Cardiovascular adaptations and inflammation in marathon runners

KONSTANTINOS TSAROUHAS[1], CHRISTINA TSITSIMPIKOU[2], ANTONIOS SAMARAS[3], CHRISTOS SARAVANIS[4], GENOVEFA KOLOVOU[5], FLORA BACOPOULOU[6], DEMETRIOS A. SPANDIDOS[7] and DIMITRIOS KOURETAS[8]

[1]Department of Cardiology, University Hospital of Larissa, 41110 Larissa; [2]General Chemical State Laboratory of Greece, 11521 Athens; [3]Department of Cardiology, General Hospital of Veria, 59100 Veria; [4]6th Department of Cardiology, Hygeia Hospital, 15123 Athens; [5]Cardiometabolic Center, Metropolitan Hospital, 18547 Piraeus; [6]Center for Adolescent Medicine and UNESCO Chair on Adolescent Health Care, First Department of Pediatrics, National and Kapodistrian University of Athens, Aghia Sophia Children's Hospital, 11527 Athens; [7]Laboratory of Clinical Virology, School of Medicine, University of Crete, 71003 Heraklion; [8]Department of Biochemistry-Biotechnology, School of Health Sciences, University of Thessaly, 41500 Larissa, Greece

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Abstract. Long-distance running has become increasingly popular. Cardiovascular adaptations to exercise are relevant to the specific sports and this is also the case in long-distance running. Significant changes regarding inflammatory and endothelial markers along with indices of oxidative stress are observed in marathon and ultra-marathon runners. However, data linking inflammatory marker levels with cardiovascular adaptations to marathon running are limited. The aim of the present study was to describe the cardiovascular adaptations observed in a group of ultra-marathon runners and the association with a series of inflammatory and endothelial markers measured in their plasma. A total of 43 ultra-marathon runners were assessed by echocardiography and a treadmill exercise test. Blood samples were used for tumor necrosis factor-α (TNF-α), asymmetric dimethylarginine (ADMA), interleukin (IL)-6, IL-10, C-reactive protein, creatine phosphokinase (CPK) and oxidative stress indice measurements. Ultra-marathon runners who presented augmented left ventricular (LV) end diastolic diameters >55 mm had higher ADMA values (1.07±0.07 vs. 0.99±0.08 μmol/ml, P<0.01) and lower CPK values (192.5±21.3 vs. 219.1±37.3 mg/dl, P<0.05) compared with those with normal LV diameters. Runners with a moderate and severe abnormal indexed LV mass >131 g/m^2 had statistically significant higher TNF-α values compared with runners, with mildly elevated and a normal LV mass indexed (16.2±1.42 vs. 14.0+1.16 pg/ml, P<0.05). Runners with an abnormal left atrial volume index (LAVI; >29 ml/m^2) had higher IL-6 values compared with runners with a normal LAVI (1.09+0.19 vs. 0.99±0.08 pg/ml, P<0.05). ROC curves analysis revealed that ADMA values were able to predict an abnormal LV diameter detected by echocardiography [P<0.05; area under the curve (AUC), 0.763], while TNF-α values could predict an abnormal LV mass in marathon runners (P<0.05; AUC, 0.78). On the whole, the present study demonstrates that, in ultra-marathon runners, cardiovascular adaptations to running are characterized by a specific pattern of changes in inflammatory and endothelial markers, which in turn can be used to predict the occurrence of the observed adaptations.

Correspondence to: Dr Christina Tsitsimpikou, General Chemical State Laboratory of Greece, 16 Anastasiou Tsocha Street, Ampelokipi, 11521 Athens, Greece
E-mail: chtsitsi@yahoo.com

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Introduction

Long-distance running has become increasingly popular and participation in marathons and ultra-marathons (longer than the traditional marathon, usually 50-100 km) is also increasing. It is estimated that 349,000 individuals in Europe and 414,000 individuals in North America race in marathons annually (1). Running is considered a favorable exercise for the cardiovascular system, and epidemiological research has demonstrated that 1 h of running extends life expectancy by 7 h (2).

On the other hand, running has a considerable effect on myocardial morphology. Running long distances as part of a daily routine leads to cardiovascular adaptations, which are crucial both for the conditioning of the cardiovascular system and enhancing running performance.

Cardiovascular adaptations to exercise are relevant to the specific sports and in the case of long-distance running, the reported cardiovascular remodeling primarily involves the increase in the bi-ventricular diameter, left ventricular (LV)

myocardial thickness, LV mass and the volume of both atria, while systolic and diastolic function remain intact. The aforementioned changes are considered to be more pronounced in highly trained individuals (3). Recent studies have demonstrated that at an early stage of long-distance training and particularly during the first marathon, runners present mostly concentric biventricular remodeling, which has been found to be less pronounced than it was previously considered (1,4,5). Eccentric LV hypertrophy is considered to occur later, probably even after years of training.

At the same time, significant changes regarding inflammatory markers are observed in marathon and ultra-marathon runners compared with sedentary individuals and at individual levels during the race (pre and post). Exercise is associated with temporal muscle damage, which in turn is the origin of local inflammation. Leucocytes are accumulated and a systemic inflammatory response is induced (6). The systemic inflammatory response comprises leukocytosis and an acute-phase response (4-7), which is characterized by the increased release of cortisol, adrenocorticotropic hormone, cytokines and acute phase proteins, such as C-reactive protein (CRP).

