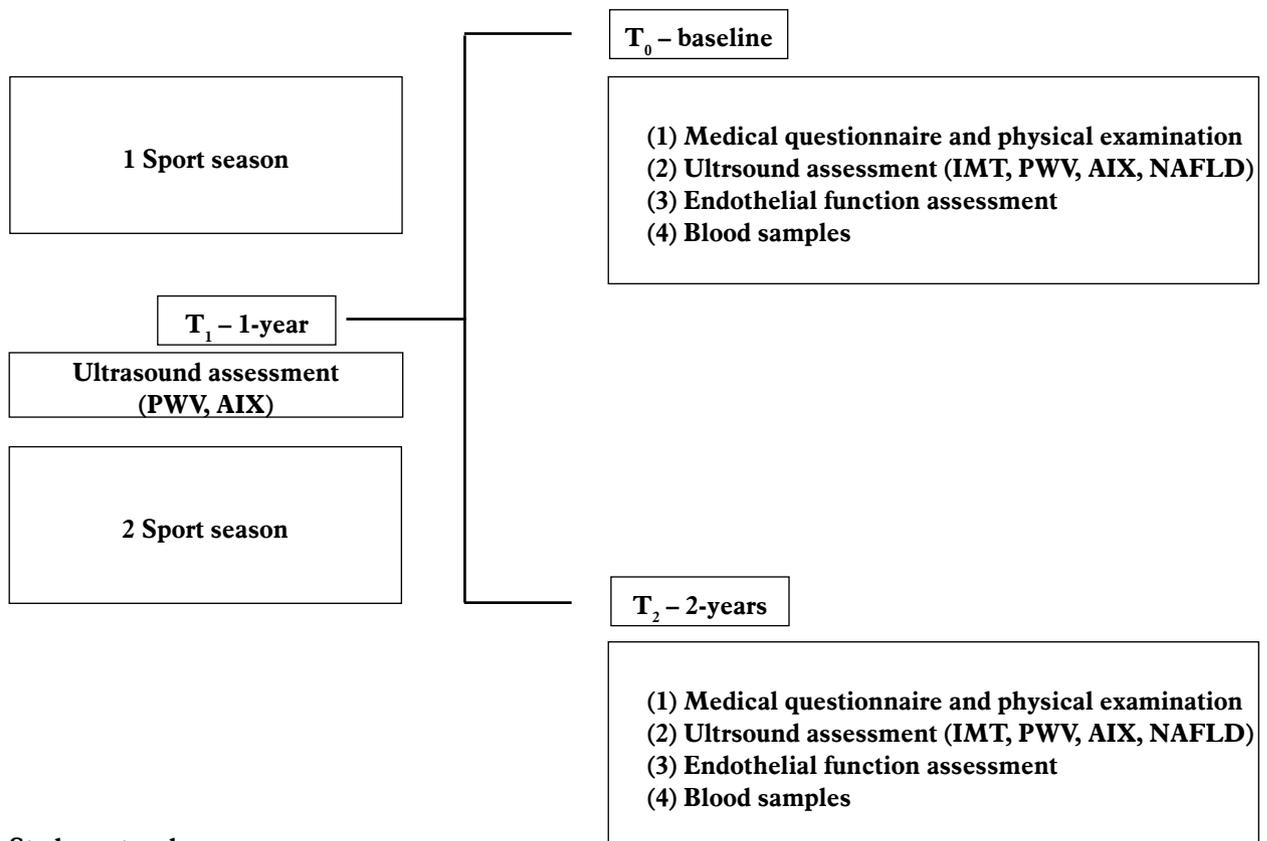


Figure. Study protocol.

Echographic evaluation was performed by a MyLab 70 XVisionEsaote machine (Esaote Medical Systems, Italy), equipped with a dedicated software for IMT and PWV assessment – radiofrequency data technology involving RF quality intima-media thickness (RFQIMT) and RF quality arterial stiffness (RFQAS).<sup>17,18</sup>

We measured the IMT of the right and the left common carotid arteries (c-IMT) 1 cm proximal to the carotid dilation with B-mode ultrasonography, using a computerized probe with a 7.5-MHz transducer. Femoral IMT (f-IMT) was sampled in the far wall of a 1 cm-long arterial segment proximal to the femoral bifurcation.

