Article

Left Cardiac Remodelling assessed by Echocardiography is Associated with Rho-kinase Activation in Long-distance Runners

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Abstract: This single-blind and cross-sectional study evaluated the role of Rho-kinase (ROCK) as a biomarker of the cardiovascular remodelling process assessed by echocardiography in competitive long-distance runners (LDR) during the training period before a marathon race. Thirty-six healthy male LDR (37.0±5.3 years; 174.0±7.0 height; BMI: 23.8±2.8; $\dot{V}O_2$-peak: 56.5±7.3 mL·kg^{-1}·min^{-1}) were separated into two groups according to previous training level: high-training (HT, n=16) ≥100 km·week^{-1} and low-training (LT, n=20) ≥70 and <100 km·week^{-1}. Also, twenty-one healthy nonactive subjects were included as a control group (CTR). A transthoracic echocardiography was performed and ROCK activity levels in circulating leukocytes were measured at rest (48-hr without exercising) the week before the race. HT group showed higher left ventricular mass index (LVMi) and left atrial volume index (LAVi) than other groups (p<0.05, for both), also higher levels of ROCK activity were found in LDR (HT=6.17±1.41 vs CTR=1.64±0.66 (p<0.01); vs LT=2.74±0.84; (p<0.05)). In LDR a direct correlation between ROCK activity levels and LVMi (r=0.83; p<0.001), and LAVi (r=0.70; p<0.001) were found. In conclusion, in male competitive long-distance runners, the load of exercise implicated in marathon training is associated with ROCK activity levels and the left cardiac remodelling process assessed by echocardiography.

Keywords: *Athlete´s heart; Cardiac biomarkers; Echocardiography; Exercise; Functional cardiac capacity.*

1. Introduction

Physical exercise plays a fundamental role in cardiovascular prevention and significantly reduces global mortality [1]. This benefit is associated with different mechanisms linked to structural changes or "adaptation" of the heart [2]. The combination of these adaptations, known as cardiovascular remodelling, involves changes at molecular and

cellular levels which translated into structural, electric and functional cardiovascular modifications [3].

The cardiac remodelling process can occur early during the training process [4]. Highly trained runners experienced these changes with greater prevalence and intensity, which in most cases they are benign and reversible [2], a condition called "athlete`s heart" [5], that includes increased bi-ventricular diameter, left ventricle (LV) parietal thickness, LV mass and bi-atrial volume with systolic and normal diastolic function [6]. Also, the athlete`s heart is accompanied by electrical remodelling, secondary to a higher vagal tone [2, 7]. The majority of these changes are a physiological adaptation to exercise; however, some patterns may overlap with channelopathies or cardiomyopathies [8], LV hypertrophy criteria are present in as many as 70% of highly trained athletes [9], and only 12% showed criteria for right ventricular hypertrophy [10]. The electrocardiogram interpretation in athletes showed benign changes in 80% of the cases and possibly pathological changes in less than 5% [7-8].

Advances in image techniques have allowed a better characterization of the athlete's heart, identifying a broad spectrum of changes. Although these changes allow a better performance in the face of intense efforts [11], some predisposed athletes subjected to high training loads may show a potentially adverse cardiac remodelling, a condition called "Phidippides" cardiomyopathy [6]. This adverse cardiac remodelling process is not frequent and is characterized by LV hypertrophy associated with myocardial fibrosis [9], increased coronary atheromatosis, greater right ventricular remodelling and extreme right and left atrial (LA) dilatation [12-13], which the uncertainty of whether there is a maximum limit of training considered safe, and if it is possible to identify an individual limit for each athlete [14]. Recent studies using cardiac magnetic resonance has shown myocardial fibrosis in triathletes with very high training loads and in those who present hypertensive pressure response to exercise [9].

