ORIGINAL ARTICLE

Effects of Endurance Training on Myocardial Hypertrophy and Ventricular Function in a Transgenic Mouse Model with Sympathetic Hyperactivity

Efectos del ejercicio intenso sobre la hipertrofia miocárdica y la función ventricular en un modelo de ratón transgénico con hiperactividad simpática

LUCIANA WILENSKY, NADIA L. MARTÍNEZ NAYA, JAZMIN KELLY, PABLO CASSAGLIA, MARÍA E. ARUANNO, BRUNO BUCHHOLZ[MTSAC], ISAAC L. MOGUNOVSKY MICHELL, CELINA MORALES[MTSAC], GERMÁN E. GONZÁLEZ

ABSTRACT

Background: Previous studies have shown that endurance training (ET) reduces inotropic, chronotropic and lusitropic reserve in normal mice.
Objective: The aim of this study was to evaluate the effect of endurance training on the inotropic and chronotropic reserve of transgenic mice with sympathetic hyperactivity induced by overexpression of the cardiac GSα protein.
Methods: Endurance training consisted in two daily 90-min sessions, 6 days/week, during 4 weeks. Four experimental groups were formed: 1) non-transgenic sedentary (nonTG Sed); 2) transgenic sedentary (TG Sed); 3) nonTG+ET and 4) TG+ET.
Results: Endurance training induced myocardial hypertrophy [left ventricular weight (g)/tibial length (mm)] from 5.3±0.3 and 5.5±0.2 in nonTG Sed and TG Sed to 6.8±0.1 and 6.8±0.3 in nonTG+ET and TG+ET, respectively ($p<0.05$ nonTG Sed vs. nonTG+ET and TG Sed vs. TG+ET). Isoproterenol administration (56 ng/kg) increased +dP/dtmax by 63±10% in nonTG Sed ($p<0.05$ vs. baseline), 34±2% in TG Sed ($p<0.05$ vs. baseline and $p<0.05$ vs. nonTG Sed), 36±7% in non TG+ET ($p<0.05$ vs. baseline) and 36±7% in TG+ET ($p<0.05$ vs. baseline). Heart rate (beats/min) increased from 301±15 to 528±37 in nonTG Sed ($p<0.05$ vs. baseline), from 519±57 to 603±41 in TG Sed, from 300±16 to 375±20 in nonTG+ET ($p<0.05$ vs. baseline) and from 484±18 to 515±21 in TG+ET. Interstitial collagen was similar among groups.
Conclusions: These results suggest that endurance training decreases inotropic and chronotropic reserve without generating structural changes associated to pathological hypertrophy. The presence of sympathetic hyperactivity does not modify this response.

Key words: Exercise Tolerance - Physical Conditioning, Animal - Sympathetic Nervous System - Isoproterenol

RESUMEN

Introducción: En estudios previos mostramos que el ejercicio intenso (EI) reduce la reserva inotrópica, cronotrópica y lusitrópica en ratones normales.
Objetivo: Evaluar el efecto del ejercicio intenso sobre la reserva inotrópica y cronotrópica en un modelo de ratones transgénicos con sobreexpresión cardíaca de la proteína Gsα, que induce un cuadro de hiperactividad simpática.
Material y métodos: El ejercicio consistió en dos sesiones diarias de 90 minutos de natación, 6 días/semana durante 4 semanas. Se utilizaron cuatro grupos experimentales: 1: sedentario no transgénico (noTG Sed); 2: sedentario TG (TG Sed); 3: noTG+EI y 4: TG+EI.
Resultados: El ejercicio indujo el desarrollo de hipertrofia miocárdica [índice peso del ventrículo izquierdo (g)/longitud de la tibia (mm)] desde 5,3±0,3 y 5,5±0,2 en noTG Sed y TG Sed a 6,8±0,1 y 6,8±0,3 en noTG+EI y TG+EI, respectivamente ($p<0,05$ noTG Sed vs. noTG+EI y TG Sed vs. TG+EI). La administración de isoproterenol (56 ng/kg) incrementó la +dP/dtmáx 63% ±10% en noTG Sed ($p<0,05$ vs. basal); 34% ±2% en TG Sed ($p<0,05$ vs.basal y $p< 0,05$ vs. noTG Sed); 36% ±7% en noTG+EI ($p<0,05$ vs. basal) y 36% ±7% en TG+EI ($p<0,05$ vs.basal). La frecuencia cardíaca aumentó de 301±15 a 528±37 latidos/min en noTG Sed ($p<0,05$ vs. basal), de 519±57 a 603±41 latidos/min en TG Sed, de 300±16 a 375±20 en noTG+EI ($p<0,05$ vs. basal) y de 484±18 a 515±21 en TG+EI. El colágeno intersticial fue similar entre los grupos.
Conclusiones: Estos resultados sugieren que el ejercicio intenso disminuye la reserva inotrópica y cronotrópica sin generar cambios estructurales vinculados a la hipertrofia patológica. La presencia de hiperactividad simpática no modifica esta respuesta.