For each subject, we used the maximum c-IMT and f-IMT between right and left values for statistical analysis, considering c-IMT values over 0.9 mm and f-IMT values over 1.2 mm as pathologic results, according to current guidelines.<sup>11,13</sup>

The Esaote machine software automatically assessed the vascular diameter of the carotid and femoral arteries.

Arterial stiffness was expressed as local PWV; as for IMT, PWV of right and left common carotid arteries was measured 1 cm proximal to the carotid dilation; f-PWV was measured in the far wall of a 1 cm-long arterial segment proximal to the femoral bifurcation. The cut-off value for c-PWV and f-PWV was considered 12 m/s, according to current literature;<sup>11,13</sup> for statistical analysis, we considered the maximum c-PWV and f-PWV.

The evaluation of AIX was performed at the common carotid and femoral arteries, simultaneous to the ultrasound investigation, in order to obtain local AIX values.<sup>18</sup> MyLab 70 XVisionEsaote software automatically executed the AIX

analysis; algorithm and analysis were performed according to a previously described method.<sup>19</sup> For statistical analysis, we used the maximum carotid and femoral AIX values for each subject.

Instrumental assessments were performed, following recommendations for standardization of subject conditions, as described elsewhere.<sup>12, 19–21</sup>

We obtained ankle-brachial index by the ratio tibial and brachial systolic blood pressure, sampled by Doppler ultrasound; we considered the value of more than 0.9 as the cut-off value for the diagnosis of peripheral arterial disease (PAD) (pathological value, ABI < 0.9), according to current guidelines.<sup>12</sup>

The presence and the severity of NAFLD, was assayed by using the real-time electronic 3.75 MHz convex-type probe. The same operator (M.B.), who was unaware of the subjects' medical history and/or laboratory findings, performed all the exams.

The presence and degree of NAFLD was assessed semi-quantitatively according to validated scoring systems, with minor modifications,<sup>22</sup> according to a scale ranging from 0 to 8 points, on the basis of different items, which are:

- (1) liver–kidney differences (0–3 points),
- (2) deep attenuation (0–1),
- (3) blurring of diaphragm (0–1),
- (4) of the hepatic vein (0–1),
- (5) of gallbladder wall (0–1),
- (6) the presence of focal sparing (0–1).

We diagnosed NAFLD when the total score was more than 0.

#### 2.4. Endothelial function assessment

Endothelial function was measured by peripheral arterial tonometry (PAT) using the EndoPAT 2000 device (Itamar Medical LTD Caesarea, Israel), according to the method detailed in previous work.<sup>14</sup> Results of PAT devices were provided automatically by the software as an absolute number, the reactive hyperemia index (RHI), described elsewhere.<sup>14</sup> In this study, accordingly to current literature and previous papers, PAT results were expressed as a natural logarithm of RHI values (LnRHI).

#### 2.5. Statistical analysis

We used SPSS for Windows v. 19 for database construction and statistical analyses. We reported categorical variables as frequencies and percentages; we evaluated analysis of data distribution using the  $\chi^2$  test (statistical significance,  $P < 0.05$ ). Continuous variables were expressed as mean  $\pm$  standard deviation (SD).

According to the guidelines of the European Society of Hypertension,<sup>11,12</sup> normal values for ABI, IMT, and PWV were considered to be more than or equal to 0.9, c-IMT – less than 0.9, f-IMT less than 1.2, and both c-PWV and f-PWV less than 12 m/s. Values for AIX were averaged through six consecutive heartbeats; value of less than 0.9 was considered pathological for the presence of PAD.<sup>12</sup>

Independent samples *t* tests were used to examine differences between athletes and control subjects.

We used analysis of variance (ANOVA) to explore the effect of continuous covariates on IMT, PWV, AIX, ABI and LnRHI values, and NAFLD score.

The relationship between continuous variables (clinical and biochemical variables) was tested using the Pearson correlation test.