The physiological and eventually pathophysiological mechanisms linked to adverse cardiac remodelling in high-performance athletes are not completely clear. In this regard, the dynamics of different biomarkers related to tissue damage, inflammation, oxidative stress and cardiac remodelling has been studied in athletes of different disciplines with no clear significance [6]. A novel pathway related to cell survival and cardiac adaptation to stress is rho-kinase (ROCK) activation [15]. This kinase exerts its role by acting on the cytoskeleton, regulating cell motility (migration), adhesion and proliferation, assuming a leading role in mediating cardiac remodelling; [15-16] in different clinical scenarios [17-18]. The dynamic of ROCK activation and their association with cardiac remodelling in endurance athletes who run high-demanding careers has not been previously studied. Thus, the primary objective of this study was to evaluate the activity of ROCK and its association with the cardiac remodelling assessed by echocardiography in competitive long-distance runners (LDR) with different training loads before a high-demanding competition (marathon).

2. Materials and Methods

Thirty-six males recreational LDR were recruited previously to a marathon race (Santiago, 42.2-km). The participants were included 16 weeks before the competition, in the training period called "optimal phase", where the volume of training is increased by running more distance by week. The inclusion criteria were: (i) age between 18 and 50 years to minimize possible cardiovascular event linked to competition, (ii) participation in three or more completed marathons previously in the last five years, (iii) recreational status to obtain a more diverse sample. The exclusion criteria were: (i) presence of any morbidity or disease that alter plasma levels of ROCK (e.g., arterial hypertension, dyslipidemia, insulin resistance, smoking or alcohol consumption habit, renal or liver dysfunction, neoplasia, chronic respiratory and cardiac diseases); and (ii) use of ani-hypertensive, anorexic, anti-depressant, and or antibiotics medication. In addition, a control group of healthy and non-active, sedentary subjects (n=21) was included. The study was approved by the Ethics

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Committee of Pontificia Universidad Católica de Chile in observance of the Declaration of Helsinki on experimentation in humans' beings (project nº 16082603). Written informed consent was obtained from the subjects prior to any procedure.

The study was cross-sectional with single-blind to researchers responsible for analyzing the serum samples, performing the echocardiographic reports and the statistical analysis; they did not know to which group each subject belonged. The participants were divided according to training volume before the marathon competition in high trained (HT ≥ 100 km·week^{-1}, n=16) and low trained (LT ≥70 and <100 km·week^{-1}, n=20). To define the training intensity, a previously validated *ad-hoc* questionnaire was used. The 100 km limit was set based on training protocols of our sports cardiology group. **Figure 1** shows the study design and assessments performed.

