ORIGINAL ARTICLE

Effects of Strenuous Exercise on Baseline Ventricular Function and Inotropic, Chronotropic and Lusitropic Response in Mice

LUCIANA WILENSKY[1, 3], GERMÁN E. GONZÁLEZ[1, 4], VERÓNICA D'ANUNZIO[1], FEDERICO MATORRA[1, 3], VIRGINIA PÉREZ[1], FEDERICO A. GULLACE[2], ELIANA M. CICALE[2], PILAR BÁRCENA BARBEIRA[1], CELINA MORALES[MTSAC, 1], RICARDO J. GELPI[MTSAC, 1, 4]

Received: 11/21/2012
Accepted: 02/06/2013

Address for reprints:
Prof. Dr. Ricardo J. Gelpi
Instituto de Fisiopatologia Cardiovascular
Departamento de Patología
Facultad de Medicina, UBA
Pte. J.E. Uriburu 950 - Piso 2
(1114) Buenos Aires
e-mail: rgelpi@fmed.uba.ar

ABSTRACT

Background
Mild to moderate exercise reduces cardiovascular risk factors, improves pre-existing pathological conditions and develops adaptive cardiac hypertrophy. However, the myocardial response to strenuous exercise is scarcely known.

Objective
The aim of this study was to evaluate baseline ventricular function and myocardial reserve (inotropic, chronotropic and lusitropic response to the β-adrenergic agonist isoproterenol) in vivo and in vitro in mice following strenuous exercise.

Methods
Three-month old male FVB mice were used. The protocol exercise consisted in 90 min swimming sessions twice a day, 6 days/week for 4 weeks. Two experimental groups were studied: 1) sedentary group, with no exercise; and 2) exercise group, with full strenuous swimming protocol.

Results
At the end of the protocol, left ventricular mass increased by 27.9±4% with preserved baseline left ventricular function. In vivo and in vitro myocardial response to isoproterenol decreased with no changes in interstitial collagen.

Conclusions
Under our experimental conditions, a strenuous swimming protocol produced moderate cardiac hypertrophy with adaptive and maladaptive hypertrophic characteristics. Although baseline ventricular function was preserved with no changes in interstitial collagen, inotropic, chronotropic and lusitropic reserve decreased.

Rev Argent Cardiol 2013;81:107-112. http://dx.doi.org/10.7775/rac.v81.i2.1891

Key words > Exercise, Hypertrophy, Ventricular Function.

Abbreviations >

bpm	Beats per minute	**ISO**	Isoproterenol
CPP	Coronary perfusion pressure	**LV**	Left ventricle
+dP/dt$_{max}$	Positive first derivative of left ventricular pressure	**LVDP**	Left ventricular diastolic pressure
		LVSP	Left ventricular systolic pressure
-dP/dt	Negative first derivative of left ventricular pressure	**TL**	Tibial length
		t_{63}	isovolumic relaxation time t_{63}
HR	Heart rate		

[MTSAC] Full Member of the Argentine Society of Cardiology
[1] Institute of Cardiovascular Pathophysiology, Department of Pathology, School of Medicine, Buenos Aires University, Argentina
[2] Central Animal Care House, School of Veterinary Science, Buenos Aires University, Argentina
[3] Doctoral Fellow, National Scientific and Technical Research Council (CONICET)
[4] Member of the Research Career at the National Scientific and Technical Research Council (CONICET)

INTRODUCTION

It is well known that exercise has beneficial effects reducing cardiovascular risk factors as hypertension, diabetes mellitus, overweight, endothelial dysfunction and dyslipidemia. (1) As a result of exercise, the heart develops physiological remodeling with myocardial hypertrophy in response to chronic hemodynamic loading, (2) and increased left ventricular diameter (3) depending on the type of stimuli. (4) Exercise may exert beneficial effects on pre-existing conditions, as hypertension, myocardial infarction and heart failure. (1) In this sense, Garciarena et al. demonstrated that moderate exercise produces a beneficial adaptation in spontaneously hypertensive rats, converting pathological into physiological hypertrophy, inhibiting apoptosis and improving ventricular function. (5)