We used multiple linear regression models to investigate the relative influence of relevant factors; univariate linear regression analyses included age, heart rate, systolic and diastolic pressure, body mass index, height, weight, family history for cardiovascular disease, soccer participation, player position, training intensity, femoral artery diameter and liver steatosis. A multivariate linear regression analysis was performed after correction for training intensity, weight, height and soccer participation, in order to determine the influence of various predictors on vascular function markers.

A *P* value less than 0.05 was considered to indicate statistical significance.

#### 2.6. Sample size

We examined anticipated means and SDs from previous literature of similar populations. We calculated sample size with 90% power to detect a variation in IMT of 0.2 mm, and in PWV of 3 m/s between two groups at a two-sided significance level of 5%. To satisfy these conditions we would need a total population of at least 50 subjects.

### 3. RESULTS

#### 3.1. Study population: clinical characteristics and biochemical parameters

Characteristics of the study population are listed in Table 1. None of the participants reported clinically relevant problems during the follow-up.

We did not find pathological values for anthropometric or physical parameters at  $T_0$  and at  $T_2$ , as reported in table 1; athletes and controls did not show significant differences in such parameters in comparison to the  $T_0$ , respectively.

A significant difference for heart rate at  $T_2$ , as seen at  $T_0$ , was reported; in particular, athletes showed a significantly lower heart rate in comparison to controls ( $60.5 \pm 2.8$  vs.  $71.3 \pm 3.4$ ;  $P < 0.0001$ ), as seen at  $T_0$ .

Biochemical parameters were within normal ranges for both controls and athletes; significant differences for haematocrit, HDL-c, and triglycerides, were confirmed at  $T_2$ . No differences regarding renal and liver function and fasting glucose were found between the 2 study groups at  $T_0$  and at  $T_2$ .

#### 3.2. Ankle brachial index and intima-media thickness at common carotid and femoral arteries

Detailed description of IMT values and arterial stiffness values at different three times was showed in Table 2. All subjects showed IMT values at the common carotid and femoral arteries within normal values.

**Table 1. Characteristics of study population at the beginning of follow up (T<sub>0</sub>) and at the end of the 2-year follow-up (T<sub>2</sub>).**

Variable	T <sub>0</sub>			T <sub>2</sub>		
	Athletes n = 50	Controls n = 40	P value	Athletes n = 50	Controls n = 40	P value
Anthropometric parameters						
Age, mean±SD, y	30.3±2.7	30.1±3.2	0.7	32.2±2.3	32.1±2.8	0.7
Heart rate, bpm	60.5 ± 2.8	72.1 ± 3.1	<b>&lt;0.0001</b>	61.7 ± 3.1	72.1± 2.8	<b>&lt;0.0001</b>
Waist circumference, cm	83.72±4.11	84.6±4.88	0.07	82.55±3.88	84.11±3.79	0.06
BMI, mean±SD (kg/m <sup>2</sup> )	22.9±1.8	23.4±1.43	0.5	23.2±1.7	22.8±1.9	0.5
SBP, mm Hg	121.4±3.6	122.6±4.2	0.6	124.3±2.8	122.1±3.7	0.5
DBP, mm Hg	72.8±4.5	71.8±5.8	0.7	71.2±4.2	74.4±5.1	0.7
Mean blood pressure, mmHg	96.6±24.9	96.65±25.5	0.7	96.6±24.9	97.75±25.6	0.7
Biochemical parameters						
Haematocrit, %	41.68±2.12	45.7±2.99	<b>0.03</b>	41.72±2.55	45.66±3.19	<b>0.02</b>
Haemoglobin, g/dL	14.64±0.16	14.12±0.55	0.07	14.11±0.53	14.16±0.76	0.06
Fibrinogen, mg/dL	373±32.7	371±40.1	0.08	355±33.9	391±43.2	0.07
Glucose, mg/dL	81±11	84±15	0.7	82±13	84±17	0.7
Total cholesterol, mg/dL	182.5±12.3	184.7±12.4	0.2	182.7±12.4	183.7±13.7	0.2
HDL-c, mg/dL	67.7±14.8	52.7±12.6	<b>0.02</b>	68.7±14.9	53.7±12.7	<b>0.02</b>
LDL-c, mg/dL	95±19	119±28	<b>&lt;0.05</b>	95±28	117±22	<b>&lt;0.05</b>
Triglycerides, mg/dL	94.1±18.8	112.4±21.5	<b>0.03</b>	93.7±21.11	115.6±23.6	<b>0.04</b>
Serum creatinine, mg/dL	0.61±0.11	0.62±0.08	0.4	0.62±0.04	0.61±0.05	0.4
AST, U/L	17±3	16±3	0.4	18±4	16±4	0.4
ALT, U/L	16±4	13±6	0.5	17±5	14±5	0.5
GGT, U/L	14±5	15±5	0.6	16±4	16±5	0.6