Palabras clave: Tolerancia al ejercicio - Condicionamiento físico animal - Sistema nervioso simpático - Isoproterenol

Rev Argent Cardiol 2015;83:501-505. http://dx.doi.org/10.7775/rac.v83.i6.7093

Received: 08/24/2015 - *Accepted:* 09/30/2015

Address for reprints: Dr. Germán E. González - Departamento de Patología - Facultad de Medicina, Universidad de Buenos Aires - Pte. Uriburu 950, 2. Piso - CABA, Argentina - e-mail: gegonzal@fmed.uba.ar

Department of Pathology, School of Medicine,Universidad de Buenos Aires
[MTSAC] Full Member of the Argentine Society of Cardiology

Abbreviations

beats/min	Beats per minute	**mRNA**	Messenger ribonucleic acid
+dP/dt$_{máx}$	Maximum time derivative of pressure	**nonTG**	Non transgenic
ET	Endurance training	**RV**	Right ventricle
HR	Heart rate	**Sed**	Sedentary
ISO	Isoproterenol	**TG**	Transgenic
LV	Left ventricle	**TL**	Tibial length

INTRODUCTION

Different studies have shown that regular physical activity prevents cardiac diseases and that chronic exercise attenuates the main cardiovascular risk factors. (1) Depending on the type, exercise can be associated with mild cardiac dilation, which represents a favorable adaptation of the heart to compensate increased functional demand, allowing it to preserve or even increase ventricular function. (2, 3) Previous studies indicated that low-intensity exercise may delay the development of heart failure and improve survival in spontaneously hypertensive rats, suggesting that moderate to mild exercise has beneficial effects. (4) However, experimental protocols of chronic endurance training (ET) performed in rats, showed that the animals developed cardiac hypertrophy with pathologic characteristics, increasing pro-fibrotic markers, right ventricular fibrosis, arrhythmia susceptibility and ventricular dysfunction. (5) In this sense, despite exercise is recommended as an efficient therapeutic strategy for different cardiovascular pathologies, the intensity/response relationship is still poorly understood and the mechanisms have not been fully investigated. (6) We have previously shown that ET reduces inotropic, chronotropic and lusotropic reserve in normal mice. (7) Since sympathetic activity is part of the physiology of exercise, the aim of this study was to analyze whether the decrease in inotropic and chronotropic reserve produced by ET was present in a transgenic model of sympathetic hyperactivity submitted to an ET protocol.