Analogous to inflammatory markers, marathon runners exhibit evidence of endothelial function alterations. Nitric oxide (NO) handling appears to be the main topic of research in marathon runners.

The aim of the present study was to describe the cardiovascular adaptations of a group of ultra-marathon runners along with the measurements of inflammatory and endothelial function indices, and to further determine the predictive ability of these markers as regards cardiovascular adaptation in ultra-marathon runners.

Patients and methods

Study subjects. A total of 43 ultra-marathon runners were assessed by echocardiography at rest (at least 2 days after a training session or race). Runners were interviewed by the attending physician/cardiologist regarding their training sessions, and the kilometres run per week and their dietary habits, as well as a full medical history were recorded. All subjects were non-smokers, had no medical history of hypertension and were not receiving anti-hypertensive or anti-inflammatory medication. All participants were subjected to measurements of height and body weight (BW) and transthoracic echocardiography. All athletes consented to undergo an assessment of body composition and treadmill exercise testing.

A written informed consent to participate in the study was provided by all participants involved. The procedures were in accordance with the Helsinki Declaration of 1975 and approval was received by the Human Subjects Committee of the University of Thessaly, Larissa, Greece and the General Hospital of Giannitsa (Giannitsa, Greece), where all medical practices were conducted (7).

In the morning and after resting in the supine position for at least 30 min, fasting venous blood samples were drawn from all runners enrolled in this study, centrifuged (1,000-2,000 x g for 10 min, 4-7˚C) within 30 min from collection and stored at -20˚C. Tumor necrosis factor-α (TNF-α), interleukin (IL)-6) and IL-10 levels were measured using the IMMULITE® 1000TNF-α (sensitivity, 1.7 pg/ml; upper limit of the working range, 1,000 pg/ml; mean intra-assay variation, 3.2%), the IMMULITE® 1000IL-6 (sensitivity, 2 pg/ml; upper limit of the working range, 1,000 pg/ml; mean intra-assay variation, 4.65%) assays (Siemens) and the human high sensitivity HS IL-10 solid-phase sandwich ELISA kit (analytical sensitivity, 0.05 pg/ml; assay range, 0.39-25.0 pg/ml; intra-assay variation, 6.8%) from Thermo Fischer Scientific, Inc. Asymmetric dimethylarginine (ADMA) levels were measured using an ADMA-ELISA kit (DLD Diagnostika GMBH) (sensitivity, 0.05 μmol/l; upper limit of the working range, 5.0 μmol/l; mean intra-assay variation, 6.05%). CRP levels were determined using immunoturbidimetry and creatine phosphokinase (CPK) levels using the N-acetylcysteine (CK-NAC) method (340 nm). For the two latter assays, the COBAS INTEGRA 400 automated system by Roche Diagnostics, Inc. and all relevant diagnostic reagents of the same company were used (8). Oxidative stress values have already been previously published by the authors in the framework of the assessment of the effects of nutrient supplementation on the pathophysiological profile of marathon runners (7).

Echocardiography. Two experienced cardiologists-ultrasonographers performed the transthoracic echocardiographic examination using commercially available ultrasound systems (Vivid I; GE Medical) with a 1.5 to 4 MHz phased-array transducer. The same echo settings and acquisition protocols were applied. All images were subsequently analyzed in a random order in order to avoid bias. M-mode echocardiography or 2D images were used to calculate left heart dimensions (9). LV mass was calculated using the Devereux formula (9) and was then indexed to the calculated body surface area using the Mosteller formula (10). The LV ejection fraction (LVEF) was estimated using Simpson's biplane approach (9) Right heart dimensions were obtained as previously described by Rudski *et al* (11). The frame rate for tissue Doppler (TDI) measurements was >100/sec. The transmitral pw-Doppler inflow at the tips of the mitral leaflets was measured to obtain E wave velocity (12). TDI measurements were assessed in the apical 4-chamber view. Peak early diastolic (E′), late diastolic (A′) and systolic (S′) velocities were measured at the basal septum (13). In order to assess the global myocardial function of each chamber, the LV and right ventricular (RV) myocardial performance index were determined (14,15). The tricuspid annular longitudinal velocity of excursion (RV S′) was assessed using pulsed-wave TDI placed in the tricuspid annulus (11).

Treadmill exercise test. All runners were submitted to the exercise stress test on the day of their echocardiography examination. Treadmill exercise testing was performed until exhaustion on a treadmill with the use of an ergometer (Ultima Series, Medgraphics, Ltd.) applying the Bruce protocol (16). Exercise duration, metabolic equivalents of stress test, maximum heart rate, heart rate change at the first minute of exercise and heart rate recovery (HRR) time were recorded. The Athens QRS score was also calculated for each stress test (16).

Statistical analysis. All data are presented as the mean ± standard deviation (SD). Statistical analyses were performed with SPSS version 23 (IBM Corp.). Independent t-tests were used

Table I. Demographics, specific training characteristics and biochemical/oxidative stress indices of the study population.