Comments: Continuous variables are expressed as mean ± SD. Bold values show significant P values.

**Table 2. Instrumental markers of atherosclerosis and vascular compliance at the beginning of follow up (T<sub>0</sub>), 1 year after the beginning (T<sub>1</sub>) and at the end of the 2-year follow-up (T<sub>2</sub>).**

Variable	T <sub>0</sub>			T <sub>1</sub>			T <sub>2</sub>		
	Athletes n = 50	Controls n = 40	P value	Athletes n = 50	Controls n = 40	P value	Athletes n = 50	Controls n = 40	P value
c-IMT, μm	407.4 ± 62.2	462.6 ± 52.8	<b>&lt;0.0001</b>	–	–	–	442.3 ± 77.1 <sup>a</sup>	573.7 ± 49.7 <sup>b</sup>	<b>&lt;0.0001</b>
f-IMT, μm	445.4 ± 103.1	530.1 ± 84.0	<b>&lt;0.0001</b>	–	–	–	458.4 ± 111.9 <sup>a</sup>	596.2 ± 82.5 <sup>b</sup>	<b>&lt;0.0001</b>
c-PWV, m/s	5.87 ± 0.80	6.61 ± 1.02	<b>0.001</b>	6.10 ± 0.77	6.98 ± 0.9	<b>0.04</b>	6.26 ± 1.21 <sup>c</sup>	7.15 ± 1.13 <sup>c</sup>	<b>0.001</b>
f-PWV, m/s	8.94 ± 1.29	7.88 ± 1.37	<b>0.004</b>	9.45 ± 1.01	7.95 ± 1.21	<b>0.04</b>	10.87 ± 1.20 <sup>f</sup>	8.12 ± 1.43 <sup>e</sup>	<b>&lt;0.0001</b>
Carotid diameter, mm	7.72 ± 0.94	7.17 ± 0.85	<b>0.03</b>	–	–	–	7.89 ± 0.73 <sup>a</sup>	7.39 ± 0.93 <sup>a</sup>	<b>0.03</b>
Femoral diameter, mm	8.23 ± 0.74	7.72 ± 0.92	<b>0.014</b>	–	–	–	8.31 ± 0.55 <sup>a</sup>	7.99 ± 0.76 <sup>a</sup>	<b>0.01</b>
Carotid thickness and diameter ratio	0.053 ± 0.03	0.070 ± 0.13	<b>0.01</b>	–	–	–	0.055 ± 0.03 <sup>a</sup>	0.067 ± 0.04 <sup>a</sup>	<b>0.01</b>
Femoral thickness and diameter ratio	0.055 ± 0.04	0.069 ± 0.05	<b>0.02</b>	–	–	–	0.056 ± 0.04 <sup>a</sup>	0.070 ± 0.04 <sup>a</sup>	<b>0.02</b>
ABI	1.1 ± 0.03	1.1 ± 0.11	0.9	–	–	–	1.1 ± 0.021 <sup>a</sup>	1.1 ± 0.031 <sup>a</sup>	0.9
Carotid AIX, %	4.02 ± 6.16	7.82 ± 5.18	<b>0.003<sup>c</sup></b>	4.63 ± 6.05	7.92 ± 7.31	<b>0.01</b>	4.79 ± 6.35 <sup>e</sup>	7.85 ± 5.41 <sup>e</sup>	<b>0.004<sup>c</sup></b>
Femoral AIX, %	8.54 ± 10.15	6.09 ± 7.91	<b>0.042<sup>d</sup></b>	9.52 ± 5.67	6.33 ± 6.42	<b>0.01</b>	10.77 ± 8.28 <sup>f</sup>	6.44 ± 8.05 <sup>e</sup>	<b>0.001<sup>d</sup></b>
Liver steatosis (0–8 point score)	0.5 ± 0.6	2.4 ± 0.5	<b>&lt;0.05</b>	–	–	–	0.5 ± 0.8 <sup>a</sup>	2.4 ± 0.5 <sup>a</sup>	<b>&lt;0.05</b>
LnRHI	0.66 ± 0.5	0.92 ± 0.7	<b>0.001</b>	–	–	–	0.56 ± 0.6 <sup>b</sup>	0.93 ± 0.6 <sup>a</sup>	<b>0.001</b>