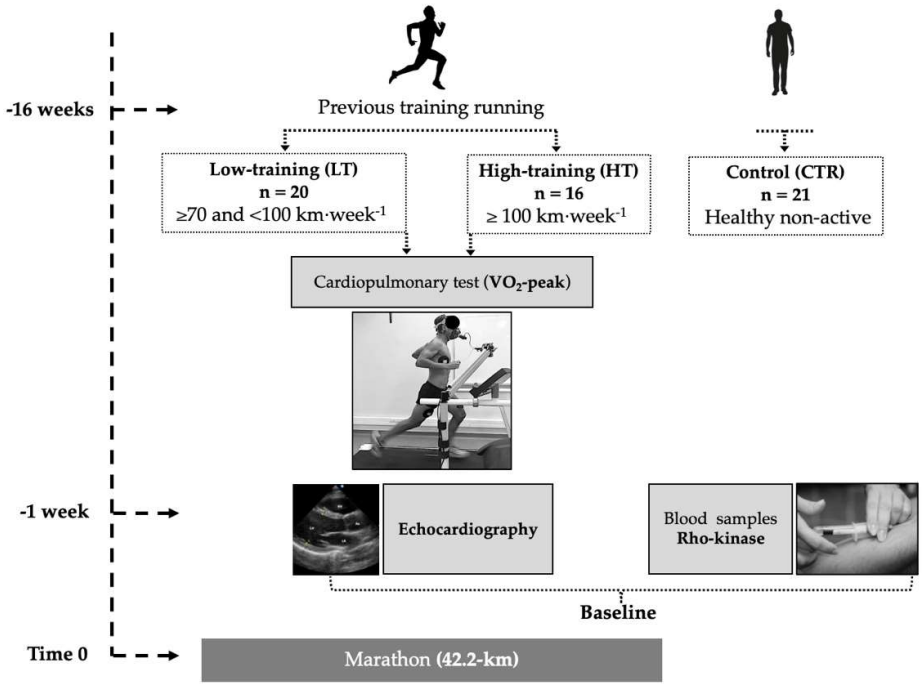

Figure 1. Study design scheme.

Through a venipuncture of the antecubital fossa, peripheral blood mononuclear cells were extracted to determine ROCK activity one week before the marathon competition. ROCK activity was determined in mononuclear cells, using protocols previously described [17]. Briefly, ROCK activation is determined by measuring the phosphorylation of a direct ROCK target, the myosin phosphatase target subunit 1 (MYPT1) of the myosin light chain phosphatase of the light chain of myosin by Western-blot. ROCK activity was expressed by the quotient between phosphorylated (p-MYPT1) and total MYPT1 (t-MYPT1). We previously showed in an experimental model that ROCK activity in circulating leukocytes reflects activation of this signaling pathway in the myocardium and aortic wall [19].

The transthoracic echocardiography (TTE) was performed in all participants using Vivid I echocardiography equipment (General Electric Healthcare, Horten, Norway) with a 2.5/5 MHz sector transducer. Traditional views were acquired from the windows: parasternal, apical and subcostal for the quantification of the left and right heart chambers according to the American Society of Echocardiography [20]. LV diastolic function was evaluated using transmitral filling waves and mitral annulus tissue Doppler. Also, LV

longitudinal deformation (longitudinal strain) was carried out using four, three and two chambers' views optimized to achieve >60 frames per second. Images were stored for further analysis by an expert, blinded echocardiographer using the manufacturer's software (EchoPAC, version BT12; GE Healthcare, Horten, Norway).

The physical performance of LDR was assessed by the cardiopulmonary test (peak aerobic capacity, $\dot{V}O_2$-peak) at the end of the "optimal phase" training period. All LDR were instructed not to perform physical activity 48 h before the measurement and avoid intakes of alcohol, caffeine, or other stimulants and food for at least three hours before. The $\dot{V}O_2$-peak test was measured on a treadmill ergometer (HP Cosmos, Traunstein, Germany) until voluntary exhaustion, despite oral breathing (respiratory quotient, 1.20±0.05). The exercise protocol consisted of a 3-min rest, 5-min warm-up (8 km·h^{-1}), and subsequent increase of 2 km·h^{-1} every 150 s, until all criteria for stopping the test were met. Ventilatory data were analysed *breath-by-breath* using open-circuit spirometry and were expressed under standard temperature, pressure and dry (STPD) conditions (MasterScreen CPX, Jaeger™, Germany). Before each test, the gas analyser and the volume transducer were calibrated according to the manufacturer's instructions.

The normality of the data was evaluated using the Shapiro-Wilk test. The ANOVA and Student t-test were used to compare groups. The Pearson correlation test was used for assessing the association between ROCK activity levels and echocardiographic parameters linked to the cardiac remodelling process. The statistical software used was GraphPad Prism 8.0 (GraphPad Software Inc., San Diego, USA). A value of $p<0.05$ was considered statistically significant.

3. Results

Fifty-seven participants were consecutively included (age 37.4±6.1 years). The LDR achieved values of $\dot{V}O_2$-peak according to their health status (LT: 52.5±8.1 mL·kg^{-1}·min^{-1} vs HT: 58.5±5.3 mL·kg^{-1}·min^{-1}; p=0.02). HT group completed the marathon race in less time (231±39 vs 197±33 min vs 231±39 LT, p=0.03). **Table 1** shows the participants` characteristics.