Normal heart function depends on the ability of the heart to keep an adequate stroke volume, not only under baseline conditions but also as a response to different stimuli, as β-adrenergic stimulation. (3, 6) This becomes more relevant in the heart subjected to exercise, as it implies a significant adrenergic stimulus for the heart which has to be capable of generating an adequate response. Therefore, it is noteworthy that few studies have evaluated the heart response to β-adrenergic stimulation after chronic exercise. In this sense, Vitiello et al. (7) showed that rats subjected to prolonged strenuous exercise had reduced baseline function with preserved response to isoproterenol (ISO). However, the exercise used in this research was strenuous and acute. Therefore, the goal of the present research was to evaluate baseline ventricular function and inotropic, chronotropic and lusitropic reserve in mice subjected to a protocol of strenuous chronic exercise. As chronic exercise may modify autonomic regulation and myocardial loading conditions which may be related to changes in ventricular function, the second goal of this study was to evaluate baseline cardiac response and myocardial reserve in an isolated isovolumic heart model .This model, where the strict control of variables which regulate myocardial function, such as ventricular volume, heart rate and pH are kept constant, allowed independence from changes induced by chronic exercise.

METHODS

Animals and exercise protocol

This study was approved by the Institutional Committee for the Care and Use of Laboratory Animals, University of Buenos Aires (Resolution N° 957 2012), and all the procedures were performed in agreement with the Guide for the Care and Use of Laboratory Animals of the United States National Academy of Sciences, updated by the American Physiological Society (APS). Adult male FVB mice (3 months old) were housed in small groups (n = 4/5) in cages and adapted to a 12:12-h light/dark cycle at a temperature of 22°C with ad libitum access to food and water at all times except during swimming sessions.

Swimming was chosen as exercise protocol as it is a mixed exercise. The adaptation period started with 20 minutes of exercise that was increased daily until the animals achieved the total stipulated time. (8) The animals from each experimental protocol swam in groups in a pool measuring 40 × 70 × 30 cm. A heating system kept the water temperature between 30 and 32ºC. (9) To prevent the animals from floating during the swimming session, homogeneous water bubbling was produced in all the system to generate constant and strenuous swimming throughout the protocol. (10) The exercise protocol consisted of 90 min sessions twice a day, 6 days a week for 4 weeks (including the adaptation week). (11, 12)

Experimental groups

The animals were assigned to two groups:

- Group 1: sedentary group. These mice were not subjected to exercise and were kept in their cages until euthanasia.
- Group 2: exercise group. These mice were subject ed to strenuous exercise and were kept in their cages until euthanasia.

Assessment of baseline left ventricular function and response to isoproterenol

In vivo study: once the protocol ended, the animals were weighed and anesthetized with ketamine (100 mg/kg) and xilacin (2.5 mg/kg). The right carotid artery was dissected and a heparinized catheter was introduced into the left ventricle (LV). The left jugular vein was then dissected and a catheter was introduced to administer intravenous ISO (56 ng/kg). Following a 10-min equilibration period, baseline left ventricular systolic and diastolic pressure (LVSP and LVDP; mmHg), its first derivative ($+dP/dt_{max}$ and $–dP/dt$; mmHg/s) and heart rate (HR; bpm) were recorded. The same variables were recorded in each group after the administration of ISO using an analog-to-digital-converter (National Instruments) with software for data acquisition and analysis.

In vitro study: once the protocol ended, another group of animals were weighed and anesthetized with sodium pentobarbital (150 mg/kg) and unfractionated heparin (500 IU/kg) was administered. Then, the aorta was isolated and cannulated with a 21G cannula. The hearts were excised and perfused according to the Langendorff technique with a Krebs- Henseleit buffer solution containing (in mM): 118.5 NaCl, 4.7 KCl, 24.8 $NaHCO_3$, 1.2 KH_2PO4, 1.2 Mg SO_4, 1.5 $CaCl_2$ and 10 glucose. This solution was bubbled with 95% O2 and 5% CO_2 (pH = 7.4) at 37°C. A latex balloon was introduced into the LV and connected to a catheter (P50) and a pressure transducer (Deltram II, Utah Medical System). Two electrodes were inserted and connected to a pacemaker to achieve a constant HR of 472 ± 30 bpm. Coronary perfusion pressure (CPP) was also recorded with a pressure transducer connected to the perfusion line. All the hearts were perfused at a constant flow of 4.00 ± 0.27 ml/min. The coronary artery flow was adjusted to obtain a CPP of 73.1 ±

3.1 mm Hg during the initial stabilization period and was kept constant throughout the experiment. Real time ventricular pressure and CPP were recorded and maximal dP/dt (dP/dt_{max}, mmHg/min) and isovolumic relaxation time (t_{63}, ms) were calculated. In this way, ventricular function was evaluated at baseline and after the administration of ISO.