Parameter	No. of subjects or average ± standard deviation value
Sex	
Male	39
Female	4
Age, years[a]	44.9±11.3
Body surface area (BSA, m^2)[a]	1.89±1.7
Weight (kg)[a]	74.7±10.1
Height (cm)[a]	176.3±8
Training experience (years)[a]	7.2±5.3
Training (/week)[a]	61.9±26
Training sessions/week[a]	4.4±1.3
Smoking	
No	40
Yes	3
IL-6 (pg/ml)[a]	1.05±0.15
TNF-a (pg/ml)[a]	15.5±1.38
IL-10 (pg/ml)[a]	2.06±0.22
CRP (mg/l)[a]	1.77±0.35
CPK (mg/dl)[a]	212.1±34.93
ADMA (μmol/ml)[a]	1.01±0.09
GSH (μmol/l)[a,b]	30.6±11.5
Carbonyls (nmol/g protein)[a,b]	0.68±0.18
TBARS (μmol/l)[a,b]	6.95±1.36
TAC (mmol DPPH/l)[a,b]	0.97±0.12

[a]Values are presented as the average ± standard deviation; [b]oxidative stress values have already been published previously by the authors (7).

Table II. Echocardiographic parameters and the main indices of the treadmill exercise tests of the marathon runners of the study population.

Parameter	Value
Echocardiographic measurements[a]	
LV end diastolic diameter (mm)	52.9±4.83
LV Septal thickness (mm)	8.93±1.84
Posterior LV wall diameter (mm)	10.7±1.93
RWT	0.380±0.100
LV EDV (ml)	97.5±32.3
LV EDV indexed (ml/m^2)	51.4±15.1
LV mass (g)	244±56.3
LV mass indexed (g/m^2)	128±25.3
Male	132±21.9
Female	91.9±32.2
LA volume (ml)	49.8±16.9
Left atrial volume index (LAVI) (ml/m^2)	26.2±8.94
RV end diastolic mid diameter (mm)	36.3±5.24
RA volume (ml)	48.6±19.8
LV EF (%)	74.7±8.33
LV MPI	0.370±0.0600
RV MPI	0.390±0.110
Early mitral inflow velocity (E) (cm/sec)	0.890±0.250
Lateral mitral e′ (cm/sec)	13.6±2.63
E/a ratio	2.07±0.500
E/e′ ratio	6.63±2.14
RV TDI S wave (cm/sec)	15.8±2.24
Treadmill exercise test measurements[1]	
Exercise stress test duration (sec)	19.5±4.13
Heart rate at rest (beats/min)	71.1±12.3
Maximum heart rate (beats/min)	175±14.6
Heart rate increase in the 1st minute of exercise (beats)	19.7±7.81
Heart rate recovery (beats)	47.3±16.4
Metabolic equivalents (METS)	18.1±5.62
Athens QRS score	10.9±6.32

[a]Values are presented as the average ± standard deviation. RWT, relative wall thickness; LV MPI: left ventricle myocardial performance index; mitral (E), mitral annular early diastolic velocity (E′); LV EDV, left ventricular end-diastolic volume; LV EF, left ventricular ejection fraction; LA volume, left atrial maximum volume; RV MPI, right ventricular myocardial performance index; RV TDI S wave, right ventricle peak systolic velocity of tricuspid annulus by tissue Doppler imaging.

to compare mean values between groups. Logistic regression analysis was performed to investigate the association between monitored biochemical parameters and myocardial adaptations. Differences between categorical variables were assessed using the Chi-squared test. A P-value <0.05 was considered to indicate a statistically significant difference. ROC curves were constructed for the evaluation of the predictive ability of the pro-inflammatory and anti-inflammatory biomarkers studied in predicting runners' myocardial adaptations to exercise.

Results

The demographic and specific training characteristics of the study population along with the inflammatory indices, biochemical markers and oxidative stress levels are presented in Table I. The echocardiographic parameters and the main indices of the treadmill exercise tests the marathon runners underwent are presented in Table II.

Ultra-marathon runners, who presented with augmented LV end-diastolic diameters (9) >55 mm, had higher ADMA values (1.07±0.07 vs. 0.99±0.08 μmol/ml, P<0.01) and lower CPK values (192.5±21.3 vs. 219.1±37.3 mg/dl, P<0.05) compared with those with normal LV diameters. In addition, runners with increased absolute LV mass values >225 g presented with higher TNF-α values compared with runners with a normal LV mass (15.9±1.40 vs. 14.7±1.02 pg/ml, P<0.05). Table III presents the levels of inflammatory and endothelial

Table III. Inflammatory and endothelial dysfunction markers (presented as the average ± standard deviation) in ultra-marathon runners with abnormal LV diameter or mass.

	Abnormal LV diameter (>55 mm)			Abnormal LV mass (>225 g)		
Parameter	Yes	No	P-value	Yes	No	P-value
No. of subjects	11	28		27	12	
Sex (n)						
Male	11	24	0.186[a]	27	8	**0.002[a]**
Female	0	4		0	4	
IL-6 (pg/ml)	1.06±0.12	1.02±0.16	0.47[b]	1.02±0.10	1.07±0.23	0.33[b]
IL-10 (pg/ml)	2.11±0.14	2.05±0.25	0.40[b]	2.05±0.19	2.10±0.30	0.54[b]
TNF-α (pg/ml)	15.6±1.61	15.6±1.33	0.87[b]	15.9±1.40	14.7±1.02	**0.02[b]**
ADMA (μmol/ml)	1.07±0.07	0.99±0.08	**0.005[b]**	1.02±0.08	1.00±0.09	0.49[b]
CRP (mg/l)	1.80±0.31	1.77±0.38	0.83[b]	1.71±0.35	1.94±0.32	0.07[b]
CPK (mg/dl)	192±21.3	219±37.2	**0.033[b]**	209 ±30.6	217±45.6	0.6[b]
Age (years)	47.3±7.4	41.9±10.4	0.12[b]	46.0±9.32	37.7±9.00	**0.01[b]**