Table 1. Participant`s characteristics.

Variables	Groups			*p*-value
	CTR (n=21)	LT (n=20)	HT (n=16)	
Age (years)	35 ± 4	39 ± 5	37 ± 6	0.32
Height (cm)	175 ± 6	174 ± 6	172 ± 7	0.47
Weight (kg)	72 ± 9	73 ± 8	69 ± 8	0.09
Body surface (m^2)	1.89 ± 0.13	1.88 ± 0.12	1.82 ± 0.13	0.08
Creatinine (mg·dL^{-1})	0.99 ± 0.11	0.97 ± 0.10	0.98 ± 0.09	0.63
Hematocrit (%)	42 ± 3	43 ± 3	43 ± 2	0.87
Sodium (mEq/L)	142 ± 2	142 ± 2	142 ± 3	0.44
AST (U/L)	26 ± 7	28 ± 8	29 ± 9	0.67
Uric acid (mg/dL)	5.2 ± 0.8	5.0 ± 0.9	5.6 ± 0.9	0.17
$\dot{V}O_2$-peak (ml·kg^{-1}·min^{-1})	-	52.5 ± 8.1	58.5 ± 5.3	**0.02***
Running experience (years)	-	15 ± 3	17 ± 3	0.81
Time training per week (hours)	-	14 ± 2	19 ± 2	**0.01***
Training intensity (%HR máx., 220-age)	-	82 ± 2	81 ± 3	0.78

Data are reported as the mean ± SD; Student's t test, * $p < 0.05$. Abbreviations. CTR: Control; LT: Low training (≥70 and <100 km·week^{-1}); HT: High training (≥100 km·week^{-1}); AST: Aspartate amino transferase; $\dot{V}O_2$-peak: Peak oxygen consumption.

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The activity of ROCK was different between the groups. HT showed highest ROCK activation (6.17±1.41 vs 2.74±0.84 LT (p=0.002), and vs 1.64±0.66 CTR (p=0.001)) (**Figure 2**).

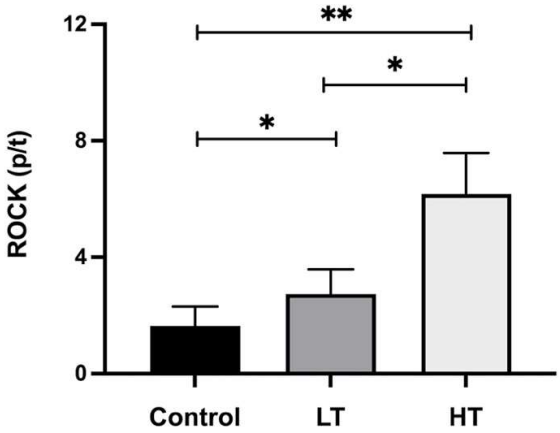

Figure 2. ROCK activity levels by group.
Abbreviations: ROCK: Rho kinase activity expressed as p-MYPT1/t-MYPT1 ratio; LT: low training (≥70 and <100 km·week^{-1}); HT: high training (≥100 km·week^{-1}).

Regarding the quantification of the cardiac chambers by TTE, the results are shown in **Table 2**.

Table 2. Heart chambers quantification.