Morphometric evaluation

Once ventricular function was recorded, the mice were euthanized and necropsy was performed. The complete cardiopulmonary block was removed and the LV, right ventricle (RV), left atrium and right atrium were dissected and weighed. The LV was fixed in buffered formalin to perform histological staining for histomorphometric studies. Tibial length (TL) was also measured. As opposed to body weight, which can increase with training, TL is not modified by exercise and was used to calculate the left ventricular weight (LVW)-to-tibial length (LVW/TL) ratio, an index of cardiac hypertrophy.

Quantification of percent LV collagen

Once the LV was fixed in buffered formalin and embedded in paraffin, semi-serial sections were stained using Picrosirius Red which differentiates collagen (red) from non-collagen tissue (yellow) without considering perivascular collagen. Collagen quantification was assessed by colorimetry using Image-Pro Plus 6.0 and expressed as percent collagen in all the left ventricle per field.

Statistical analysis

Results were expressed as mean ± standard error of the mean.

A t test was used for inter-group comparisons and a p value < 0.05 was considered as statistically significant. All calculations were performed using Sigma STAT32 software.

RESULTS

Necropsy results in Table 1 show that left ventricular mass, evaluated by LVW, as well as LVW/body weight and LVW/TL hypertrophy ratios were significantly higher in the exercise group vs. the sedentary group.

Table 2 describes baseline ventricular function values in vivo before ISO. Heart rate, LVSP, LVDP, +dP/dt_{max} and t_{63} were similar in both groups. Figure 1 shows in vivo baseline ventricular function and the response of +dP/dt_{max}, HR and t_{63} to the administration of ISO. Baseline +dP/dt_{max} (Fig.1, panel A) did not show significant differences in the study groups (5314.5 ± 404.9 vs. 6297.1 ± 499.5 mmHg/s), while the administration of ISO increased in both groups, though the increase was significantly lower in the group subjected to exercise (35.6 ± 7.0 vs. 63.2 ± 9.6%, $p<0.05$). Fig.1, panel B shows that baseline HR was similar (301 ± 15 vs. 300 ± 16 bpm), and increased after the administration of ISO in both groups. However, this increase was significantly lower in the group subjected to exercise (27.7 ± 5.3 vs. 66.8 ± 8.9%, $p < 0.001$). Finally, baseline relaxation time (Fig.1, panel C) was similar in both groups (8.32 ± 0.72 vs. 9.27 ± 0.64 ms), while ISO decreased t_{63} only in the sedentary group (8.32 ± 0.72 vs. 5.61 ± 0.31 ms, $p<0.004$), suggesting increased relaxation velocity in this group, and not in the exercise group (9.27±0.64 vs. 8.43 ±.0.76 ms). Thus, t_{63} percent variation showed a significant reduction in the sedentary group compared to the exercise group (9.82 ± 3.16%, vs. 29.93 ± 6.07%, $p<0.011$).

In the in vitro, isolated, perfused hearts, baseline +dP/dt_{max} (Fig. 2, panel A) did not evidence significant differences in the groups with and without exercise (1998.4 ± 149.3 vs. 2308.6 ± 409.1 mmHg/s). ISO produced a significant increase in the sedentary group (1998.4 ± 149.3 vs. 2951 ± 232.1 mmHg/s, $p<0.003$), but not in the exercise group (2308.6 ± 409.1 vs. 2882.8 ± 532.3 mmHg/s), resulting in a significantly lower percent increase of the exercise group compared to the sedentary group (49 ± 7.2% vs. 63.2 ± 9.6%, $p<0.05$).