METHODS

Male, 3-month-old mice (31±1 g) corresponding to the non-transgenic FVB strain (nonTG) and the TG strain overexpressing the specific cardiac Gsα protein were used. Gsα protein overexpression was achieved through the insertion of the sequence contiguous to the promoter gene of the α-myosin heavy chain expressed in cardiomyocytes, using the lines showing 38-fold mRNA increase and 2.8 elevation of Gsα protein content. This genetic modification is phenotypically evident by the characteristic sympathetic hyperactivity of the model, described as a significant increase of heart rate and contractility. (8, 9)

Experimental protocol

An intense exercise protocol consisting of two daily 90-min sessions, 6 days a week, was performed during 4 weeks. Mice were placed in a 40×70×30 cm pool with water maintained at 30-32 ºC via a temperature stabilizer. (10) At the beginning of the protocol, the animals were adapted for a week, starting with a 20-minute swimming period which was extended in the successive days to reach the complete protocol time. (11-13) The animals were housed at 20-22 ºC with 12 h light/darkness cycles and ad libitum food and water availability.

Experimental groups

Four experimental groups were formed: 1) Control nonTG sedentary group (n=14): animals did not perform exercise and were kept in their respective cages until euthanasia; 2) Control TG sedentary group (n=5): animals did not perform exercise and were kept in their respective cages until euthanasia; 3) nonTG+ET (n=14): animals performed the ET protocol previously described, and 4) TG+ET (n=16): TG mice performed the ET protocol previously described.

After completion of the protocol period all the experimental groups underwent in vivo baseline ventricular function and inotropic and chronotropic reserve studies followed by complete autopsy.

In vivo ventricular function studies

After completion of the protocol period, the nonTG (n=8), TG (n=3), nonTG+ET (n=9) and TG+ET (n=8) groups were weighed and anesthetized with ketamine (100 mg/kg) and xylazine (2.5 mg/kg). The right carotid artery was dissected and a heparinized catheter was inserted and advanced into the left ventricle (LV). The left jugular vein was also dissected and another catheter was inserted for intravenous bolus injection of isoproterenol (ISO, 56 ng/kg). After stabilization (10 minutes), baseline left ventricular pressure, its first derivative (+dP/dt$_{max}$, mmHg/s) and heart rate (HR, beats/min) were recorded. (14) The same variables were recorded for each group after ISO administration, using a computer equipped with an analog to digital converter (National Instruments) and software for data acquisition and analysis. (15)

Assessment of cardiac hypertrophy

After the ventricular function study, the animals were sacrificed and the corresponding autopsy was done. The complete cardiopulmonary block was removed and the LV, the RV and both atria were dissected and weighed. The LV was fixed in formaldehyde buffer for later inclusion in paraffin and staining for collagen quantification. Body weight was also recorded before and after the exercise protocol and tibial length (TL) was measured to calculate hypertrophy coefficients (LV/body weight (BW) and LV/TL). (16) Both indexes were considered and compared, as BW evidenced great variability with training; however, TL is a growth index that does not change with exercise.

Quantification of interstitial collagen

Interstitial collagen was quantified with colorimetric technique in histological sections of nonTG (n=5), TG (n=3), nonTG+ET (n=7) and TG+ET (n=7) groups stained with picrosirius red, using Image-Pro Plus 6.0 digital image analyzer. Results were expressed as percent collagen in the total LV per field. Perivascular collagen was not considered in any group. (17)

Statistical analysis

Data are expressed as mean±standard error of the mean. Sigma STAT32 software package was used for data statistical analyses and ANOVA followed by Bonferroni to compare among groups. A p value <0.05 was considered statistically significant.

Ethical considerations

All the experiments were performed according to the National Institute of Health "Guide for the Care and Use of Laboratory Animals" (NIH Publication 85-3, revised 1985).

RESULTS

All the animals that started finished the exercise protocol without any deaths from exhaustion or other causes.

Endurance training significantly increased the myocardial hypertrophy index similarly in both groups (nonTG+ET and TG+ET) (Table 1).

In the in vivo ventricular function study, we confirmed that baseline HR (Figure 1) and myocardial contractility ($+dP/dt_{max}$, Figure 2) were significantly increased in TG animals (sedentary (Sed) and exercise), in response to the sympathetic hyperactivity of the experimental model.