Comments: All continuous variables are expressed as mean±SD. Bold values showed significant p values. <sup>a</sup> not statistically significant difference in comparison to T<sub>0</sub>; <sup>b</sup> statistically significant difference in comparison to T<sub>0</sub>, P < 0.05; <sup>c</sup> after correction for heart rate, AIXr, at T<sub>0</sub> (P = 0.003), and at T<sub>1</sub> (P = 0.004); <sup>d</sup> after correction for heart rate, AIXr, at T<sub>0</sub> (P = 0.042), and at T<sub>1</sub> (P = 0.001); <sup>e</sup> P for trend not significant; <sup>f</sup> P for trend less than 0.01.

At  $T_0$ , we found a significant difference in several vascular markers between athletes and controls. In particular, we found that athletes had lower values of IMT at carotid and femoral arteries ( $P < 0.0001$  and  $P < 0.0001$ , respectively); moreover, they showed significantly higher values of diameter of carotid and femoral arteries ( $P = 0.03$  and  $P = 0.014$ , respectively), in comparison to the other subjects.

A  $T_2$ , athletes showed a slightly, not statistically significant, increase in IMT values at carotids and femoral arteries in comparison to  $T_0$  (Figure); conversely, we found a statistically significant difference regarding IMT values between  $T_2$  and  $T_0$  (Figure), in control subjects. At the end of follow up, athletes confirmed significantly lower IMT values at carotids and femoral arteries in comparison to controls (Table 2).

All subjects showed normal values regarding ABI, with no significant differences between the two groups at  $T_2$  (Table 2), and no differences in comparison to  $T_0$  values in the two different groups (Table 2).

At  $T_0$  we found a positive correlation between HR and c-IMT ( $r = 0.527$ ,  $P < 0.0001$ ) and between HR and f-IMT ( $r = 0.539$ ,  $P < 0.0001$ ), confirmed at the end of follow up ( $T_2$ ), respectively ( $r = 0.499$ ,  $P < 0.0001$ ) and ( $r = 0.522$ ,  $P < 0.0001$ ). Positive correlation between fasting glucose and c-IMT found at  $T_0$  ( $r = 0.402$ ,  $P = 0.034$ ) and f-IMT ( $r = 0.398$ ,  $P = 0.042$ ), was confirmed at  $T_2$  ( $r = 0.432$ ,  $P = 0.04$  and  $r = 0.377$ ,  $P = 0.04$ , respectively).

### 3.3. Arterial stiffness at common carotid and femoral arteries

Data on instrumental markers of vascular compliance are listed in Table 2. At baseline ( $T_0$ ), after one year ( $T_1$ ) and at the end of follow up ( $T_2$ ), all subjects showed c-PWV and f-PWV values within normal ranges, according to current guidelines.<sup>18</sup>

In particular, athletes showed significantly lower c-PWV values than controls at three times, as reported in Table 2. The differences in these values between athletes and controls, at each different time ( $T_0$ ,  $T_1$  and  $T_2$ ), were statistically significant. We showed a progressive increase in c-PWV values at different times, both in athletes and controls, but these differences were not statistically significant ( $P > 0.05$ ).

We provided significant results regarding f-PWV. In particular, athletes showed significantly higher values of femoral PWV at each time in comparison to controls ( $T_0$ :  $P = 0.004$ ;  $T_1$ :  $P = 0.04$ ;  $T_2$ :  $P < 0.0001$ ), and the progressive increase in f-PWV values during the follow-up was statistically significant ( $P < 0.05$ ). Moreover, at  $T_2$  athletes showed an increase in f-PWV values of 1.91 m/s, equal to 21.3%, in comparison to 0.23 m/s of control subjects (2.91%).