Fig. 2, panel B shows that baseline t_{63} was similar in both groups (44.71 ± 1.9 vs. 41.17 ± 1.43 ms), while ISO decreased t_{63} only in the sedentary group, indicat-

Table 1. Morphometric values of the experimental group

Groups	Body weight		LV weight (mg)	TL (mm)	LVW/BW (mg/g)	LVW/TL (mg/mm)	Lung (mg)
	Before (g)	After (g)					
Sedentary (n=9)	---	31±0.9	99±7	18±0.1	3.2±0.2	5.4±0.4	185±10.2
Exercise (n=14)	32±0.1	31±0.6	125±4 *	18±0.1	4.1±0.1 *	6.9±0.2*	210±10.5

Body weight before and after exercise. LV weight: left ventricular weight. TL: tibial length. LVW/BW: left ventricular weight to body weight ratio. LVW/TL: left ventricular weight to tibial length ratio. Lung: lung weight. * Significance $p<0.05$ vs. sedentary

Baseline ventricular function	HR (bpm)	LVSP (mmHg)	EDLVP (mmHg)	+dP/dt$_{max}$ (mmHg/s)	t$_{63}$ (ms)
Sedentary (n=8)	301±16	83±3	8.97±0.6	5315±405	8.3±0.7
Exercise (n=9)	298±15	102±9	8.55±1.6	6297±500	9.3±0.6

HR: Heart rate. bpm: beats per minute. LVSP: Left ventricular systolic pressure. LVDP: Left ventricular diastolic pressure. +dP/dt$_{max}$: First derivative of left ventricular pressure. t$_{63}$: isovolumic relaxation index t$_{63}$.

Table 2. Baseline values of ventricular function in the experimental groups.

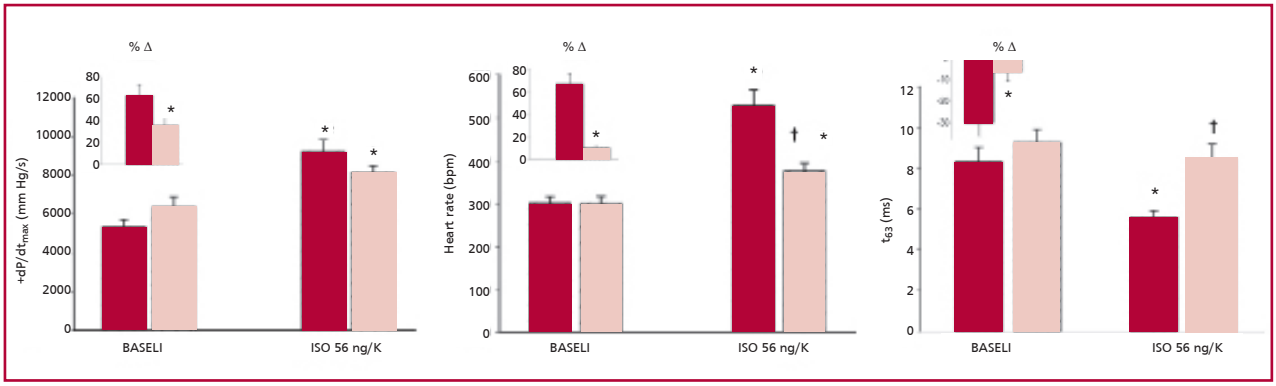

Fig. 1. Baseline values and inotropic, chronotropic and lusitropic response to ISO (56 ng/kg) of the two in vivo experimental groups. A. +dP/dt$_{max}$ graph, where the blue bars represent the sedentary group and the light blue bars represent the exercise group. The first two bars show baseline values for each group, and the second bars show the response to ISO. B. Heart rate graphs, where the first two bars show baseline values for each group, and the second bars show the response to ISO. C. Isovolumic relaxation graphs, evaluated by the index t$_{63}$, where the first two bars show baseline values for each group, and the second bars show the response to ISO. *p<0.05 vs. baseline; †p<0.05 vs. sedentary + ISO.

ing increased relaxation velocity (39.4 ± 1.6 vs 44.7 ± 1.9 ms, p<0.044), with no changes in the exercise group (41.17 ± 1.43 vs. 39.4 ± 1.59 ms). This resulted in a significantly lower percent decrease in the exercise group (4.3 ± 1.7 vs. 11.7 ± 1.9%, p<0.024).