Heart rate in nonTG (nonTG Sed: 301±15 vs. nonTG+ET: 300±16 beats/min) as in TG mice submitted to exercise was not modified with respect to their corresponding baseline values (TG Sed: 519±57 vs. TG+ET: 484±18 beats/min) (Figure 1A). Isoproterenol administration only increased HR in nonTG groups (nonTG Sed: from 301±15 to 528±37 beats/min, and nonTG+ET: from 300±16 to 375±20 beats/min, p<0.05), with no significant changes in TG groups (TG Sed: from 519±57 to 603±41 beats/min and TG+ET: from 484±18 to 515±21 beats/min). The percent increase in HR (Figure 1B) revealed a significantly lower response in TG Sed, nonTG+ET and TG+ET groups compared with the nonTG Sed group (TG Sed: 13.7%±4.5%; nonTG+ET: 27.7%±5.3 %; TG+ET: 9.9%±1.3%, p<0.05).

Exercise did not modify baseline contractile state, assessed through $+dP/dt_{max}$, in nonTG (nonTG Sed: 5315±382 mmHg/s, nonTG+ET: 6350±531 mmHg/s), nor in TG (TG Sed: 7681±513 mmHg/s, TG+ET: 7495±219 mmHg/s) groups (Figure 2). Myocardial contractility did not increase significantly with ISO administration in all the experimental groups (nonTG Sed: from 5314±382 mmHg/s to 9218±605 mmHg/s; TG: from 7681±513 mmHg/s to 10084±421 mmHg/s; nonTG+ET: from 6350±531 mmHg/s to 8128±374 mmHg/s and TG+ET: from 7495±219 mmHg/s to 10226±679 mmHg/s; p<0.05 for all cases), although the percent increase was different among groups,

Table 1. Autopsy results

Groups	BW (g)		TL	LV	LV/BW	LV/TL	Lung
	Before	After	(mm)	(mg)	(mg/g)	(mg/mm)	(mg)
nonTG Sed (n=14)	-	31.1±1	18.3±0.1	97.5±4.2	3.12±0.21	5.3±0.2	183±7
TG Sed (n=5)	-	30.8±1	17.9±0.3	97.8±5	3.2±0.03	5.5±0.3	192±19
nonTG+ET (n=14)	31.5±1	30.5±1	18.2±0.1	124.9±4*	4.1±0.1*	6.8±0.1*	210±11
TG+ET (n=16)	32.4±1	32.6±1	18.3±0.1	124.2±4&	3.8±0.1&	6.9±0.2&	210±9

nonTG: Non transgenic. TG: Transgenic. Sed: Sedentary. ET: Endurance training. BW: Body weight before and after exercise. TL: Tibial length. LV: Left ventricular weight. LV/BW: Left ventricle/body weight ratio. LV/TL: Left ventricle/tibial length ratio. Lung: Lung wet weight. * p<0.05 vs. nonTG; & p<0.05 vs. TG.