We observed a similar trend for carotid and femoral AIX values; in particular, athletes showed significantly higher values at each time in comparison to controls ( $T_0$ :  $P = 0.042$ ;  $T_1$ :  $P = 0.01$ ;  $T_2$ :  $P = 0.001$ , respectively), and the progressive increase in f-AIX values during the follow-up was statistically significant ( $P < 0.05$ ).

We found a positive significant correlation between c-PWV and HR at  $T_0$  ( $r = 0.410$ ,  $P = 0.01$ ) and at  $T_2$  ( $r = 0.4720$ ,  $P = 0.01$ ), and a positive significant correlation

between c-PWV and fasting glucose at  $T_0$  ( $r = 0.389$ ,  $P = 0.032$ ) and at  $T_2$  ( $r = 0.412$ ,  $P = 0.01$ ).

### 3.4. Liver steatosis

Liver steatosis was absent in both athletes and controls, but controls showed significantly higher values in comparison to athletes, at  $T_0$  ( $P < 0.05$ ) and at  $T_2$  ( $P < 0.05$ ). We found a positive correlation between NAFLD and BMI values ( $r = 0.376$ ;  $P = 0.01$ ) and between NAFLD and triglycerides ( $r = 0.402$ ;  $P = 0.01$ ).

### 3.5. Endothelial function assessment

Findings of endothelial function were reported in Table 2. Athletes presented lower LnRHI values in comparison to controls at baseline. At the end of follow up ( $T_2$ ), we showed a reduction of LnRHI values of athletes.

### 3.6. Univariate and Multivariate regression analyses

We performed univariate linear regression analyses, in order to identify predictors of femoral-PWV in the whole study population (controls and athletes), evaluated at  $T_2$ .

Participation in soccer, height, weight, and training intensity were significant associated to high f-PWV values at the univariate linear regression analyses.

In the multivariate analyses, after correction for training intensity, weight, height and soccer participation, only soccer participation ( $\beta = 0.4$ ,  $P = 0.0001$ ) was an independent predictor for high f-PWV values.

## 4. DISCUSSION

The beneficial effects of physical activity on cardiovascular risk were extensively reported. Leifer et al., investigating several biochemical markers, such as fasting insulin, triglycerides, and HDL cholesterol, and resting systolic blood pressure in a case-control study, showed a significant improvement of these parameters in the exercise group.<sup>23</sup> Moreover, Matelot et al. showed that endurance training was able to ameliorate markers of cardiovascular health in athletes, regardless of the age at which physical activity is initiated.<sup>24</sup> A follow-up study on former Finnish male athletes showed that elite athletes have 5–6 years additional life expectancy, when compared to men who were healthy as young adults, and these findings were partially explained by the lower rates of smoking in athletes than controls.<sup>25</sup>

However, several studies reported conflicting results regarding effects of strenuous physical activity on vascular function and structure. In particular, Borriore et al.<sup>26</sup> showed a high prevalence of homocysteine plasma levels in athletes, compared with a control group, suggesting that it would represent an adaptation to training, without excluding the possibility of a secondary vascular damage. Moreover, Berge et al. suggested that the type and intensity of training may contribute towards higher blood pressure values, and that the presence of hypertension was associated with hyper-

trophic cardiac remodelling in elite athletes.<sup>27</sup> Mohlenkamp et al. showed that veteran marathon runners presented higher coronary artery calcium scores respect to non-running controls, matched for Framingham risk scores;<sup>17</sup> similarly, in male marathon runners a significant high prevalence of carotid and peripheral atherosclerosis was found.<sup>18</sup>

On the contrary, a recent work, investigating von Willebrand factor antigen levels as indicator of endothelial function, proposed that strenuous exercise did not lead to endothelial activation or dysfunction in well-trained elite soccer players, producing, on the contrary, a beneficial effect on the endothelium of these players.<sup>2</sup>

Based on these premise, the aim of our research was to provide an integrated assessment of morphological and functional vascular indicators, providing a scenario of cardiovascular risk profile in elite athletes, in particular evaluating the effects of two consecutive sports seasons.