				Groups
	CTR (n = 21)	LT (n = 20)	HT (n = 16)	p-value
Left cardiac cavities				
Interventricular septum (mm)	7.6 ± 0.8	9.0 ± 1.6	10.2 ± 1.2*	*< 0.001*
Posterior wall (mm)	7.6 ± 0.8	8.5 ± 1.2	9.3 ± 2.1*	*0.01*
Diastolic diameter (mm)	46 ± 4	50 ± 5	58 ± 4*	*0.04*
Systolic diameter (mm)	30 ± 3	30 ± 4	33 ± 5	0.40
Ejection fraction (%)	57 ± 4	55 ± 6	54 ± 3	0.11
LV mas index (g·m^{-2})	58 ± 11	78 ± 18	106 ± 27*	*< 0.001*
LA diameter (mm)	33 ± 4	34 ± 3	36 ± 4	0.22
LA area (cm^2)	19 ± 5	22 ± 4	25 ± 3.5*	*< 0.001*
LA volumen index (ml·m^{-2})	25 ± 9	30 ± 11	42 ± 8*	*< 0.001*
Global LV longitudinal strain (%)	-21 ± -2.0	-19.6 ± -1.6	-19.5 ± -2.4	0.11
E wave (cm·sec^{-1})	77 ± 15	84 ± 12	78 ±13	0.21
A wave (cm·sec^{-1})	48 ±16	53 ± 10	50 ±12	0.43
Deceleration time (msec)	200 ± 66	229 ± 65	233 ± 65	0.18
e´ lateral (cm·sec^{-1})	15 ± 1.8	15 ± 2.5	15 ± 2.3	0.70

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e´ medial (cm·sec^{-1})	11 ± 1.8	10 ± 2.0	10 ± 2.0	0.75
Right ventricle				
TAPSE (mm)	25.4 ± 3.3	25.6 ± 4.7	25.8 ± 3.0	0.16
FAC (%)	52.5 ± 3.9	57.3 ± 4.6	56.4 ± 3.7	0.07

Data are reported as the mean ± SD; ANOVA test (HT vs other groups), * $p < 0.05$. Abbreviations. CTR: Control; LT: Low training (≥70 and <100 km·week^{-1}); HT: High training (≥100 km·week^{-1}); LV: left ventricle; LA: left atrium; é: mitral annulus tissue Doppler; TAPSE: tricuspid annulus plane systolic excursion; FAC: fractional area change.

The HT group showed significantly larger LV linear dimensions than the other groups. Also, they showed a significantly increased LV mass index and LA volume index (**Figure 3**). LV diastolic function and right ventricle parameters were similar between groups.

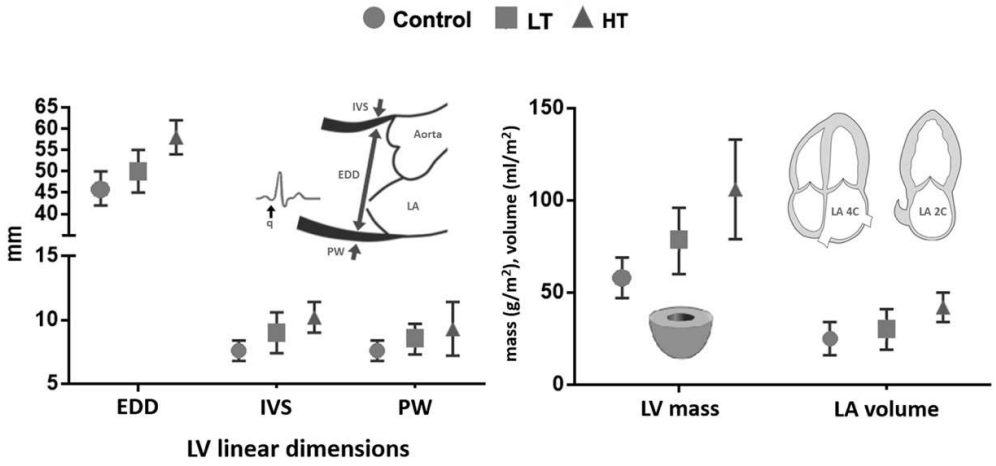

Figure 3. Quantification of left heart chambers.
Abbreviations: LT: low training (≥70 and <100 km·week^{-1}); HT: high training (≥100 km·week^{-1}); EDD: end diastolic diameter; IVS: inter ventricular septum; PW: posterior wall; LV: left ventricle; LA: left atrium.