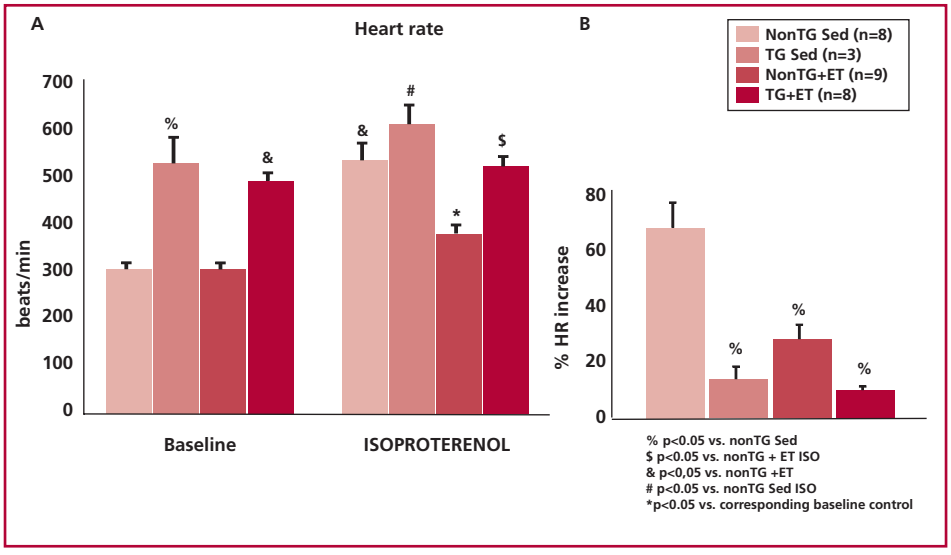

Fig. 1. Baseline and isoproterenol (ISO) (56 ng/Kg) chronotropic response in the experimental groups submitted to in vivo ventricular function studies, showing both baseline and ISO response values. **A.** The first 4 bars shows baseline heart rate (HR), and the second 4 bars the response to ISO. **B.** Percent increase for each of the experimental groups. % p<0.05 vs. nonTG; & p<0.05 vs. nonTG+ET; * p<0.05 vs. the corresponding baseline value; # p<0.05 vs. nonTG+ISO. HR: Heart rate. nonTG: Non transgenic. TG: Transgenic. Sed: Sedentary. ET: Endurance training.

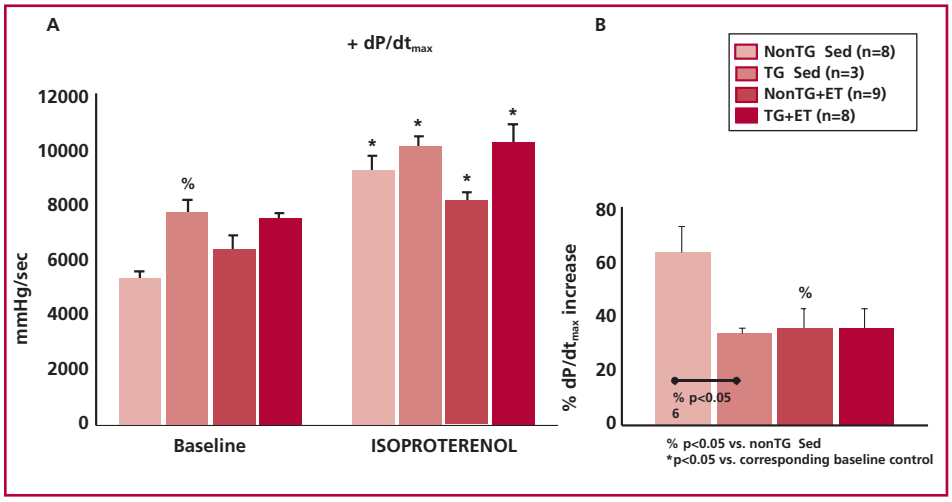

Fig. 2. Baseline and inotropic response to isoproterenol (ISO) administration (56 ng/kg) in the experimental groups submitted to in vivo ventricular function studies. **A.** The first 4 bars show baseline $+dP/dt_{max}$, and the second 4 bars the response to ISO. **B.** Percent increase for each of the experimental groups. % p<0.05 vs. nonTG; * p<0.05 vs. the corresponding baseline value. nonTG: Non transgenic. TG: Transgenic. Sed: Sedentary. ET: Endurance training. $+dP/dt_{max}$: Maximum time derivative of pressure.

with a significant decrease in the response to ISO in TG Sed (34%±2%), nonTG+ET (36%±7%) and TG+ET (36%±7%) groups, compared with nonTG Sed (63%±10%, p<0.05 vs. all the other groups) (Figure 2B).

Interstitial collagen was similar in all the experimental groups (nonTG Sed: 2.25%±0.30%, TG Sed: 2.50%±0.4%, nonTG+ET: 2.46%±0.30% and TG+ET: 2.65%±0.54%) (Figure 3).