[a]Differences were detected using the Chi-squared test; [b]differences were detected using an independent t-test. Values in bold font indicate statistically significant differences. IL, interleukin; ADMA, asymmetric dimethylarginine; CRP, C-reactive protein; CPK, creatine phosphokinase.

dysfunction markers in ultra-marathon runners according to the presence of abnormal LV diameter or mass. Runners with 'abnormal' LV diastolic volumes >155 ml exhibited lower CPK values compared with runners with normal LV diastolic volumes (197±10.1 vs. 222±35.4 mg/dl, P<0.05). As regards the LV volume, runners with increased volumes followed more training sessions per week and covered more kilometers on a weekly basis, compared with runners with normal LV volumes (5.3±1.1 vs. 3.8±1.2 sessions/week, P<0.05 and 78.8±22.5 vs. 57.2±27.2 km/week, P<0.05, respectively). Finally, runners with augmented left atrium (LA) volumes >58 ml presented lower IL-10 values compared with runners with normal left atrial volumes (1.97±0.17 vs. 2.14±0.23 pg/ml, P<0.05).

In the present study, runners with an abnormal RV diameter (17) (mid RV segment) >34 mm were those who had been training for a greater number of years, covering more kilometers per week and following more training sessions per week (9.5±7.4 vs. 4.8±3.3 training years, P<0.05; 69.7±29.6 vs. 52.3±15.55 km/week, P<0.05; 4.7±1.5 vs. 3.7±0.7 training sessions/week, P<0.01, respectively). The runners' diet habits, characteristics of exercise stress test (such as exercise duration, heart rate change in the first minute of exercise or heart rate recovery time in the first minute of recovery-HRR), were not associated with alterations in RV dimensions. Specifically, bread daily consumption did not differ in marathon runners with an abnormal RV diameter compared to runners with normal values (2.7±1.5 portions vs. 2.5±1.6 portions, P=0.49), nor carbohydrates consumption (4.8±4.4 vs. 5.2±6.1 portions per week, P=0.82), nor meat consumption (2.6±1.5 vs. 2.3±0.8 portions per week, P=0.46). Exercise test duration did not differ between athletes with an abnormal RV diameter compared to runners with normal values (20.5±4.4 vs. 18.3±2.2 min, P=0.1) nor did heart rate change in the first minute of exercise (18.9±7.7 vs. 21.1±7.1beats, P=0.41), nor did HRR (46.2±15.1 vs. 52.8±18.8 beats, P=0.24).

HRR in an exercise maximum stress test is a reliable marker of the balance between parasympathetic and sympathetic nervous system and is associated with years of physical exercise and good cardiorespiratory fitness. Runners usually tend to have better HRR values compared with individuals who lead a sedentary lifestyle (18). In the present study, 'normal' HRR values were considered as >35 beats according to the findings of Mann *et al* (19). The present study found that runners with lower than usual HRR values (<35 beats) had lower exercise duration at the exercise stress test compared with runners with usual HRR (17.6±2.2 min vs. 20.5±4.6 min, P<0.05), while they did not differ in years of training, kilometers run or training sessions per week.

The majority (72%) of ultra-marathon runners of the present study presented an abnormal LV mass indexed to BSA >115 g/m^2 (9). Runners with abnormal LV mass indexed did not present statistically significant differences in IL-10, IL-6, TNF-A, ADMA, CPK, CRP levels compared with runners with normal LV mass indexed. However, runners with moderate and severe abnormal indexed LV mass >131 g/m^2 had statistically significant higher TNF-α values compared with runners with mildly elevated and normal LV mass indexed (16.2±1.42 vs. 14.0+1.16 pg/ml, P<0.05).

Runners with an abnormal left atrial volume index (9) (LAVI) >29 ml/m^2 had higher IL-6 values compared with runners with a normal LAVI (1.09+0.19 vs. 0.99±0.08 pg/ml, P<0.05).

Elite marathon runners of the present study, as defined according to their training status (i.e., kilometers covered per week >55) (20), presented specific differences from the remaining marathon runners with a less intense training program (<40 km/week). Elite runners were characterized by higher RV end-diastolic diameters (37.8±5.6 vs. 33.2±3.4 mm, P=0.009), right atrium volume (52±23.3 vs. 39.6±9.7 ml, P=0.045) and a lower maximum heart rate achieved at the

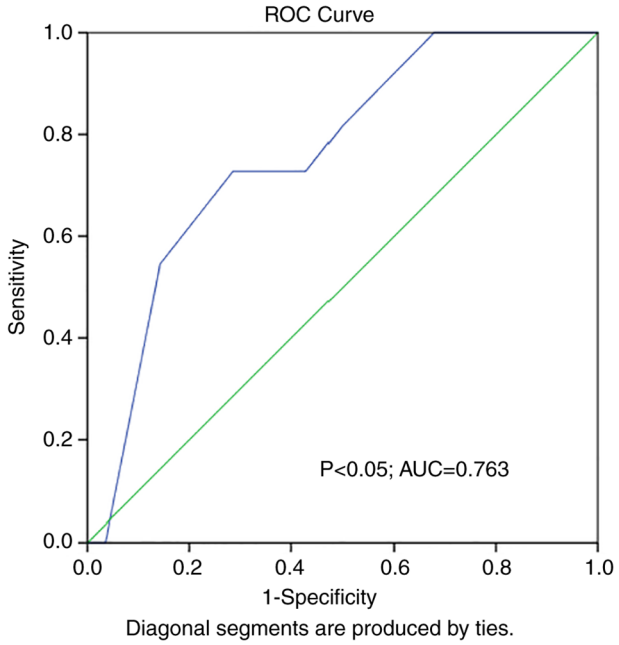

Figure 1. ROC curve of asymmetric dimethylarginine for the prediction of abnormal left ventricular diameters in marathon runners. AUC, area under the curve.