Figure 3 shows that there were no significant differences in interstitial collagen in both groups (2.3 ± 0.3 vs.2.5 ± 0.3%).

DISCUSSION

In the present study, we have shown that mice subjected to a chronic swimming protocol, equivalent to strenuous exercise, developed moderate cardiac hypertrophy of 27.9±4.0% with no changes in collagen matrix. Baseline ventricular systolic function and isovolumic relaxation were similar in the exercise group and in the sedentary group. When the animals subjected to the swimming protocol underwent β-adrenergic stimulation with ISO, inotropic, chronotropic and lusitropic reserve decreased. Inotropic reserve and lusitropic reserve were evaluated both in vivo in the anesthetized animal, and in vitro with an isolated isovolumic Langendorff perfused heart model. As this model requires a pacemaker to keep a constant heart rate, the chronotropic reserve was evaluated only in vivo. Of interest, although many studies have evaluated ventricular function during exercise, (3, 5, 7, 9, 13) most of them have only assessment of baseline ventricular function without considering myocardial reserve. This aspect is particularly important, as the ability of the myocardium to respond to the metabolic requirements secondary to different activities of daily life needs an adequate capacity of response to sympathetic stimulation mediated by catecholamines which, in other words, represents the ability to react to an extra-stimulus. (14-16) Only one published study evaluated the inotropic and lusitropic reserve in animals subjected to strenuous exercise. (7) These authors found that baseline ventricular function was reduced in the exercise group compared to control and that inotropic and lusitropic reserve was preserved after the administration of ISO. These animals were subjected to a protocol of strenuous exercise for a short period of time (7 days), and probably the myocardium did not have enough time to adapt to chronic exercise. The type and duration of exercise in this protocol was different from the one used in our study, making comparisons difficult. However, these methodological disparities might explain the different results.