Among LDR, a direct correlation between ROCK activation in circulating leukocytes, measured by p-MYPT1/t-MYPT1 ratio, and left cardiac remodelling, evaluated by LV mass index (**Figure 4a**) and LA volume index, were found (**Figure 4b**).

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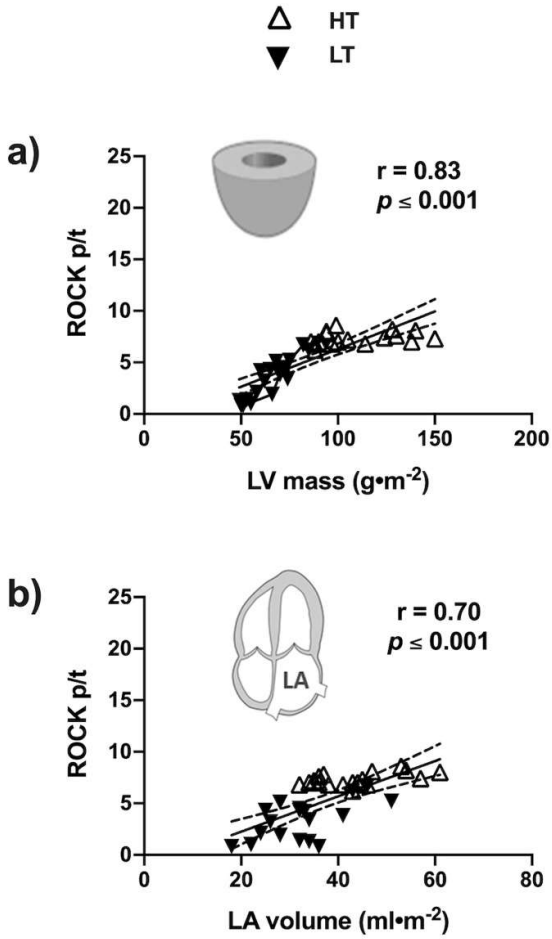

Figure 4. Correlation between ROCK activity and heart remodeling parameters.
(a) ROCK-activity (expressed as p-MYPT1/t-MYPT1 ratio) and LV mass index; **(b)** ROCK-activity and LA volume index.
Abbreviations: LV, left ventricle; LA, left atrium.

4. Discussion

The main results of this study are that HT runners showed higher ROCK activity in circulating leukocytes than LT runners and physically healthy and non-active participants. Also, the HT group showed more significant LV diastolic diameter, LV wall thickness, and LV indexed mass, with normal deformation properties and systo-diastolic function; also, in HT group LA volume was bigger but with normal filling pressures. Among LDR, the ROCK activation levels in circulation leukocytes were correlated with the LV and LA remodelling as evaluated by indexed LV mass and LA volume.

Previous reports revealed that athletes who participate in competitively endurance sports showed a heart remodelling process similar to our findings. Athletes showed an increased LA volume and LV mass with normal systolic and diastolic function; these structural changes were more pronounced in those with a more intensive training protocol [6, 12-13];. A meta-analysis in athletes with high training regime showed that acute and prolonged exercise was associated with larger LV volumes and a relative lower LV ejection fraction post intensive exercise [21]. In this study, the HT group showed a greater left cardiac remodelling process, which potentially gives athletes a better performance in

a highly demanding race [11]; however, these changes could be related to a theoretically excessive and deleterious remodelling process [13], which is in keeping with other reports showing inflammatory and cell remodelling biomarkers elevation in athletes whose final significance are not fully clarified [22-23].