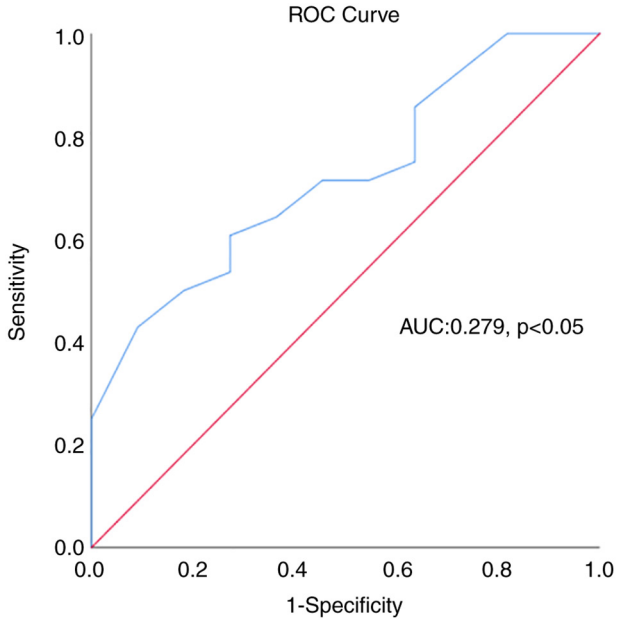

Figure 2. ROC curve for the predictive ability of CPK on abnormal left ventricular diameters in marathon runners. AUC, area under the curve.

treadmill stress test (171.3±11.9 vs. 190.2±12.5 beats/min, P<0.001). However, they did not present any significant differences in the biochemical or oxidative stress indices measured in the present study.

ROC curves were constructed to evaluate the ability of ADMA to predict the presence of abnormal LV diameter in marathon runners. ROC curve analysis revealed statistical significance [P<0.05; area under the curve (AUC), 0.763] (Fig. 1). At the same time, ROC curve analysis for the predictive ability of CPK regarding the presence of an augmented LV diameter in marathon runners also yielded statistical significance (P<0.05), although with a low predictive ability (AUC: 0.28) (Fig. 2).

Logistic regression analysis was performed for the prediction of the presence of an abnormal LV diameter in echocardiography in ultra-marathon runners. The model presented statistical significance (P<0.001, Chi-squared=25.8) and could explain 69.6% of the variance of the abnormal LV diameter presence (Nagelkerke R Square) and correctly classify 87% of the athletes. As shown in Table IV, IL-6, ADMA, height and age, but not IL-10, significantly contributed to the model.

ROC curve analysis was also performed to evaluate whether TNF-α can predict abnormal LV mass in runners and statistically significant findings were obtained (P<0.05, AUC: 0.78) (Fig. 3).

Logistic regression analysis was performed for the prediction of the presence of abnormal LV mass in echocardiography in ultra-marathon runners. The model presented statistical significance (P<0.001, Chi-squared=28.4) and could explain 79.6% of the variance of the abnormal LV mass presence (Nagelkerke R Square) and correctly classify 91.4% of the athletes. As presented in Table V, TNF-α, and not age or weight significantly contributed to the model.

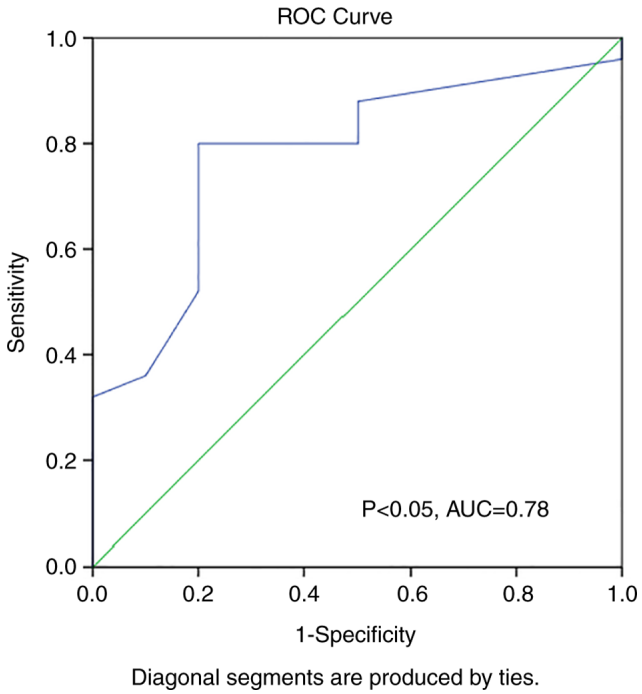

Figure 3. ROC curve for the predictive ability of TNF-α on abnormal left ventricular mass in marathon runners. AUC, area under the curve.

ROC curve analysis for the predictive ability of training sessions and kilometers covered per week on runners' abnormal RV dimensions produced nearly significant results (P=0.05, AUC: 0.702; P=0.502, AUC:0.691, respectively). However, the number of training sessions and kilometers run per week appear to be important for runners' LV adaptations, as ROC curve analysis of the said parameters on predicting abnormal LV volumes produced significant results (P<0.05, AUC: 0.835; and P<0.05, AUC: 0.802, respectively) (Fig. 4).