Another important aspect to consider is the type

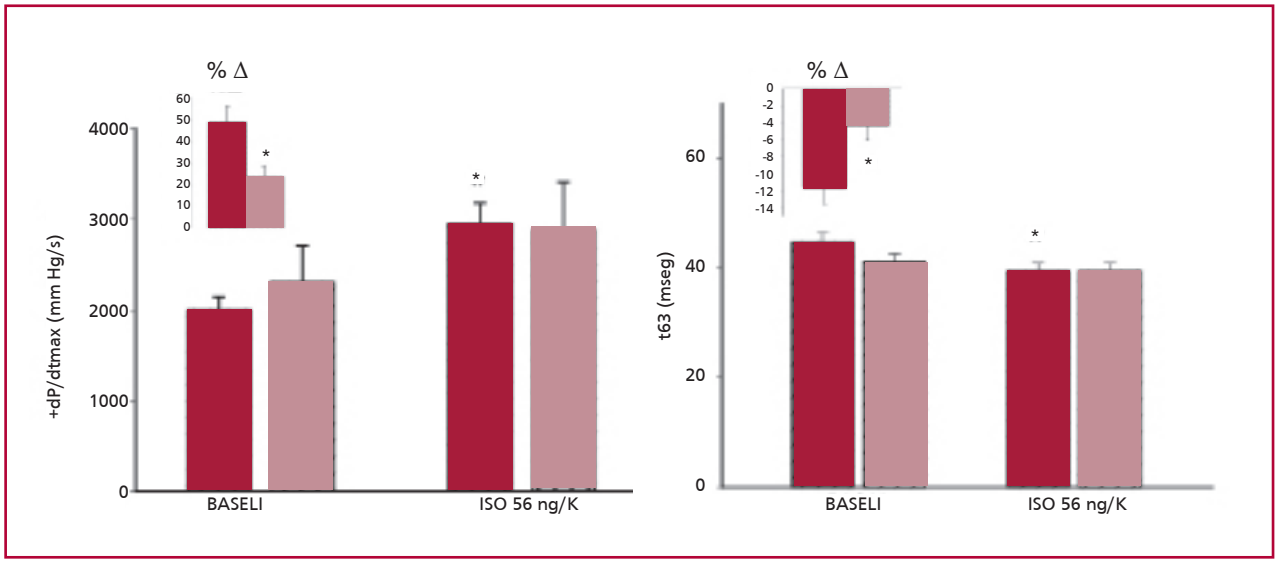

Fig. 2. Baseline values and inotropic, chronotropic and lusitropic response to ISO (56 ng/kg) of the two in vitro experimental groups. A. $+dP/dt_{max}$ graph, where the blue bars represent the sedentary group and the light blue bars represent the exercise group. The first two bars show baseline values for each group, and the second bars show the response to ISO. B. Isovolumic relaxation graph, evaluated by the index t_{63}, where first two bars show baseline values for each group, and the second bars show the response to ISO. * $p<0.05$ vs. baseline.

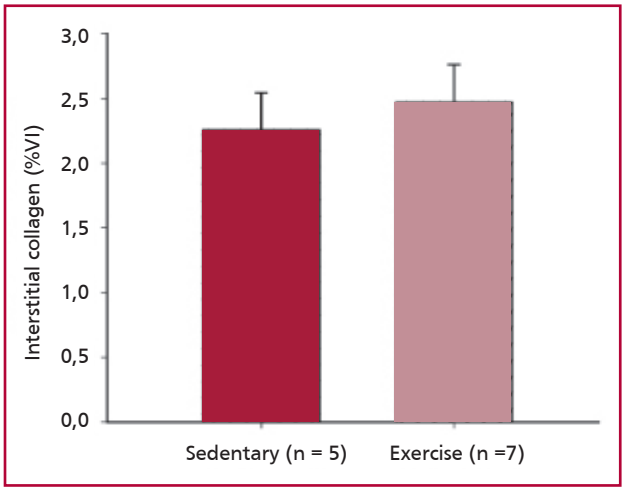

Fig. 3. Bar graph representing the two experimental groups for quantification of interstitial collagen (percent collagen in the LV per field). The blue bar represents the sedentary group and the light blue bar the exercise group.

of myocardial hypertrophy developed after chronic exercise. In this sense, there is general agreement that exercise is a beneficial intervention for the heart because it develops a "physiological" or adaptive hypertrophy, which is clearly different from the "pathological" or maladaptive hypertrophy secondary to different cardiac conditions, of which hypertension (5,17) and aortic stenosis (18) stand out as the most important. Adaptive hypertrophy is characterized by preserved ventricular function, absence of changes in collagen matrix and greater capillary density that is proportional to the increase in myocyte size. On the other hand, maladaptive hypertrophy presents normal or depressed ventricular function with reduced myocardial reserve, increased collagen matrix and decreased capillary density. (19)

During the last years, it has been noted that the major determinant of hypertrophy is not only the type of stimulus (exercise for adaptive hypertrophy or pressure or volume overload for maladaptive hypertrophy) but also the intensity and type of exercise. (20) Experimental evidence suggests that mild to moderate exercise stimulates adaptive hypertrophy, (5, 21) while strenuous exercise seems to generate maladaptive hypertrophy with the functional and structural characteristics previously described. Interestingly, the strenuous swimming protocol performed by mice in our study (9, 11) induced moderate hypertrophy with both adaptive and maladaptive characteristics, as the animals not only preserved systolic and diastolic ventricular function and normal collagen matrix but presented significant reduction in inotropic, chronotropic and lusitropic reserve, as seen in maladaptive hypertrophy. As opposed to strenuous exercise, myocardial reserve has been well studied in maladaptive hypertrophy secondary to hypertension or aortic stenosis. (22, 23)

CONCLUSIONS

Our results show that mice subjected to strenuous exercise with a swimming protocol have different functional and structural aspects which do not allow a clear characterization of the type of hypertrophy attained in the study, as it presented features of adaptive hypertrophy and reduced inotropic, chronotropic and lusitropic reserve characteristic of maladaptive hypertrophy.