The importance of our work is related with several aspects. Firstly, we have conducted an integrated assessment of micro and macro-vascular patterns, in order to provide a comprehensive evaluation of cardiovascular risk, investigating vascular districts directly or indirectly related to cardiovascular risk, as surrogate markers. In addition, the results we have shown indicate that intense physical activity has a beneficial effect on global cardiovascular risk, identified by carotid IMT, endothelial function and liver steatosis, but at the same time exerts negative effects on some vascular districts heavily stressed by the specific physical activity, as reported by the increase in the compliance of femoral arteries.

In this study we evaluated the effect of two consecutive seasons of aerobic sports on markers of vascular function in 50 athletes and 40 healthy controls. All subjects showed anthropometrical, biochemical and ultrasound findings within normal values, according to standard international cut-offs. Baseline biochemical values were compatible with a better profile for sportsmen with respect to controls, in particular referring to lower plasma levels of triglycerides and LDL-c, and higher levels of HDL-c. This difference remained statistically significant even at subsequent checks ( $T_2$ ). The difference in HR between athletes and controls, statistically significant even at the end of follow up, may express both an increase in basal vagal tone and an adequate cardiopulmonary performance, typical of an adequately trained subject. This condition was associated to a favourable cardiovascular profile, as extensively reported in literature.<sup>28,29</sup>

Therefore, participation at two consecutive sport seasons was able to strengthen the positive effect of physical active on anthropometrical features and lipid profile, associated with constant training and an optimized cardiopulmonary performance.

Similar considerations can be made regarding findings of intima media thickness at carotid and femoral arteries; lower values found in athletes at  $T_0$ ,  $T_1$  and  $T_2$  checks, were associated to the positive effect of physical activity on morphological early marker of vessel damage.<sup>30,31</sup> In particular, after two sport seasons we found that the progression of c-IMT and f-IMT values in athletes was significantly slower than in controls.

Regarding PWV, we showed conflicting results at femoral artery districts, opposite to those collected in the carotids. In particular, the significantly higher increase in compliance values at femoral level than those at the carotid level could be the result of a selective workout of the lower limbs. We presented similar findings about AIX values at the femoral district. Data reported on compliance values, in the context of chronic exercise activity, could infer a sport-specific phenomenon. Several studies confirmed the positive effects of sports on central PWV in sedentary subjects,<sup>32,33</sup> however the negative effects of resistance training on vascular compliance are known. Repeated and extremely selective physical activity alters the structure of the femoral arteries, forcing the change of wall constituents and the elastic elements, and finally compliance properties.<sup>34</sup>

At baseline, athletes showed lower endothelial function values in comparison to control group; moreover, we showed a reduction in LnRHI values in athletes at  $T_2$ . This effect was previously explained;<sup>14</sup> in this work we showed a progressive damage on endothelial function, caused by chronic strenuous physical activity.

As known, a reduction in vascular compliance is an independent marker of atherosclerosis and a predictor of cardiovascular events and all-cause mortality in different populations.<sup>35</sup> Previous works has established an association between strenuous exercise and an increased inflammatory burden and arterial stiffness.<sup>36,37</sup>

Therefore, our findings showed that a relatively increased of arterial stiffness is present in young athletes and affects lower limbs as a sport-specific effect. The progression of femoral stiffness could contribute to the increased cardiovascular risk, as reported in retired sportsmen who practiced strenuous resistance activities.

Moreover, the relatively elevated PWV were recorded during sport season and after training sessions, and we cannot rule out that it could be an acute and reversible change. Therefore, further studies are necessary to clarify the timing of these changes and, above all, whether they are reversible and only related to the ongoing physical activity, or whether they can trigger progressive changes can trigger a vicious circle even after the termination of the sport activity.

## 5. CONCLUSIONS

The worsening of femoral pulse-wave velocity and stiffness values at femoral arteries could be the result of a sport specific phenomenon. Physical activity differentially affects vascular beds in elite athletes, and in elicited district by strenuous exercise, we reported the progression of early markers of atherosclerosis.

## Conflict of interest

None.

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