Table IV. Logistic regression analysis for the prediction of the presence of abnormal LV diameter as detected by echocardiography in ultra-marathon runners.

							95% CI OR	
Parameter	B	SE	Wald χ^2	df	P-value	OR	Ll	UL
IL-6 (pg/ml)	-9.61	4.71	4.16	1	**0.041**	0.00	0.00	0.68
IL-10 (pg/ml)	-5.96	3.68	2.62	1	0.105	0.01	0.00	3.50
Height (cm)	-0.40	0.16	6.27	1	**0.012**	0.66	0.49	0.91
Age (years)	-0.14	0.07	3.91	1	**0.048**	0.86	0.75	0.99
ADMA (μmol/ml)	-25.55	11.05	5.34	1	**0.021**	0.00	0.00	0.02

Values in bold font indicate statistically significant differences. B, unstandardized regression weight; SE, standard error (of B); OR, odds ratio; CI, confidence interval; df, degrees of freedom; LL, lower limit; UL, upper limit.

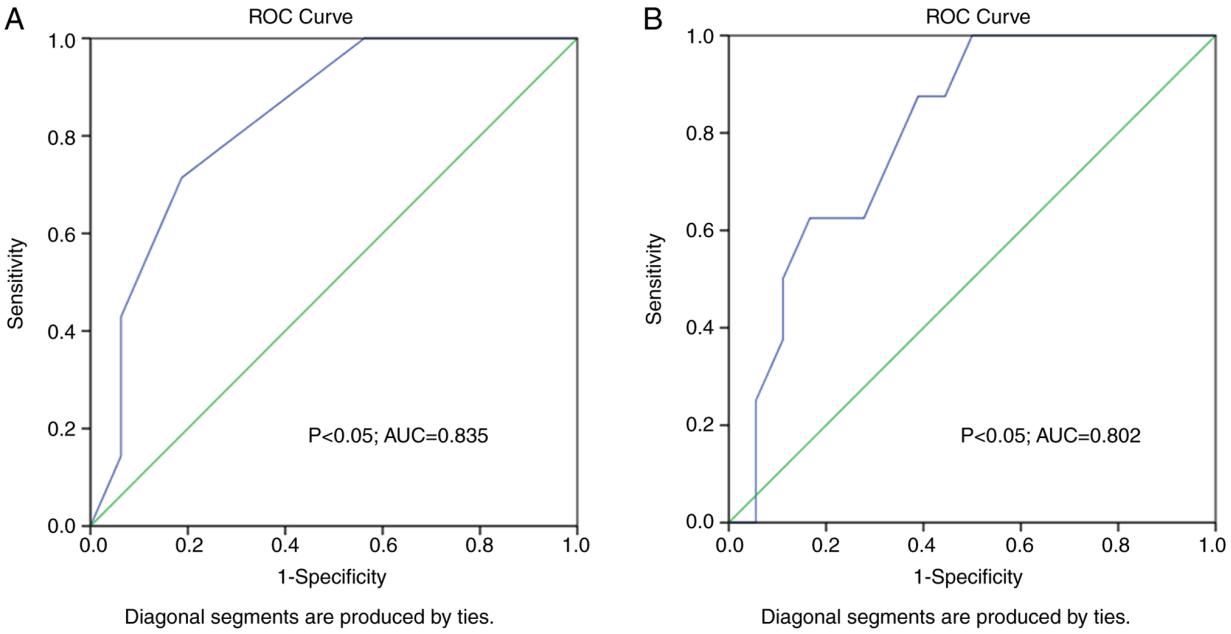

Figure 4. (A) ROC curve of training sessions per week for the prediction of abnormal left ventricular volumes in marathon runners. (B) ROC curve of kilometers run per week for the prediction of abnormal left ventricular volumes in marathon runners. AUC, area under the curve.

Logistic regression analysis was performed for the prediction of the presence of an abnormal LV volume in echocardiography in ultra-marathon runners. The model presented statistical significance (P<0.001, Chi-squared=9.52) and could explain 47.9% of the variance of the abnormal LV volume presence (Nagelkerke R Square) and correctly classify 82.6% of the athletes. As shown in Table VI, the number of training sessions per week, and not the kilometers ran per week or weight significantly contributed to the model.

Discussion

Muscle damage, glycogen deficiency and oxidative stress are all characteristics of strenuous exercise. In addition to these, the release of endotoxins, and the increase of plasma cortisol and catecholamine levels are observed. The subsequent increase in the levels of pro-inflammatory cytokines is considered to be both a trigger and a consequence of the said phenomena (6,21). Exercise intensity and the availability of energy sources are the main determinants of the tight regulation of IL-6 levels in response to exercise (22).

Circulating monocytes or fatigued contracting muscle express TNF-α, although its main source is macrophages (23). IL-6 has both pro- and anti-inflammatory properties and is induced by exercise. IL-6 induction along with the cytokine inhibition (IL-1ra) by exercise partly counteracts the increase in TNF-α levels. However, strenuous exercise triggers oxidative stress, which in turn impairs intracellular signaling and induces inflammation with an increase in pro-inflammatory cytokine expression and the disruption of anti-inflammatory cytokine production (24).

Previous studies have shown that strenuous exercise and specifically marathon running is associated with an increase in blood levels of specific inflammatory markers, namely IL-6, high-sensitivity CRP and TNF-α (23,25).