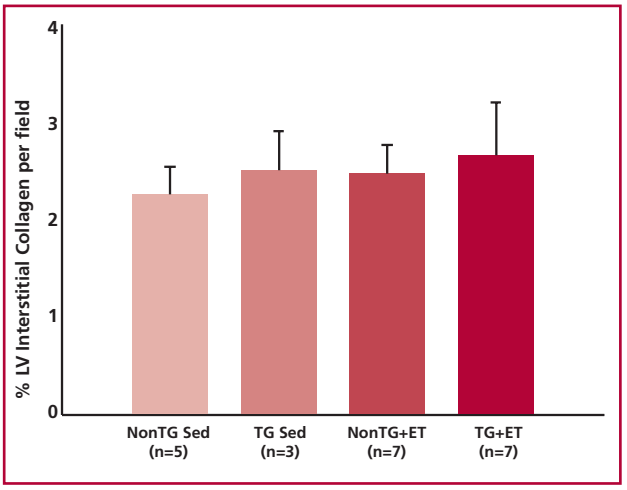

Fig. 3. Interstitial collagen assessed in histological sections stained with picrosirius red in all the experimental groups. There were no significant differences in all the groups studied. LV: Left ventricular. nonTG: Non transgenic. TG: Transgenic. Sed: Sedentary. ET: Endurance training.

DISCUSSION

The present study showed that ET in normal mice, as well as in those with specific cardiac Gsα overexpression produces a significant impairment of inotropic and chronotropic reserve with no changes in interstitial collagen. Moreover, results showed that the decreased inotropic reserve in normal animals was similar to that seen in both TG groups, suggesting that the effect of ET did not enhance the decrease of contractile reserve in TG mice. In agreement with previous studies (4, 5, 7, 10), no deaths from exertion were observed in the groups that performed ET, probably due to the previous week of adaptation before starting the protocol, during which they got used to the environment and improved their aerobic capacity.

In previous studies we showed that ET decreases inotropic, chronotropic and lusotropic reserve in mice. (7) The present work extends these findings analyzing ET-induced inotropic and chronotropic reserve impairment in a TG model with specific cardiac sympathetic hyperactivity. Thus, the present study showed that overexpression of the cardiac protein Gsα increases HR as well as contractility in baseline conditions. However, these animals developed myocardial hypertrophy secondary to ET, similarly to nonTG animals. Also, a decreased inotropic and chronotopic reserve in nonTG+ET mice was observed, not generating the expected cardioprotection. Moreover, TG+ET animals preserved their functional parameters compared with their sedentary control (TG Sed), as these started from higher values than the nonTG Sed group, without modification of the values in response to beta-adrenergic stimulation.

In the in vivo ventricular function assessment, the changes in HR could modify contractility, and since the TG model has a significant increase in HR, these data should be confirmed evaluating ventricular function and inotropic reserve in an in vitro model allowing a strict control of variables, as for example constant HR, ventricular volume and coronary flow. (18, 19)

In our experimental conditions, the hearts of animals that were submitted to exercise (nonTG and TG) developed myocardial hypertrophy without fibrosis, suggesting that, at least from a structural point of view, exercise produced adaptive hypertrophy. (6)

However, while it is well known that exercise activates the sympathetic nervous system, (20) the myocardial response to different levels of physical activity in the presence of sympathetic stimulation has not been identified. Our data suggest that although the TG model shows increased contractility and HR, this does not produce a greater deleterious effect, which is otherwise observed in nonTG animals, where a decreased inotropic and chronotropic reserve outweighs the beneficial effect of exercise.

CONCLUSIONS

Our results indicate that ET comparable to beta-adrenergic stimulation decreases in a non-additive way the contractile reserve without generating structural changes associated to maladaptive hypertrophy.

Conflicts of interest

None declared. (See authors' conflicts of interest forms in the website/Supplementary material).

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