RESUMEN

Efectos del ejercicio intenso sobre la función ventricular basal y la respuesta inotrópica, cronotrópica y lusitrópica en ratones

Introducción
El ejercicio leve a moderado reduce los factores de riesgo cardiovascular, mejora estados patológicos previamente establecidos y produce el desarrollo de hipertrofia cardíaca adaptativa. Sin embargo, la respuesta del miocardio frente a un tipo de ejercicio intenso no es del todo conocida.

Objetivo
Estudiar la función ventricular basal y la reserva miocárdica (respuesta inotrópica, cronotrópica y lusitrópica frente a un agonista ß-adrenérgico como el isoproterenol) luego de un tipo de ejercicio intenso tanto in vivo como in vitro en ratones.

Material y métodos
Se utilizaron ratones macho de tres meses de edad de la cepa FVB. El protocolo de ejercicio consistió en dos sesiones diarias de 90 minutos de natación, 6 días/semana durante 4 semanas. Se conformaron dos grupos experimentales: 1) Sedentario: no realiza ejercicio y 2) Ejercicio: realiza protocolo completo de natación intenso.

Resultados
Al finalizar el protocolo hubo un incremento de la masa ventricular izquierda del 27,9% ± 4%, con función ventricular basal conservada. Sin embargo, hubo una disminución de la respuesta miocárdica al isoproterenol tanto in vivo como in vitro, sin observarse modificaciones en el colágeno intersticial.

Conclusiones
En nuestras condiciones experimentales, el protocolo de natación, con características de ejercicio intenso, produjo una hipertrofia cardíaca moderada con características mixtas de hipertrofia adaptativa y no adaptativa. Si bien la función basal se mantuvo conservada y no hubo cambios en el colágeno intersticial, se observó una disminución en la reserva inotrópica, cronotrópica y lusitrópica.

Palabras clave > Ejercicio - Hipertrofia - Función ventricular

Conflicts of interest
None declared.

Acknowledgments
We are grateful to histotechnologists Ana Chiaro and Victor Hugo Tomasi and to Natalia Wulf, technician at the Central Animal Care House, Institute of Cardiovascular Pathophysiology, School of Medicine, University of Buenos Aires, for their collaboration in sample care and processing. We also appreciate the cooperation of Dr Pablo Cassaglia and of the students Gabriel H. Amaya, Luz Peña and Florencia Lalor.