Table V. Logistic regression analysis for the prediction of the presence of abnormal LV mass as detected by echocardiography in ultra-marathon runners.

							95% CI OR	
Parameter	B	SE	Wald χ^2	df	P-value	OR	LL	UL
Age (years)	-0.28	0.16	3.20	1	0.073	0.75	0.54	1.02
TNF-α (pg/ml)	-2.06	1.02	4.01	1	**0.045**	0.13	0.02	0.95
Weight (kg)	-0.28	0.15	3.30	1	0.069	0.75	0.55	1.02

Values in bold font indicate statistically significant differences. B, unstandardized regression weight; SE, standard error (of B); OR, odds ratio; CI, confidence interval; df, degrees of freedom; LL, lower limit; UL, upper limit.

Table VI. Logistic regression analysis for the prediction of the presence of abnormal LV volume as detected by echocardiography in ultra-marathon runners.

							95% CI OR	
Parameter	B	SE	Wald χ^2	df	P-value	OR	LL	UL
Training sessions per week	-2.36	1.18	3.99	1	0.046	0.09	0.01	0.95
KM per week	0.05	0.04	1.48	1	0.223	1.05	0.97	1.15
Weight (kg)	-0.08	0.07	1.17	1	0.278	0.92	0.81	1.06

B, unstandardized regression weight; SE, standard error (of B); OR, odds ratio; CI, confidence interval; df, degrees of freedom; LL, lower limit; UL, upper limit.

ADMA is an analogue of L-arginine and is produced via methylation from L-arginine (L-Arg) by protein arginine methyltransferase type I. ADMA is a marker and also a determinant of endothelial system dysfunction, as it directly inhibits endothelial NO synthase (eNOS) and reduces the bioavailability of NO through the activation of the vascular renin-angiotensin system, and as a consequence of an increased production of reactive oxygen species (ROS). NO is one of the major endothelium-derived vaso-active substances; thus, increased ADMA levels lead to an impairment of the NO regulation of vascular tone (26). Emerging evidence suggests that NO plays a role in heart muscle response to mechanical stimulus in the form of chronic volume or pressure overload. NO deficiency is considered to induce myocardial hypertrophy and remodeling, as in the case of hypertension or in animal models of hemodynamically overloaded circulation (27). A recent study on patients undergoing coronary artery bypass graft also demonstrated that ADMA levels measured in pericardial fluid were positively associated with end-diastolic and end-systolic LV diameters, and negatively with LV ejection fraction (28). In the present study, ultra-marathon runners with increased LV end diastolic diameters, had elevated ADMA levels, while ROC curve analysis revealed that ADMA values could predict the presence of abnormal LV diameter at echocardiography.

Thus, renin-angiotensin system (RAS) activation in the arterial wall could be induced by increased ADMA values. Angiotensin II plays a central role in RAS. Angiotensin II has growth hormone properties. Reduced NO levels and local RAS activation can synergistically lead to cardiac hypertrophy (28). The majority of ultra-marathon runners monitored in the present study indeed presented an abnormal LV mass indexed to BSA >115 g/m^2, which was not associated with ADMA levels. However, runners with moderate and severe abnormal indexed LV mass >131 g/m^2 had statistically significant higher TNF-α values and ROC curve analysis revealed that TNF-α values could predict the presence of abnormal LV mass in marathon runners. Animal models of experimental hypertension using aortic banding for the induction of pressure overload showed that TNF-α/TNFR1 signaling are crucial for the development of myocardial hypertrophy, as there is an association between hypertrophy and myocardial TNF-α levels (29,30). TNF-α myocardial signaling is considered to be concentration-dependent (31) and at an early stage of hypertrophy, there is recent evidence to suggest that it is cardioprotective (32).

There are limited reports on the mechanistic insight of a possible link between inflammation and cardiovascular adaptations, and no solid evidence is provided thus far (6,33). Running in humans is associated with increased cardiovascular activity and increased ventricular pressure. B-type natriuretic peptide (BNP) and its cleaved inactive NH2-terminal fragment (NTproBNP) are secreted by ventricles in response to cardiomyocyte stress produced by volume or pressure overload (34). Although BNP elevations in healthy individuals, in the context of increased ventricular pressure, were reported >20 years ago (35), there is a long debate whether elevations in the levels of this biomarker after running is an epiphenomenon

or a warning sign of possible cardiac damage. BNP and NT-proBNP levels at rest in endurance athletes are similar to their untrained and age-matched peers, but increase 5- to 10-fold after exercise in subjects participating in endurance exercise events (34). Exercise duration and not intensity affect BNP and NT-proBNP release. BNP and NT-proBNP levels increase the most with exercise in the least trained athletes, suggesting that the acute increase may help initiate a training response.

Although the role of the primary transcriptional response factor for hypoxic adaptation of the skeletal muscle, the hypoxia inducible factor (HIF)-1α, during endurance training, has been widely studied (36,37) relevant reports on cardiac muscle were not found. Since HIFs are key oxygen sensors that mediate the ability of the cell to cope with decreased oxygen tension, a persistently activated hypoxic response in the heart as occurs during pressure overload or tachypacing may stabilize HIF-1α levels (36). In addition, mitochondria are implicated in multiple HIF-dependent and -independent pathways through the production of mitochondrial ROS and HIF-1-mediated adaptations influence lactate production, transport and metabolism. In that sense, similar studies on heart muscle may shed light on training adaptations and energy demands of the heart during exercise.