The ROCK signaling pathway participates in cell survival and cardiac hypertrophy, cardiac fibrosis, and cell apoptosis [16]. Some athletes may develop an extreme cardiac remodelling process, including atrial structure and function changes [12-13], worse ventricular performance post-effort [6], and appearance of arrhythmias in the long-term follow-up [24]. Regarding the physiopathology of the previous process, the ROCK signaling pathway could play an important role in electrical properties changes in atrial tissue (connexin expression), as Chen *et al.* (2018) showed studying the LA appendage in patients with and without atrial fibrillation [25]. In our study, the HT group showed higher: left cardiac cavities remodelling, ROCK activity and sports performance than other groups; probably these pathways related to cell survival to stress and cardiac remodelling allow to athletes a better adaptation and performance to extreme efforts, but there could be an individual point for each subject in which a poor adaptation may be observed. This "point" could lead to an extreme cardiac remodelling process and the risk of future arrhythmias [6] and cardiac dysfunction.

Athletes showed a systemic and cellular adaptation to mechanical, inflammatory and metabolic stress caused by regular and intense exercise training that generates adaptation mechanisms, such as cardiac remodelling and changes in cardiac biomarkers expression [26], so that more trained athletes can perform higher workloads, obtaining better performance in highly demanding competitions; however, some subjects could experiment extreme and potentially not adaptive changes [6, 13]. The maladaptive changes could be partially explained by experimental models of prolonged intense exercise that showed an increased expression of tumour growth factor-β1 (TGF-β1) in atrial and right ventricular tissue related to myocardial stiffness and myocardial fibrosis [27]. Genetic influences in ROCK activation cannot be ruled in the formation of cardiac fibrosis associated to remodelling process induced by intense exercise and regular physical training load. In this regard, normal rodents with genetically higher angiotensin-converting enzyme levels have increased ROCK cascade activation simultaneously in the heart and circulating leukocytes, and they develop higher LV fibrosis levels in response to isoproterenol [28].

Therefore, we suggest that these processes of conditioning and adaptation to continuous effort could reach in individual athletes a "maladaptation" over time, triggering an adverse remodelling process which would be explained by the concept of "hormesis" that is defined as an adaptive cellular response to stressors resulting in a biphasic dose-response: low doses are related to a beneficial adaptation while high doses result in an adverse effect in some specific subjects. Currently, it is unknown precisely the real meaning of these changes and the behavior of cardiac remodelling biomarkers in apparently non-pathological conditions and whether they predispose and partly explain the development of arrhythmias in this subgroup. Moreover, ROCK activity in circulating leukocytes, which reflects activation of this pathway in the myocardium [19], is related to adverse cardiac remodelling in patients with hypertension and cardiac failure [17-18] making more complex the interpretation of our findings.

The main limitations of our study were the reduced number of participants recruited, the exclusive participation of male athletes and that our conclusions are potentially applicable exclusively to long-distance runners. Also, the presence of myocardial fibrosis with cardiac resonance was not evaluated. We assumed that athletes had a normal systo-diastolic function and normal deformation (global longitudinal strain) of the left ventricle, only with echocardiographic assessment.

There is no doubt that moderate exercise training leads to cardiovascular good health and longevity [1]. However, there is increasing evidence that more intense exercise can produce potentially excessive cardiac remodelling and arrhythmic events in the long-term

follow-up. So, it is essential to have available and accessible clinical tools and biomarkers, that help identify those individuals with a greater risk.

5. Conclusions

In male long-distance competitive runners, the load of exercise implicated in marathon training (overload cardiac volume) is associated with ROCK activity levels and the left cardiac remodelling process assessed by echocardiography.

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Institutional Review Board Statement: This study was approved by the ethics committee of the Pontificia Universidad Católica de Chile (Institutional Review Board, protocol number 16082603, date of approval: 5 June 2016). The study was carried out according to the Declaration of Helsinki for human experimentation, and we confirm that the study meets the journal's ethical standards.

Informed Consent Statement: All the participants were informed of the purpose, protocol, and procedures before informed consent was obtained from them.

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Conflicts of Interest: The authors declare no conflict of interest.

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