REFERENCES

1. Powers SK, Lennon SL, Quindry J, Mehta JL. Exercise and cardioprotection. Curr Opin Cardiol 2002;17:495-502. http://doi.org/dzczzv
2. Grossman W. Cardiac hypertrophy: useful adaptation or pathologic process? Am J Med 1980;69:576-84. http://doi.org/cg7h7c
3. Benito B, Gay-Jordi G, Serrano-Mollar A, Guasch E, Shi Y, Tardif J-C, et al. Cardiac arrhythmogenic remodeling in a rat model of long-term intensive exercise training. Circulation 2011;123:13-22. http://doi.org/dhgwsv
4. McMullen JR, Jenning GL. Diferences between pathological and physiological cardiac hypertrophy: novel therapeutic strategies to treat heart failure. Clin Exp Pharmacol Physiol 2007;34:255-62. http://dx.doi.org/10.1111/j.1440-1681.2007.04585.x
5. Garciarena CD, Pinilla OA, Nolly MB, Laguens RP, Escudero EM, et al. Endurance training in the spontaneously hypertensive rat. Hypertension 2009;53:708-14. http://doi.org/cwqvjk
6. Weeks KL, McMuller JR. The athlete's heart vs. the failing heart: can signaling explain the two distinct outcomes? Physiology 2011;26:97-105. http://doi.org/d2bw2d
7. Vitiello D, Boissiere J, Doucende G, Gayrard S, Polge A, Faure P, et al. Adrenergic receptors desensitization is not involved in exercise-induced cardiac fatigue: NADPH oxidase-induced oxidative stress as a new trigger. J Appl Physiol 2011;111:1242-8. http://doi.org/cmq3jm
8. Madeiros A, Oliveira EM, Gianolla R, Casarini DE, Negrao CE, Brum PC. Swimming training increases cardiac vagal activity and induces cardiac hypertrophy in rats. Braz J Med Biol Res 2004;37:1909-17.
9. Evangelista FS, Brum PC, Krieger JE. Duration-controlled swimming exercise training induce cardiac hypertrophy in mice. Braz J Med Biol Res 2003;36:1751-1759. http://doi.org/bvw42v
10. Batista EC, Batista EC, Ramalho JDS, Reis FCG, Barros CC, Moraes MR, et al. Swimming training exacerbates pathological cardiac hypertrophy in B2 receptor-deficient mice. Int Immunopharmacol 2008;8:271-5. http://doi.org/b4m8rq
11. Ikeda H, Shiojima I, Ozasa Y, Yoshida M, Holzenberger M, Kahn CR, et al. Interaction of myocardial insulin receptor and IGF receptor signaling in exercise-induced cardiac hypertrophy. J Mol Cell Cardiol 2009;47:664-75. http://doi.org/b4m8rq
12. Kaplan ML, Cheslow Y, Vikstrom K, Malhotra A, Geenen DL, Nakouzi A, et al. Cardiac adaptations to chronic exercise in mice. Am J Physiol 1994;267:1167-73. http://doi.org/dzfbhz
13. Hafstad AD, Boardman NT, Lund J, Hagve M, Khalid AM, Wisloff U, et al. High intensity interval training alters substrate utilization and reduce oxygen consumption in the heart. J Appl Physiol 2011;111:1235-41. http://doi.org/d8npwr
14. Marmor A, Schneeweiss A. Prognostic value of non invasively obtained left ventricular contractile reserve in patients with severe heart failure. J Am Coll Cardiol 1997;29: 422-8. http://doi.org/c76vns
15. Naqvi T, Goel RK, Forrester JS. Myocardial contractile reserve on dobutamine echocardiography predicts late spontaneous improvement in cardiac function in patients with recent onset idiopathic cardiomyopathy. J Am Coll Cardiol 1999;34:1537-44. http://doi.org/c76vns
16. Hees PS, Fleg JL, Mirza ZA, Ahmed S, Siu CO, Shapiro EP. Effects of normal aging on left ventricular lusitropic, inotropic, and chronotropic responses to dobutamine. J Am Coll Cardiol 2006;47:1440-7. http://doi.org/c76vns
17. Liu W, Jim J, Prehar S, Oceandy D, Kimura TE, Lei M, et al. Cardiac-specific deletion of Mkk4 reveals its role in pathological hypertrophic remodeling but not in physiological cardiac growth. Circ Res 2009;104:905-14http://doi.org/cbrr8d
18. Golia G, Milano AD, Dodonov M, Bergamini C, Faggian G, Tomezzoli A, et al. Influence of myocardial fibrosis on left ventricular hypertrophy in patients with symptomatic severe aortic stenosis. Cardiology 2011;120:139-45. http://doi.org/fxphmh
19. Hittinger L, Shannon RP, Bishop SP, Gelpi RJ, Vatner SF. Subendomyocardial exhaustion of blood flow reserve and increased fibrosis in conscious dogs with heart failure. Circ Res 1989;65:971-80. http://doi.org/kwr
20. Pelliccia A, Maron MA, Maron BJ. Assessment of left ventricular hypertrophy in a trained athlete: differential diagnosis of physiologic athlete's heart from pathologic hypertrophy. Prog Cardiovasc Dis 2012;54:387-96. http://doi.org/kws
21. Ellison GM, Waring CD, Vicinanza C, Torella D. Physiological cardiac remodelling in response to endurance exercise training: cellular and molecular mechanisms. Heart 2012;98:5-10. http://doi.org/fh7vm3
22. Mills GD, Kubo H, Harris DM, Berretta RM, Piacentino V, Houser SR. Phosphorylation of phospholamban at threonine-17 reduces cardiac adrenergic contractile responsiveness in chronic pressure overload-induced hypertrophy. Am J Physiol Heart Circ Physiol 2006;291:H61-70. http://doi.org/dkxzxx
23. Pinz I, Tian R, Belke D, Swanson E, Dillmann W, Ingwall JS. Compromised myocardial energetics in hypertrophied mouse hearts diminish the beneficial effect of overexpressing SERCA2a. J Biol Chem 2011;286:10163-8. http://doi.org/c9zc33.