Cardiac volume overload characterizes endurance sports. It is associated with LV and left atrium dilation, and an increase in relative wall thickness and LV mass. No systolic and diastolic dysfunction has been found in this specific remodeling observed in aerobic dynamic exercise (38,39). Previous studies have revealed an association between left atrial size and IL-6 levels (40,41), mainly in the setting of atrial fibrillation. Atrial myocardial stretch is believed to induce IL-6 expression (42). In the present study, runners with an abnormal left atrial volume index had higher IL-6 values compared with runners with a normal LAVI.

On the other hand, RV volume augmentation and dysfunction have been reported following a marathon race in previous studies on (ultra-)endurance athletes (43-45). RV dilation is considered to be associated with bradycardia and an increased venous return post-exercise in marathon runners, also connected with an increased ventricular systolic function (46,47). Ultra-marathon runners have been found to have an increased RV end-diastolic area and RV fractional area changing compared with marathon runners (48). In the present study, runners with an abnormal RV diameter were those who had been training more years and with a more demanding training program, indicating an elite running status.

A well-known theory advocates that at exercise, the pulmonary circulation presents a lower rate of decrease in vascular resistance in comparison to the systemic circulation and as a result, the stroke work at aerobic exercise required by the right ventricle to be achieved in order to sustain adequate flow is higher than the left ventricle, thus explaining both the more pronounced acute and chronic effects of marathon running on RV structure and function (44). The right ventricle is considered more vulnerable to fatigue after prolonged exercise and the hemodynamic theory attributes the said fatigue to the increased ventricular load imposed on the right ventricle, which is additionally increased with higher exercise volumes and intensity (43).

In amateur athletes training and those participating in marathon or half-marathon runs, an increased sympathetic drive has been found which outlasts the period of exercise, and this cardiac sympathetic modulation potentially is linked to adverse cardiovascular prognosis (49). Runners of the present study with lower than usual HRR values (<35 beats) had lower exercise duration at the exercise stress test.

Volume overload observed in marathon athletes is the cause of the augmented LV and RV chambers. High dynamic exercise also leads to an increase in LV thickness in relation to volume, leading to LV eccentric hypertrophy (50).

The study by Arbab-Zadeh *et al* (4) demonstrated that in previously sedentary individuals who began training in order to participate in a marathon, the RV volume increased according to training intensity from the beginning of the training, while the LV volume increased only after 6 months. The LV and RV mass responded with hypertrophy to marathon training. In the first 6 months, concentric hypertrophy was noted, and after this time point, higher intensity and prolonged running training led to eccentric hypertrophy as the LV dilated (4). In the present study, in agreement with the study by Arbab-Zadeh *et al* (4), ultra-marathon runners presented mild eccentric myocardial hypertrophy. It was found that in middle-aged marathon runners trained with a mild program covering 40k m per week, less pronounced myocardial adaptations were observed despite the fact that peak oxygen consumption during cardiopulmonary exercise test was increased at the end of the 18-week training period (5).

In young athletes, criteria have been developed to distinguish a physiological adaptation of cardiac morphology and function to exercise (‘athlete's heart’) from early cardiovascular disorders (51). In older-aged adults characterized by a higher prevalence of cardiovascular risk factors and possible subclinical cardiac disease, the differentiation of athlete's heart from early cardiac disease may be more challenging. Increases in LV mass and LV volume may not only represent a response to exercise, but are also dependent on age and blood pressure. In addition, a left ventricular hypertrophy without an increase in volume may be an indicator for early subclinical cardiac alterations in response to risk factor exposure (52). The findings of the present study may help to distinguish physiological adaptation to exercise from alterations in response to cardiovascular risk factors and aging and protect athletes from sudden cardiovascular events (53). In this regard, other imaging techniques may be very helpful in distinguishing cardiovascular adaptations, such as myocardial magnetic resonance imaging, which is a valuable tool to classify myocardial hypertrophy, as a manifestation of cardiovascular remodeling due to exercise or a sign of underlying pathology (54,55).

In conclusion, the present study demonstrated that, in ultra-marathon runners, cardiovascular adaptations to running developed in combination with specific patterns of inflammatory and endothelial alterations. The levels of such biochemical markers may, in their turn, be used to predict the occurrence of the said cardiovascular adaptations.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Authors' contributions

KT, AS and CT organized and performed the research, collected relevant information, wrote the manuscript and performed overall project management. CT performed the statistical analysis, data assessment and manuscript preparation. GK, AS, DK and DAS performed the statistical analysis and the evaluation of the results, and were involved in the preparation and writing of the research article. FB, DK and CS reviewed the manuscript and comprehensively assessed the study design and the data analysis, prepared and wrote the manuscript, organized the references and reviewed the current study. KT, CS and GK confirm the authenticity of all the raw data. All authors have read and approved the final version of this manuscript.

Ethics approval and consent to participate

Written informed consent to participate in the study was provided by all participants involved. The procedures were in accordance with the Helsinki declaration of 1975 and approval was received by the Human Subjects Committee of the University of Thessaly, Larissa, and the General Hospital of Giannitsa (city of Giannitsa), where all medical practices were conducted.

Patient consent for publication

Not applicable.

Competing interests

DAS is the Editor-in-Chief for the journal, but had no personal involvement in the reviewing process, or any influence in terms of adjudicating on the final decision, for this article. The other authors declare that they have no competing interests.

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